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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

AveMix 02 CS
(FAD-2020-0055; CRL/200046)

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-2020-0055 - CRL/200046**
Name of Feed Additive:	***AveMix 02 CS***
Active Agent (s):	**Endo-1,4-beta-xylanase (EC 3.2.1.8)** **Endo-1,3(4)-beta-glucanase (EC 3.2.1.6)** **Polygalacturonase (EC 3.2.1.15)**
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: Date:	**Stefano Bellorini** **25/05/2021**
Report approved by: Date:	**Christoph von Holst** **26/05/2021**

EXECUTIVE SUMMARY

AveMix 02 CS is a *feed additive* currently authorised (4a14) for weaned piglets by Commission Regulation (EC) No 527/2011 under the category/functional group 'zootechnical additives'/'digestibility enhancers', according to Annex I of Regulation (EC) No 1831/2003. In the current application a renewal of the *feed additive* authorisation under Article 14 of the Regulation (EC) No 1831/2003 is requested for weaned piglets.

According to the Applicant, the *feed additive* contains three active substances: *endo-1,4-beta-xylanase* (EC 3.2.1.8) produced by *Trichoderma reesei SIMMONS* (MUCL 49755); *endo-1,3(4)-beta-glucanase* (EC 3.2.1.6) produced by *Trichoderma reesei SIMMONS* (MUCL 49754) and *polygalacturonase* (EC 3.2.1.15) produced by *Aspergillus aculeatus* (CBS 589.94).

The activity of *endo-1,4-beta-xylanase* is expressed in *xylanase* units (XU), where one XU unit is the amount of enzyme which releases 1 micro-mol of reducing sugar (xylose equivalent) per minute from xylan of oat spelt at 50 °C, pH 4.8. The activity of *endo-1,3(4)-beta-glucanase* is expressed in *beta-glucanase* units (BGU), where one BGU unit is the amount of enzyme which releases 1 micro-mol of reducing sugar (cellobiose equivalent) per minute from beta-glucan of barley at 50 °C, pH 5.0. The activity of *polygalacturonase* is expressed in *pectinase* (PGLU) units, where one PGLU unit is the amount of enzyme which releases 1 micro-mol of reducing sugar (glucose equivalent) per minute from polymethylgalacturonic acid at 35 °C and pH 4.8.

The *feed additive* is intended to be marketed as a brownish powder (*AveMix 02 CS*) and as a brown liquid (*AveMix 02 CS L*) formulation, having the following guaranteed minimum activities: (i) for *endo-1,4-beta-xylanase* 21400 XU/g (solid formulation) and 10700 XU/g (liquid formulation) (ii) for *endo-1,3(4)-beta-glucanase* 12300 BGU/g (solid formulation) and 6150 BGU/g (liquid formulation) and (iii) for *polygalacturonase* 460 PGLU/g (solid formulation) and 230 PGLU/g (liquid formulation).

The *feed additive* is intended to be incorporated into *premixtures* (solid formulation) or directly into *feedingstuff*s (solid and liquid formulations) to obtain the minimum target activities of 2140 XU/kg *feedingstuffs* for *endo-1,4-beta-xylanase*, 1230 BGU/kg *feedingstuffs* for *endo-1,3(4)-beta-glucanase* and 46 PGLU/kg *feedingstuffs* for *polygalacturonase*.

For the quantification of *xylanase*, *beta-glucanase* and *pectinase* activities in the *feed additive* and *premixtures* the Applicant submitted single-laboratory validated and further verified colorimetric methods based on the enzymatic hydrolysis of the respective substrates and the colour formation of the released reducing sugar with 3,5-dinitrosalicylic acid (DNS).

Furthermore, for the quantification of *xylanase* and *beta-glucanase* activities in *feedingstuffs* the Applicant submitted additional single-laboratory validated and further verified colorimetric methods based on the quantification of the water soluble dye fragments produced by the action of the enzymes on commercially available substrates (Megazyme) while for the quantification of *pectinase* activity in *feedingstuffs* the Applicant submitted a single-laboratory validated and further verified viscosimetric method.

