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## Safety and efficacy of Beltherm MP/ML (endo-1,4-beta-xylanase) as a feed additive for piglets, pigs for fattening and other porcine species

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### Abstract

Beltherm MP/ML contains endo-1,4-beta-xylanase and it is to be used as a zootechnical additive for weaned piglets, pigs for fattening and minor porcine species for fattening. The safety and efficacy of the additive when used for poultry species have been evaluated previously by the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel). In that assessment, the safety for the consumers, environment and the users was assessed. The applicant has requested for the authorisation in new species/categories. The Panel considered that the new use would not introduce hazards that have not already been considered. Therefore, it was concluded that the additive is safe for the consumers and the environment. Considering the safety for the user, the Panel concluded that the additive, in either form, is not toxic by inhalation or irritant for skin or eye but it is considered a potential respiratory sensitiser. The Panel could not conclude on the dermal sensitisation potential of the additive. In a trial with weaned piglets, the animals tolerated well a 200-fold the level recommended in feed by the applicant; therefore, the Panel concluded that the additive is safe for weaned piglets, pigs for fattening and minor porcine species at the recommended level. Based on the data provided to support the efficacy of the additive in weaned piglets and pigs for fattening, the Panel concluded that the additive has the potential to be efficacious at the recommended level in those species/categories. The conclusions on the safety and efficacy for weaned piglets and pigs for fattening were extrapolated to minor porcine species.

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## 1. Introduction

### 1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003<sup>1</sup> establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Puratos<sup>2</sup> for authorisation of the product Beltherm (endo-1,4-beta-xylanase), when used as a feed additive for weaned piglets, pigs for fattening and other porcine species (category: zootechnical additives; functional group: digestibility enhancers).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 12 January 2018.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the product Beltherm MP/ML (endo-1,4-beta-xylanase), when used under the proposed conditions of use.

### 1.2. Additional information

The product under assessment presents endo-1,4-beta-xylanase activity and is produced by fermentation with a genetically modified strain of *Bacillus subtilis*. The FEEDAP Panel adopted two scientific opinions on the safety and efficacy of Beltherm MP/ML (endo-1,4-beta-xylanase) as a feed additive for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding, turkeys for breeding purposes and minor poultry species (EFSA FEEDAP Panel, 2017, 2019).

## 2. Data and methodologies

### 2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier<sup>3</sup> in support of the authorisation request for the use of Beltherm MP/ML as a feed additive.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active substance in animal feed. The Executive Summary of the EURL report can be found in Annex A.<sup>4</sup>

### 2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Beltherm MP/ML (endo-1,4-beta-xylanase) is in line with the principles laid down in Regulation (EC) No 429/2008<sup>5</sup> and the relevant guidance documents: Guidance on zootechnical additives (EFSA FEEDAP Panel, 2012), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011), Technical Guidance: Extrapolation of data from major species to minor species regarding the assessment of additives for use in animal nutrition (EFSA, 2008), Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use (EFSA GMO Panel, 2011) and the Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018).

<sup>1</sup> Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.

<sup>2</sup> Puratos, Industrialaan 25, 1702 Groot-Bijgaarden, Belgium.

<sup>3</sup> FEED dossier reference: FAD-2017-0061.

<sup>4</sup> The full report is available on the EURL website: <https://ec.europa.eu/jrc/sites/jrcsh/files/finirep-fad-2016-0010-beltherm.pdf>

<sup>5</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.

### 3. Assessment

The additive Beltherm MP/ML contains endo-1,4-beta-xylanase (Enzyme Commission number: 3.2.1.8 xylanase) and it is intended to be used in feed for weaned piglets and pigs for fattening as a zootechnical additive (digestibility enhancer).

#### 3.1. Characterisation of the additive

The additive has been characterised and described in full in previous opinions (EFSA FEEDAP Panel, 2017, 2019). It is available in two forms, solid and liquid, which ensure a minimum activity of 500 ADXU<sup>6</sup>/g product. The xylanase present in the additive is produced by a genetically modified strain of *B. subtilis* which is deposited at the Belgian Co-ordinated Collections of Microorganisms, University of Ghent, with the deposition number BCCM-LMG S-27588.

The applicant has now provided new data on the stability in premixtures aimed to be added to feed for pigs.<sup>7</sup> Three batches of the xylanase were mixed with a complete premixture and stored for 6 months at room temperature (not specified); no enzyme activity loss was found after 6 months.

The additive is to be used in feed for weaned piglets, pigs for fattening and other porcine species at a level of 100 ADXU/kg feed.

