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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® Spiky
(FAD-2013-0030; CRL/130022)

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-2013-0030 - CRL/130022**
Name of Product:	***Rovabio® Spiky***
Active Agent (s):	**Endo-1,4-β-xylanase (EC 3.2.1.8) & endo-1,3 (4) β-Glucanase (EC 3.2.1.6)**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA) Geel, Belgium**
Report prepared by:	**María José González de la Huebra**
Report checked by: Date:	**Piotr Robouch (EURL-FA)** **11/04/2014**
Report approved by: Date:	**Christoph von Holst** **11/04/2014**

EXECUTIVE SUMMARY

In the current application authorisation is sought under article 4(1) for *Rovabio® Spiky*, 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, authorisation is sought for the use of the *feed additive* for chickens for fattening; chickens reared for laying and minor poultry species (for fattening and reared for laying).

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

- One *xylanase (*or *β-glucanase)* viscosity unit (U) is defined as the amount of *xylanase (*or *β-glucanase)* that hydrolizes wheat arabinoxylan (or barley β-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 *β-glucanase)* DNS unit corresponds to the amount of *xylanase (*or *β-glucanase)* which liberate from the birchwood xylan (or barley β-glucan) one μmol 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 in powder (*Rovabio® Spiky AP*) and liquid (*Rovabio® Spiky LC*) formulations, having the following guaranteed minimum activities:

- for *xylanase*: 22000 U/g or 2300 DNS units/g and 5500 U/ml or 570 DNS units /ml, and
- for *β-glucanase*: 15200 U/g or 1600 DNS units /g and 3800 U/ml or 400 DNS units /ml.

The carrier in the solid formulation is wheat flour, while sorbitol, potassium sorbate and demineralized water are used for the liquid formulation. The *feed additive* is intended to be incorporated into *premixtures* and/or complete *feedingstuffs* to obtain a minimum *xylanase* and *β-glucanase* activity of 1100 and 760 U/kg, respectively.

For the quantification of *xylanase* and *β-glucanase* activities in the *feed additive*, *premixtures* and *feedingstuffs*, the Applicant submitted a single-laboratory validated and further verified viscosimetry methods. Furthermore, the Applicant submitted alternative colorimetric (DNS) methods for the quantification of *xylanase* and *β-glucanase* activities in the *feed additive*. In the viscosimetry methods the *endo-1,4-β-xylanase (*or the *endo-1,3 (4)-β-glucanase)* catalyses the hydrolysis of xylosidic (or glycosidic) bonds in the wheat arabinoxylan (or barley β-glucan) substrate to yield *xylose (*or *glucose*) and reduces consequently the viscosity of the sample solution. The colorimetric methods are based on the enzymatic hydrolysis of the

birchwood xylan (or barley β-glucan) and the consequent colour formation of released sugar with 3,5-dinitrosalicylic acid (DNS) at pH 4.0 (or pH 5.0) and 50 °C. Based on the acceptable performance characteristics the EURL recommends for official control the validated and further verified viscosimetry methods for the determination of *xylanase* and *β-glucanase* in the *feed additive, premixtures* and *feedingstuffs* together with the alternative colorimetric (DNS) methods for the determination of *xylanase* and *β-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) is not considered necessary.

KEYWORDS

Endo-1,4-β-xylanase (EC 3.2.1.8) and *endo-1.3(4)-β-glucanase* (EC 3.2.1.6), *Rovabio® Spiky*, "zootechnical additives"/"digestibility enhancers", *chickens and minor poultry species for fattening and reared for laying*

1. BACKGROUND

In the current application authorisation is sought under article 4(1) for *Rovabio® Spiky*, 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, authorisation is sought for the use of the *feed additive* for chickens for fattening; chickens reared for laying and minor poultry species (for fattening and reared for laying).

