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## Safety of the fermentation product of *Aspergillus oryzae* NRRL 458 (Amaferm<sup>®</sup>) as a feed additive for dairy cows (Biozyme Inc.)

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Mojca Fašmon Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Noël Dierick, Miguel Prieto Maradona, Jaume Galobart, Elisa Pettenati and Montserrat Anguita

### Abstract

Amaferm<sup>®</sup> is a fermentation product produced by *Aspergillus oryzae* NRRL 458, containing alpha-amylase and cellulase enzyme activities, authorised for use as a feed additive for dairy cows. In 2016, the applicant requested for the renewal of the authorisation and the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued an opinion at that regard in 2020. In that opinion, the Panel could not confirm the previously drawn conclusions (EFSA, 2006) regarding the safety of the production strain, and consequently could not confirm the safety of the additive for the target species and consumers. In the current submission, the applicant provided supplementary information that allowed the Panel to conclude on the identity of the production strain, redefine the specifications of the additive, and finally to conclude on its safety. Therefore, the new data provided permit to conclude that Amaferm<sup>®</sup> complies with the conditions of the authorisation. However, the Panel noted that there is the need to change the specification and description of the cellulase and amylase units in the authorisation act. The data provided in the previous (EFSA FEEDAP Panel, 2020) and the current assessments support that Amaferm<sup>®</sup> remains safe under the approved conditions for target species, consumers and the environment. The additive is non-irritant to skin and eyes, or a dermal sensitiser but should be considered a potential respiratory sensitiser.

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## 1. Introduction

### 1.1. Background and Terms of Reference as provided by the requestor

Regulation (EC) No 1831/2003 establishes rules governing the Community authorisation of additives for animal nutrition and, in particular, Article 9 defines the terms of the authorisation by the Commission.

The applicant, van Eys, J.E. (acting on behalf of Biozyme Inc.),<sup>1</sup> is seeking a Community authorisation of Fermentation product of *Aspergillus oryzae* NRRL 458 (Amaferm) as a feed additive to be used as a digestibility enhancer for dairy cows (Table 1).

**Table 1:** Description of the substances

<b>Category of additive</b>	Zootechnical additives
<b>Functional group of additive</b>	Digestibility enhancers
<b>Description</b>	Fermentation product of <i>Aspergillus oryzae</i> NRRL 458 (Amaferm®)
<b>Target animal category</b>	Dairy cows
<b>Applicant</b>	Van Eys, J.E. (Acting on behalf of Biozyme Inc.) <sup>1</sup>
<b>Type of request</b>	New Opinion

On 28 January 2020, the Panel on Additives and Products or Substances used in Animal Feed of the European Food Safety Authority ("Authority"), in its opinion on the product, could not conclude on the safety for the target species and consumers. Regarding the enzyme activities in the fermentation product of fermentation product of *Aspergillus oryzae* NRRL 458 (Amaferm), its taxonomic classification, and uncertainty remains regarding the presence of viable cells/spores in the final product and weaknesses and limitation in the methods of analysis were noted. After the discussion with the Member States on the Standing Committee, it was suggested to check for the possibility to provide supplemental data.

The Commission gave the possibility to the applicant to submit complementary information in order to complete the assessment and to allow a revision of Authority's opinion. The new data have been received on 17 August 2020 and are attached to this letter.

In view of the above, the Commission asks the Authority to deliver a new opinion on fermentation product of *Aspergillus oryzae* NRRL 458 (Amaferm) as a feed additive for dairy cows, based on the additional data submitted by the applicant.

### 1.2. Additional information

The fermentation product of *A. oryzae* NRRL 458 (Amaferm®) under assessment is authorised as a zootechnical additive (digestibility enhancers) for dairy cows.<sup>2</sup>

The European Food Safety Authority (EFSA) adopted a scientific opinion on the safety and efficacy of the product for dairy cows and cattle for fattening (EFSA, 2006). In 2020, the FEEDAP Panel assessed the application for the renewal of the authorisation and concluded that the data provided by the applicant did not allow the Panel to conclude that Amaferm® complies with the conditions of the authorisation (EFSA FEEDAP Panel, 2020). In this regard, the FEEDAP Panel could not confirm the previously drawn conclusions regarding the identity of the production strain and the safety of the resulting product. Moreover, weaknesses and limitations were identified in the methods of analysis for the active substances submitted in the context of the renewal of the authorisation and on the specifications of the additive.