#### 3.2. Safety

The safety of the production strain, and the safety of the additive for consumers and the environment were established in a previous opinion (EFSA FEEDAP Panel, 2017). Considering the safety for the user the Panel concluded that the additive, in either form, is not toxic by inhalation or irritant for skin or eye but it is considered a potential respiratory sensitiser. The Panel could not conclude on the dermal sensitisation potential of the additive. The FEEDAP Panel is not aware of any new information that would lead it to reconsider the conclusions drawn previously on the safety for the consumers, users and the environment. Moreover, the FEEDAP Panel considers that the new use requested by the applicant would not introduce hazards/risks not considered in previous assessments.

Since the application includes new species/categories the safety for these new target species should be assessed.

##### 3.2.1 Safety for weaned piglets and pigs for fattening

The applicant did not formally submit data at this regard on the basis that the additive is produced by a production strain that belongs to a species which qualifies for the Qualified Presumed Safety (QPS) assessment for safety. The FEEDAP Panel considers that for the enzyme products, even if produced by a species that qualifies for the QPS assessment, data supporting the safety for the target species is needed.

Among the efficacy trials submitted, one was identified as suitable to provide data regarding the safety for the target species (trial 5 in Section 3.3.1). A total of 144 male and female piglets (Pietrain × (Landrace × Duroc), 21 days old) were distributed in groups of 4 pigs (2 males and 2 females) in a total of 36 floor pens and allocated to four dietary treatments (9 replicates per treatment).<sup>8</sup> At arrival, the animals received from day 21 to day 26 of life a post-weaning adaptation diet (medicated feed). The piglets received the experimental diets from day 26 of life (mean body weight 9.6 kg). Two basal diets (prestarter and starter) based on wheat, milk whey, rye, barley and soya bean meal were either not supplemented (control) or supplemented with the additive to provide 100 (1×), 200 or 20,000 (200×) ADXU/kg feed. The enzyme activity was confirmed by analysis. The feed was offered in pelleted form and *ad libitum* for 42 days. General health status and mortality were monitored throughout the study. Body weight and cumulative feed intake were measured on days 14, 28 and 42 of study (body weight at the beginning as well) and feed to gain ratio was calculated. An analysis of variance (ANOVA) was performed on the data obtained, differences between the mean groups were checked with Dunnett's test and Duncan's multiple range test. The model included the effect of the diet and the block (location) and differences were considered significant at a level of at least  $p < 0.05$ .

<sup>6</sup> One ADXU is defined as the amount of enzyme which liberates one micromole of reducing sugars (xylose equivalents) from beechwood xylan per minute at pH 6.0 and at 70°C.

<sup>7</sup> Technical dossier/Section II/Annex 29.

<sup>8</sup> Technical dossier/Section IV/Annex IV.2.5.

A significant effect on the daily feed intake was observed showing that the animals receiving the 200-fold diet ate more feed than those in the control group. No other differences were observed in the parameters measured, with a final body weight of 27 kg, daily body weight gain of 425 g and a feed to gain ratio of 1.50. The product was tolerated well by the weaned piglets up to 200-fold the recommended level. Therefore, the FEEDAP Panel concludes that the additive is safe at the recommended level. This conclusion can be extended to pigs for fattening and extrapolated to minor porcine species (weaned and for fattening).

### 3.3. Efficacy

#### 3.3.1. Efficacy for weaned piglets

The applicant provided a total of five long-term trials. The details on the design of the studies are presented in Table 1 and the main results in Table 2. The five studies were conducted with weaned piglets and included groups with a non-supplemented diet (control) or with diets containing the xylanase under assessment. In all studies, the recommended level of 100 ADXU/kg feed was tested. The intended enzyme activities were confirmed by analysis (see Table 1) and showed values close to the intended dosage, with the exception of the recommended dose in trial 4 which was 178 ADXU/kg feed (78% higher than the intended). In trials 4 and 5, 21-day-old weaned piglets received a medicated feed before the start of the trial. In all studies, males and females were used, in trials 1, 2 and 3 the animals were sex separated, while in trials 4 and 5 pens included males and females. The animals were under study for at least 42 days (43 days in case of trial 4). The health and mortality were monitored throughout the study and the body weight and feed intake were recorded. Feed to gain ratio was calculated. Trial 5 included the analysis of the digestibility of the diets but did not include data on the metabolisable energy and therefore the data on the digestibility was not considered further in the assessment. The performance data of the individual trials were analysed with an ANOVA.

The results are summarised in Table 2. In general, no differences were observed in any of the parameters evaluated with the exception of the feed to gain ratio in trial 2 which was better for the piglets that received the additive at the recommended level of 100 ADXU/kg feed.

