SCIENTIFIC OPINION

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Safety and efficacy of a feed additive consisting of endo-1,4-β-xylanase produced by *Komagataella phaffii* DSM 33574 (Xylamax) for chickens and turkeys for fattening, chickens reared for laying/breeding, turkeys reared for breeding and minor poultry species for fattening or raised to the point of lay (BioResource international, Inc.)

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Abstract

Following a request from the European Commission, the EFSA Panel on Additives and Products of Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of the product containing endo-1,4-β-xylanase produced by *Komagataella phaffii* DSM 33574 (Xylamax) as a zootechnical additive in chickens for fattening, chickens reared for laying/breeding, turkeys for fattening, turkeys reared for breeding and minor poultry species for fattening or raised to the point of lay. 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. Based on the no observed adverse effect level identified in a subchronic oral toxicity study in rats, the Panel concluded that Xylamax is safe for all poultry species for fattening or reared to the point of lay. Considering the production strain and the results obtained in the genotoxicity studies, the Panel concluded that the additive is safe for the consumers. The Panel also concluded that Xylamax is not irritant to the skin but should be considered a potential eye irritant and a respiratory sensitiser. No conclusions could be drawn on the potential of the additive to cause skin sensitisation. The use of the product as a feed additive is of no concern for the environment. The FEEDAP Panel concluded that the additive has the potential to be efficacious at 10,000 XU/kg feed in chickens for fattening. This conclusion was extended/extrapolated to chickens reared for laying/breeding, turkeys for fattening, turkeys reared for breeding and minor poultry species for fattening or raised to the point of lay.

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

Requestor: European Commission

Question number: EFSA-Q-2021-00254

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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 BioResource International, Inc. represented in the EU by Pen & Tec consulting S.L.U.[2] for the authorisation of the additive consisting of endo-1,4-beta-xylanase produced by *Komagataella phaffii* DSM 33574 (Xylamax), when used as a feed additive for chickens for fattening, chickens reared for laying/breeding, turkeys for fattening, turkeys reared for breeding and minor poultry species for fattening or raised to the point of lay[3] (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 27 May 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-beta-xylanase produced by *K. phaffii* DSM 33574 (Xylamax), when used under the proposed conditions of use (see Section 3.1.5).

1.2. Additional information

The additive consisting of endo-1,4-ß-xylanase produced by *K. phaffii* DSM 33574 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* DSM 33574 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.[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 *K. phaffii* DSM 33574 (Xylamax) 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 assessment of the safety of feed additives for the consumer (EFSA FEEDAP

[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] BioResource International, Inc. (BRI) 4,222 Emperor Blvd, Suite 460; Durham, NC 27703, USA; represented in EU by Pen & Tec consulting S.L.U., Plaza Ausias March 1, 4th floor D10, 08195, Sant Cugat del Vallès, Barcelona, Spain.
[3] Following the request for Supplementary information dated 11 October 2021, the applicant clarified the target species for which the application was made, restricting it to minor poultry species.
[4] FEED dossier reference: FAD-2021-0033.
[5] The full report is available on the EU Science Hub website: https://joint-research-centre.ec.europa.eu/publications/fad-2021-0033_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.

Panel, 2017a), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018a), Guidance on the characterisation of microorganisms used as 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 contains endo-1,4-β-xylanase (IUBMB EC 3.2.1.8; xylanase) produced by *K. phaffii* DSM 33574 and it is intended to be used as a as a zootechnical additive (functional group: digestibility enhancers) for chickens for fattening, chickens reared for laying/breeding, turkeys for fattening, turkeys reared for breeding and minor poultry species for fattening or raised to the point of lay. The additive under assessment will be hereafter referred to as Xylamax.

3.1. Characterisation

3.1.1. Characterisation of the production organism

The additive is produced by the genetically modified strain of *K. phaffii* (formerly *Pichia pastoris*) which is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) with deposition number DSM 33574.[7]

The taxonomical identification of the production strain as *K. phaffii* was confirmed by bioinformatic analysis of the whole genome sequence (WGS) data of the production strain DSM 33574.[8] [redacted]

3.1.1.1. Information related to the genetically modified microorganism

Characterisation of the parental or recipient microorganism

[redacted] (Sturmberger et al., 2016). The taxonomical identification of the parental/recipient strain as *K. phaffii* was confirmed [redacted] (see Section 3.1.1).