## 2. Data and methodologies

### 2.1. Data

The present assessment is based on data submitted by the applicant in the form of supplementary information<sup>3</sup> to a previous application on the same additive.<sup>4</sup> The FEEDAP Panel used the data

<sup>1</sup> On 10th December 2021 EFSA was informed about the change of the EU representative for Biozyme Inc. from Mr van Eys to Pen&Tec Consulting SLU.

<sup>2</sup> Commission Regulation (EC) No 537/2007 of 15 May 2007 concerning the authorisation of the fermentation product of *Aspergillus oryzae* (NRRL 458) (Amaferm) as a feed additive. OJ L 128, 16.5.2007, p. 13.

<sup>3</sup> FEED dossier reference: FAD-2020-0064.

<sup>4</sup> FEED dossier reference: FAD-2016-0055.

provided by the applicant together with data from other sources, such as previous risk assessments by EFSA.

The European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active substances in animal feed was released in the context of the previous assessment.<sup>5</sup> In the current submission the applicant provided a new method for the determination of cellulase activity in the feed additive and the EURL amended the report accordingly.<sup>6</sup>

## 2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety of the fermentation product(s) of *A. oryzae* NRRL 458 (Amaferm®) is in line with the principles laid down in Regulation (EC) No 429/2008<sup>7</sup> and the relevant guidance documents: Guidance on the renewal of the authorisation of feed additives (EFSA FEEDAP Panel, 2013).

## 3. Assessment

The fermentation product of *A. oryzae* NRRL 458 (Amaferm®) under assessment is authorised as a zootechnical additive (digestibility enhancers) for dairy cows. The additive is authorised for use in dairy cows from 85 to 300 mg of additive/kg complete feed (12% moisture content, equivalent to 2 to 6 g/cow per day).

The additive is authorised as a mixture of 4–5% of the fermentation product of *A. oryzae* NRRL 458, with 94–95% wheat bran and 1% stainless steel grit (containing 5% cobalt carbonate) as a microtracer. The composition of the additive was described in full in 2006 by the FEEDAP Panel (EFSA, 2006), and the new formulation without the stainless-steel grit was described in the assessment done in 2020 (EFSA FEEDAP Panel, 2020).

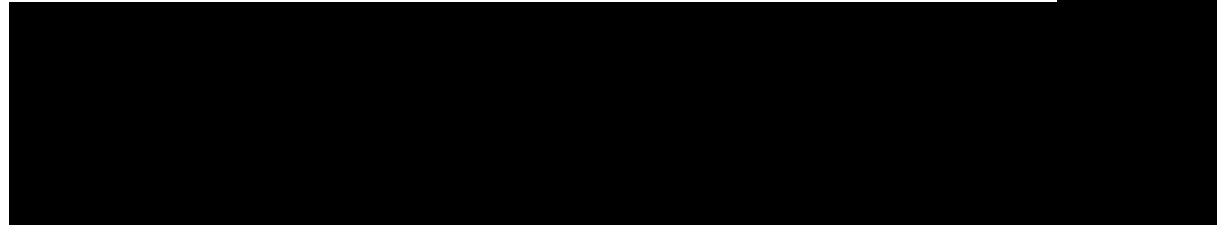
From the data provided by the applicant for the renewal of the authorisation, uncertainty remained on the identification of the production strain as *A. oryzae*. Consequently, the Panel considered that the information provided by the applicant did not fulfil the minimum requirements to support that the additive remains safe under the approved conditions for target species, consumers and the users. Moreover, comments on the methods of analysis of the active substances and on the specifications of the additive were made by the Panel (EFSA FEEDAP Panel, 2020).

The applicant provided supplementary information regarding the identification of the production strain, presence of viable cells in the additive and clarifications on the enzyme activities present in the additive, including the method of analysis of cellulase.

