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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

Rovabio® Advance
(FAD-2020-0004; CRL/190057)

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-2020-0004 - CRL/190057**
Name of Product:	***Rovabio® Advance***
Active Agent (s):	**Endo-1,4-beta-xylanase (EC 3.2.1.8) & endo-1,3 (4)-beta-glucanase (EC 3.2.1.6)**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA) JRC Geel, Belgium**
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Report checked by: Date:	**Zigmas Ezerskis** **03/11/2020**
Report approved by: Date:	**Christoph von Holst** **04/11/2020**

EXECUTIVE SUMMARY

In the current application an authorisation is sought under Article 4(1) for *Rovabio® Advance*, under the category/functional 4(a) "zootechnical additives"/"digestibility enhancers" according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, the authorisation is sought for the use of the *feed additive* for pigs for fattening and weaned piglets.

According to the Applicant, the *feed additive* contains two active substances: *endo-1,4-beta-xylanase* and *endo-1,3(4)-beta-glucanase*, produced from *Talaromyces versatilis* (IMI 378536) and (DSM 26702), respectively. The enzymatic activity for both enzymes is expressed by the Applicant in viscosity (U) and DNS units, where:

- One *xylanase (*or *beta-glucanase)* viscosity unit (U) is defined as the amount of *xylanase (*or *beta-glucanase)* that hydrolyses wheat arabinoxylan (or barley *beta*-glucan), reducing the solution viscosity, in order to change the relative fluidity by one dimensionless unit per minute, at pH 5.5 and 30 ^{o}C
- One *xylanase* (or *beta-glucanase)* DNS unit corresponds to the amount of *xylanase (*or *beta-glucanase)* which liberate from the xylan (or barley beta-glucan) substrate one micromol of *xylose* (or *glucose*) per minute at 50 ^{o}C and pH 4.0 (or pH 5.0)

The product is intended to be marketed as powder (*Rovabio® Advance POWDER*) and liquid (*Rovabio® Advance LIQUID*) formulations, having the following guaranteed minimum activities:

- for *xylanase*: 36000 U/g or 3740 DNS units/g and 9000 U/ml or 940 DNS units/ml, and
- for *beta-glucanase*: 25000 U/g or 2600 DNS units/g and 6250 U/ml or 650 DNS units/ml.

The *feed additive* is intended to be incorporated into *premixtures* and/or complete *feedingstuffs* to obtain a minimum *xylanase* and *beta-glucanase* activities of 1800 and 1250 viscosity U/kg, respectively.

When expressing the enzymatic activity in terms of viscosity units a viscometric method is applied, while for the enzymatic activity in DNS units a colorimetric methods is used.

For the quantification of *xylanase* and *beta-glucanase* activities in the *feed additive*, *premixtures* and *feedingstuffs*, the Applicant submitted single-laboratory validated and further verified viscometric methods. In these methods the *endo-1,4-beta-xylanase (*or the *endo-1,3(4)-beta-glucanase)* catalyses the hydrolysis of xylosidic (or glycosidic) bonds in the wheat arabinoxylan (or barley beta-glucan) substrate to yield xylose (or glucose) and reduces consequently the viscosity of the sample solution.

Furthermore, the Applicant submitted alternative colorimetric (DNS) methods for the quantification of *xylanase* and *beta-glucanase* activities in the *feed additive*. The colorimetric methods are based on the enzymatic hydrolysis of the xylan (or barley beta-glucan) substrate by *xylanase* (or *beta-glucanase*) and the consequent colour formation of the released sugars with 3,5-dinitrosalicylic acid (DNS) at pH 4.0 (or pH 5.0) and 50 ^{o}C.

