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## Assessment of the application for renewal of authorisation of AveMix<sup>®</sup> XG 10 (endo-1,4-beta-xylanase and endo-1,3(4)-beta-glucanase) for chickens for fattening

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### Abstract

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the assessment of the application for renewal of authorisation of AveMix<sup>®</sup> XG 10 (endo-1,4-beta-xylanase and endo-1,3(4)-beta-glucanase) for chickens for fattening. The applicant has provided evidence that the additive currently in the market complies with the existing conditions of authorisation. There is no new evidence that would lead the FEEDAP Panel to reconsider its previous conclusions. Thus, the Panel concludes that the additive remains safe for the target species, consumer and the environment under the authorised conditions of use. Regarding user safety, the additive is not considered to be a dermal or eye irritant but it is a dermal and respiratory sensitiser. There is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

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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 14(1) of that Regulation lays down that an application for renewal shall be sent to the Commission at the latest one year before the expiry date of the authorisation.

The European Commission received a request from AVEVE NV<sup>2</sup> for renewal of the authorisation of the product AveMix® XG 10 (endo-1,4-beta-xylanase and endo-1,3(4)-beta-glucanase), when used as a feed additive for chickens for fattening (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 14(1) (renewal of the authorisation). 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 20 December 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 AveMix® XG 10 (endo-1,4-beta-xylanase and endo-1,3(4)-beta-glucanase), when used under the proposed conditions of use (see Section 3.1.1).

### 1.2. Additional information

The additive AveMix® XG 10 is a preparation of endo-1,4-beta-xylanase and endo-1,3(4)-beta-glucanase produced by *Trichoderma longibrachiatum* (formerly identified as *Trichoderma reesei*) Culture Collection–Mycothèque de l'Université Catholique de Louvain (MUCL)/Belgian Coordinated Collections of Microorganisms (BCCM) 49754 and *T. longibrachiatum* (formerly identified as *Trichoderma reesei*) MUCL/BCCM 49755.

The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) has issued six opinions on the product. The first opinion was on the safety and efficacy of AveMix® XG 10 as a feed additive for chickens for fattening (EFSA, 2009) and the second was on the modification of the terms of the authorisation of the product for this species (EFSA FEEDAP Panel, 2010). The third and fourth were on the safety and efficacy for weaned piglets and laying hens and minor poultry species (EFSA FEEDAP Panel 2011, 2012). The fifth and sixth were on the safety and efficacy of AveMix® XG 10 for turkeys for fattening and for pigs for fattening and minor porcine species (EFSA FEEDAP Panel, 2013a,b).

The additive is authorised for use in chickens for fattening,<sup>3,4</sup> weaned piglets,<sup>5</sup> laying hens and minor poultry species for fattening and laying<sup>6</sup> and in pigs for fattening and minor porcine species (other than *Sus scrofa domesticus*) and turkeys for fattening.<sup>7</sup>

<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> AVEVE NV Aarschotsesteenweg 84 3012 Leuven, Belgium.

<sup>3</sup> Commission Regulation (EC) No 1091/2009 of 13 November 2009 concerning the authorisation of an enzyme preparation of endo-1,4-beta-xylanase produced by *Trichoderma reesei* (MUCL 49755) and endo-1,3(4)-beta-glucanase produced by *Trichoderma reesei* (MUCL 49754) as a feed additive for chickens for fattening (holder of authorisation Aveve NV). OJ L 299, 14.11.2009, p. 6.

<sup>4</sup> Commission Regulation (EU) No 335/2011 of 7 April 2011 amending Regulation (EC) No 1091/2009 as regards the minimum content of the enzyme preparation of endo-1,4-beta-xylanase produced by *Trichoderma reesei* (MUCL 49755) and endo-1,3(4)-beta-glucanase produced by *Trichoderma reesei* (MUCL 49754) as a feed additive in feed for chickens for fattening. OJ L 94, 8.4.2011, p. 14.

<sup>5</sup> Commission Implementing Regulation (EU) No 1088/2011 of 27 October 2011 concerning the authorisation of an enzyme preparation of endo-1,4-beta-xylanase produced by *Trichoderma reesei* (MULC 49755) and endo-1,3(4)-beta-glucanase produced by *Trichoderma reesei* (MULC 49754) as a feed additive for weaned piglets (holder of authorisation Aveve NV).

<sup>6</sup> Commission Implementing Regulation (EU) No 989/2012 of 25 October 2012 concerning the authorisation of endo-1,4-beta-xylanase produced by *Trichoderma reesei* (MULC 49755) and endo-1,3(4)-beta-glucanase produced by *Trichoderma reesei* (MULC 49754) as a feed additive for laying hens and minor poultry species for fattening and laying (holder of authorisation Aveve NV). OJ L 297, 26.10.2012, p. 11.

<sup>7</sup> Commission Implementing Regulation (EU) No 1040/2013 of 24 October 2013 concerning the authorisation of a preparation of endo-1,4-beta-xylanase produced by *Trichoderma reesei* (MUCL 49755) and endo-1,3(4)-beta-glucanase produced by *Trichoderma reesei* (MUCL 49754) as a feed additive for pigs for fattening and minor porcine species for fattening other than *Sus scrofa domesticus* and turkeys for fattening (holder of authorisation Aveve NV).

