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Feed Additives

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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 endo-1,4-beta-xylanase, endo-1,4-beta-glucanase and xyloglucan-specific-endo-1,4-beta-1,4-glucanase
(FAD-2021-0036; CRL/200083)

European Union Reference Laboratory
Feed Additives

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-2021-0036 - CRL/200083**
Name of Feed Additive:	***Preparation of endo-1,4-beta-xylanase, endo-1,4-beta-glucanase and xyloglucan-specific-endo-1,4-beta-1,4-glucanase***
Active Agent (s):	**endo-1,4-beta-xylanase (EC 3.2.1.8)** **endo-1,4-beta-glucanase (EC 3.2.1.4)** **xyloglucan-specific-endo-1,4-beta-1,4-glucanase (EC 3.2.1.151)**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC Geel, Belgium**
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Report checked by: Date:	**Zigmas Ezerskis** **29/11/2021**
Report approved by: Date:	**Christoph von Holst** **29/11/2021**

EXECUTIVE SUMMARY

In the current application an authorisation of a *preparation of endo-1,4-beta-xylanase, endo-1,4-beta-glucanase and xyloglucan-specific-endo-1,4-beta-1,4-glucanase* is sought under Article 4 under the category/functional group 4(a) "zootechnical additives"/"digestibility enhancers" according to Annex I of Regulation (EC) No 1831/2003. The authorisation is sought for the use of the *feed additive* for poultry species, ornamental birds and piglets (weaned and suckling).

According to the Applicant, the *feed additive* contains three active substances: (i) *endo-1,4-beta-xylanase* (EC 3.2.1.8), (ii) *endo-1,4-beta-glucanase* (EC 3.2.1.4) and (iii) *xyloglucan-specific-endo-beta-1,4-glucanase* (EC 3.2.1.151), which are produced by *Trichoderma citrinoviride B-125 (DSM 33578)*.

The activity of: (i) *endo-1,4-beta-xylanase* is expressed in endo-pentosanase units (EPU), where one EPU unit is the amount of enzyme, which liberates 0.0083 micromoles of reducing sugars (xylose equivalents) from oat spelt xylan per minute at pH 4.7 and 50 °C; (ii) *endo-1,4-beta-glucanase* is expressed in cellulase units (CU), where one CU unit is the amount of enzyme that liberates 0.128 micromoles of reducing sugars (glucose equivalents) from barley beta-glucan per minute at pH 4.5 and 30 °C; and (iii) *xyloglucan-specific-endo-beta-1,4-glucanase* is expressed in xyloglucanase unit (XGU), where one XGU unit is the amount of enzyme that releases low-molecular fragments from dyed xyloglucan in amount equal to the amount of such fragments liberated from 1 unit enzyme standard under the conditions of the assay (50 °C and pH 4.5). The *feed additive* is intended to be marketed as solid and liquid formulations having the following guaranteed minimum activities: 15000 EPU/g for *endo-1,4-beta-xylanase*, 1000 CU/g for *endo-1,4-beta-glucanase* and 1000 XGU/g for *xyloglucan-specific-endo-beta-1,4-glucanase*. The solid product is intended to be incorporated through *premixtures* or directly into *feedingstuff*s while the liquid formulation should be applied after the pelleting process by spraying it on the pellets to obtain a minimum content for *endo-1,4-beta-xylanase*, *endo-1,4-beta-glucanase* and *xyloglucan-specific-endo-beta-1,4-glucanase* respectively of 1500 EPU, 100 CU and 100 XGU/kg *feedingstuffs*.

For the quantification of the *endo-1,4-beta-xylanase* activity in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted single-laboratory validated and further verified colorimetric 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.

For the quantification of the *endo-1,4-beta-glucanase* activity in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted a single-laboratory validated and

further verified colorimetric method based on the quantification of water soluble dyed fragments produced by the action of *endo-1,4-beta-glucanase* on commercially available azurine cross-linked cellulose substrate from Megazyme.