### 3.1. Characterisation of the production strain and absence of viable cells

In the previous assessment (EFSA FEEDAP Panel, 2020), the FEEDAP Panel could not conclusively determine the species of the production strain since the data provided did not allow to distinguish between *A. oryzae* and *A. flavus* (potentially toxigenic and pathogen). Moreover, uncertainty remained on the presence of viable cells/spores of the production strain in the final product (additive). Owing to the uncertainty on these two aspects, a conclusion on the compliance with the conditions of the authorisation or on the safety of the additive could not be drawn.

In the current submission, whole genome sequence data of the production strain



<sup>5</sup> The full report is available on the EURL website: <https://ec.europa.eu/jrc/sites/default/files/finrep-fad-2016-0055-amaferm.pdf>

<sup>6</sup> The full report is available on the EURL website: [https://ec.europa.eu/jrc/sites/default/files/finrep\\_rev\\_addendum\\_fad-2020-0064-amaferm.pdf](https://ec.europa.eu/jrc/sites/default/files/finrep_rev_addendum_fad-2020-0064-amaferm.pdf)

<sup>7</sup> Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

<sup>8</sup> FAD-2020-0064/Enclosure 1.

<sup>9</sup> FAD-2020-0064/Supplementary information July 2021/SIN Reply.

[REDACTED] allow to conclude that the production strain belongs to the *A. oryzae* clade.

In the first assessment for the renewal (EFSA FEEDAP Panel, 2020), no viable cells were detected

[REDACTED]<sup>10</sup> However, the information was deemed as not sufficient to conclude, owing to the short incubation time. In the current assessment, two new data sets were provided. [REDACTED]

Considering the overall data provided in the current and the previous assessments, the Panel is not in the position to fully conclude on the absence of viable cells/spores of the production strain in the additive.

The production strain has been identified as *A. oryzae*. Although the herein data provided do not allow to conclusively exclude the presence of viable cells/spores of the production strain in the additive, in the previous assessment (EFSA FEEDAP Panel, 2020), no antimicrobial activity was found in the final additive, and mycotoxins that may be potentially produced by strains of the *A. oryzae* clade (including ochratoxin A, aflatoxin and fumonisin) were not detected. Based on the above data, the Panel did not identify safety concerns related to the possible presence of viable cells/spores of the production strain in the additive.

### 3.2. Characterisation of the additive

In the assessment for the renewal of the authorisation (EFSA FEEDAP Panel, 2020), the Panel noted that enzyme activities given, 3 IU<sup>12</sup> cellulase/g and 40 IU<sup>13</sup> amylase/g, were not in line with the enzyme activities reported in the previous EFSA opinion (EFSA, 2006). Following a request for clarification, the applicant confirmed that the new minimum specifications for Amaferm® should be 0.3 mIU<sup>14</sup> cellulase/g and 20 mIU<sup>15</sup> amylase/g.<sup>16</sup> The applicant provided analytical data from four batches demonstrating compliance with these new minimum specifications (average for cellulase was 0.78 mIU/g and for amylase 33 mIU/g). Data from other batches analysed in the context of the EURL assessment of the methods also showed compliance with the minimum specifications for the enzyme activity.<sup>17</sup> The Panel also noted that the performance of the method of analysis for cellulase does not allow to reliably quantify the enzyme activity in the feed additive at the minimum specifications. Moreover, no methods for the detection of the cellulase and amylase in premixtures and feed were made available by the applicant. Finally, the Panel considered that no comparison was possible with the enzyme activities present in the additive now and those established at the time of the first assessment and, therefore, the Panel could not compare the composition of the additive to the one previously assessed/authorised. In the current submission, the applicant provided clarifications on the differences in the two enzyme activities present in the additive reported previously (EFSA FEEDAP

<sup>10</sup> FAD-2016-0055/Technical dossier/Supplementary information June 2018/Annex 7.

<sup>11</sup> FAD-2020-0064/Enclosure 2.

<sup>12</sup> Commission Regulation (EC) No 537/2007: one IU refers to the cellulase that liberates 1 micromole of glucose per minute from carboxymethylcellulose at pH 6.5 and at 39°C.

<sup>13</sup> Commission Regulation (EC) No 537/2007: one IU refers to the amylase that liberates 1 micromole of glucose per minute from potato starch at pH 6.5 and at 39°C.

