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Safety and efficacy of a feed additive consisting of endo-1,4-β-xylanase produced by *Komagataella phaffii* ATCC PTA-127053 (Xygest™ HT) for poultry (Kemin Europa N.V.)

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, Boet Glandorf, Paul Brantom, Montserrat Anguita, Jaume Galobart, Paola Manini, Jordi Ortuño, Elisa Pettenati, Fabiola Pizzo, Jordi Tarrés and Joana Revez

Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of endo-1,4-β-xylanase produced by *Komagataella phaffii* ATCC PTA-127053 (Xygest™ HT) as a zootechnical feed additive for poultry. The production strain is genetically modified. No viable cells nor recombinant DNA of the production strain were detected in the final product. Therefore, the Panel concluded that the additive does not pose any safety concern regarding the production strain. The FEEDAP Panel concluded that Xygest™ HT is considered safe for chickens for fattening at 30,000 U/kg and in laying hens at 45,000 U/kg, with a wide margin of safety. These conclusions can be extended to chickens reared for laying/breeding and extrapolated to all poultry species for fattening and reared for laying/breeding and to ornamental birds. The conclusions on laying hens are extrapolated to all laying poultry species. The use of Xygest™ HT in animal nutrition is of no concern for consumer safety and this feed additive is considered safe for the environment. The Panel also concluded that Xygest™ HT is not irritant to the eyes and skin but should be considered a dermal and a respiratory sensitiser. The Panel concluded that the additive Xygest™ HT has a potential to be efficacious for laying hens under the proposed conditions of use and this conclusion can also be extrapolated to all laying poultry species. The Panel could not conclude on the efficacy of the product for chickens for fattening due to insufficient data.

Keywords: zootechnical additives, digestibility enhancers, Xygest™ HT, endo-1,4-β-xylanase, poultry, safety, efficacy

Requestor: European Commission

Question number: EFSA-Q-2021-00098

Correspondence: feedap@efsa.europa.eu

Panel members: 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 and Ruud Woutersen.

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Table of contents

1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] 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 feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Kemin Europa N.V.[2] for the authorisation of the additive consisting of endo-1,4-β-xylanase produced by *Komagataella phaffii* ATCC PTA-127053[3] (Xygest™ HT), when used as a feed additive for poultry (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 8 July 2021.

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 feed additive consisting of endo-1,4-β-xylanase produced by *K. phaffii* ATCC PTA-127053 (Xygest™ HT), when used under the proposed conditions of use (see **Section 3.1.6**).

1.2. Additional information

The additive consisting of endo-1,4-β-xylanase produced by *K. phaffii* ATCC PTA-127053 (Xygest™ HT) is not authorised as a feed additive in the European Union.

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[4] in support of the authorisation request for the use of the product consisting of endo-1,4-β-xylanase produced by *K. phaffii* ATCC PTA-127053 (Xygest™ HT) 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 endo-1,4-β-xylanase in animal feed. The Executive Summary of the EURL report can be found in Annex A.[5]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of the product consisting of endo-1,4-β-xylanase produced by *Komagataella phaffii* ATCC PTA-127053 (Xygest™ HT) is in line with the principles laid down in Regulation (EC) No 429/2008[6] and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018a), Guidance on the characterisation of microorganisms used as

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on the additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] Kemin Europa N.V., Toekomstlaan 42, 2020 Herentals, Belgium.
[3] KMI000000 corresponding to the deposition number of PTA-127053 provided by the applicant.
[4] FEED dossier reference: FAD-2020-0110.
[5] The full report is available on the EU Science Hub website: https://joint-research-centre.ec.europa.eu/publications/fad-2020-0110_en
[6] 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.

feed additives or as production organisms (EFSA FEEDAP Panel, 2018b) and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

3. Assessment

The product containing endo-1,4-β-xylanase (IUBMB EC 3.2.1.8; xylanase) produced by *K. phaffii* ATCC PTA-127053 (Xygest™ HT) is intended to be used as a zootechnical additive (functional group: digestibility enhancers) for poultry. The additive under assessment will be hereafter referred to as Xygest™ HT.