Description of the genetic modification

[redacted][9]

[7] Technical dossier/Section II/Annexes_Sect_II/Annex_II_2_1a.
[8] Technical dossier/Section II/Annexes_Sect_II/Annex_II_2_1b, Annex_II_2_1d and Annex_II_2_1f.
[9] Technical dossier/Section II/Annexes_Sect_II/Annex_II_2_1b and Supplementary information September 2021/ 2_EFSA_SIn_6JUL21_reply.

[redacted][10]

3.1.2. Manufacturing process

The enzyme contained in the additive Xylamax is produced by fermentation with *K. phaffii* DSM 33574. [redacted][11]

The applicant declared that no antibiotics are used during the manufacturing process of the additive.

3.1.3. Characterisation of the additive

The additive is available in solid form and is specified to contain a minimum xylanase activity of 150,000 XU[12]/g of product. [redacted][13] The batch-to-batch variation was studied in eight batches and the mean value was 165,675 XU/g product, ranging from 151,889 to 183,175 XU/g.[14] The results demonstrated compliance with the specifications set.

Three batches of the additive were analysed for chemical impurities which included arsenic (range 0.292–0.373 mg/kg), cadmium (0.360–0.388 mg/kg), lead (0.215–0.295 mg/kg), mercury (< 0.018 mg/kg) and the following mycotoxins which showed values below the corresponding limits of quantification (LOQs): aflatoxins (B1, G1, B2, G2, M1 and M2), deoxynivalenol, fumonisins (B1 and B2), HT-2 toxin, T-2 toxin, ochratoxin A and zearalenone.[15]

Polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), coplanar dioxin-like polychlorinated biphenyls (DL-PCBs) and non-DL PCBs (ICES-6) were analysed in three batches.[16] The calculated (upper bound) levels of the sum of dioxins ranged from 0.03 to 0.20 ng WHO-PCDD/F-TEQ/kg. The calculated (upper bond) value of the sum of DL-PCBs was 0.001 ng WHO-PCB-TEQ/kg (in all three batches). The sum of non-DL-PCBs was < 20 ng/kg.

[redacted][17] Moreover, three batches of the additive were analysed for the presence of residues of methanol, isopropanol and ethanol [redacted] In all three batches, the analysis showed results below the LOQ (< 10 mg/kg).[18]

The applicant set specifications for microbiological contamination which include moulds ($\leq 10^3$ colony forming units (CFU)/g), *E. coli* (≤ 10 CFU/g), coliforms (≤ 10 CFU/g) and *Salmonella* (not detected in 25 g). These specifications were confirmed in the analysis of three batches: yeasts (< 10 CFU/g), moulds (< 10 CFU/ g except 10 CFU/g in one batch tested), aerobic plate count (range 430–940 CFU/g), total coliforms (< 10 CFU/g), *E. coli* (< 10 CFU/g) and *Salmonella* spp. (not detected in 25 g).[19] Three additional batches were tested for the presence of Enterobacteriaceae and the result in all three batches was < 10 CFU/g.[18]

The detected impurities do not raise safety concerns.

[10] Technical dossier/Section II/Annexes_Sect_II/Annex_II_2_1b.
[11] Technical dossier/Section II/Annexes_Sect_II/Annex_II_3_1 and Annex_II_3b.
[12] One XU unit is the amount of enzyme which releases 1 nano-mol of reducing sugar (xylose equivalent) per second from xylan of beechwood at 50 °C and pH 6.0.
[13] Technical dossier/Supplementary information September 2021/Annexes/Annex_II_1_3a.
[14] Technical dossier/Section II/Annexes_Sect_II/Annex_II_1_3b and Annex_II_1_4a.
[15] Technical dossier/Section II/Annexes_Sect_II/Annex_II_1_4a and Supplementary information September 2021/2_EFSA_Sin_6JUL21_reply. Limit of quantification (LOQ) in mg/kg was 0.018 for mercury and in μg/kg was 0.5 for aflatoxins (B1, G1, B2, G2, M1 and M2), 5 for lead and cadmium, 100 for deoxynivalenol and HT-2 toxin, 25 for fumonisins (B1 and B2), 10 for T-2 toxin and arsenic, 1 for ochratoxin A and 30 for zearalenone.
[16] Technical dossier/Supplementary information September 2021/Annexes/Annex_II_1_4a.
[17] Technical dossier/Section II/Annexes_Sect_II/Annex_II_1_4b.
[18] Technical dossier/Supplementary information September 2021/Annexes/ Annex_II_1_4f.
[19] Technical dossier/Section II/Annexes_Sect_II/Annex_II_1_4a.