Based on the acceptable performance characteristics the EURL recommends for official control (1) the single-laboratory validated and further verified colorimetric (DNS) methods for the determination of *endo-1,4-beta-xylanase*; *endo-1,3(4)-beta-glucanase* and *polygalacturonase* in the *feed additive* and *premixtures* (2) the colorimetric (Megazyme) methods for the determination of *1,4-beta-xylanase* and *endo-1,3(4)-beta-glucanase* in *feedingstuffs* and (3) the viscosimetric method, for the determination of *polygalacturonase* in *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

AveMix 02 CS; endo-1,4 beta-xylanase (EC 3.2.1.8)*; endo-1,3(4)-beta-glucanase* (EC 3.2.1.6)*; polygalacturonase (EC. 3.2.1.15); Trichoderma reesei SIMMONS; Aspergillus aculeatus; digestibility enhancer; weaned piglets.*

1. BACKGROUND

AveMix 02 CS is a *feed additive* currently authorised (4a14) for weaned piglets by Commission Regulation (EC) No 527/2011 [1] under the category/functional group 'zootechnical additives'/'digestibility enhancers' according to Annex I of Regulation (EC) No 1831/2003. In the current application a renewal of the *feed additive* authorisation under Article 14 of the Regulation (EC) No 1831/2003 is requested for weaned piglets [2].

According to the Applicant, the *feed additive* contains three active substances: *endo-1,4-beta-xylanase* (EC 3.2.1.8) produced by *Trichoderma reesei SIMMONS* (MUCL 49755), *endo-1,3(4)-beta-glucanase* (EC 3.2.1.6) produced by *Trichoderma reesei SIMMONS* (MUCL 49754) and *polygalacturonase* (EC 3.2.1.15) produced by *Aspergillus aculeatus* (CBS 589.94) [3].

The activity of *endo-1,4-beta-xylanase* is expressed in *xylanase* units (XU), where one XU unit is the amount of enzyme which releases 1 micro-mol of reducing sugar (xylose

equivalent) per minute from xylan of oat spelt at 50 °C, pH 4.8. The activity of *endo-1,3(4)-beta-glucanase* is expressed in *beta-glucanase* units (BGU), where one BGU unit is the amount of enzyme which releases 1 micro-mol of reducing sugar (cellobiose equivalent) per minute from beta-glucan of barley at 50 °C, pH 5.0. The activity of *polygalacturonase* is expressed in *pectinase* (PGLU) units, where one PGLU unit is the amount of enzyme which releases 1 micro-mol of reducing sugar (glucose equivalent) per minute from polymethylgalacturonic acid at 35 °C and pH 4.8 [4].

The *feed additive* is intended to be marketed as a brownish powder (*AveMix 02 CS*) and as a brown liquid (*AveMix 02 CS L*) formulations, having the following guaranteed minimum activities [4]:

- for *endo-1,4-beta-xylanase* 21400 XU/g (solid formulation) and 10700 XU/g (liquid formulation);
- for *endo-1,3(4)-beta-glucanase* 12300 BGU/g (solid formulation) and 6150 BGU/g (liquid formulation) and
- for *polygalacturonase* 460 PGLU/g (solid formulation) and 230 PGLU/g (liquid formulation)

AveMix 02 CS (solid formulation) is intended to be incorporated through *premixtures* or directly into *feedingstuff*s while *AveMix 02 CS L* (liquid formulation) should only be applied after the pelleting process by spraying it on the pellets [5]. The Applicant recommended a dose of 100 g *AveMix 02 CS* per tonne of *feedingstuffs* and 200 g *AveMix 02 CS L* per tonne of *feedingstuffs* to obtain minimum *xylanase* activity of 2140 XU / kg *feedingstuffs;* minimum *beta-glucanase* activity of 1230 BGU / kg *feedingstuffs* and minimum *pectinase* activity of 46 PGLU / kg *feedingstuffs* [5].

Note: The EURL has evaluated analytical methods for the determination of *endo-1,4-beta-xylanase* (EC 3.2.1.8); *endo-1,3(4)-beta-glucanase* (EC 3.2.1.6) and *polygalacturonase* (EC 3.2.1.15) in the frame of previous dossiers [5].