## 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>8</sup> in support of the request on the renewal of the authorisation for the use of AveMix® XG 10 (endo-1,4-beta-xylanase and endo-1,3(4)-beta-glucanase) as a feed additive.

The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment are valid and applicable for the current application.<sup>9</sup>

### 2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of AveMix® XG 10 (endo-1,4-beta-xylanase and endo-1,3(4)-beta-glucanase) is in line with the principles laid down in Regulation (EC) No 429/2008<sup>10</sup> and the Guidance on the renewal of the authorisation of feed additives (EFSA FEEDAP Panel, 2013c).

## 3. Assessment

The additive AveMix® XG 10 is a preparation of endo-1,4-β-xylanase and endo-1,3(4)-β-glucanase produced by two non-genetically modified strains of *T. longibrachiatum* (formerly *T. reesei*) (MUCL 49754 and MUCL 49755). It is authorised for use in feed for chickens for fattening, turkeys for fattening, laying hens and minor poultry species for fattening and laying, weaned piglets, pigs for fattening and minor porcine species for fattening as a zootechnical additive (functional group: digestibility enhancers).

The applicant is requesting the renewal of the authorisation of the additive for chickens for fattening.

### 3.1. Characterisation of the additive

The additive is authorised with a minimum activity of 40,000 XU<sup>11</sup>/g and 9,000 BGU<sup>12</sup>/g. The applicant stated that the manufacturing process and the composition of the additive have not been modified since the original authorisation.

The additive is marketed in two different forms, a solid (AveMix® XG 10) and a liquid (AveMix® XG 10 L). The solid formulation, AveMix® XG 10 contains the enzyme preparation (27–30%) and soybean meal (68–73%). The liquid formulation, AveMix® XG 10 L, is a brown liquid that contains the enzyme preparation (68–73%) and 18–23% of the carrier material (63% water and 37% sorbitol). The two formulations ensure a minimum activity of 40,000 XU and 9,000 BGU/g. The analysis of five batches of each form<sup>13</sup> produced in 2018, showed compliance with these specifications.

The active ingredients are produced by fermentation with two non-genetically modified strains of *T. longibrachiatum* (formerly *T. reesei*) deposited at the Belgian Co-ordinated Collections of Micro-organisms culture collection with deposition numbers MUCL 49754 and MUCL 49755.

The taxonomic identification of the production strains was confirmed by comparing partial sequences [REDACTED] and using an up to date taxonomic framework. [REDACTED]

In the previous authorisation the strains producing the xylanase and glucanase activities were identified as *Trichoderma reesei*. In the current application the producing strains are the same as the ones already authorised and are not genetically modified. Their name in the culture collection BCCM

<sup>8</sup> FEED dossier reference: FAD-2018-0068.

<sup>9</sup> The full report is available on the EURL website: <https://ec.europa.eu/jrc/sites/jrcsh/files/FinRep-FAD-2009-0062.pdf>

<sup>10</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>11</sup> 1 XU is the amount of enzyme which releases 1 μmol of reducing sugar (xylose equivalent) per minute from xylan of oat spelt at pH 5,0 and 50°C.

<sup>12</sup> 1 BGU is the amount of enzyme which releases 1 μmol of reducing sugar (cellobiose equivalent) per minute from β-glucan of barley at pH 4,8 and 50°C.

<sup>13</sup> Technical dossier/Section II/Annex II.3.14-18 for the solid and II.3.19-23 for the liquid form.

changed from *Trichoderma reesei* to *Trichoderma longibrachiatum* without changing the accession number.<sup>15</sup>

Analytical data of six recent batches showed that the product does not contain antimicrobial activities.<sup>16</sup> The Panel notes that the method<sup>17</sup> used to screen the antibiotic activity is not compliant with the provisions of the applicable Guidance (EFSA FEEDAP Panel, 2018). Although *Trichoderma* species produce peptaibols with antimicrobial activity, they are not known of producing antibiotics of clinical and veterinary importance (AINIA Technology Centre, 2017). Therefore, the Panel assumes that the presence of antibiotic activity in the final product is unlikely.

Microbiological quality of the additive was assayed in three production batches of each form of the additive produced in 2018.<sup>19</sup> Measurements included enterobacteria (< 10 CFU/g), yeasts and filamentous fungi (< 100 CFU/g), *Bacillus cereus* (< 100 CFU/g) and *Salmonella* (absence in 25 g).