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

- One *xylanase (*or *β-glucanase)* viscosity unit (U) is defined as the amount of *xylanase (*or *β-glucanase)* that hydrolizes wheat arabinoxylan (or barley β-glucan), reducing the solution viscosity, in order to change the relative fluidity by one dimensionless unit per minute, at pH 5.5 and 30 °C.
- One *xylanase* (or *β-glucanase)* DNS unit corresponds to the amount of *xylanase (*or *β-glucanase)* which liberate from the birchwood xylan (or barley β-glucan) one μmol of *xylose* (or *glucose*) per minute at 50 °C and pH 4.0 (or pH 5.0).

The product is intended to be marketed in powder (*Rovabio® Spiky AP*) and liquid (*Rovabio® Spiky LC*) formulations, having the following guaranteed minimum activities [2,3]:

- for *xylanase*: 22000 U/g or 2300 DNS units/g and 5500 U/ml or 570 DNS units /ml, and
- for *β-glucanase*: 15200 U/g or 1600 DNS units /g and 3800 U/ml or 400 DNS units /ml.

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

The *feed additive* is intended to be incorporated into *premixtures* and/or complete *feedingstuffs* to obtain a minimum *xylanase* and *β-glucanase* activity of 1100 and 760 U/kg, respectively.

Note: The EURL previously evaluated the analytical methods for the determination of *endo-1,4-β-xylanase* (EC 3.2.1.8) and *endo-1,3 (4)-β-glucanase* (EC 3.2.1.6) in the frame of the *Rovabio® Excel* dossier (FAD 2010-0189) [4].

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009, 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® Spiky* and their suitability to be used for official controls in the frame of the authorisation were evaluated.

3. EVALUATION

Identification /Characterisation of the feed additive

Qualitative and quantitative composition of impurities in the additive

When required by EU legislation, analytical methods for official control of undesirable substances in the additive (e.g. arsenic, cadmium, lead, mercury, aflatoxin B1 and dioxins) are available from the respective European Union Reference Laboratories [5]

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

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

In the viscosimetry method the *endo-1,4-β-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 viscosimeter at pH 5.5 and 30 ^{o}C.

The *feed additive* (0.6 g for the powder and 0.9 g for the liquid formulation) is weighed and extracted in water at room temperature. If needed, the extract is filtered and/or further diluted to be falling within the 5-15 min test measuring interval. The substrate (4.8 ml of 0.25 % wheat arabinoxylan) is placed in a disposable sterile tube, sonicated for 5 s and let at 30 ^{o}C for at least 5 min. Then 0.2 ml of the *feed additive* extract is added to the incubated substrate and the analysis is performed. The *xylanase* activity is quantified against a linear regression curve prepared from enzyme standards with known activities prepared in pure solutions (external calibration) [6].

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

The *feed additive* sample (0.6 g for the powder and 0.9 g for the liquid formulation) is weighed, extracted in water at room temperature and, if needed, further diluted to be falling within the linear range of the method. The substrate (1.75 ml of 1.5 % birchwood xylan solution) is placed into a tube and equilibrated at 50 ^{o}C for at least 5 min. Then 250 µl 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 2 ml of 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 let cool down to room temperature in a water bath. The reaction products are finally determined by colorimetry at 540 nm using a standard *xylose* calibration curve. The calculated enzymatic activity is then expressed in *xylose* equivalents [7].

Table 1: Performance characteristics obtained by the Applicant for the determination of *xylanase* activity in the *Rovabio* products (*Excel and Spiky*) applying the viscosimetry and colorimetry (DNS) methods.

Method	RSDr (%)		RSD_{ip} (%)		R_{Rec} (%)	
	Spiky* [10]	Excel	Spiky* [10]	Excel	Spiky [11]	Excel [11]
Visco	1.7-2.7	1.7-3.6 [8]	4.8-6.2	3.6 [8]	98.2-102	98.9-109
DNS	1.1-2.1	0.4-1.8 [9]	1.3-2.4	2.7 [9]	87.3-103	95.0-104

RSD_r; RSD_{ip}: relative standard deviation for *repeatability* and *intermediate precision*; R_{Rec}: *Recovery rate*
*Calculated by EURL from [10]

At first the Applicant submitted experimental data related to *Rovabio Excel* (subject to another authorisation). Supplementary information was later provided, upon request by the EURL-FA, to demonstrate the applicability of the two methods (viscosimetry & colorimetry) to the two products (i.e. *Rovabio® Spiky* and *Rovabio® Excel*). Table 1 shows that the performance characteristics reported [8,9,10,11] for the determination of *xylanase* activity in the two products are in good agreement, thus extending the scope of the two methods to *Rovabio® Spiky* samples.