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 *AveMix 02 CS* 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 xylanase activity:

For the quantification of the *xylanase* activity in the *feed additive* and *premixtures* the Applicant submitted a single-laboratory validated and further verified colorimetric method based on the enzymatic hydrolysis of the oat spelt xylan substrate and the colour formation of the released reducing sugar with 3,5-dinitrosalicylic acid (DNS) at pH 4.8 and 50 °C [6].

The *feed additive* sample is weighed, mixed with citrate buffer, filtered and further diluted. The xylan substrate is added to an aliquot of the *feed additive* extract and incubated at 50 °C for 15 min. After this time the reaction is stopped by adding the DNS solution. The reagent-blank and the standards undergo a similar procedure, but without incubation, while for the sample-blank the enzyme solution is added after the reaction is stopped by DNS. Finally, a glucose standard solution is added to all tubes. The tubes are then boiled for 10 min, cooled down, diluted with water and centrifuged. The supernatants are taken and the reaction products are determined by colorimetry at 550 nm using a xylose standard calibration curve. The calculated enzymatic activity is expressed in xylose equivalents according to the unit definition for this enzyme [6]. The method was in-house validated [7] and further verified [8] by the Applicant. Relevant performance characteristics for the *feed additive* from the validation and verification studies recalculated by the EURL [9] are depicted in Table 1.

The Applicant did not provide validation and/or verification data for the determination of *endo-1,4-beta-xylanase* in the *premixtures*. However, in the frame of the stability studies [10] the Applicant applied the proposed method for different *premixtures*. Precision values, recalculated by the EURL [11], are similar to those calculated for the *feed additive* demonstrating thus the suitability of the method for *premixtures*.

For the quantification of *endo-1,4-beta-xylanase* in *feedingstuffs* the Applicant submitted another single-laboratory validated and further verified colorimetric method, based on the quantification of the water soluble dye fragments produced by the action of xylanase on a commercially available azurine cross-linked wheat arabinoxylan substrate (Megazyme) at pH 4.3 and 50 °C [12].

Table 1: Performance characteristics for the quantification of *xylanase* activity in the *feed additive, premixtures* and *feedingstuffs*

	Feed additive		***Feedingstuffs***		
	Validation [7]	**Verification [8]**	**Validation [12]**	**Verification [8]**	**Verification [8]**
Activity, XU/kg	19756200	24768000	6846	2525	4551
RSD_r, (%)[1]	2.6	8.7	3.8	6.7	11.4
RSD_{ip}, (%)[1]	5.6	16.8	3.9	9.3	14.9
R_{Rec}, (%)	105	121	104	98	99

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

[1]recalculated by EURL [9]

5 g *feedingstuffs* are mixed with 50 ml of 0.1 M acetate buffer (pH 4.3), stirred for 10 min and centrifuged. Three tubes, each containing 10 ml feed extract are supplemented with 0, 0.5 and 1.0 ml xylanase standard solution prepared from *AveMix 02 CS* with known *xylanase* activity expressed in XU units and available from the Applicant upon request. 0.5 ml aliquots are equilibrated at 50 °C for 3 min, the azurine cross-linked wheat arabinoxylan substrate is then added, and incubated at 50 °C for 30 min. The reaction is stopped by adding 3.0 ml of a stop solution (Trizma base solution 2 % (w/v)). Samples are vortex mixed, let to cool down for 5 min, filtered and the absorbance of the filtrate is measured against a blank at 590 nm [12].

Table 1 presents relevant performance characteristics for *feedingstuffs* recalculated by the EURL and based on experimental data obtained in the frame of the validation [12] and verification [8] studies. Additionally the Applicant estimated a limit of quantification (LOQ) of 1878 XU/kg *feedingstuffs* [12].

Measurement of beta-glucanase activity:

For the quantification of *beta-glucanase* activity in the *feed additive* and *premixtures* the Applicant submitted a single-laboratory validated and further verified colorimetric method based on the enzymatic hydrolysis of the barley beta-glucan substrate and the colour formation of the released reducing sugar with 3,5-dinitrosalicylic acid (DNS) at pH 5.0 and 50 °C [13].