3.1. Characterisation

3.1.1. Characterisation of the production organism

The additive is produced by a genetically modified strain of *Komagataella phaffii* which is deposited in the American Type Culture Collection with deposition number ATCC PTA-127053.[7]

The taxonomical identification of the production strain as *K. phaffii* was confirmed by whole genome sequence (WGS)-based analyses of the production strain ATCC PTA-127053. [redacted][9]

No acquired antibiotic resistance genes were identified [redacted]

3.1.1.1. Information related to the genetically modified microorganism

Characterisation of the recipient microorganism

[redacted]

Description of the genetic modification

[redacted]

3.1.2. Manufacturing process

The active substance of Xygest™ HT is produced by fermentation with *K. phaffii* ATCC PTA-127053. [redacted]

[7] Technical dossier/Section II/Annex II_1, Annex II_2 and Supplementary Information March 2022/Annex SIn3.
[8] Technical dossier/Section II/Annex II_91.
[9] Technical dossier/Section II/Annex II_91, Annex II_92a and Annex II_92b.
[10] Technical dossier/Section II/Annex II_91 and Supplementary Information November 2021/Annex SIn17.
[11] Technical dossier/Section II/Annex II_99.
[12] Technical dossier/Section II/Annex II_97.
[13] Technical dossier/Section II/Annex II_91 and Annex II_92C.
[14] Technical dossier/Section II/Annex II_92C.

[redacted][16]

3.1.3. Characterisation of the additive

The additive is available in solid form and ensures a guaranteed minimum xylanase activity of 3,000,000 U[17]/g of product. Xygest™ HT contains dried ferment (11–20%), maize starch (78–87%) and calcium carbonate (0.5–2%). Analytical data on batch-to-batch variation was provided for seven batches and the mean value was 3,243,248 U/g product, ranging from 3,017,356 to 3,546,859 U/g.[18]

Seven batches of the additive were analysed for chemical contamination including lead, mercury, cadmium and arsenic, mycotoxins and dioxins. [redacted]

Polychlorinated dibenzodioxins and dibenzofurans (PCDDs and PCDFs) and coplanar dioxin-like polychlorinated biphenyls (DL-PCBs) were analysed in three batches and were below the corresponding LOQs. The calculated (upper bound) levels of the sum of dioxins (88% d.w.) was 0.096 ng WHO-PCDD/F-TEQ/kg, while the sum of DL-PCBs was 0.032 ng WHO-PCB-TEQ/kg and the sum of dioxins and DL-PCBs was 0.13 ng WHO-PCDD/F-PCB-TEQ/kg. In the same batches, the sum of non-DL PCBs ranged between 0.45 and 0.51 μg/kg.[22]

The presence of hexachlorocyclohexane (HCH) and dichlorodiphenyltrichloroethane (DDT) pesticides was determined in three batches,[23] while organochlorine and organophosphorus pesticides were investigated in four batches.[24] Results showed all values below the respective LOQs.[25]

[redacted]

The applicant set specifications for microbiological contamination [redacted][16]