For the quantification of *xyloglucan-specific-endo-beta-1,4-glucanase* activity in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted a single-laboratory validated and further verified colorimetric method based on the quantification of soluble dyed labelled fragments produced at pH 4.5 and 50°C by the action of *xyloglucan-specific-endo-beta-1,4-glucanase* on commercially available xyloglucan substrate from Megazyme.

Based on the acceptable performance characteristics presented the EURL recommends for official control the single-laboratory validated and further verified colorimetric methods submitted by the Applicant for the determination of the activity of *endo-1,4-beta-xylanase*, *endo-1,4-beta-glucanase* and *xyloglucan-specific-endo-beta-1,4-glucanase* 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

Huvezym® neXo 100 G; Huvezym® neXo 100 L; preparation of endo-1,4-beta-xylanase (EC 3.2.1.8); endo-1,4-beta-glucanase (EC 3.2.1.4); xyloglucan-specific-endo-beta-1,4-glucanase (EC 3.2.1.151); Trichoderma citrinoviride B-125 (DSM 33578); zootechnical additives digestibility enhancer; poultry species, ornamental birds and piglets (weaned and suckling).

1. BACKGROUND

In the current application an authorisation of a *preparation of endo-1,4-beta-xylanase, endo-1,4-beta-glucanase and xyloglucan-specific-endo-1,4-beta-1,4-glucanase* is sought under Article 4(1) under the category/functional group 4(a) "zootechnical additives"/"digestibility enhancers" according to Annex I of Regulation (EC) No 1831/2003 [1]. The authorisation is sought for the use of the *feed additive* for poultry species, ornamental birds and piglets (weaned and suckling) [2].

According to the Applicant, the *feed additive* contains three active substances: (i) *endo-1,4-beta-xylanase* (EC 3.2.1.8), (ii) *endo-1,4-beta-glucanase* (EC 3.2.1.4) and (iii) *xyloglucan-specific-endo-beta-1,4-glucanase* (EC 3.2.1.151), which are produced by the non-genetically modified strain *Trichoderma citrinoviride B-125 (DSM 33578)* [3].

The activity of: (i) *endo-1,4-beta-xylanase* is expressed in endo-pentosanase units (EPU), where one EPU unit is the amount of enzyme which liberates 0.0083 micromoles of reducing

sugars (xylose equivalents) from oat spelt xylan per minute at pH 4.7 and 50 °C [4,5]; (ii) *endo-1,4-beta-glucanase* is expressed in cellulase units (CU), where one CU unit is the amount of enzyme that liberates 0.128 micromoles of reducing sugars (glucose equivalents) from barley beta-glucan per minute at pH 4.5 and 30 °C [6,7]; and (iii) *xyloglucan-specific-endo-beta-1,4-glucanase* is expressed in xyloglucanase unit (XGU), where one XGU unit is the amount of enzyme that releases low-molecular fragments from dyed xyloglucan in amount equal to the amount of such fragments liberated from 1 unit enzyme standard under the conditions of the assay (50 ºC and pH 4.5) [8].

The *feed additive* is intended to be marketed as a brownish granular powder (*Huvezym® neXo 100 G*) and as a yellowish to brown liquid (*Huvezym® neXo 100 L*) formulations, having the following guaranteed minimum activities: 15000 EPU/g for *endo-1,4-beta-xylanase*, 1000 CU/g for *endo-1,4-beta-glucanase* and 1000 XGU/g for *xyloglucan-specific-endo-beta-1,4-glucanase* [9].

Huvezym® neXo 100 G (solid formulation) is intended to be incorporated through *premixtures* or directly into *feedingstuff*s while *Huvezym® neXo 100 L* (liquid formulation) should be applied after the pelleting process by spraying it on the pellets [10,11]. The Applicant recommended to include the *feed additive* in *feedingstuffs* in order to obtain a minimum content for *endo-1,4-beta-xylanase*, *endo-1,4-beta-glucanase* and *xyloglucan-specific-endo-beta-1,4-glucanase* respectively of 1500 EPU, 100 CU and 100 XGU/kg *feedingstuffs* [10].