Based on the acceptable performance characteristics the EURL recommends for official control (1) the single-laboratory validated and further verified viscometric methods for the determination of *xylanase* and *beta-glucanase* in the *feed additive*, *premixtures* and *feedingstuffs* and (2) the alternative colorimetric (DNS) methods for the determination of *xylanase* and *beta-glucanase* in the *feed additive.*

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 (EC 3.2.1.8) and *endo-1.3(4)-beta-glucanase* (EC 3.2.1.6), *Rovabio® Advance*, zootechnical additives, digestibility enhancers, pigs for fattening and weaned piglets

1. BACKGROUND

In the current application an authorisation is sought under Article 4(1) (new feed additive) for *Rovabio® Advance*, under the category/functional 4(a) "zootechnical additives"/"digestibility enhancers" according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1,2]. Specifically, the authorisation is sought for the use of the *feed additive* for pigs for fattening and weaned piglets.

According to the Applicant, the *feed additive* contains two active substances: *endo-1,4-beta-xylanase* (EC 3.2.1.8) and *endo-1,3(4)-beta-glucanase* (EC 3.2.1.6), produced from *Talaromyces versatilis* (IMI 378536) and (DSM 26702), respectively [3]. The enzymatic activity for both enzymes is expressed by the Applicant in viscosity (U) and DNS units, where:

- One *xylanase (*or *beta-glucanase)* viscosity unit (U) is defined as the amount of *xylanase (*or *beta-glucanase)* that hydrolyses wheat arabinoxylan (or barley beta-glucan), reducing the solution viscosity, in order to change the relative fluidity by one dimensionless unit per minute, at pH 5.5 and 30 ^{o}C.

- One *xylanase* (or *beta-glucanase)* DNS unit corresponds to the amount of *xylanase (*or *beta-glucanase)* which liberate from the xylan (or barley beta-glucan) substrate one micromol of xylose (or glucose) equivalents per minute at 50 °C and pH 4.0 (or pH 5.0).

The product is intended to be marketed as powder (*Rovabio® Advance POWDER*) and liquid (*Rovabio® Advance LIQUID*) formulations, having the following guaranteed minimum activities [2,3]:

- for *xylanase*: 36000 U/g or 3740 DNS units/g and 9000 U/ml or 940 DNS units /ml, and
- for *beta-glucanase*: 25000 U/g or 2600 DNS units /g and 6250 U/ml or 650 DNS units /ml.

The carrier in the solid formulation is wheat flour or maltodextrin while sorbitol, potassium sorbate and demineralised water are used for the liquid formulation [3].

The *feed additive* is intended to be incorporated in complete *feedingstuff*s through *premixtures* to obtain minimum *xylanase* and *beta-glucanase* activities of 1800 and 1250 U/kg, respectively.

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

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 *Rovabio® Advance* 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)

According to the Applicant the only difference between *Rovabio® Advance* and *Rovabio® Spiky*, currently authorised by Commission Implementing Regulations (EU) 2015/661; 2015/2304 and 2017/210, is the level of the *endo-1,4-beta-xylanase* and *endo-1,3(4)-beta glucanase* activity in the solid and liquid forms of the *feed additive* [3]. Consequently, the

Applicant provided the same experimental data already submitted in the frame of a previous dossier (*Rovabio® Spiky*) [5].

Given the fact that i) the *endo-1,4-beta-xylanase* and *endo-1,3(4)-beta glucanase* activities in *Rovabio® Advance* are higher than the ones in *Rovabio® Spiky* and ii) the minimum content in *feedingstuffs* of *Rovabio® Spiky* is lower than the ones proposed for *Rovabio® Advance* [3,5], the EURL considers that all the methods and the validation data provided in the frame of the *Rovabio® Spiky* dossier [5] are also applicable for *Rovabio® Advance*.

Furthermore, in the frame of the homogeneity and stability studies the Applicant applied the above-mentioned methods to the product (*Rovabio® Advance*) [6] *premixtures* [7] and *feedingstuffs* [8] thus confirming the suitability of the methods for *Rovabio® Advance*.

Measurement of xylanase activity

For the quantification of the *xylanase* activity in the *feed additive* the Applicant submitted two single-laboratory validated and further verified methods based on (i) viscometry and (ii) colorimetry (DNS).

In the viscometric method the *endo-1,4-beta-xylanase* catalyses the hydrolysis of xylosidic linkages in the wheat arabinoxylan substrate to yield xylose, and reduces consequently the viscosity of sample solution. The decrease in viscosity of the sample solution, expressed in terms of a drop time, is determined using a falling ball viscometer at pH 5.5 and 30 ^{o}C.