[15] Technical dossier/Section II//Annex II_101 and Supplementary Information November 2021/Annex Sin4.
[16] Technical dossier/Supplementary Information November 2021/Annex SIn14.
[17] One U unit is the amount of enzyme that releases 0.0067 μmol of reducing sugar (xylose equivalent) per minute and per gram of enzyme product at 50 °C and pH 5.3.
[18] Technical dossier/Section II//Annex II_3 - Annex II_9.
[19] Limits of quantification (LOQs): cadmium (0.010 mg/kg), lead (2 mg/kg), mercury (0.50 mg/kg); aflatoxins (B1, G1, B2, G2) (2.0 μg/kg); deoxynivalenol (100 μg/kg); fumonisins (B1 and B2) (50 μg/kg); ochratoxin A (5.0 μg/kg); zearalenone (10 μg/kg).
[20] Technical dossier/Section II/Annex II_40 - Annex II_46.
[21] Technical dossier/Section II/Annex II_11 - Annex II_39.
[22] Technical dossier/Supplementary Information November 2021/Annex SIn16
[23] Technical dossier/Section II/Annex II_49 - Annex II_51.
[24] Technical dossier/Section II/Annex II_52 - Annex II_58.
[25] Limits of quantification (LOQs): 0.01 mg/kg for HCH and DDT pesticides; 7.1 or 9.5 μg/kg for Organoclhorine pesticides; 7.1 or 9.5 μg/kg Organophosphorus pesticides.
[26] Technical dossier/Supplementary Information November 2021/Annex SIn9, Annex SIn10 and Annex SIn11.
[27] Technical dossier/Supplementary Information November 2021/Annex SIn13.
[28] Technical dossier/Section II/Annex II_59, Annex II_63, Annex II_67, Annex II_71, Annex II_73, Annex II_75 and Annex II_77.
[29] Technical dossier/Section II/Annex II_61, Annex II_65, Annex II_69, Annex II_71, Annex II_73, Annex II_76 and Annex II_78.
[30] Technical dossier/Section II/Annex II_60, Annex II_64, Annex II_68, Annex II_71, Annex II_73, Annex II_75 and Annex II_77.
[31] Technical dossier/Section II/Annex II_62, Annex II_66, Annex II_70, Annex II_72 and Annex II_74.

The detected amounts of the above described impurities do not raise safety concerns.

The presence of viable cells of the production strain in the final additive was analysed in triplicate [redacted] No viable cells of the production strain were found in the final product; however, the Panel has reservations on the acceptability of this data set due to the method not fully following the recommendations listed in the FEEDAP Guidance (EFSA FEEDAP Panel, 2018b). In a second data set, 10-g samples from each batch in triplicate were suspended in 590 mL of 0.9% NaCl. Thereafter, 60 mL of this suspension (corresponding to 1 g of sample) was filtered over a mixed cellulose ester (MCE) filter (0.45 μm) and incubated at 30°C for 5 days on YPG agar plates with 16 mg/L ciprofloxacin and 16 mg/L gentamycin. A positive control (spiked sample) was included.[34] Results showed that no viable cells of the production strain were found in the final product.

The presence of recombinant DNA from the production strain was tested in three batches of Xygest™ HT, each in triplicate.[35] [redacted] No DNA of the production strain was detected.

3.1.4. Physical properties of the additive

The additive is a powder [redacted] The same three batches were analysed for particle size distribution by laser-diffraction method;[38] the results showed that particles < 100 μm, < 50 μm and < 10 μm were on average 30.6% (range 29.7–31.2%), 13.0% (range 12.2–14.2%) and 1.3% (range 1.2–1.7%), respectively.

3.1.5. Stability and homogeneity

The stability of the additive was studied in samples (50 g) from three batches stored in plastic containers at 4°C (up to 12 months), 22°C (up to 20 months) and 40°C (up to 6 months). Negligible losses were seen at 4°C and 22°C while losses up to 34% were seen at 40°C.[39]

The stability of the additive (one batch) was tested in a commercial vitamin/mineral premixture when added at 450 mg/kg and stored at room temperature.[40] Negligible losses were seen after 6 months.

The stability of the additive under assessment (one batch) was studied in a complete feed for layers (finisher) in mash and pelleted forms when added at 30 mg/kg feed (corresponding to 90,000 U/kg feed) and stored at room temperature for 3 months. The basal diet consisted of maize, wheat and soybean meal.[41] The mash feed supplemented with the additive was pelleted at 90°C for 30 s. The heat treatment led to a loss of activity of 16%. Losses at the end of the storage period were negligible in mash and 13% and pelleted feed.