For the determination of **xylanase activity in *premixtures* and *feedingstuffs*** the Applicant submitted a single-validated and further verified viscosimetry method.

The *premixture* sample (4.5 g) is weighed and extracted in sodium acetate buffer solution (pH 5.5) at room temperature, and submitted to the procedure described above for the *feed additive* [12].

The *feedingstuffs* (10 g) is extracted with a citrate buffer solution (pH 3.3) in presence of bovine serum albumin (BSA) for 30 min. An aliquot of the extract is then centrifuged, filtered and further diluted. The substrate (3.2 ml of 0.3 % wheat arabinoxylan) is placed in a disposable sterile tube, sonicated for 5 s and let at 30 °C for at least 5 min. Then 0.8 ml of the final *feedingstuffs* extract is added to the incubated substrate and the analysis is performed. The quantification is carried out via a calibration curve of a reference *endo-1,4-β-xylanase* with a known activity (determined applying the viscosimetric method for feed additives described above). 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-β-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 and allows for 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 [13].

Table 2: Performance characteristics for the determination of *xylanase* activity in *premixtures* (PM) and *feedingstuffs* (FS) containing one of the *Rovabio* products (*Excel* or *Spiky*) by viscosimetry, obtained in the frame of the validation (Val) and verification (Ver) studies.

Matrices	RSD_r (%)			RSD_{ip} (%)		R_{Rec} (%)		
	Excel		Spiky	Excel		Excel		Spiky
	Val*	Ver	Ver*	Val*	Ver	Val*	Ver	Ver*
PM [14]	1.7-4.9	2.4-5.6	2.3 [11]	3.2	4.6	87.1-102	89.8-103	88.9-100 [11]
FS [15]	2.0-6.3	0.7-5.0	2.4-4.3 [11]	5.5-6.1	1.6-4.7	96.1-104	92.9-104	98.4-114 [11]

RSD_r; RSD_{ip}: relative standard deviation for *repeatability* and *intermediate precision*; R_{Rec}: *Recovery rate*
*Studies carried out by the Applicant

The performance characteristics obtained in the frame of the validation and verification studies for the determination of *xylanase* activity in *premixtures* and *feedingstuffs* containing *Rovabio® Excel* (Table 2) [14,15] are in good agreement with (i) those reported in the frame of the a previous dossier (FAD 2010-0189) [4] and (ii) those obtained when analysing *premixtures* and *feedingstuffs* containing the *feed additive* investigated (*Rovabio® Spiky)* [11], thus demonstrating the extension of the scope of the method to the latter samples. Furthermore, a limit of quantification (LOQ) of 65 U/kg *feedingstuffs* was reported by the Applicant [11].

Based on the presented performance characteristics the EURL recommends for official control the single-laboratory validated and further verified viscosimetry methods for the determination of *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*.

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

In the viscosimetry method the *endo-1,3(4)-β-glucanase* catalyses the hydrolysis of glycosidic bonds in the barley β-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 viscosimeter at pH 5.5 and 30 ^{o}C.

The *feed additive* (0.6 g for the powder and 0.9 g for the liquid formulation) is weighed and extracted in sodium acetate buffer (pH 5.5) at room temperature. If needed, the extract is filtered and/or further diluted to be falling within the 5-15 min test measuring interval. 3 ml of water and 1 ml substrate (1% barley β-glucan) are placed in a disposable sterile tube, sonicated for 5 s and let at 30 ^{o}C for at least 5 min. Then 1 ml of the *feed additive* extract is

added to the substrate at 30 °C and the analysis is performed. The *β-glucanase* activity is quantified against a linear regression curve prepared from enzyme standards with known activities prepared in pure solutions (external calibration) [16].