The *feed additive* sample is weighed, mixed with acetate buffer, stirred, filtered and further diluted. The beta-glucan substrate is added to an aliquot of the *feed additive* extract and incubated at 50 °C for 10 min. After this time the reaction is stopped by adding the DNS solution. The reagent-blank and the standards undergo a similar procedure, but without incubation while for the sample-blank the enzyme solution is added after the reaction is stopped by DNS. Finally, a glucose standard solution is added to all tubes. The tubes are then boiled for 10 min, cooled down and diluted with water. The supernatants are taken and the reaction products determined by colorimetry at 550 nm using a cellobiose standard calibration

curve. The calculated enzymatic activity is expressed in cellobiose equivalents according to the unit definition for this enzyme [13]. The method was in-house validated [14] and further verified [8] by the Applicant. Relevant performance characteristics for the *feed additive* from the validation and verification studies recalculated by the EURL [15] are depicted in Table 2.

The Applicant did not provide validation and/or verification data for the determination of *endo-1,3(4)-beta-glucanase* in the *premixtures*. However, in the frame of the stability studies [10] the Applicant applied the proposed method for different *premixtures*. Precision values, recalculated by the EURL [11], are similar to those calculated for the *feed additive* demonstrating thus the suitability of the method for *premixtures*.

For the quantification of *endo-1,3(4)-beta-glucanase* in *feedingstuffs* the Applicant submitted another single-laboratory validated and further verified colorimetric method, based on the quantification of the water soluble dye fragments produced by the action of beta-glucanase on a commercially available azo barley glucan substrate (Megazyme) at pH 4.6 and 40 °C [16].

Three 25 g *feedingstuffs* samples are supplemented with the appropriate amount of *AveMix 02 CS,* having a known *beta-glucanase* activity expressed in BGU units and available from the Applicant upon request, to get 0, 50 and 100 mg *AveMix 02 CS / kg feedingstuffs*. The samples are then mixed with 0.4 mM acetate buffer (pH 4.6), stirred for 30 min, filtered and diluted with the buffer. The *feedingstuffs* extracts (0.5 ml aliquots) and a blank are then incubated at 40 °C for 210 min with the azo barley glucan substrate. The reaction is stopped by adding 3.0 ml of a stop solution (industrial methylated spirits/methoxyethanol) and centrifuged. Samples are vortex mixed, cooled down, centrifuged and the absorbance of the supernatant is measured against a blank at 590 nm [16].

Table 2 presents relevant performance characteristics for *feedingstuffs* recalculated by the EURL and based on experimental data obtained in the frame of the validation [14] and verification [8] studies. Additionally the Applicant estimated a limit of quantification (LOQ) of 222 BGU/kg *feedingstuffs* [16].

Table 2: Performance characteristics for the quantification of *beta-glucanase* activity in the *feed additive, premixtures* and *feedingstuffs*

	Feed additive		***Feedingstuffs***		
	Validation [14]	**Verification [8]**	**Validation [16]**	**Verification [8]**	**Verification [8]**
Activity, BGU/kg	13473400	14139200	1087	1386	3073
RSD_r, (%)[1]	5.7	5.8	5.0	4.0	5.1
RSD_{ip}, (%)[1]	5.7	9.8	5.7	4.0	5.3
R_{Rec}, (%)	107	100	109	100	100

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

[1]recalculated by EURL [15]

Measurement of pectinase activity

For the quantification of *pectinase* activity in the *feed additive* and *premixtures* the Applicant submitted a single-laboratory validated and further verified colorimetric method based on the enzymatic hydrolysis of the pectin substrate and the colour formation of the released reducing sugar with 3,5-dinitrosalicylic acid (DNS) at pH 4.8 and 35 °C [17].

The *feed additive* sample is weighed, mixed with citrate buffer, stirred, filtered and further diluted. The pectin substrate is added to an aliquot of the *feed additive* extract and incubated at 35 °C for 20 min. After this time the reaction is stopped by adding the DNS solution. The reagent-blank and the standards undergo a similar procedure, but without incubation while for the sample-blank the enzyme solution is added after the reaction is stopped by DNS. The tubes are then boiled for 10 min, cooled down, diluted with water and centrifuged. The supernatants are taken and the reaction products determined by colorimetry at 550 nm using a glucose standard calibration curve. The calculated enzymatic activity is expressed in glucose equivalents according to the unit definition for this enzyme [17]. The method was in-house validated [18] and further verified [8] by the Applicant. Relevant performance characteristics for the *feed additive* recalculated by the EURL [19] from the validation and verification studies are depicted in Table 3.