The presence of viable cells of the production strain in the final additive was investigated in three batches of Xylamax analysed in triplicate.[20] [redacted] Therefore, 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 Xylamax, each tested in triplicate.[21] [redacted] No recombinant DNA of the production strain was detected.

The additive appears as a light grey powder and it has a bulk density of 930 kg/m^3.[22] The dusting potential of three batches of the additive was determined using the Stauber–Heubach method and showed values on average of 12,210 mg/m^3 (range 12,114–12,341 mg/m^3).[23] The same three batches were analysed for particle size distribution by laser-diffraction; the results showed that particles < 100 μm, < 50 μm, < 10 μm and < 1 μm were on average 63.2% (range 59.5–69.8%), 43.1% (range 39.9–48.4%), 12.4% (range 11.2–14.0%) and 1.4% (range 1.3–1.6%), respectively.

3.1.4. Stability and homogeneity

The shelf life of three batches of the additive (each analysed in triplicate, initial enzyme activity 200,000 XU/g) was studied in samples stored at 30°C in packaging bags up to 24 months. Losses of enzyme activity after 24 months ranged between 18.8% and 21.1%.[24]

The additive is not intended to be incorporated via a premixture.[25] The stability of the xylanase in feed was evaluated in one batch of the additive (analysed in triplicate) when added at 70 mg/kg to a mash standard soy/maize-based feed for chickens for fattening.[26] The mash feed supplemented with the additive was pelleted at 85°C for 30 s to study the effect of the heat treatment. The pelleting process led to a loss of activity of 30%. Samples of the mash and pelleted feed were stored for 3 months at 25°C and 60% relative humidity (in paper bags) and losses at the end of the storage period were 5% and 4.5% in mash and pelleted feed, respectively.

The capacity for homogeneous distribution of the additive in feed was studied in 10 subsamples of mash and pelleted feeds described above and the coefficients of variation were 6% and 2%, respectively.[26]

3.1.5. Conditions of use

The additive is intended for use in feed for chickens for fattening, chickens reared for laying/breeding, turkeys for fattening, turkeys reared for breeding and minor poultry species for fattening or raised to the point of lay at a proposed minimum level of 10,000 XU/kg feed. The additive is not to be used in premixtures.

3.2. Safety

3.2.1. Safety of the production microorganism

The production organism 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, 2007; EFSA BIOHAZ Panel, 2020). The production strain was well identified and

[20] Technical dossier/Section II/Annexes_Sect_II/Annex_II_1_4c and Annex_II_1_4e.
[21] Technical dossier/Section II/Annexes_Sect_II/Annex_II_1_4d.
[22] Technical dossier/Section II/Annexes_Sect_II/Annex_II_1_5a.
[23] Technical dossier/Section II/Annexes_Sect_II/Annex_II_1_5b.
[24] Technical dossier/Section II/Annexes_Sect_II/Annex_II_4_1.
[25] Technical dossier/Supplementary information September 2021/2_EFSA_Sin_6JUL21_reply.
[26] Technical dossier/Section II/Annexes_Sect_II/Annex_II_4_2.

differed from the recipient strain by expressing [redacted] The production strain contains [redacted] However, no viable cells nor recombinant DNA of the production strain were detected in the final product.

Therefore, the additive does not pose any safety concern regarding the production strain.

3.2.2. Toxicological studies

Toxicological studies are not required for fermentation products produced by a genetically modified microorganism for which the recipient strain is considered by EFSA to qualify for the QPS approach to safety assessment and for which the genetic modification raises no concerns. The genetic modification introduced [redacted]; however, this is not expected to have an impact on the toxicological profile of the production strain. Therefore, from this point of view, the production strain is presumed as safe. The applicant submitted genotoxicity studies and a subchronic oral toxicity study to support the safety of the additive and are presented here below. All the toxicological studies were performed with the intermediate concentrate form of xylanase ([redacted]) prior to the addition of the carriers.