Note: The EURL has already evaluated analytical methods for the determination of *endo-1,4-beta-xylanase* (EC 3.2.1.8) and *endo-1,4-beta-glucanase* (EC 3.2.1.4) in the frame of several dossiers [12].

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 a *preparation of endo-1,4-beta-xylanase, endo-1,4-beta-glucanase and xyloglucan-specific-endo-1,4-beta-1,4-glucanase* 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)

Measurement of endo-1,4-beta-xylanase activity

For the quantification of the *endo-1,4-beta-xylanase* activity in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted single-laboratory validated and further verified colorimetric 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 [13]. The methods were already submitted for a different (FAD-2010-0001) authorised *feed additive* (4a1617) [5,14]. Consequently, the Applicant proposed the same standard operating procedures and provided the same validation and verification studies already submitted in the frame of the previous dossier [14].

For the determination of the activity of *endo-1,4-beta-xylanase* in the *feed additive* and in *premixtures*, the samples are prepared by extracting 1.0 g in 100 ml of acetate buffer. After centrifugation the sample solution is diluted with buffer to a concentration corresponding within 400 – 800 EPU/ml. After an incubation of 30 min at pH 4.7 and 50 °C, the reaction is stopped with 2-Amino-2-(hydroxymethyl)propane-1,3-diol (TRIS) solution. The rate of dye release is measured on a spectrophotometer at 590 nm and quantified against a reference enzyme standard with certified activity, available from the applicant upon request.

For the determination of the activity of *endo-1,4-beta-xylanase* in *feedingstuffs*, the Applicant proposes a single laboratory validated and further verified method based on standard additions. The feed sample (10 g) is extracted 15 min with 200 ml acetate buffer containing the spiking solution. After centrifugation, the solution is incubated at 50 °C and pH 4.7 for 150 min. The reaction is stopped by addition of TRIS solution and the absorbance is measured at 590 nm. The quantification is carried out by means of standard addition technique, where subsamples are taken and (i) a part of these subsamples are analysed as such and (ii) the other sub-samples are analysed after being fortified with known amounts of the reference standard. The following method performance characteristics were derived from the validation and verification studies as re-calculated by the EURL:

- for the *feed additive*: a relative standard deviation for *repeatability* (RSD_r) ranging from 1.9 to 3.3 %, a relative standard deviation for *intermediate precision* (RSD_{ip}) ranging from 1.9 to 3.3 % and a *recovery rate* (R_{Rec}) ranging from 101 to 104 %;

- for *premixtures*: a RSD_r ranging from 3.2 to 8.2 %, a RSD_{ip} of 3.2 % and a R_{Rec} ranging from 96 to 103 %; and
- for *feedingstuffs*: a RSD_r ranging from 7.6 to 16 %, a RSD_{ip} ranging from 8.9 to 16 % and a R_{Rec} ranging from 93 to 112 %.

Furthermore, a limit of detection (LOD) and a limit of quantification (LOQ) of 107 and 358 EPU/kg of *feedingstuffs*, respectively, were re-calculated by the EURL, based on the validation data provided by the Applicant [14].

Given the fact that i) the guaranteed minimum *endo-1,4-beta-xylanase* activity in the current product, ii) the proposed minimum *endo-1,4-beta-xylanase* activity in *feedingstuffs* and iii) the definition of the *endo-1,4-beta-xylanase* unit (EPU) is equivalent to the one proposed for the former evaluated dossier, the EURL considers that all the methods, validation and verification studies provided in the frame of the *FAD-2010-0001* dossier are also applicable and suitable for the current application. Furthermore, in the frame of the homogeneity and stability studies the Applicant satisfactory applied the above-mentioned methods to the current product, *premixtures* and *feedingstuffs* containing the *preparation of endo-1,4-beta-xylanase, endo-1,4-beta-glucanase and xyloglucan-specific-endo-1,4-beta-1,4-glucanase*, thus confirming the suitability of these methods [15,16].