**Table 1:** Design of the studies performed with weaned piglets

Trial	Total no animals (animals/replicate) Replicates/treatment	Breed Sex Age at start	Diet composition (form)	Enzyme activity (ADXU/kg)	
				Intended	Analysed
1 <sup>(a)</sup>	288 (12) 12	Pietrain × (Landrace × Large White) ♀,♂ (sex separated) 28 days	Wheat, barley, rye, soya bean meal, whey powder (pellets)	0 100	0 77
2 <sup>(b)</sup>	576 (12) 24	Duroc × (Landrace × Large White) ♀,♂ (sex separated) 28 days	Wheat, barley, rye, soya bean meal, whey powder (pellets)	0 100	0 85
3 <sup>(c)</sup>	320 (10) 16	Pietrain × (Landrace × Large White) ♀,♂ (sex separated) 28 days	Wheat, barley, rye, soya bean meal, whey powder (pellets)	0 100	0 95
4 <sup>(d)</sup>	216 (6) 9	Pietrain × (Landrace × Large White) ♂,♀ 26 days	Wheat, milk whey, rye, barley, soya bean meal (pellets)	0 100 200 400	0 178 191 409

Trial	Total no animals (animals/replicate) Replicates/treatment	Breed Sex Age at start	Diet composition (form)	Enzyme activity (ADXU/kg)	
				Intended	Analysed
5 <sup>(e)</sup>	144 (4) 9	Pietrain × (Landrace × Duroc) ♂,♀ 26 days	Wheat, milk whey, rye, barley, soya bean meal (pellets)	0 100 200 20,000	0 95 225 21,500

(a): Technical dossier/Section IV/Annex IV.2.1.

(b): Technical dossier/Section IV/Annex IV.2.2.

(c): Technical dossier/Section IV/Annex IV.2.3.

(d): Technical dossier/Section IV/Annex IV.2.4.

(e): Technical dossier/Section IV/Annex IV.2.5.

**Table 2:** Effects of Beltherm xylanase on the performance of weaned piglets

Trial	Enzyme activity (ADXU/kg)	Daily feed intake (g)	Body weight (kg)		Daily body weight gain (g)	Feed to gain ratio	Dead and culled (%)
			Initial	Final			
1	0	542	7.9	24.2	387	1.40	1.4
	100	533	7.9	24.4	391	1.36	0.7
2	0	652 <sup>a</sup>	7.3	25.1	424	1.55 <sup>a</sup>	2.1
	100	605 <sup>b</sup>	7.3	24.5	411	1.48 <sup>b</sup>	2.8
3	0	492	6.8	21.7	354	1.39	3.1
	100	493	6.8	21.9	360	1.37	2.5
4	0	591	6.6	25.6	442	1.34	0
	100	574	6.6	25.1	431	1.33	1.9
	200	575	6.6	25.2	433	1.33	1.9
	400	582	6.6	26.7	444	1.31	9.3
5	0	614 <sup>b</sup>	9.6	27.1	416	1.47	2.8
	100	631 <sup>ab</sup>	9.7	27.8	431	1.46	0
	200	619 <sup>b</sup>	9.7	26.7	407	1.53	0
	20,000	663 <sup>a</sup>	9.6	28.1	441	1.51	0

<sup>a,b</sup>: Values within one column for the same study with different superscripts are different ( $p < 0.05$ ).

The applicant pooled the data of the trials and considered control and 100 ADXU/kg feed groups. Upon request, the applicant excluded trial 4 due to a high enzyme activity in the diet with the recommended dose.<sup>9</sup> An ANOVA was done including the treatment, the sex, the trial (nested into sex) and the interaction between trial (nested into sex) and treatment and considering the initial body weight of the piglets.<sup>10</sup> The final body weight was 23.6 and 23.7 kg for control and 100 ADXU/kg feed, respectively (corresponding to an average daily weight gain of 379 and 381 g/day) and the average daily feed intake was 554 and 546 g/day, respectively. The feed conversion ratio was 1.45 for control and 1.42 for the group receiving 100 ADXU/kg feed. The statistical analysis showed no treatment effect on the final body weight and on the feed intake, but a significant and positive effect of the xylanase on the feed conversion ratio. Therefore, the Panel concludes that the additive has the potential to be efficacious in weaned piglets as a zootechnical additive.

### 3.3.2. Efficacy for pigs for fattening

The applicant provided four long-term trials. The details on the design of the studies are presented in Table 3 and the main results in Table 4. The four studies were conducted with growing pigs and

<sup>9</sup> Technical dossier/Supplementary information July 2018/ Annex 2.