3.2.2.1. Genotoxicity

Bacterial reverse mutation test

In order to investigate the potential of the test item to induce gene mutations in bacteria, the Ames test was performed according to Organisation for Economic Co-operation and Development (OECD) testing guideline (TG) 471, and to Good Laboratory Practice (GLP) principles, [redacted][27] No increase in the number of revertant colonies was observed in any tester strain and experimental condition. The FEEDAP Panel concluded that the test item did not induce gene mutations in bacteria under the experimental conditions applied in the study.

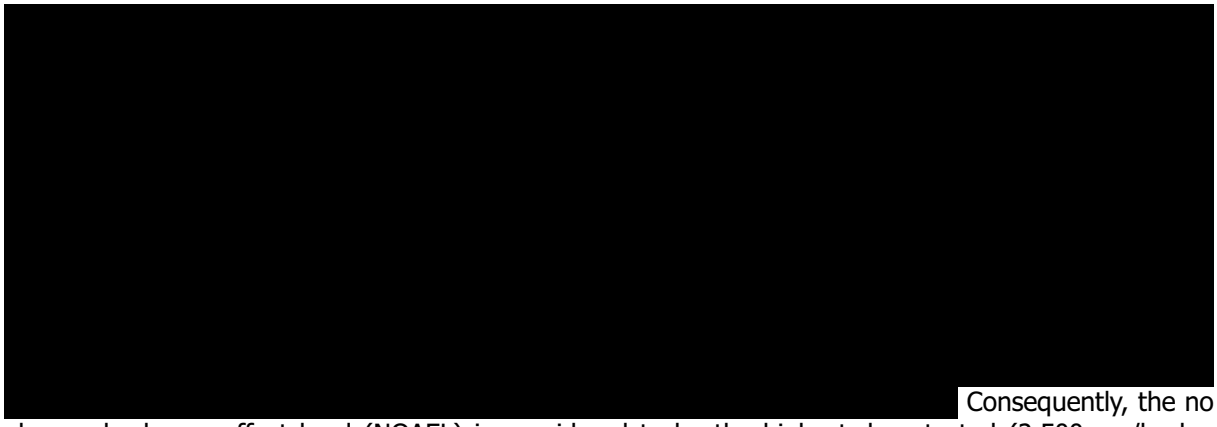
In vitro Mammalian Micronucleus Assay

To evaluate the potential clastogenic and aneugenic activity of the test item, an *in vitro* micronucleus test was performed [redacted] in compliance with OECD TG 474 and to GLP principles.[28] [redacted] The Panel notes that the absolute concentrations of concentrated xylanase in the diluted test material were not provided. However, as the genotoxicity studies were used as supporting information only, the FEEDAP Panel considers that further data were not needed. Under the conditions of the study, the test item did not exhibit either clastogenic or aneugenic effects.

Repeated dose toxicity study

[redacted][29] The study was conducted according to GLP and in compliance with OECD TG 408.

[27] Technical dossier/Supplementary information January 2022/Annex_III_2_2_2a.
[28] Technical dossier/Supplementary information January 2022/Annex_III_2_2_2b.
[29] Technical dossier/Supplementary information January 2022/Annex_III_2_2_3.



Consequently, the no observed adverse effect level (NOAEL) is considered to be the highest dose tested (2,500 mg/kg bw per day, corresponding to 1,292,410 XU/kg bw per day).

Conclusion on the toxicological studies

The FEEDAP Panel concludes that the intermediate product used for the formulation of the additive did not show any genotoxicity potential. Moreover, the results obtained in a subchronic oral toxicity study raised no concerns regarding the product and allowed to derive a NOAEL of 1,292,410 XU/kg bw per day.

3.2.3. Safety for the target species

No tolerance studies in relevant target species were submitted. In order to support the safety of the additive for the target species, the applicant referred to the 90-day toxicity study that has been described above (see Section 3.2.2). The NOAEL identified (1,292,410 XU/kg bw per day) was used to calculate the maximum safe level in feed for chickens and turkeys for fattening in accordance with the procedure described in the Guidance on the safety for the target species (EFSA FEEDAP Panel, 2017b). The calculated maximum safe concentration in feed was 143,964 XU/kg complete feed for chickens for fattening and 192,766 XU/kg complete feed for turkeys for fattening. These values are higher than the recommended use level of 10,000 XU/kg feed for poultry species for fattening or reared for laying. Therefore, the Panel concludes that the additive is safe for all poultry species for fattening or reared to the point of lay.