The capacity for homogeneous distribution of the additive in feed was studied in ten-subsamples of the complete feed descried above (mash form). The coefficient of variation was 6%.[42]

[32] Technical dossier/Section II/Annex II_79 - Annex II_88.
[33] Technical dossier/Section II/Annex II_79.
[34] Technical dossier/Supplementary Information November 2021/Annex SIn18.
[35] Technical dossier/Section II/Annex II_89.
[36] Technical dossier/Supplementary Information November 2021/Annex SIn19.
[37] Technical dossier/Section II/Annex II_127.
[38] Technical dossier/Section II/Annex II_127 and II_128.
[39] Technical dossier/Section II/Annex II_102 and II_103.
[40] Technical dossier/Section II/Annex II_104 and Annex II_106.
[41] Technical dossier/Section II/Annex II_104 and Annex II_105.
[42] Technical dossier/Section II/Annex II_104 and Annex II_107.

3.1.6. Conditions of use

The additive is intended for use in feed for poultry at a proposed minimum level of 30,000 U/kg of complete feed for growing poultry and 45,000 U/kg of complete feed for laying poultry.

3.2. Safety

3.2.1. Safety of the production strain, for the consumer and environment

The recipient strain from which the production organism was derived belongs to *K. phaffii*, which is considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to safety assessment when used for enzyme production (EFSA BIOHAZ Panel, 2007, 2020). The production strain was well identified, differed from the parental strain by four [redacted] and contains no acquired antibiotic resistance genes. Therefore, the genetic modification does not raise safety concerns. Moreover, neither viable cells nor DNA of the production strain were detected in the final product. Therefore, the additive does not pose any safety concern regarding the production strain. Considering all the above, the FEEDAP Panel concludes that the additive is safe for the consumers.

Regarding the environment, no cells and no DNA of the production strain were detected in the final product and the active substance of the additive is a protein, and as such will be degraded/inactivated during passage through the digestive tract of animals or in the environment. Therefore, the additive raises no concerns for the environment.

3.2.2. Safety for the target species

Two tolerance trials, one with chickens for fattening and one with laying hens, were submitted to support the safety for the target species.

3.2.2.1. Chickens for fattening

[redacted]

[43] Technical dossier/Section III/Annex III_1, Annex III_2 and Annex III_3.
[44] Technical dossier/Section III/Annex III_9.
[45] Carcass weight, breast meat weight, legs (thigh) meat weight and weights of liver, heart, gizzard and intestine.
[46] Kidney, bursa of Fabricius, thymus and spleen.
[47] Technical dossier/Supplementary Information May 2022/Annex 2.
[48] Technical dossier/Supplementary Information November 2021/Annex SIn23.

3.2.2.2. Laying hens

3.2.2.3. Conclusion on safety for the target species

Based on tolerance studies in chickens for fattening and laying hens, the FEEDAP Panel concludes that the additive is safe in chickens for fattening at 30,000 U/kg and in laying hens at 45,000 U/kg, with margins of safety of 300× and 25×, respectively. The conclusions on chickens for fattening are extended to chickens reared for laying/breeding and extrapolated to all poultry for fattening and reared for laying/breeding and to ornamental birds. The conclusions on laying hens are extrapolated to all laying poultry species.

3.2.3. Safety for the user

3.2.3.1. Effect on respiratory system

No specific tests were submitted on the effects of the additive on the respiratory system. However, based on the proteinaceous nature of the active substance of the additive, it is considered as a respiratory sensitiser.

3.2.3.2. Effect on eyes and skin

An acute eye irritation/corrosion study with Xygest™ HT was performed according to GLP and OECD Test Guideline (TG) 405.[53] Under the conditions of the study, the test item did not produce any signs of irritation/corrosion and is considered as non-irritant to the eyes.

An acute skin irritation/corrosion study was performed according to GLP and OECD TG 404.[54] Under the conditions of the study the test item did not produce any signs of dermal irritation and is considered as non-irritant to the skin.