<sup>10</sup> Technical dossier/Supplementary information November 2018.



included groups with a non-supplemented diet (control) or diets containing the xylanase from Beltherm. In all studies, the recommended level of 100 ADXU/kg feed was tested. The intended enzyme activities were confirmed by analysis (see Table 3), with a very low enzyme activities in trial 2 for the recommended level. In all studies, males and females were used. The animals were under study for at least 77 days. The health and mortality were monitored throughout the study and the body weight and cumulative feed intake were recorded. Feed to gain ratio was calculated. The data from the individual studies were analysed with an ANOVA.

The results are presented in Table 4. No significant effects were found in any of the parameters studied.

**Table 3:** Design of the studies performed with pigs for fattening

Trial	Total no animals (animals/replicate) Replicates/treatment	Breed Sex Duration of the study	Diet composition (form)	Enzyme activity (ADXU/kg)	
				Intended	Analysed
1 <sup>(a)</sup>	160 (5) 8	PIC × (Landrace × Duroc) ♀,♂ (3 female, 2 male per pen) 77 days	Barley, wheat, rapeseed, soya (pellets)	0	0
				100	78
				200	141
				400	274
2 <sup>(b)</sup>	128 (4) 8	Pietrain × (Landrace × Large White) ♀,♂ (sex separated) 103 days	Wheat, barley, rye, soya bean meal (pellets)	0	0
				100	47
				200	140
				400	342
3 <sup>(c)</sup>	72 (3) 12	Pietrain × (Landrace × Large White) ♀,♂ (sex separated) 99 days	Wheat, rye, soya bean meal (pellets)	0	0
				100	85
4 <sup>(d)</sup>	240 (10) 8	Duroc × (Landrace × Large White) ♂,♀ (sex separated) 94 days	Wheat, barley, soya bean meal, rapeseed meal (pellets)	0	0
				100	80
				200	175

(a): Technical dossier/Section IV/Annex IV.3.1.

(b): Technical dossier/Section IV/Annex IV.3.2.

(c): Technical dossier/Section IV/Annex IV.3.3.

(d): Technical dossier/Section IV/Annex IV.3.4.

**Table 4:** Effects of Beltherm xylanase on the performance of pigs for fattening

Trial	Enzyme activity (ADXU/kg)	Daily feed intake (g)	Body weight (kg)		Daily body weight gain (g)	Feed to gain ratio	Dead and culled (%)
			Initial	Final			
1	0	2,048	37.4	106	885	2.32	2.5
	100	2,081	37.4	109	926	2.25	2.5
	200	2,109	37.4	110	932	2.26	2.5
	400	2,090	37.4	110	935	2.24	2.5
2	0	1,838	26.3	109.3	805	2.29	0
	100	1,821	26.3	109.7	809	2.25	0
	200	1,850	26.4	109.3	805	2.30	3.1
	400	1,848	26.3	109.9	811	2.28	0
3	0	1,608	25.7 <sup>a</sup>	94.2	692	2.32	0
	100	1,617	25.6 <sup>b</sup>	95.0	701	2.31	0
4	0	1,639	25.6	97.7	767	2.14	2.5
	100	1,646	25.6	98.7	777	2.12	2.5
	200	1,631	25.7	94.3	731	2.24	5.1

a,b: Values within one column for the same study with different superscripts are different ( $p < 0.05$ ).



The applicant pooled the data of trials and considered control and 100 ADXU/kg feed groups. An ANOVA was done including the treatment, the sex, the trial (nested into sex) and the interaction between trial (nested into sex) and treatment.<sup>11</sup> The final body weight was 102 and 103 kg for control and 100 ADXU/kg feed, respectively (corresponding to an average daily weight gain of 798 and 817 g/day) and the average daily feed intake was 1,812 and 1,823 g/day, respectively. The feed conversion ratio was 2.27 for control and 2.242 for the group receiving 100 ADXU/kg feed. Therefore, the Panel concludes that the additive has the potential to be efficacious as a zootechnical additive in pigs for fattening at 100 ADXU/kg feed.

### 3.3.2.1. Conclusions on efficacy for the target species

The Panel concludes that the additive has the potential to be efficacious as a zootechnical additive in weaned piglets and pigs for fattening at 100 ADXU/kg feed. The conclusion is extrapolated to minor porcine species (weaned and fattening).

## 3.4. 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>12</sup> and Good Manufacturing Practice.

## 4. Conclusions

Beltherm is safe for the target species at the recommended level and no safety concerns are expected for the consumers of food products derived from animals fed with the additive or for the environment. The additive, in either form, is not toxic by inhalation or irritant for skin or eye but it is considered a potential respiratory sensitiser. The Panel cannot conclude on the dermal sensitisation potential of the additive.