The colorimetric method is based on the enzymatic hydrolysis of the barley β-glucan and the consequent colour formation of released sugar with 3,5-dinitrosalicylic acid (DNS) at pH 5.0 and 50 °C.

The *feed additive* sample (0.6 g for the powder and 0.9 g for the liquid formulation) is weighed, extracted in water at room temperature and, if needed, further diluted to be falling within the linear range of the method. The substrate (1.5% barley β-glucan solution) is placed into a tube and equilibrated at 50 °C for at least 5 min. Then 250 µl of the *feed additive* extract is added and incubated at 50 °C for 10 min. After this time the reaction is stopped by adding 2 ml of 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 15 min and then let 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 then expressed in *glucose* equivalents [17].

As for *xylanase*, the Applicant submitted first experimental data related to *Rovabio® Excel*. Supplementary information was later provided, upon request by the EURL-FA, to demonstrate the applicability of the two methods (viscosimetry & colorimetry) to the two products (i.e. *Rovabio® Spiky* and *Rovabio® Excel*). Table 3 shows that the performance characteristics reported [10,11,18,19] for the determination of *β-glucanase* activity in the two products are in good agreement, thus extending the scope of the two methods to *Rovabio® Spiky* samples.

Table 3. Performance characteristics obtained by the Applicant for the determination of *β-glucanase* activity in the *Rovabio* products (*Excel* and *Spiky*) applying the viscosimetry and colorimetry (DNS) methods.

Method	RSDr (%)		RSD_{ip} (%)		R_{Rec} (%)	
	Spiky* [10]	Excel	Spiky* [10]	Excel	Spiky [11]	Excel [11]
Visco	1.8-8.6	2.4-3.6 [18]	3.5-8.2	3.1 [18]	96.4-105	96.7-117
DNS	0.9-1.6	1.0-2.7 [19]	0.8-2.0	3.9 [19]	94.1-105	96.5-107

RSD_r; RSD_{ip}: relative standard deviation for *repeatability* and *intermediate precision;* R_{Rec}*: Recovery rate*
*Calculated by EURL from [10]

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

The *feedingstuffs* (10 g) is extracted with a buffer solution (0.1M (4-Morpholine Ethane Sulfonic acid)**,** pH 6.0; 1 % lauryl sulphate) 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). 3 ml of water and 1 ml substrate (1% barley β-glucan) are placed in a disposable sterile tube, sonicated for 5 s and let at 30 ^{o}C for at least 5 min. Then 1 ml of the *feedingstuffs* extract is added to the substrate at 30 ^{o}C and the analysis is performed. The quantification is carried out via a calibration curve of a reference *endo-1,3 (4)-β-glucanase* with a known activity (determined applying the viscosimetric method for the *feed additive* described above). 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)-β-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 and allows for 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 [20].

The performance characteristics obtained in the frame of the validation and verification studies for the determination of *β-glucanase* activity in *feedingstuffs* containing *Rovabio® Excel* (Table 4) [21] are in good agreement with (i) those reported in the frame of the a previous dossier (FAD 2010-0189) [4] and (ii) those obtained when analysing *feedingstuffs* containing the *feed additive* investigated (*Rovabio® Spiky)* [11], thus demonstrating the extension of the scope of the method to the latter samples. Furthermore, a limit of quantification (LOQ) of 218 U/kg *feedingstuffs* was reported by the Applicant [11].

Table 4: Performance characteristics for the determination of *β-glucanase* activity in *feedingstuffs* (FS) containing one of the *Rovabio* products (*Excel* or *Spiky*) by viscosimetry, obtained in the frame of the validation (Val) and verification (Ver) studies.