The Applicant did not provide validation and/or verification data for the determination of *polygalacturonase* in *premixtures*. However, in the frame of the stability studies [10] the Applicant applied the proposed method for different *premixtures* samples. Precision values, recalculated by the EURL [11], are similar to those calculated for the *feed additive* demonstrating thus the suitability of the method for *premixtures*.

For the quantification of the *pectinase* activity in *feedingstuffs* the Applicant submitted another single-laboratory validated and further verified viscosimetric method, based on viscosity reduction as a result of enzymatic hydrolysis by the action of *polygalacturonase* on the pectin substrate [20].

Table 3: Performance characteristics for the quantification of *pectinase* activity in the *feed additive, premixtures* and *feedingstuffs*

	Feed additive		***Feedingstuffs***		
	Validation [18]	**Verification [8]**	**Validation [20]**	**Verification [8]**	**Verification [8]**
Activity, PGLU/kg	807000	623600	61.4	77.5	129.4
RSD_r, (%)[1]	7.4	5.4	9.0	9.3	8.5
RSD_{ip}, (%)[1]	16.2	8.0	9.0	9.3	12.2
R_{Rec}, (%)	105	90	113	91	93

RSD_r; RSD_{ip}: relative standard deviation for *repeatability* and *intermediate precision;* R_{Rec}*: recovery rate*
[1]recalculated by EURL [19]

The *feedingstuffs* sample (25 g) is mixed with a 0.1 M acetic acid solution (pH 4.0) at 22 °C for 30 min. The extract is then centrifuged. Three tubes, each containing 3 ml feed extract are supplemented with 0, 0.05, 0.1 and 0.2 ml of a *polygalacturonase* standard solution prepared from *AveMix 02 CS* with known *pectinase* activity expressed in PGLU units and available from the Applicant upon request. An aliquot of the extracts are incubated with the pectin substrate at 50 °C for 4 h. The viscosity is then measured at 50 °C. The reagent-blank is prepared by replacing the 3 ml *feedingstuffs* extract by water [21].

Table 3 presents relevant performance characteristics for *feedingstuffs* recalculated by the EURL and based on experimental data obtained in the frame of the validation [21] and verification [8] studies. Additionally the Applicant estimated a limit of quantification (LOQ) of 28 PLGU/kg *feedingstuffs* [21].

All the validation and verification studies provided by the Applicant have been carried out using the solid form of the feed additive (*AveMix 02 CS).* However, in the frame of the stability and homogeneity studies [10] the Applicant applied the proposed methods for the liquid formulation of the *feed additive* (*AveMix 02 CS L)* and for *feedingstuffs* containing *AveMix 02 CS L* proving thus the suitability of the methods also for the liquid formulation.

Based on the performance characteristics available, the EURL recommends for official control the single-laboratory validated and further verified colorimetric and viscosimetric methods described above for the quantification of *xylanase, beta-glucanase* and *pectinase* activities 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 concluded that (1) the single-laboratory validated and further verified colorimetric (DNS) methods for the determination of *endo-1,4-beta-xylanase*; *endo-1,3(4)-beta-glucanase* and *polygalacturonase* in the *feed additive* and *premixtures* (2) the colorimetric (Megazyme) methods for the determination of *1,4-beta-xylanase* and *endo-1,3(4)-beta-glucanase* in *feedingstuffs* and (3) the viscosimetric method, for the determination of *polygalacturonase* in *feedingstuffs* are suitable for official control.