A Guinea Pig Maximisation Test according to Magnusson and Kligman was conducted according to GLP and OECD TG 406.[55] Under the conditions of the test the additive showed patchy erythema after

[49] Technical dossier/Section III/Annex III_10, Annex II_11 and Annex II_12, Technical dossier/Supplementary Information November 2021/Annex SIn24 and Technical dossier/Supplementary Information May 2022/Annex 4.
[50] Technical dossier/Section III/Annex III_14.
[51] Red blood cells count (RBC), packed cell volume (PCV), mean corpuscular volume (MCH), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBC), differential leucocyte count, platelet count, prothrombin time, fibrinogen, sodium, potassium, chloride, calcium, magnesium, total protein, albumin, globulin, glucose, urea, uric acid, cholesterol, creatinine, bilirubin total, phosphate, amylase, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, gamma-glutamyltransferase, alkaline phosphatase and creatine kinase.
[52] Liver, kidneys, spleen, adrenal gland, thyroid gland, lungs, heart, pancreas, small intestine, caecum, thymus and ovary.
[53] Technical dossier/Section III/Annex III_16.
[54] Technical dossier/Section III/Annex III_15.
[55] Technical dossier/Section III/Annex III_17.

the induction phase in two out of 10 guinea pigs but no skin reaction in any animal after the challenge phase. The FEEDAP Panel noted that the study did not follow the recommendation of the relevant OECD TG regarding the minimum number of animals to be used, however given the nature of the test material and since the results were already positive on a reduced number of animals, the test compound should be considered a skin sensitiser.

3.2.3.3. Conclusions on safety for the user

The additive is considered not to be irritant to eyes and skin but is considered a dermal and respiratory sensitiser.

3.3. Efficacy

3.3.1. Chickens for fattening

A total of four trials with chickens for fattening (including the tolerance trial) with a similar design were submitted. However, two could not be further considered for the following reasons: one had a high mortality ([redacted])[56] and the husbandry conditions did not reflect the way chickens for fattening should be raised in the EU (for the lighting programme) according to the Directive 2007/43/EC[57], and the other study (short-term) was discarded due to the inadequacy of the endpoints provided to support the efficacy.[58]

The details on the study design of the remaining trials are provided in Table 1. [redacted]

[56] Technical dossier/Section IV/Annex IV_21-Annex IV_24.
[57] Council Directive 2007/43/EC of 28 June 2007 laying down minimum rules for the protection of chickens kept for meat production, OJ L 182 12.7.2007, p. 19.
[58] Technical dossier/Section IV/Annex IV_15-Annex IV_20.

Taking all the evidence together, two studies demonstrated a positive effect of the additive on performance in chickens for fattening at the recommended dose of 30,000 U/kg feed. In the absence of a third study with significant positive results, the FEEDAP Panel is not in the position to conclude on the efficacy of Xygest™ HT for chickens for fattening, and consequently for growing poultry.