The *feed additive* is weighed and extracted in water at room temperature. If needed, the extract is filtered and/or further diluted in order to be within the measuring interval of 5 to 15 min. The substrate (wheat arabinoxylan) is placed in a disposable sterile tube, sonicated for 5 s and let at 30 ^{o}C for at least 5 min. After this, an aliquot of the *feed additive* extract is added to the incubated substrate for further analysis [5,10].

The colorimetric method is based on the enzymatic hydrolysis of the xylan substrate and the colour formation of the released sugar with 3,5-dinitrosalicylic acid (DNS) at pH 4.0 and 50 ^{o}C.

Table 1: Performance characteristics obtained by the Applicant for the determination of *xylanase* activity in the *Rovabio®* products (*Excel, Spiky* and *Advance*) by applying the viscometric (VISCO) and colorimetric (DNS) methods.

Method	Activity (U/g or U/ml)	RSDr (%)			RSD_{ip} (%)			R_{Rec} (%)	
		Advance[1]	Spiky[2]	Excel[3]	Advance[1]	Spiky[2]	Excel[3]	Spiky[2]	Excel[3]
VISCO	5443-57134	0.9-6.1	1.7-2.7	1.7-3.7	2.1-6.7	4.8-6.2	3.6	98-102	95-109
DNS	626-6797	1.5-1.6	1.1-2.1	0.4-2.6	1.6-1.7	1.3-2.4	2.7	87-103	95-104

RSD_r; RSD_{ip}: relative standard deviation for *repeatability* and *intermediate precision*; R_{Rec}: *Recovery rate*
[1]Calculated by EURL [9], [2]Data from [5], [3]Data from Annexes_II_6_2, II_6_4, II_6_6 and II_6_8

The *feed additive* sample is weighed, extracted in water at room temperature and, if needed, further diluted to be within the linear range of the method. The xylan substrate is placed into a tube and equilibrated at 50 °C for at least 5 min. Then, an aliquot of the *feed additive* extract is added and incubated at 50 °C for 10 min. After this time the reaction is stopped by adding the DNS solution. The blank samples undergo a similar procedure, but the enzyme solution is added after the reaction is stopped by DNS. All the samples are then incubated at 95 °C for 5 min and let cool down to room temperature in a water bath. The reaction products are finally diluted with water and determined by colorimetry at 540 nm using a xylose standard calibration curve. The calculated enzymatic activity is expressed in xylose equivalents according to the DNS unit definition for this enzyme [11].

The validation and verification data provided by the Applicant are related to *Rovabio® Excel* (subject to another authorisation). Additionally, in the frame of the *Rovabio® Spiky* dossier previously evaluated by the EURL, the Applicant demonstrated the applicability of the two methods (viscometic and colorimetric) also to this product [5]. Table 1 shows acceptable performance characteristics reported for the determination of *xylanase* activity in the three *Rovabio®* products, thus further extending the scope of the two methods to *Rovabio® Advance*.

The EURL was aware of the lack of a commercially available birch xylan substrate. This is commonly solved for the quantification of the *endo-1,4-beta-xylanase* activity by a change of substrate, from birch to beech xylan. As the birch xylan substrate was stated in the procedure submitted for the quantification of the *xylanase* activity in *Rovabio® Advance* [11], the Applicant, upon EURL request, provided i) confirmation that the substrate has been changed from birch to beech xylan for *Rovabio® Advance;* ii) an updated procedure for the quantification of the *xylanase* activity in *Rovabio® Advance* [12] and iii) experimental evidences demonstrating that the change of the substrate from birch to beech xylan did not have a significant impact on the determination of the *endo-1,4-beta-xylanase* activity in the product of this dossier [13].