Based on the above mentioned considerations and 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 determination of the activity of *endo-1,4-beta-xylanase* in the *feed additive*, *premixtures* and *feedingstuffs.*

Measurement of endo-1,4-beta-glucanase activity

For the quantification of the *endo-1,4-beta-glucanase* activity in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted a single-laboratory validated and further verified colorimetric method based on the quantification of water soluble dyed fragments produced by the action of *endo-1,4-beta-glucanase* on commercially available azurine cross-linked cellulose substrate from Megazyme [17]. The method was already submitted for a different (FAD-2010-0062) authorised *feed additive* (4a1616) [7,18]. Consequently, the Applicant proposed the same standard operating procedures and provided the same validation and verification studies already submitted in the frame of the previous dossier [18]. The activity of the samples is calibrated against reference enzyme standards with known activity determined at the definition conditions of the activity unit, available from the Applicant upon request. The traceability to the standard provided by the Applicant ensures the comparability of the measurement results.

For the determination of the activity of *endo-1,4-beta-glucanase* in the *product* and *premixtures*, 1 g of the sample is suspended in 200 mL acetate buffer, stirred for 15 min and centrifuged. The substrate tablets are added to an aliquot of the supernatant and the mixture is incubated in a water bath at 50°C for 2.5 h. The reaction is stopped by adding 6 mL TRIS solution. The solutions are cooled at room temperature for 10 min, shaken again and filtered through filter paper. The absorbance of the standard and sample solutions is measured with a spectrophotometer at 590 nm against water, using an external calibration. For samples of *feedingstuffs*, similar extraction and incubation conditions were applied using the technique of standard additions, starting with 10 g samples spiked with different amounts of standards.

The following method performance characteristics were derived from the validation and verification studies as re-calculated by the EURL:

- for the *feed additive*: a RSD_r ranging from 1.1 to 2.2 %, a RSD_{ip} ranging from 1.9 to 2.3 % and a R_{Rec} ranging from 97 to 103 %;
- for *premixtures*: a RSD_r ranging from 3.8 to 6.1 %, a RSD_{ip} ranging from 4.2 to 6.1 % and a R_{Rec} ranging from 92 to 118 %; and
- for *feedingstuffs*: a RSD_r ranging from 8.0 to 9.7 %, a RSD_{ip} ranging from 8.0 to 9.8 % and a R_{Rec} ranging from 96 to 100 %.

Furthermore, a LOD and a LOQ of 17 and 58 CU/kg *feedingstuffs*, respectively, were re-calculated by the EURL based on the validation data provided by the Applicant [18].

Given the fact that i) the guaranteed minimum *endo-1,4-beta-glucanase* activity in the current product and ii) the proposed minimum *endo-1,4-beta-glucanase* activity in *feedingstuffs* are above the established LOQ and iii) the definition of the *endo-1,4-beta-glucanase* unit (CU) is equivalent to the one proposed for the former evaluated dossier, the EURL considers that all the methods, validation and verification studies provided in the frame of the *FAD-2010-0062* dossier are also applicable and suitable for the current application. Furthermore, in the frame of the homogeneity and stability studies the Applicant satisfactory applied the above-mentioned methods to the current *product*, *premixtures* and *feedingstuffs* containing the *preparation of endo-1,4-beta-xylanase, endo-1,4-beta-glucanase and xyloglucan-specific-endo-1,4-beta-1,4-glucanase*, thus confirming the suitability of these methods [15,16].

Based on the above mentioned considerations and 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 determination of the activity of *endo-1,4-beta-glucanase* in the *feed additive*, *premixtures* and *feedingstuffs*.

Measurement of xyloglucan-specific-endo-beta-1,4-glucanase activity

For the quantification of *xyloglucan-specific-endo-beta-1,4-glucanase* activity in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted a single-laboratory validated and further verified colorimetric method based on the quantification of soluble dyed labelled fragments produced at pH 4.5 and 50 °C by the action of *xyloglucan-specific-endo-beta-1,4-glucanase* on commercially available xyloglucan substrate from Megazyme [19-24].