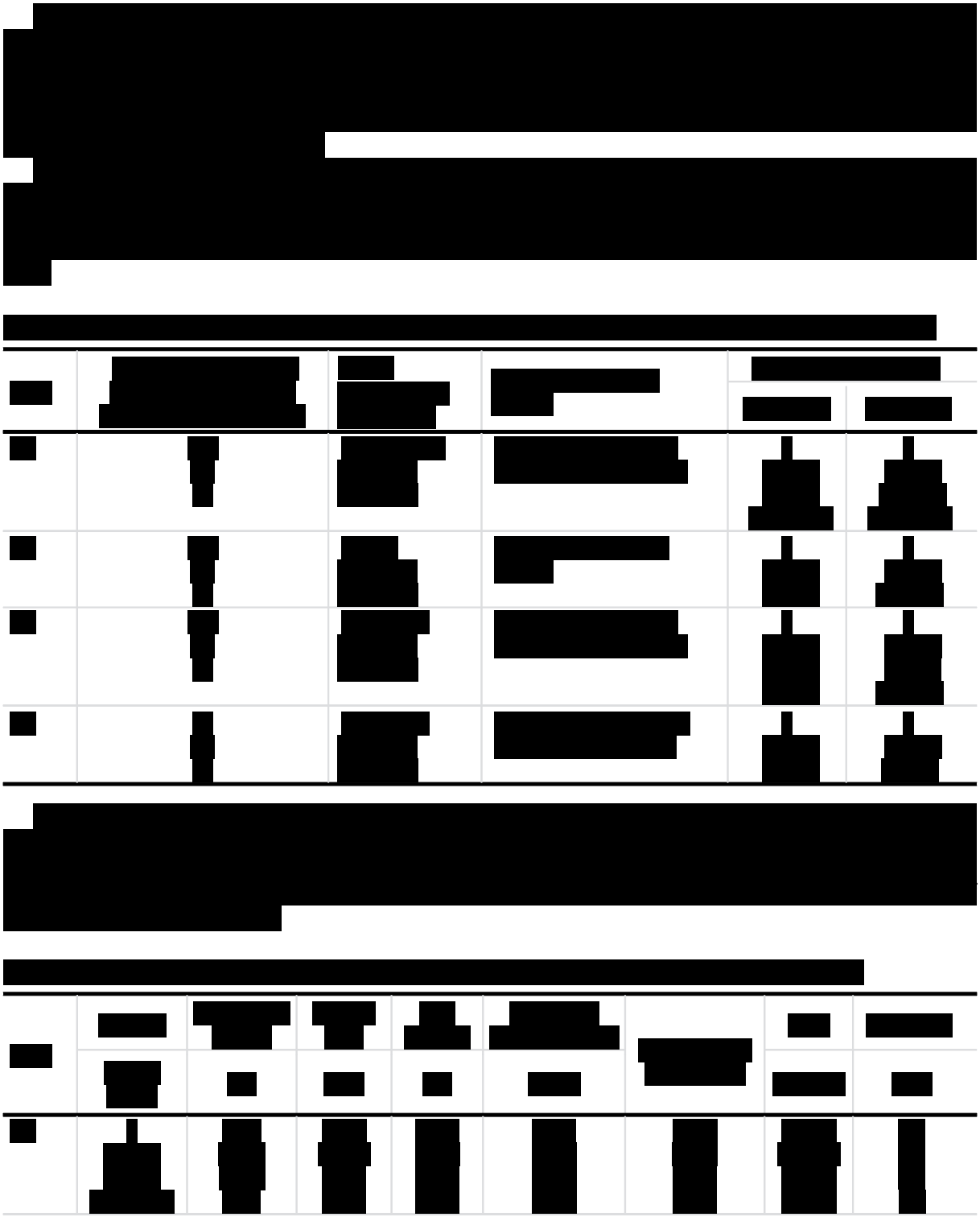
3.3.2. Laying hens

A total of five trials with laying hens were submitted (including the tolerance trial), four long-term trials (duration of 84 days) and one short-term study (duration 28 days) sharing a common design. However, one study[62] could not be further considered due to a lack of data (e.g. daily egg mass per hen and confirmation of level of additive used) and the husbandry conditions which did not reflect the husbandry conditions for laying hens in the EU (space granted per hen) according to the Directive 1999/74/EC.[63]

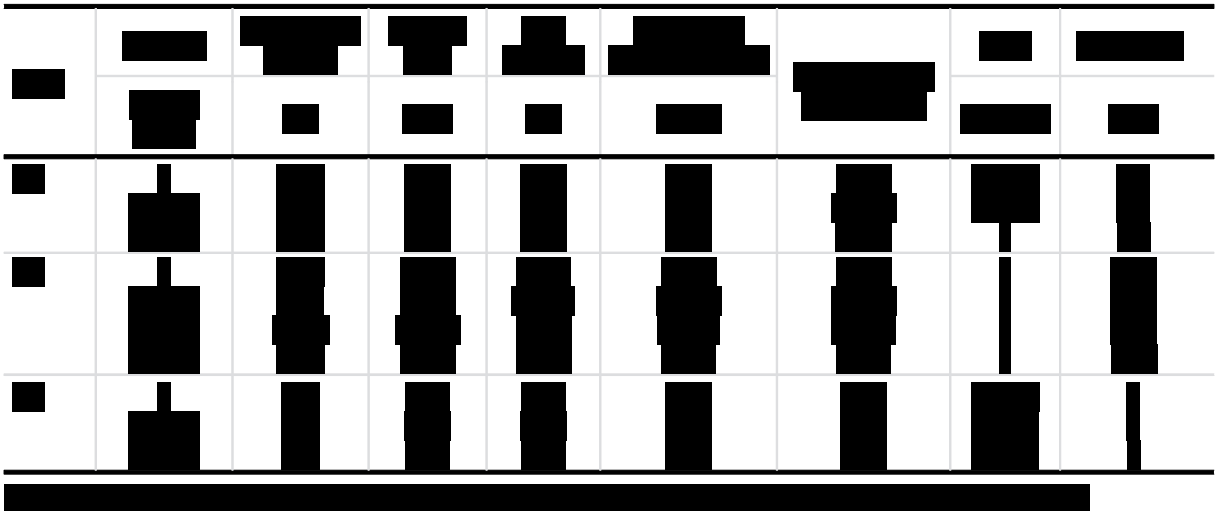
The details on the study design are provided in Table 3.