Table 2: Performance characteristics for the determination of *xylanase* activity in *feedingstuffs* (FS) containing one of the *Rovabio* products (*Excel, Spiky or Advance*) by applying the viscometric method

Matrix	Activity (U/kg)	RSD_r (%)				RSD_{ip} (%)		R_{Rec} (%)		
		Excel[1]		Spiky[2]	Advance[3]	Excel[1]		Excel[1]		Spiky[2]
		Val	Ver	Ver	Ver	Val	Ver	Val	Ver	Ver
FS	1835-3892	2.0-6.3	0.7-5.0	2.4-4.3	4.6-6.8	5.5-6.1	1.6-4.7	96-104	93-104	98-114

RSD_r; RSD_{ip}: relative standard deviation for repeatability and intermediate precision; R_{Rec}: Recovery rate;
Val: Validation; Ver: Verification; [1]Data from Annexes_II_6_18 and II_6_19; [2] Data from [5]; [3]Data from [8]

For the determination of xylanase in *feedingstuffs* the Applicant submitted a single-laboratory validated and further verified viscometric method.

The sample of *feedingstuffs* is extracted with a citrate buffer solution (pH 3.3) containing bovine serum albumin (BSA). An aliquot of the extract is then centrifuged and the supernatant is further filtered. The substrate solution (wheat arabinoxylan) is placed in a disposable sterile tube, sonicated for a few seconds and let rest at 30 ^{o}C for at least 5 min. Then, an aliquot of the filtered supernatant is added to the incubated substrate for further analysis. This experimental protocol is applied to treated *feedingstuffs* (containing the *feed additive*) and to untreated control samples (with no added enzymes). The activity of the added *endo-1,4-beta-xylanase* is determined by subtracting the activity of the control sample from the total activity. Such blank correction is only possible when control samples are available allowing the determination of the added enzyme activity. Whenever a control feed sample is not available the EURL considers this method suitable for official control to determine the total enzyme activity [14].

Table 2 shows the performance characteristics obtained for the determination of the *xylanase* activity in *feedingstuffs* containing the three *Rovabio®* products.

Furthermore, a limit of quantification (LOQ) of 912 viscosity U/kg *feedingstuffs* was reported by the Applicant [15].

For the quantification of the xylanase activity in *premixtures* the Applicant proposed a solid dilution of *premixtures* with corn meal. After mixing thoroughly the ground pseudo-feed sample was suspended in lauryl sulfate buffer (pH = 6.0), stirred and centrifuged. The obtained supernatant is then filtered and analysed according to the above described procedure for *feedingstuffs* [14].

The EURL recommends for official control the single-laboratory validated and further verified viscometric methods for the determination of the *xylanase* activity in the *feed additive*, *premixtures* and *feedingstuffs* together with the alternative colorimetric (DNS) method for the determination of *xylanase* in the *feed additive*.

Measurement of beta-glucanase activity

For the quantification of the *beta-glucanase* activity in the *feed additive* the Applicant submitted two single-laboratory validated and further verified methods based on (i) viscometry and (ii) colorimetry (DNS).

In the viscometric method the *endo-1,3(4)-beta-glucanase* catalyses the hydrolysis of glycosidic bonds in the barley beta-glucan substrate to yield glucose, 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 5.5 and 30 ^{o}C.

Table 3. Performance characteristics obtained by the Applicant for the determination of beta-glucanase activity in the *Rovabio products (Excel, Spiky* and *Advance*) by applying the viscometric (VISCO) and colorimetric (DNS) methods.

Method	Activity (U/g or U/ml)	RSD_r (%)			RSD_{ip} (%)			R_{Rec} (%)	
		Advance[1]	Spiky[2]	Excel[3]	Advance[1]	Spiky[2]	Excel[3]	Spiky[2]	Exce[3]l
VISCO	3903-56417	2.2-3.7	1.8-8.6	1.8-3.9	2.2-3.7	3.5-8.2	3.1	96-105	97-117
DNS	443-5996	0.1-1.2	0.9-1.6	1.0-3.0	0.7-1.9	0.8-2.0	3.9	94-105	97-107

RSD_r; RSD_{ip}: relative standard deviation for *repeatability* and *intermediate precision; R_{Rec}: Recovery rate*
[1]Calculated by EURL [9]; [2]Data from [5]; [3]Data from Annexes_II_6_10, II_6_12, II_6_14, and II_6_ 16

The *feed additive* is weighed and extracted in sodium acetate buffer (pH 5.5) at room temperature. If needed, the extract is filtered and/or further diluted in order to be within the measuring interval of 5 to 15 min. The substrate solution (barley beta-glucan) is placed in a disposable sterile tube, sonicated for 5 s and let at 30 ^{o}C for at least 5 min. Then, an aliquot of the extract is added to the substrate at 30 ^{o}C for further analysis [16].