1 g of the *feed additive* sample is mixed with acetate buffer (pH 4.5), stirred and further diluted. The extract is left to decant and a sub-sample is centrifuged. The supernatant is used for preparing a series of additional dilutions with acetate buffer (0.03 to 0.06 XGU/ml). For *premixtures* and *feedingstuffs*, the extraction is carried out starting from 40 g of sample. For *premixtures* the samples are milled (particle size < 0.5 mm) and 1 g is homogenised with 39 g of maize meal. The samples are mixed with acetate buffer (pH 4.5), stirred and diluted. The extract is left to decant, a subsample is centrifuged, filtered and transferred into a clean test tube. In case of high expected activity, the analysed samples maybe additionally diluted (0.03 to 0.06 XGU/ml). The *xyloglucan* substrate is added to the tubes (reference solutions, samples and control tubes) and the mixture is incubated in a water bath at 50 °C. After 10 min, 1 ml of enzyme solutions are transferred in each tube and incubated for 2.5 h. The reaction is stopped by adding 6 mL TRIS solution and vortex-mixed. The solutions are cooled at room temperature, shaken and filtered. The absorbance of the standard and sample solutions is measured with a spectrophotometer at 590 nm using an external calibration.

The following method performance characteristics were presented by the Applicant within the validation and verification studies:

- for the *feed additive*: a RSD_r ranging from 2.8 to 3.3 %, a RSD_{ip} ranging from 3.0 to 3.5 % and a R_{Rec} ranging from 94 to 100 %;
- for *premixtures*: a RSD_r ranging from 3.7 to 7.0 %, a RSD_{ip} of 6.7 % and a R_{Rec} ranging from 95 to 107 %; and
- for *feedingstuffs*: a RSD_r ranging from 3.3 to 6.1 %, a RSD_{ip} ranging from 4.5 to 5.9 % and a R_{Rec} ranging from 96 to 109 %.

Furthermore, a LOD and a LOQ of 7 and 18 XGU/kg *feedingstuffs*, respectively, were determined by the Applicant [24].

Based on the performance characteristics available, the EURL recommends for official control the single-laboratory validated and further verified colorimetric method for the quantification of *xyloglucan-specific-endo-beta-1,4-glucanase* 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)

The evaluation of corresponding methods of analysis is not relevant for the present application.

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: (i) the single-laboratory validated and further verified colorimetric method 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 substrate from Megazyme for the quantification of the *endo-1,4-beta-xylanase* activity in the *feed additive*, *premixtures* and *feedingstuffs*; (ii) the single-laboratory validated and further verified colorimetric method based on the quantification of water soluble dyed fragments produced by the action of *endo-1,4-beta-glucanase* on commercially available azurine cross-linked cellulose substrate from Megazyme for the quantification of the *endo-1,4-beta-glucanase* activity in the *feed additive*, *premixtures* and *feedingstuffs*; and (iii) the single-laboratory validated and further verified colorimetric method based on the quantification of soluble dyed labelled fragments produced at pH 4.5 and 50 °C by the action of *xyloglucan-specific-endo-beta-1,4-glucanase* on commercially available xyloglucan substrate from Megazyme for the quantification of *xyloglucan-specific-endo-beta-1,4-glucanase* activity in the *feed additive*, *premixtures* and *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the determination of *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-beta-xylanase* from azurine cross-linked wheat arabinoxylan substrate

For the determination of *endo-1,4-beta-glucanase* activity in the *feed additive*, *premixtures* and *feedingstuffs*:

- colorimetric method based on the quantification of water soluble dyed fragments (azurine) produced by the action of *endo-1,4-beta-glucanase* on azurine-crosslinked cellulose

For the determination of *xyloglucan-specific-endo-beta-1,4-glucanase* activity in the *feed additive*, *premixtures* and *feedingstuffs*:

- colorimetric method based on the quantification of soluble dyed labelled fragments produced by the action of *xyloglucan-specific-endo-beta-1,4-glucanase* on xyloglucan substrate

One EPU unit is the amount of enzyme which liberates 0.0083 micromoles of reducing sugars (xylose equivalents) from oat spelt xylan per minute at pH 4.7 and 50 °C.