[redacted]

[59] Technical dossier/Section IV/Annex IV_1-Annex IV_10.
[60] Technical dossier/Section IV/Annex IV_11-Annex IV_14.
[61] Technical dossier/Section IV/Annex IV_1-Annex IV_10 and Supplementary Information November 2021/AnnexSIn23.
[62] Technical dossier/Section IV/Annex IV_45-Annex IV_49 and Supplementary Information November 2021/FAD-2020-0110.
[63] Council Directive 1999/74/EC of 19 July 1999 laying down minimum standards for the protection of laying hens. OJ L 203 3.8.1999, p. 53.



[64] Technical dossier/Section IV/ Annex IV_25, Annex IV_26, Annex IV_27, Annex IV_28, Annex IV_29, Annex IV_30 and Annex IV_31.
[65] Technical dossier/Section IV/ Annex IV_33, Annex IV_34, Annex IV_35, Annex IV_36, Annex IV_37, Annex IV_38 and Supplementary Information November 2021/Annex SIn28 and Annex Sin29.
[66] Technical dossier/Section IV/Annex IV_50, Annex IV_51, Annex IV_52, Annex IV_53 and Annex IV_54
[67] Technical dossier/Section IV/Annex IV_39, Annex IV_40, Annex IV_41, Annex IV_42, Annex IV_43 and Annex IV_44.
[68] Technical dossier/Section IV/Annex IV_25, Annex IV_26, Annex IV_27, Annex IV_28, Annex IV_29, Annex IV_30 and Annex IV_31.



The Panel concludes that the additive has the potential to be efficacious by improving the feed to egg mass ratio and/or the AME at the recommended use level in laying hens. The conclusions on laying hens are extrapolated to all laying poultry species.

3.3.3. Conclusions on efficacy

Owing to the lack of sufficient data, the Panel cannot conclude on the efficacy for chickens for fattening. The Panel concludes that the additive has the potential to be efficacious for laying hens when added to feed at 45,000 U/kg feed and this conclusion can also be extrapolated to all laying poultry species.

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[71] and Good Manufacturing Practice.

4. Conclusions

The additive under assessment, Xygest™ HT, does not pose any safety concern regarding the production strain. Its use in animal nutrition under the conditions of use proposed is considered safe for the consumers and for the environment.

The FEEDAP Panel concludes that the additive is safe for chickens for fattening at the 30,000 U/kg and in laying hens at 45,000 U/kg, with a wide margin of safety. The conclusions on chickens for fattening are extended to chickens reared for laying/breeding and extrapolated to all poultry for fattening and reared for laying/breeding and to ornamental birds. The conclusions on laying hens are extrapolated to all laying poultry species.

On the basis of the studies submitted the additive is considered not to be irritant to eyes and skin but is considered a dermal sensitiser and a respiratory sensitiser, although exposure by inhalation is unlikely.

The Panel cannot conclude on the efficacy for chickens for fattening. The Panel concludes that the additive has the potential to be efficacious for laying hens when added to feed at 45,000 U/kg feed and this conclusion can also be extrapolated to all laying poultry species.