The colorimetric method is based on the enzymatic hydrolysis of the barley beta-glucan. The reaction products are determined by colorimetry after reaction of the reducing groups with 3,5-dinitrosalicylic acid (DNS) at pH 5.0 and 50 ^{o}C [17].

The *feed additive* sample is weighed, extracted in water at room temperature and, if needed, further diluted to be within the linear range of the method. The substrate (1.5 % barley beta-glucan solution) is placed into a tube and equilibrated at 50 ^{o}C for at least 5 min. Then, an aliquot of the *feed additive* extract is added and incubated at 50 ^{o}C for 10 min. After this time the reaction is stopped by adding the DNS solution. The blank samples undergo a similar procedure, but the enzyme solution is added after the reaction is stopped by DNS. All the samples are then incubated at 95 ^{o}C for 15 min and left to cool down to room temperature in a water bath. The reaction products are finally determined by colorimetry at 540 nm using a standard glucose calibration curve. The calculated enzymatic activity is expressed in glucose equivalents according to the DNS unit definition for this enzyme [17].

As for *xylanase*, the validation and verification data provided by the Applicant are related to previous *Rovabio® Excel* and *Robavio® Spiky* dossiers [5]. Table 3 shows that the performance characteristics reported for the determination of the *beta-glucanase* activity in the three products are in good agreement, thus extending the scope of the two methods to *Rovabio® Advance*.

For the quantification of *beta-glucanase* in *feedingstuffs*, the Applicant submitted a single-laboratory validated and further verified viscometric method.

The sample of *feedingstuffs* is extracted with a buffer solution (0.1 M of 4-morpholine ethane sulfonic acid**,** pH 6.0 and 1 % lauryl sulfate) in a cold water bath. An aliquot of the extract is then centrifuged, filtered and further diluted with an acetate buffer solution (pH 5.5). A

diluted substrate solution (barley beta-glucan) is placed in a disposable sterile tube, sonicated for a few seconds and kept at 30 °C for at least 5 min. Then an aliquot of the extract is added to the substrate at 30 °C and the analysis is performed. This experimental protocol is applied to treated *feedingstuffs* (containing the *feed additive*) and to untreated control samples (with no added enzymes). The activity of the added *endo-1,3(4)-beta-glucanase* is determined by subtracting the activity of the control sample from the total activity. Such blank correction is only possible when control samples are available allowing the determination of the added enzyme activity. Whenever a control feed sample is not available the EURL considers this method suitable for official control to determine the total enzyme activity [18].

Table 4 shows the performance characteristics obtained for the determination of the *beta-glucanase* activity in *feedingstuffs* containing the three *Rovabio®* products.

Furthermore, a limit of quantification (LOQ) of 236 U/kg *feedingstuffs* was reported by the Applicant [19].

For the quantification of the *beta-glucanase* activity in *premixtures* the Applicant proposed a solid dilution of *premixtures* with corn meal. After mixing thoroughly the ground pseudo-feed sample was suspended in lauryl sulfate buffer (pH 6.0), stirred and centrifuged. The obtained supernatant is then filtered and analysed according to the above described procedure for *feedingstuffs* [18].

Based on the performance characteristics presented the EURL recommends for official control the single-laboratory validated and further verified viscometric methods for the determination of the *beta-glucanase* activity in the *feed additive*, *premixtures* and *feedingstuffs* together with the alternative colorimetric (DNS) method for the determination of *beta-glucanase* in the *feed additive*.