3.2.4. Safety for the consumer

The enzyme is produced by a genetically modified strain of *K. phaffii*; this species is considered to qualify for the QPS approach to safety assessment when used for enzyme production. The identity of the strain was established, and the genetic modification of the production strain raises no concerns as regards to the toxicological profile of the production strain. Therefore, the production strain is presumed safe for production purposes and no concerns would raise for the consumer from the fermentation product obtained from this strain. The results obtained in the genotoxicity studies and the 90-day study support this conclusion. The FEEDAP Panel concludes that the use of Xylamax in animal nutrition under the proposed conditions of use is safe for the consumers.

3.2.5. Safety for the user

3.2.5.1. Effects on respiratory system

Based on the dusting potential of the additive (highest measured value: 12,341 mg/m^3), the FEEDAP Panel considers that exposure of users by inhalation is very likely. Due to the proteinaceous nature of the active substance of the additive, the additive is considered to be a respiratory sensitiser.

3.2.5.2. Effects on eyes and skin

The skin irritation potential of Xylamax was tested in a study performed according to GLP and OECD TG 439.[30] The study showed that the additive is not a skin irritant.

[30] Technical dossier/Section III/Annexes_Sect.III/Annex_III_3_1_2a.

The eye irritation potential of Xylamax was tested in a study performed according to GLP and OECD TG 492.[31] The results of the test were inconclusive; therefore, the FEEDAP Panel considers the product as a potential eye irritant.

No information on skin sensitisation potential was provided, therefore the FEEDAP Panel cannot conclude on the skin sensitisation potential of the additive.

3.2.5.3. Conclusions on safety for the user

Xylamax is not a skin irritant but should be considered a potential eye irritant and a respiratory sensitiser. No conclusions can be drawn on the potential of the additive to cause skin sensitisation.

3.2.6. Safety for the environment

The production strain and its DNA were not detected in the final product. The additive does not raise safety concerns for the environment with regard to the genetic modification of the production strain. The active substance of the additive is a protein, and as such will be degraded/inactivated during the passage through the digestive tract of animals or in the environment. Therefore, no risks to the environment are expected and no further environmental risk assessment is required.

3.3. Efficacy

To support the efficacy of the additive the applicant submitted four trials in chickens for fattening, two designed as balance trials[32] and the other two as long-term trials[33] which also included measurements on the apparent metabolisable energy content of the diets. The applicant also submitted five published papers (Flores et al., 2017; Nusairat et al., 2018; Duarte et al., 2019, 2020; Nusairat and Wang, 2020).

3.3.1. Efficacy trials in chickens

3.3.1.1. Short-term trials

The two trials were designed as balance trials and in each of them, a total of 96 (trial 1[34]) or 72 (trial 2[35]) one-day-old male chickens for fattening (Ross 708) were distributed in 12 cages and allocated to two dietary treatments (6 replicates per treatment). During the study, the birds received one basal diet based on wheat, soya bean meal and dried distillers grains, which was offered in mash (trial 1) or crumble (trial 2) form on *ad libitum* basis. Feed was either not supplemented (control) or supplemented with Xylamax to provide 10,000 XU/kg feed (confirmed by analysis). The animals received the diets for 21 days. Animal health was monitored during the study. Feed intake and body weight were measured weekly and feed to gain ratio was calculated including the dead/culled animals. The excreta were collected for the last 3 days of the study. The diets contained an external marker and the excreta and the diets were analysed for gross energy and nitrogen contents to determine the apparent metabolisable energy adjusted for nitrogen (AMEn). The data was analysed with a one-way analysis of variance (ANOVA) and significance was set at 0.05.

The birds receiving the minimum recommended dose had significantly higher AMEn compared to the non-supplemented group in the two trials (in trial 1, 2,940 kcal/kg feed in control group and 3,054 kcal/kg feed in the group receiving the additive, the corresponding values in trial 2 were 3,154 kcal/kg feed and 3,363 kcal/kg feed).