<sup>14</sup> FAD-2016-0055: one unit of endo-1,4-beta-glucanase activity (IU) refers to the cellulase that liberates 1 micromole of glucose per minute from carboxymethylcellulose at pH 6.5 and at 38°C. 'm' stands for milli.

<sup>15</sup> FAD-2016-0055: one unit of alpha-amylase activity (IU) refers to the amylase that liberates 1 micromole of glucose per minute from potato starch at pH 6.9 and at 38°C. 'm' stands for milli.

<sup>16</sup> FAD-2016-0055: Technical dossier/Supplementary information February 2019.

<sup>17</sup> FAD-2016-0055: Technical dossier/Supplementary information February 2019/Annexes 10–15.

Panel, 2020), which were of administrative nature and including errors in the description of the method and during the assessment/authorisation process.

The explanations provided by the applicant allow to conclude that the additive shows similar composition as the one reported in the initial assessment and therefore the additive would comply with the conditions of the authorisation and no modification would be required in the conditions of use.

However, in the current submission the applicant provided a new method of analysis for the cellulase in the additive which has been evaluated by the EURL (see Section 2.1). The characteristics are presented in the EURL report and based on colorimetry and on the reaction by endo-1,4-beta-glucanase on the substrate used in the method.<sup>18</sup> The newly defined activity should read 'one CellG5 Unit (U) is the amount of enzyme in the presence of excess thermostable  $\beta$ -glucosidase, required to release one micromole of 4-nitrophenol from CellG5, in one minute under the defined assay conditions'. Since the new method leads to a new enzyme activity definition, the applicant provided data from five batches of the additive to establish the conversion factor (and correlation) between the two methods. The conversion factor established based on the data for the five batches is given as 1 mU (new method) = 48.4 mIU (old method).

Considering all the information made available by the applicant regarding the administrative errors in the amylase activity and the change in the method of analysis of the cellulase, the correct enzyme activities in the specifications of the additive would be 14.5 mU (CellG5)<sup>19</sup> cellulase/g and 20 mIU<sup>15</sup> amylase/g.

### 3.3. Safety of the additive

In the initial assessment in 2006, based on the data and knowledge available at that time, the FEEDAP Panel concluded that the additive is safe for dairy cows, for the consumers and the environment. It was also concluded that the additive is non-irritant to skin and eyes or a dermal sensitiser but should be considered a potential respiratory sensitiser.

In the application regarding the renewal of the authorisation (EFSA FEEDAP Panel, 2020), the applicant provided a literature search which the Panel considered to indicate no safety concerns from the use of the fermentation product from *A. oryzae* under assessment as a feed additive for cows. However, as uncertainty remained in the identification of the production strain as *A. oryzae* or *A. flavus*, the Panel considered that the suitability of the literature search performed was questionable until a definitive identification of the strain would be provided. Therefore, no conclusions were drawn on the safety. Since the identity of the strain has been confirmed in the supplementary information submitted and no concerns were identified, the Panel concludes that the additive remains safe under the approved conditions for the target species, consumers and the environment. The conclusions drawn by the FEEDAP Panel in 2006 on the safety for the users are retained, and therefore, it is concluded that the additive is non-irritant to skin and eyes or a dermal sensitiser but should be considered a potential respiratory sensitiser.

### 3.4. Efficacy

The additive is authorised for use in dairy cows from 85 to 300 mg of additive/kg complete feed (12% moisture content), equivalent to 2–6 g/cow per day. The present application for renewal of the authorisation does not include a proposal for amending or supplementing the conditions of the original authorisation that would have an impact on the efficacy of the additive and the composition of the additive shows similar specifications as the ones previously described. Therefore, there is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

### 3.5. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation<sup>20</sup> and Good Manufacturing Practice.

<sup>18</sup> [https://www.megazyme.com/documents/Booklet/K-CellG5-2V\\_DATA.pdf](https://www.megazyme.com/documents/Booklet/K-CellG5-2V_DATA.pdf)

<sup>19</sup> One CellG5 Unit (U) is the amount of enzyme in the presence of excess thermostable  $\beta$ -glucosidase, required to release one micromole of 4-nitrophenol from CellG5, in one minute under the defined assay conditions.