One CU unit is the amount of enzyme that liberates 0.128 micromoles of reducing sugars (glucose equivalents) from barley beta-glucan per minute at pH 4.5 and 30 °C.

One XGU unit is the amount of enzyme that releases low-molecular fragments from dyed xyloglucan in amount equal to the amount of such fragments liberated from 1 unit enzyme standard under the conditions of the assay (50 ºC and pH 4.5).

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of the *preparation of endo-1,4-beta-xylanase, endo-1,4-beta-glucanase and xyloglucan-specific-endo-1,4-beta-1,4-glucanase* 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: FORW. APPL. 1831-0035-2021 & Annex I – submission number 1615972736615-2896

[2] *Technical dossier, Section II: 2.1.2 Proposal for classification

[3] *Technical dossier, Section II: 2.1.3.1.3 Characterisation of ingredients used in the formulation of the additive

[4] *Technical dossier, Section II: Annex_II_41_Method xylanase additive premix validation

[5] Commission Implementing Regulation (EU) 2015/1043 of 30 June 2015 concerning the authorisation of the preparation of endo-1,4-beta-xylanase (EC 3.2.1.8) produced by Trichoderma citrinoviride Bisset (IM SD135) as a feed additive for chickens for fattening, turkeys for fattening, laying hens, weaned piglets, pigs for fattening and minor poultry species for fattening and for laying, and amending Regulations (EC) No 2148/2004, (EC) No 828/2007 and (EC) No 322/2009 (holder of authorisation Huvepharma NV), O.J. L 167/63, 1.7.2015

[6] *Technical dossier, Section II: Annex_II_45_method cellulase additive premix validation

[7] Commission Implementing Regulation (EU) 2015/2305 of 10 December 2015 concerning the authorisation of a preparation of endo-1,4-beta-glucanase (EC 3.2.1.4)

produced by Trichoderma citrinoviride Bisset (IM SD142) as a feed additive for chickens for fattening, minor poultry species for fattening and weaned piglets, and amending Regulations (EC) No 2148/2004 and (EC) No 1520/2007 (holder of authorisation Huvepharma NV), O.J. L 326/43, 11.12.2015

[8] *Technical dossier, Section II: Annex_II_50_method xyloglucanase additive premix feed description

[9] *Technical dossier, Section II: 2.1.3.2 Batch release specifications of the additive

[10] *Technical dossier, Section II: 2.5 Conditions of use of the additive

[11] *Technical dossier, Section II: 2.4.1.3 Stability of Huvezym® neXo in feedingstuffs for poultry

[12] EURL evaluation Reports: https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports

[13] *Technical dossier, Section II: 2.6.1.1 Methods for analysis of endo-1,4-beta-xylanase

[14] EURL evaluation Report: https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2010-0001.pdf

[15] *Technical dossier, Section II: 2.4.1 Stability

[16] *Technical dossier, section II : 2.4.2 Homogeneity

[17] *Technical dossier, Section II: 2.6.1.2 Methods for analysis of endo-1,4-beta-glucanase

[18] EURL evaluation Report: https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2010-0062.pdf

[19] *Technical dossier, Section II: 2.6.1.3 Methods for analysis of xyloglucan-specific-endo-beta-1,4-glucanase

[20] *Technical dossier, Section II: Annex_II_50_method xyloglucanase additive premix feed description

[21] *Technical dossier, Section II: Annex_II_51_method xyloglucanase additive validation

[22] *Technical dossier, Section II: Annex_II_52_method xyloglucanase premix validation

[23] *Technical dossier, Section II: Annex_II_53_method xyloglucanase feed validation

[24] *Technical dossier, Section II : Annex_II_54_method xyloglucanase additive premix feed verification

*Refers to Dossier no: FAD-2021-0036

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)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, PESCA, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Laboratoire de Rennes (SCL L35), Service Commun des Laboratoires DGCCRF et DGDDI, Rennes (FR)