[69] Technical dossier/Section IV/Annex IV_33, Annex IV_34, Annex IV_35, Annex IV_36, Annex IV_37, Annex IV_38 and Supplementary Information November 2021/Annex SIn28 and Annex SIn29.

[70] Technical dossier/Section IV/Annex IV_39, Annex IV_40, Annex IV_41, Annex IV_42, Annex IV_43 and Annex IV_44 Supplementary Information November 2021/AnnexSIn31.

[71] 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.

5. Documentation provided to EFSA/chronology

Date	Event
18/12/2020	Dossier received by EFSA. Xygest™ HT (endo-1,4-beta-xylanase (EC 3.2.1.8)). Submitted by Kemin Europa N.V.
08/01/2021	Reception mandate from the European Commission
09/07/2021	Application validated by EFSA – Start of the scientific assessment
23/07/2021	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation/target species safety/efficacy*
11/10/2021	Comments received from Member States
09/11/2021	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
29/11/2021	Reception of supplementary information from the applicant - Scientific assessment re-started
21/03/2022	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation*
28/03/2022	Reception of supplementary information from the applicant - Scientific assessment re-started
23/05/2022	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: target species safety/efficacy*
30/05/2022	Reception of supplementary information from the applicant - Scientific assessment re-started
29/06/2022	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

AME	apparent metabolisable energy
ANOVA	analysis of variance
CFU	colony forming unit
DDT	dichlorodiphenyltrichloroethane
DL-PCB	dioxin-like polychlorinated biphenyl
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
GLP	Good Laboratory Practice
HCH	hexachlorocyclohexane
IUBMB	International Union of Biochemistry and Molecular Biology
LOD	limit of detection
LOQ	limit of quantification
OECD	Organisation for Economic Co-operation and Development
PCDD	polychlorinated dibenzodioxin
PCDF	polychlorinated dibenzofuran
WGS	whole genome sequence

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of the Analysis for Xygest™

In the current application, an authorisation of *a preparation of endo-1,4-beta-xylanase (EC 3.2.1.8)* is sought under Article 4(1) for all poultry, under the category/functional group 4 (a) "zootechnical additives"/"digestibility enhancers" according to Annex I of Regulation (EC) No 1831/2003.

According to the Applicant, the *active agent* of the *feed additive* is *endo-1,4-beta-xylanase*, produced by fermentation with the genetically modified strain *Komagataella phaffii* ATCC KMI000000[3]. The activity of *endo-1,4-beta-xylanase* is expressed in xylanase units (U). One U unit is the amount of enzyme that releases 0.0067 μmol of reducing sugar (xylose equivalent) per minute and per gram of enzyme product at 50°C and pH 5.3.

The *feed additive* is intended to be marketed as a solid formulation having a guaranteed minimum *endo-1,4-beta-xylanase* activity of 3,000,000 U/g and to be incorporated directly into complete *feedingstuffs* or through *premixtures* to obtain a minimum *xylanase* activity of 30,000 or 45,000 U/kg *feedingstuffs* depending on the target species.

For the quantification of the *xylanase* activity in the *feed additive* the Applicant submitted a single-laboratory validated and further verified method based on the enzymatic hydrolysis of xylanase on the beechwood xylan substrate and the colour formation of the released reducing sugar with arsenomolybdate.

Furthermore, for the quantification of *endo-1,4-beta-xylanase* in *premixtures* and *feedingstuffs* the Applicant submitted another single-laboratory validated and further verified colorimetric method, based on the measurement on the quantification of water soluble dyed fragments produced by the action of xylanase on a commercially available (Xylazyme AXE®, Megazyme) azurine cross-linked wheat arabinoxylan substrate.

Based on the acceptable performance characteristics the EURL recommends for official control (i) the single-laboratory validated and further verified colorimetric (arsenomolybdate) method for the determination of *endo-1,4-beta-xylanase* in the *feed additive* and (ii) the colorimetric (Megazyme) method for the determination of *1,4-beta-xylanase* in *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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.