<sup>20</sup> Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.



## 4. Conclusions

The identity of the strain has been confirmed as *A. oryzae*. The presence of viable cells/spores of the production strain in the final product cannot be conclusively excluded. However, it is not considered a safety concern.

The Panel concludes that the additive remains safe under the approved conditions for the target species, consumers and the environment. The additive is non-irritant to skin and eyes, or a dermal sensitiser, but should be considered a potential respiratory sensitiser.

There is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

## 5. Recommendation

In order to consider the changes/errors in the methods and resulting enzyme activity and units, the specifications of the additive should be 14.5 mU (CellG5)<sup>19</sup> cellulase/g and 20 mIU<sup>15</sup> amylase/g.

## 6. Documentation provided to EFSA/Chronology

Date	Event
27/08/2020	Dossier received by EFSA. Fermentation product of <i>Aspegillus oryzae</i> NRRL 458. Submitted by Biozyme Inc.
24/08/2020	Reception mandate from the European Commission
27/11/2020	Request of supplementary information to the applicant in line with Article 7(3) of Commission Regulation (EC) No 1304/2003 – Scientific assessment suspended. <i>Issues: characterisation of the additive</i>
14/12/2020	Clarification teleconference during the risk assessment
25/02/2021	Reception of supplementary information from the applicant - Scientific assessment re-started
15/03/2021	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
17/05/2021	Request of supplementary information to the applicant in line with Article 7(3) of Commission Regulation (EC) No 1304/2003 – Scientific assessment suspended. <i>Issues: characterisation of the additive</i>
16/07/2021	Reception of supplementary information from the applicant - Scientific assessment re-started
10/11/2021	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

## References

- EFSA (European Food Safety Authority), 2006. Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on the safety and efficacy of the product "Amaferm" as a feed additive for dairy cows and cattle for fattening in accordance with Regulation (EC) No 1831/2003. EFSA Journal 2006;4(3):337, 17 pp. <https://doi.org/10.2903/j.efsa.2006.337>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2013. Guidance on the renewal of the authorisation of feed additives. EFSA Journal 2013;11(10):3431, 8 pp. <https://doi.org/10.2903/j.efsa.2013.3431>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos MDL, Christensen H, Dusemund B, Kos Durjava M, Kouba M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Cocconcelli PS, Dierick NA, Glandorf B, Herman L, Maradona MP, Rychen G, Saarela M and Anguita M, 2020. Scientific Opinion on the assessment of the application for renewal of the authorisation of Amaferm® (fermentation product of *Aspergillus oryzae* NRRL 458) as a feed additive for dairy cows. EFSA Journal 2020;18(2):6011, 9 pp. <https://doi.org/10.2903/j.efsa.2020.6011>

## Abbreviations

EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
RRec	recovery rate
RSDip	relative standard deviation for intermediate precision
RSDr	relative standard deviation for repeatability



## Annex A – Addendum to the Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of the Analysis for fermentation product of *Aspergillus oryzae* NRRL 458

Upon a request from DG SANTE, the EURL has evaluated the supplementary information provided in the frame of dossier FAD-2016-0055 - *fermentation product of Aspergillus oryzae* NRRL 458 (Amaferm®). The present evaluation of the analytical method for the official control of *endo-1,4-beta-glucanase* in the *feed additive* integrates the recommendations of the previously issued EURL report.

In the original dossier, the Applicant submitted for the quantification of *endo-1,4-beta-glucanase* (i.e. cellulase activity) in the *feed additive* a single-laboratory validated and further verified colorimetric method (SOP 953). Based on the insufficient performance characteristics of the method, the EURL did not consider it as suitable for official control. In response to this conclusion, the Applicant is proposing a modified colorimetric method for the quantification of *endo-1,4-beta-glucanase* in the *feed additive*, which has been validated and further verified (SOP 955). In particular, the Applicant implemented in the method protocol a change of the substrate by using a commercially available CellG5 reagent obtainable from Megazyme.

The updated measurement unit of the *endo-1,4-beta-glucanase* activity is a CellG5 Unit (U), where U is defined as “the amount of enzyme in the presence of excess thermostable  $\beta$ -glucosidase, required to release one micromole of 4-nitrophenol from CellG5 in one minute under the defined assay conditions”.