Matrix	RSD_r (%)			RSD_{ip} (%)		R_{Rec} (%)		
	Excel		*Spiky*	*Excel*		*Excel*		*Spiky*
	Val*	Ver	Ver*	Val*	Ver	Val*	Ver	Ver*
FS [21]	1.9-3.3	2.5-4.5	4.6-5.4 [11]	3.5	6.8	97-120	81-103	95.5-106 [11]

RSD_r; RSD_{ip}: relative standard deviation for *repeatability* and *intermediate precision;* R_{Rec}*: Recovery rate*
*Studies carried out by the Applicant

For the quantification of ***β-glucanase* activity in *premixtures*** the Applicant performed, in the frame of a previous dossier (FAD 2010-00189) [4], a solid dilution of the *premixtures* with a blank feed and demonstrated the applicability of the method for the *feedingstuffs* described above. Additionally, the Applicant provided similar experimental evidences for *premixtures* containing *Rovabio® Spiky* [11], thus demonstrating the extension of the scope of this method to the latter samples.

Based on the performance characteristics presentedthe EURL recommends for official control the single-laboratory validated and further verified viscosimetry methods for the determination of *β-glucanase* activity in the *feed additive*, *premixtures* and *feedingstuffs* together with the alternative colorimetric (DNS) method for the determination of *β-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) is not considered necessary.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control the validated and further verified viscosimetry methods for the determination of *xylanase* and *β-glucanase* in the *feed additive premixtures* and *feedingstuffs* and alternatively the colorimetric (DNS) methods for the determination of *xylanase* and *β-glucanase* in the *feed additive.*

Recommended text for the register entry (analytical method)

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

- viscosimetry method based on decrease in viscosimetry produced by the action of *endo-1,4-β-xylanase* on the xylan containing substrate (wheat arabinoxylan) at pH 5.5 (or pH 3.3) and 30 ^{o}C.

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

- colorimetric (DNS) method based on the enzymatic hydrolysis of the birchwood xylan pH 4.0 and 50 ^{o}C.

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

- viscosimetry method based on decrease in viscosimetry produced by the action of *endo-1.3(4)-β-glucanase* on the glucan containing substrate (barley β-glucan) at pH 5.5 and 30 ^{o}C.

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

- colorimetric (DNS) method based on the enzymatic hydrolysis of the barley β-glucan at pH 5.0 and 50 °C.

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

One *xylanase* (or *β-glucanase)* DNS unit corresponds to the amount of *xylanase (*or *β-glucanase)* which liberate from the birchwood xylan (or barley β-glucan) one µmol of *xylose* (or *glucose*) per minute at 50 °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® Spiky* 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, Ref: SANCO/G1:Forw.Appl.1831/0027-2013
[2] *Application, Proposal for Register Entry – Annex A
[3] *Technical dossier, Section II, Identity, characterisation and conditions of use of the additive; methods of analysis
[4] EURL evaluation Report FAD 2010-0189 http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2010-0189.pdf
[5] 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
[6] *Technical dossier, Section II, Annex_II_6_1
[7] *Technical dossier, Section II, Annex_II_6_5
[8] *Technical dossier, Section II, Annex_II_6_2
[9] *Technical dossier, Section II, Annex_II_6_6
[10] *Technical dossier, Section II, Annex_II_4_1
[11] *Technical dossier, Supplementary information, Applicability reports preparations
[12] *Technical dossier, Section II, Annex_II_6_21
[13] *Technical dossier, Supplementary Information, T005-i07ev _Xyl visco in feeds
[14] *Technical dossier, Section II, Annex_II_6_24
[15] *Technical dossier, Section II, Annex_II_6_28
[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_14
[19] *Technical dossier, Section II, Annex_II_6_10
[20] *Technical dossier, Section II, Annex_II_6_29
[21] *Technical dossier, Section II, Annex_II_6_32

*Refers to Dossier no: FAD-2013-0030

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was European Union Reference Laboratory for Feed Additives, IRMM, 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 (EC) No 885/2009.

8. ACKNOWLEDGEMENTS

The following National Reference Laboratories contributed to this report:

- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen. Jena (DE)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin (PL))