3.3.1.2. Long-term trials

The two long term studies[36] included at the end of the growing period measurements of the utilisation of the energy in the diets. The Panel notes that the husbandry conditions in which the birds

[31] Technical dossier/Section III/Annexes_Sect.III/Annex_III_3_1_2b.
[32] Technical dossier/Section IV/Annexes_Sect_IV/Annex_IV_2_1, Annex_IV_2_2 and Supplementary information January 2022/2_EFSA_SIn_11OCT21_reply_FINAL.
[33] Technical dossier/Section IV/Annexes_Sect_IV/Annex_IV_3_1, Annex_IV_3_2 and Supplementary information January 2022/2_EFSA_SIn_11OCT21_reply_FINAL.
[34] Technical dossier/Section IV/Annexes_Sect_IV/Annex_IV_2_1.
[35] Technical dossier/Section IV/Annexes_Sect_IV/Annex_IV_2_2.
[36] Technical dossier/Section IV/Annexes_Sect_IV/Annex_IV_3_1 and Annex_IV_3_2.

were raised did not reflect the conditions in which the birds should be raised in the EU and were not in line with Directive 2007/43/EC[37] (regarding the lighting program and/or bedding used), and therefore, disregarded the performance parameters measured in them. However, the Panel considers that the data on the energy utilisation could be retained considering that the husbandry conditions would have a lower impact on the parameters measured/endpoints.

On top of the limitations on the husbandry conditions, one of the studies[38] showed high mortality/culling (7.8%) and low growth of the animals (1,500 g compared to 2,400 g (standard of the breed)). Therefore, that study was not considered further.

In the remaining trial, a total of 780 one-day-old male chickens for fattening (Cobb 430) were distributed in 30 pens and allocated to three dietary treatments. Three basal diets (starter, grower and finisher) based on maize, soya and vegetable oil, were either not supplemented (control) or supplemented with Xylamax to provide 10,000 XU/kg feed (confirmed by analysis). The animals received the starter diet until day 14, the grower from day 15 to 28 and the finisher from day 29 to 42, in mash form and on *ad libitum* basis. On the last 3 days of study, two birds per pen were transferred to a cage and the excreta was collected for 3 days and feed intake was measured. The diets and the excreta were analysed for the content of energy to calculate the apparent metabolisable energy (AME) content of the diets. The data were analysed with a one-way ANOVA and the p-level was set at 0.05.

The AME was significantly higher in the group of birds that received the additive compared to the control group (2,437 vs. 2,521 kcal/kg feed).

3.3.2. Published papers

The applicant submitted five publications, two of which (Duarte et al., 2019, 2020) reported studies in pigs, which are not the scope of the current assessment and therefore were not considered further.

The other three publications submitted described studies in chickens for fattening which aimed at studying the effect of the additive mainly on the zootechnical endpoints. The husbandry conditions in which the birds were kept did not reflect the conditions in which the birds should be raised in the EU (i.e. under continuous light[39] or receiving higher number of hours of light[40] and the use of reused bedding[41]) and therefore were not in line with Directive 2007/63/EC. The Panel did not consider the results of the zootechnical parameters further in the assessment. However, the study from Nusairat and Wang (2020) included data on the utilisation of the energy of the diets at two different time points. The results showed that the supplementation of xylanase at 10,000 XU/kg feed improved, compared to the control, the AME and AMEn on days 21 (2,926 vs 2,889 kcal/kg feed and 2,895 vs 2,857 kcal/kg feed, respectively) and 42 (3,122 vs 3,078 kcal/kg feed and 3,095 vs 3,054 kcal/kg feed, respectively). The results are considered to support the conclusions reached in the studies described above.

3.3.3. Conclusions on efficacy

The FEEDAP Panel concludes that Xylamax has the potential to be efficacious in chickens for fattening at 10,000 XU/kg feed. This conclusion is extended to chickens reared for laying/breeding. Considering that the mode of action of xylanases is well known and can reasonably be assumed to be the same among poultry species the conclusion is extrapolated to turkeys for fattening, turkeys reared for breeding and minor poultry species for fattening or raised to the point of lay.

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[42] and Good Manufacturing Practice.