According to the Applicant, CellG5 is a reagent containing two components, namely the substrate and  $\beta$ -glucosidase. When applying the assay, *endo-1,4-beta-glucanase* of the sample reacts on the substrate, thereby releasing oligosaccharides labelled with 4-nitrophenol from the substrate. Subsequently, these oligosaccharides are hydrolysed by  $\beta$ -glucosidase from the reagent forming free 4-nitrophenol, which is measured with a spectrophotometer at 400 nm.

0.5 g of sample is extracted with an acetate buffer. After shaking for 15 min, the solution is filtered under vacuum and the enzyme extract from the sample is transferred into a 15 ml enzyme extract reaction tube. The sample is analysed in triplicate and a test of the blank is performed in parallel for each sample. 0.10 ml of CellG5 reagent substrate solution is dispensed in a sample reaction tube (blank included). The sample reaction tube and enzyme reaction tubes are pre-equilibrated at 40 °C for 3 min and subsequently 0.1 ml of the enzyme extract is added to each sample reaction tube (with the exception of the blank). The tubes are vortexed and incubated at 40 °C for 10 min. 1.5 ml of stopping reagent (2 % w/v Tris buffer pH 10) and 0.1 ml of enzyme extract are added to the blank and vortexed. The sample blank and the sample reaction tubes are transferred into a cuvette to measure the absorbance at 400 nm against distilled water in order to obtain the background and the reaction absorbances, respectively (recorded as average of the three measurements). Finally, the *endo-1,4-beta-glucanase* activity, expressed as CellG5 mU/g, is calculated from the difference of the reaction and blank absorbances in conjunction with the absorption coefficient of 4-nitrophenol, which is known for the specific spectrophotometric measurement conditions applied in the experiments.

The following performance characteristics were obtained in the frame of the validation and verification studies: a relative standard deviation for repeatability (RSDr) and intermediate precision (RSDip) ranging from 6.6 to 8.6%, a recovery rate (RRec) ranging from 96.3 to 97.8% and a limit of quantification of 7 CellG5 mU/g.

Based on the performance characteristics available the EURL recommends for official control the single-laboratory validated and further verified colorimetric method presented for the quantification of *endo-1,4-beta-glucanase* in the *feed additive*.

Given the fact that the modified method also led to a new enzyme activity definition for 1,4-beta-glucanase, there is also a need to estimate a conversion from the previously used Unit to the new Unit. According to the former definition, the guaranteed minimum enzymatic activity content of *endo-1,4-beta-glucanase* in Amaferm® is 0.3 mIU/g, where “one unit of *endo-1,4-beta-glucanase* activity (IU) refers to the cellulase that liberates 1 micromole of glucose per minute from carboxymethylcellulose at pH 6.5 and at 39 °C”.

The Applicant presented a study where 5 samples have been analysed in duplicate with the old SOP 953 and the new SOP 955 with results expressed respectively in IU and CellG5 U. The *feed additive* samples included in the study covered an activity range using the old definition from 0.43 mIU/g to 0.87 mIU/g and a conversion factor was established by dividing the measured enzyme

activity expressed in CellG5 mU by the corresponding enzyme activity expressed in mIU obtained on the same sample. The average conversion factor of 48.4<sup>21</sup> with a relative standard error of 5 % was calculated from these measurements and can be used for the conversion of the old to the new enzyme activity of *feed additives* within the activity range covered in this study.

### **Recommended text for the registry entry (analytical method)**

For the quantification of *endo-1,4-beta-glucanase* in the *feed additive*:

- colorimetric method based on the enzymatic reaction by *endo-1,4-beta-glucanase* on a CellG5 substrate

One CellG5 Unit (U) is the amount of enzyme in the presence of excess thermostable  $\beta$ -glucosidase, required to release one micromole of 4-nitrophenol from CellG5 in one minute under the defined assay conditions.

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<sup>21</sup> In the EURL report dated 15/03/2021 (Ref. Ares(2021)1860343) the average conversion factor has been accidentally specified as 48.2. In the present revised text the factor has been corrected to 48.4.