Chemical contamination was measured in three recent batches (2018) of both forms. Data were provided for heavy metals (cadmium (< 0.20 mg/kg), mercury (< 0.02 mg/kg), lead (0.62 mg/kg in the solid and < 0.50 mg/kg in the liquid form)), arsenic (< 0.50 mg/kg) and fluorine (59 mg/kg in the solid and < 40 mg/kg in the liquid form). Mycotoxins were also analysed in the same batches (aflatoxin B1 < 0.3000 µg/kg, fumonisine B1 and B2 < 100 µg/kg, sum of fumonisine B1/B2 < 200 µg/kg, HT-2 Toxin < 200 µg/kg in both forms, deoxynivalenol averaged < 0.09 mg/kg in the solid form and < 0.06 mg/kg in the liquid form, zearalenone < 5 µg/kg in the solid form and < 6.6 µg/kg in the liquid form, ochratoxin A < 0.89 µg/kg in the solid form and < 1 µg/kg in the liquid form).<sup>20</sup>

### 3.1.1. Conditions of use

The additive is currently authorised for use in feed for chickens for fattening at a minimum recommended level of 3,000 XU<sup>11</sup> and 675 BGU<sup>12</sup>/kg of complete feedingstuffs with a moisture content of 12%. The applicant states that the liquid formulation should only be incorporated into feed after pelleting.

Under the other provisions of the authorisation it is specified that: 'in the directions for use of the additive and premixture indicate (i) the storage temperature, storage life, and stability to pelleting. (ii) for use in feed rich in non-starch polysaccharides (mainly beta-glucans and arabinoxylans), e.g. containing more than 30% wheat, barley, rye and/or triticale. (iii) for safety reasons: breathing protection, glasses and gloves shall be used during handling'.

The applicant does not ask to modify these conditions of use.

## 3.2. Safety

In its previous opinion (EFSA, 2009), the FEEDAP Panel concluded that the additive was safe under the proposed conditions of use for chickens for fattening, consumers and the environment. With regards to user safety, the Panel concluded that the additive was not a dermal or eye irritant but should be considered a dermal and respiratory sensitiser.

For the current assessment, the applicant provided an additional *in vitro* micronucleus test performed in cultured human lymphocytes following the OECD Guidance 487 'In Vitro Mammalian Cell Micronucleus Test'.<sup>22</sup> The test was done with and without metabolic activation (S9 mix) with doses of 5,000; 2,500 and 1,250 µg AveMix® XG 10/ml culture medium. The results did not show any binucleated micronuclei. The positive controls performed as expected.

<sup>15</sup> Technical Dossier/Section II/Annex II.1.3.2.

<sup>16</sup> Technical dossier/Section II/Annex II.4.12.

<sup>17</sup> Technical dossier/Section II/Annex II.6.1.8.

<sup>19</sup> Technical dossier/Section II/Annex II.4.11.

<sup>20</sup> Technical dossier/Section II/Annex II.1.4.10.

<sup>22</sup> Technical Dossier/Section III/Annex III.2.2.5

The applicant stated that 'no complaints from users have been transmitted regarding adverse effects of AveMix® XG 10'.<sup>23</sup>

The applicant conducted a literature search on the safety of the additive covering the period 2006–2018 in the databases ScienceDirect, PUBMED, Google Scholar and AGRIS including search terms covering the name of the additive, the strain deposition numbers, the acronym of the culture collection in which they were deposited, terms concerning the safety and the toxicity for the target animals, the consumer, the user and the environment. The search identified 16 hits other than previous EFSA opinions. The articles retrieved dealt with a different product than the one under assessment or they did not show any adverse effects, therefore were considered not relevant.

Based on the above and the fact that the manufacturing and composition of the additive have not been modified, the FEEDAP Panel considers that there is no evidence to reconsider the conclusions reached in the previous opinion (EFSA, 2009). Therefore, the Panel concludes that the additive remains safe for chickens for fattening, consumer and the environment under the authorised conditions of use. Regarding user safety, the Panel reiterates that the additive is not considered to be a dermal or eye irritant but it is a dermal and respiratory sensitiser.

### 3.3. Efficacy

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. Therefore, there is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

### 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>24</sup> and Good Manufacturing Practice.

### 3.5 Conclusions

The applicant has provided evidence that the additive currently in the market complies with the existing conditions of authorisation.

There is no new evidence that would lead the FEEDAP Panel to reconsider its previous conclusions. Thus, the Panel concludes that the additive remains safe for chickens for fattening, consumer and the environment under the authorised conditions of use. Regarding user safety the additive is not considered to be a dermal or eye irritant but it is a dermal and respiratory sensitiser.

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

## 4. Documentation as provided to EFSA/Chronology

Date	Event
25/09/2018	Dossier received by EFSA. AveMix® XG 10. Submitted by Aveve NV
04/10/2018	Reception mandate from the European Commission
20/12/2018	Application validated by EFSA – Start of the scientific assessment
05/02/2019	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 safety</i>
20/03/2019	Comments received from Member States
13/09/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
17/03/2020	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

<sup>23</sup> Technical Dossier/Supplementary Information September 2019/Annex Supplementary information request on AveMix XG 10 20190909.pdf.

<sup>24</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.

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## Abbreviations

BCCM	Belgian Coordinated Collections of Microorganisms
CEF	EFSA Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CFU	colony forming unit
CV	coefficient of variation
DM	dry matter
EURL	European Union Reference Laboratory
MUCL	Culture Collection–Mycothèque de l'Université Catholique de Louvain