The Panel concludes that the additive has the potential to be efficacious as a zootechnical additive in weaned piglets and pigs for fattening at 100 ADXU/kg feed. The conclusion is extrapolated to minor porcine species (weaned and fattening).

## Documentation provided to EFSA

- 1) Beltherm MP/ML for pigs. November 2017. Submitted by Puratos.
- 2) Beltherm MP/ML for pigs. Supplementary information. July 2018. Submitted by Puratos.
- 3) Beltherm MP/ML for pigs. Supplementary information. August 2018. Submitted by Puratos.
- 4) Beltherm MP/ML for pigs. Supplementary information. November 2018. Submitted by Puratos.
- 5) Comments from Member States.

## Chronology

Date	Event
3/11/2017	Dossier received by EFSA
22/11/2017	Reception mandate from the European Commission
12/1/2018	Application validated by EFSA – Start of the scientific assessment
12/4/2016	Comments received from Member States
5/6/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: characterisation and efficacy</i>
13/7/2018	Reception of supplementary information from the applicant – Scientific assessment re-started
30/7/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: characterisation and efficacy</i>
10/8/2018	Reception of supplementary information from the applicant – Scientific assessment re-started

<sup>11</sup> Technical dossier/Supplementary information July 2018/Annex 3.

<sup>12</sup> Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

Date	Event
10/9/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: characterisation and efficacy</i>
13/11/2018	Reception of supplementary information from the applicant – Scientific assessment re-started
23/1/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

## References

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- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2017. Scientific Opinion on the safety and efficacy of Beltherm MP/ML (endo-1,4-beta-xylanase) as a feed additive for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding, turkeys for breeding purposes and minor poultry species. *EFSA Journal* 2017;15(7):4941, 17 pp. <https://doi.org/10.2903/j.efsa.2017.4941>
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- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2019. Scientific Opinion on the safety and efficacy of Beltherm MP/ML (endo-1,4-beta-xylanase) as a feed additive for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding, turkeys for breeding purposes and minor poultry species. *EFSA Journal* 2019;5609, 8 pp. <https://doi.org/10.2903/j.efsa.2019.5609> under publication.
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## Abbreviations

ANOVA	analysis of variance
BCCM	Belgian Co-ordinated Collections of Microorganisms
DNS	3,5-dinitrosalicylic acid
EURL	European Union Reference Laboratory
FEEDAP	Panel EFSA Panel on Additives and Products or Substances used in Animal Feed
QPS	Qualified Presumed Safety

## **Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Beltherm MP/ML**

In the current application, authorisation is sought for Beltherm<sup>®</sup> MP &ML under article 4(1) (new feed additive) under the category/functional 4(a) “zootechnical additives”/“digestibility enhancers”. Specifically, authorisation is sought for the use of the feed additive for chickens for fattening and reared for laying, turkeys for fattening, for breeding and reared for breeding and minor poultry species. According to the Applicant, endo 1,4-beta-xylanase is the active substance of Beltherm<sup>®</sup> produced by a genetically modified strain of the bacteria *Bacillus subtilis*. The Applicant expresses the xylanase enzymatic activity in ADXU units defined as “the amount of enzyme which liberates one micromole of reducing sugars (xylose equivalent) per minute from beechwood xylan at pH 6.0 and 70 °C”. The product is intended to be marketed as a granulated and liquid formulations (MP and ML) having a guaranteed minimum xylanase activity of 500 ADXU/g. The carrier in the solid formulation is wheat flour, while glycerol in acetate buffer is used for the liquid formulation. The feed additive is intended to be included through premixtures or directly in feedingstuffs to obtain a minimum activity of 100 ADXU/kg of feedingstuffs for all the target species.

For the quantification of the xylanase activity in the feed additive, the Applicant proposed a single-laboratory validated and further verified colorimetric method based on the measurement of reducing sugars (xylose equivalents) released by the action of xylanase on 3% beechwood xylan substrate in the presence of 3,5-dinitrosalicylic acid (DNS) at pH 6.0 and 70°C (DNS method).

For the quantification of the xylanase activity in premixtures and feedingstuffs, the Applicant proposed another single-laboratory validated and further verified colorimetric method, based on the quantification of water soluble dyed fragments produced by the action of xylanase on commercially available azurine cross-linked arabinoxylan substrates.

Based on the satisfactory performance characteristics, the EURL recommends for official control the proposed single-laboratory validated and further verified colorimetric methods for the quantification of the xylanase activity in the feed additive (DNS method) premixtures and feedingstuffs.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.