[37] 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.
[38] Technical dossier/Section IV/Annexes_Sect_IV/Annex_IV_3_2 and Supplementary information January 2022/2_EFSA_SIn_11OCT21_reply_FINAL and Annexes/Annex_IV_3_2 and Annex_IV_3_2b.
[39] Nusairat et al. (2018) and Nusairat and Wang (2020).
[40] Flores et al. (2017).
[41] Flores et al. (2017), Nusairat et al. (2018) and Nusairat and Wang (2020).
[42] 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.

4. Conclusions

The production strain is a genetically modified strain of *Komagataella phaffii* (DSM 33574). No 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.

Xylamax is safe for all poultry species for fattening or reared to the point of lay at the recommended level of 10,000 XU/kg feed.

The additive is safe for the consumers of food derived from animals fed with the additive.

Xylamax is not irritant to the skin but should be considered a potential eye irritant and a respiratory sensitiser. No conclusions can be drawn on the potential of the additive to cause skin sensitisation.

The use of the product as a feed additive is of no concern for the environment.

The FEEDAP Panel concludes that Xylamax has the potential to be efficacious in chickens for fattening at 10,000 XU/kg feed. This conclusion is extended to chickens reared for laying/breeding and extrapolated to turkeys for fattening, turkeys reared for breeding and minor poultry species for fattening or raised to the point of lay.

5. Documentation provided to EFSA/Chronology

Date	Event
25/03/2021	Dossier received by EFSA. Xylamax (endo-1,4-beta-xylanase produced by *Komagaetella phaffii* DSM 33574) for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding and all minor avian species. Submitted by BioResource International, Inc.
12/04/2021	Reception mandate from the European Commission
27/05/2021	Application validated by EFSA – Start of the scientific assessment
06/07/2021	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*
25/08/2021	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
30/08/2021	Comments received from Member States
07/09/2021	Reception of supplementary information from the applicant - Scientific assessment re-started
11/10/2021	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*
26/01/2022	Reception of supplementary information from the applicant - Scientific assessment re-started
29/04/2022	Reception of spontaneous information from the applicant
29/06/2022	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

AMEn	apparent metabolizable energy adjusted for nitrogen
ANOVA	analysis of variance
bw	body weight
CFU	colony forming unit
DL-PCB	dioxin-like polychlorinated biphenyl
DNS	3,5-dinitrosalicylic acid
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products of Substances used in Animal Feed
GLP	Good Laboratory Practice

LOQ	limit of quantification
NOAEL	no observed adverse effect level
PCDD	polychlorinated dibenzodioxin
PCDF	polychlorinated dibenzofuran
OECD	Organisation for Economic Co-operation and Development
QPS	qualified presumption of safety
TG	testing guideline
WGS	whole genome sequence
WHO	World Health Organization

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 endo-1,4-β-xylanase produced by *Komagataella phaffii* DSM 33574 (Xylamax)

In the current application, an authorisation of a *preparation of endo-1,4-beta-xylanase (EC 3.1.3.28)* is sought under Article 4(1) for chickens and turkeys for fattening, chickens reared for laying, turkeys reared for breeding and minor avian species (including also sporting, ornamental and exotic birds), 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 e*ndo-1,4-beta-xylanase*, produced by fermentation of the genetically modified strain *Komagaetella phaffii xyl-2* (DSM 33574). The activity of *endo-1,4-beta-xylanase* is expressed in *xylanase* units (XU). One XU unit is the amount of enzyme which releases 1 nano-mol of reducing sugar (xylose equivalent) per second from xylan of beechwood at 50 °C and pH 6.0. The *feed additive* is intended to be marketed as a solid formulation, having a guaranteed minimum *endo-1,4-beta-xylanase* activity of 15,000 XU/g and to be incorporated directly into complete *feedingstuffs* to obtain minimum *xylanase* activity of 10,000 XU/kg *feedingstuffs*.

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 the beechwood xylan substrate and the colour formation of the released reducing sugar with 3,5-dinitrosalicylic acid (DNS).

Furthermore, for the quantification of *endo-1,4-beta-xylanase* in *feedingstuffs* the Applicant submitted another single-laboratory validated and further verified colorimetric method, based on the measurement of 4-nitrophenol group produced by the action of *xylanase* on a commercially available substrate (Megazyme).

Based on the acceptable performance characteristics the EURL recommends for official control (i) the single-laboratory validated and further verified colorimetric (DNS) 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 *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.