Recommended text for the register entry (analytical method)

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

- colorimetric (DNS) method based on the enzymatic hydrolysis of the oat spelt xylan substrate

For the determination of *endo-1,4-beta-xylanase* in *feedingstuffs*:

- colorimetric method based the enzymatic reaction of endo-1,4-beta-xylanase on the azurine cross-linked wheat arabinoxylan substrate

For the determination of *endo-1,3(4)-beta-glucanase* in the *feed additive* and *premixtures*:

- colorimetric (DNS) method based on the enzymatic hydrolysis of the barley beta-glucan substrate

For the determination of *endo-1,3(4)-beta-glucanase* in *feedingstuffs*:

- colorimetric method based the enzymatic reaction of endo-1,3(4)-beta-glucanase on the azo barley glucan substrate

For the determination of *polygalacturonase* in the *feed additive* and *premixtures*:

- colorimetric (DNS) method based on the enzymatic hydrolysis of the pectin substrate

For the determination of *polygalacturonase* in *feedingstuffs*:

- viscosimetric method based on decrease in viscosity produced by action of polygalacturase on the pectin substrate

One *xylanase* unit (XU) is the amount of enzyme which releases 1 micro-mol of reducing sugar (xylose equivalent) per minute from xylan of oat spelt at 50 °C and pH 4.8.

One *beta-glucanase* unit (BGU) is the amount of enzyme which releases 1 micro-mol of reducing sugar (cellobiose equivalent) per minute from beta-glucan of barley at 50 ^{o}C and pH 5.0.

One *pectinase* unit (PGLU) is the amount of enzyme which releases 1 micro-mol of reducing sugar (glucose equivalent) per minute from polymethylgalacturonic acid at 35 °C and pH 4.8.

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *AveMix 02 CS* 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] Commission Implementing Regulation (EU) 527/2011 of 30 May 2011 concerning the authorisation of a preparation of endo-1,4-β-xylanase produced by Trichoderma reesei (MUCL 49755), endo-1,3(4)-β-glucanase produced by Trichoderma reesei (MUCL 49754) and polygalacturonase produced by Aspergillus aculeatus (CBS 589.94) as feed additive for weaned piglets (holder of the authorisation Aveve NV) O.J. L 143, 31.5.2011

[2] *Application, Reference SANTE/E5: FORW. APPL. 1831-0052-2020 & Annex I – submission number 152558602408-2638

[3] *Technical dossier, Section II: 2. Characterisation of the active substance(s)/agents

[4] *Technical dossier, Section II: 1. Identity of the additive

[5] EURL evaluation Reports:
http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2010-0189.pdf
https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2019-0029_econasext.pdf;
https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2018-0071-econasext.pdf
https://ec.europa.eu/jrc/sites/jrcsh/files/FinRep-FAD-2008-0022.pdf
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https://ec.europa.eu/jrc/sites/jrcsh/files/FinRep-FAD-2008-0011.pdf
https://ec.europa.eu/jrc/sites/jrcsh/files/finirep-fad-2014-0028-fraoctazymec.pdf
https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2016-0013_polygalacturonase.pdf
https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2020-0004-rovabioadvance.pdf
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https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2017-0053-axtra_xap_104_tpt.pdf

[6] *Technical dossier, Section II, Annex II.6.1.01

[7] *Technical dossier, Section II, Annex II.6.1.09

[8] Supplementary information, " Verification Reports - AveMix 02 CS - CRL200046.pdf"

[9] Supplementary information, "eurl_anova_stability_pm.pdf"

[10] *Technical dossier, Section II, Annex_II.4.1.08; Annex_II.4.1.09 & Annex_II.4.2.03

[11] Supplementary information, "eurl_anova_val-ver-xyl.pdf"

[12] *Technical dossier, Section II, Annex II.6.1.12

[13] *Technical dossier, Section II, Annex II.6.1.02

[14] *Technical dossier, Section II, Annex II.6.1.10

[15] Supplementary information, "eurl_anova_val-ver-gluc.pdf"

[16] *Technical dossier, Section II, Annex II.6.1.13

[17] *Technical dossier, Section II, Annex II.6.1.03

[18] *Technical dossier, Section II, Annex II.6.1.11

[19] Supplementary information, "eurl_anova_val-ver-pec.pdf"

[20] *Technical dossier, Section II, Annex II.6.1.14

*Refers to Dossier no: FAD-2020-0055

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:

- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Centro di referenza nazionale per la sorveglienza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- 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)