Table 4: Performance characteristics for the determination of *beta-glucanase* activity in *feedingstuffs* (FS) containing one of the *Rovabio®* products (*Excel*, *Spiky* or *Advance*) by applying the viscometric method

Matrix	Activity (U/kg)	RSD_r (%)				RSD_{ip} (%)		R_{Rec} (%)		
		Excel[1]		Spiky[2]	Advance[3]	Excel[1]		Excel[1]		Spiky[2]
		Val	Ver	Ver	Ver	Val	Ver	Val	Ver	Ver
FS	641-2009	1.3-4.6	2.6-5.2	4.6-5.4	7.0-11.6	3.5-6.1	5.7-6.8	97-120	81-103	96-106

RSD_r; RSD_{ip}: relative standard deviation for *repeatability* and *intermediate precision;* R_{Rec}: Recovery rate
Val: Validation; Ver: Verification; [1]Data from Annexes_II_6_22 and II_6_23; [2] Data from [5]; [3]Data from [8]

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)

An 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 viscometric methods for the determination of *xylanase* and *beta-glucanase* in the *feed additive premixtures* and *feedingstuffs* and (2) the colorimetric (DNS) methods for the determination of *xylanase* and *beta-glucanase* in the *feed additive* are suitable for official control.

Depending on whether minimum enzymes activities of the *feed additive* will be defined in the legal act exclusively by viscosity units or by DNS units as well, the EURL recommends that only a reference to the viscometric method or in addition to the colorimetric method is included in the register entry.

Recommended text for the register entry (analytical method)

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

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

Alternatively, for the determination of *endo-1,4-beta-xylanase* in the *feed additive*:

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

For the determination of *endo-1.3(4)-beta-glucanase* in the *feed additive, premixtures* and *feedingstuffs*:

- viscosimetric method based on decrease in viscosity produced by action of *endo-1.3(4)-beta-glucanase* on the glucan containing substrate (barley β-glucan)

Alternatively, for the determination of *endo-1.3(4)-beta-glucanase* in the *feed additive*:

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

One *xylanase (*or *beta-glucanase)* viscosity unit (U) is defined as the amount of *xylanase (*or *beta-glucanase)* that hydrolyses wheat arabinoxylan (or barley beta-glucan), reducing the solution viscosity, in order to change the relative fluidity by one dimensionless unit per minute, at pH 5.5 and 30 ^{o}C.

One *xylanase* (or *beta-glucanase)* DNS unit corresponds to the amount of *xylanase (*or *beta-glucanase)* which liberate from the xylan (or barley beta-glucan) substrate one micromol of xylose (or glucose) per minute at 50 ^{o}C and pH 4.0 (or pH 5.0).

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Rovabio® Advance* 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. 1831-0013-2020

[2] *Application. Application Form - Annex I. Submission number 1578584419306-2512

[3] *Technical dossier, Section II: 2.1 Identity of the additive

[4] 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

[5] EURL evaluation Report:
https://ec.europa.eu/jrc/sites/jrcsh/files/FinRep-FAD-2013-0030-RovabioSpiky.pdf

[6] *Technical dossier, Section II, Annex_II_4_1

[7] *Technical dossier, Section II, Annex_II_4_2

[8] *Technical dossier, Section II, Annex_II_4_3

[9] Supplementary Information, "eurl_anova_rovabio_advance.pdf "

[10] *Technical dossier, Section II, Annex_II_6_1

[11] *Technical dossier, Section II, Annex_II_6_5

[12] Supplementary information, "Detailed protocol T006-I09ev.pdf "

[13] Supplementary information, "substrate_comparison.pdf"

[14] *Technical dossier, Section II, Annex_II_6_17

[15] *Technical dossier, Section II, Annex_II_6_18

[16] *Technical dossier, Section II, Annex_II_6_13

[17] *Technical dossier, Section II, Annex_II_6_9

[18] *Technical dossier, Section II, Annex_II_6_21

[19] *Technical dossier, Section II, Annex_II_6_22

*Refers to Dossier no: FAD-2020-0004

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)
- Instytut Zootechniki - Państwowy Instytut Badawczy, Krajowe Laboratorium Pasz, Lublin (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)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)