SCIENTIFIC OPINION

efsa JOURNAL

ADOPTED: 6 January 2023

doi: 10.2903/j.efsa.2023.7816

Safety and efficacy of a feed additive consisting of endo-1,4-beta-xylanase produced by *Trichoderma reesei* ATCC PTA-5588, protease produced by *Bacillus subtilis* CBS 148232, and alpha-amylase produced by *Bacillus licheniformis* ATCC SD-6525 (Axtra® XAP 104 TPT) for chickens for fattening, laying hens and minor poultry species (Genencor international B.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, Noël Dierick, Maria Saarela, Rosella Brozzi, Elisa Pettenati and Montserrat Anguita

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-beta-xylanase produced by *Trichoderma reesei* ATCC PTA-5588, protease produced by *Bacillus subtilis* CBS 148232, and alpha-amylase produced by *Bacillus licheniformis* ATCC SD-6525, Axtra® XAP 104 TPT, for chickens for fattening, laying hens and minor poultry species. In the previous assessment, a series of shortcomings did not allow to conclude on the safety of the product. The shortcomings included uncertainty on the presence of viable cells of one of the production strains; uncertainty on the identity of the production strains of the [redacted] used in the manufacturing process of the protease and their presence in the final additive; and uncertainty about the test item used for the toxicological testing of the xylanase. Moreover, the Panel could not conclude on the efficacy in laying hens. The applicant submitted some new information to address some of the limitations previously identified. Moreover, the applicant declared a change in the production strain of the protease, substituting *B. subtilis* ATCC SD-2107 for *B. subtilis* CBS 148232. The Panel concluded that the additive is safe for the target species at the recommended use level (1,000 xylanase U, 100 amylase U and 2,000 protease U per kg feed). The additive is safe for the consumers of food products obtained from animals fed with the additive and raises no concerns for the environment. The Panel could not conclude on the skin/eye irritancy potential of the additive nor on its dermal sensitisation potential. Owing to the proteinaceous nature of the active substances, the additive is considered a respiratory sensitiser. The additive is efficacious in chickens for fattening, chickens reared for laying and minor poultry species up to the point of lay at the level of 2,000 xylanase U, 200 amylase U and 4,000 protease U per kg feed (double the minimum recommended use level). Owing to the lack of sufficient data, the Panel could not conclude on the efficacy of the additive for laying hens.

Keywords: zootechnical additives, digestibility enhancers, safety, efficacy, poultry

Requestor: European Commission

Question number: EFSA-Q-2020-00620

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.

Declarations of interest: If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

Acknowledgements: The Panel wishes to thank the following for the support provided to this scientific output (in alphabetical order of the last name): Working Group on Microbiology, Matteo Lorenzo Innocenti and Maria Vittoria Vettori.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Fašmon Durjava M, Kouba M, López-Alonso M, Puente SL, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Dierick N, Saarela M, Brozzi R, Pettenati E and Anguita M, 2023. Scientific Opinion on the safety and efficacy of a feed additive consisting of endo-1,4-beta-xylanase produced by *Trichoderma reesei* ATCC PTA-5588, protease produced by *Bacillus subtilis* CBS 148232, and alpha-amylase produced by *Bacillus licheniformis* ATCC SD-6525 (Axtra® XAP 104 TPT) for chickens for fattening, laying hens and minor poultry species (Genencor international B.V.). EFSA Journal 2023;21(2):7816, 13 pp. https://doi.org/10.2903/j.efsa.2023.7816

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.

Table of contents

1. Introduction

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

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

The applicant, Genencor International B. V. is seeking a Community authorisation of xylanase (EC 3.2.1.8), alfa-amylase (EC 3.2.1.1) and protease (EC 3.4.21.62) as a feed additive to be used as a digestibility enhancers for chickens for fattening, laying hens, minor poultry species (Table 1).

Table 1: Description of the substances

Category of additive	**Zootechnical additives**
Functional group of additive	Digestibility enhancers
Description	xylanase (EC 3.2.1.8), alfa-amylase (EC 3.2.1.1) and protease (EC 3.4.21.62)
Target animal category	chickens for fattening, laying hens, minor poultry species
Applicant	Genencor International B. V.
Type of request	New opinion

On June 2020, the Panel on Additives and Products or Substance used in Animal Feed of the European Food Safety Authority ("Authority"), in its opinion on the safety and efficacy of the product, could not conclude on the safety of xylanase (EC 3.2.1.8), alfa-amylase (EC 3.2.1.1) and protease (EC 3.4.21.62) for the target species, consumers and users. There was also an uncertainty on the presence of viable cells of one of the production strains in the additive. After the discussion with the Member States on the Standing Committee, it was suggested to check for the possibility to demonstrate the safety.

The Commission gave the possibility to the applicant to submit complementary information in order to complete the assessment and allow a revision of Authority's opinion. The new data have been received on 31 August 2020 and were already transmitted to EFSA by the applicant.

In view of the above, the Commission asks EFSA to deliver a new opinion on xylanase (EC 3.2.1.8), alfa-amylase (EC 3.2.1.1) and protease (EC 3.4.21.62) as a feed additive for chickens for fattening, laying hens, minor poultry species based on the additional data submitted by the applicant.

1.2. Additional information

The additive, with the trade name Axtra® XAP 104 TPT, is a preparation containing endo-1,4-beta-xylanase produced by a genetically modified strain of *Trichoderma reesei* (ATCC PTA-5588), protease produced by a genetically modified strain of *Bacillus subtilis* (ATCC SD-2107) and alpha-amylase produced by a genetically modified strain of *Bacillus licheniformis* (ATCC SD-6525).

The FEEDAP Panel adopted an opinion on this product in 2020 (EFSA FEEDAP Panel, 2020). In the context of the current assessment, the applicant has changed the production strain of the protease, substituting strain ATCC SD-2107 of *B. subtilis* for strain CBS 148232 of the same species.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of supplementary information[1] to a previous application on the same product.[2]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of endo-1,4-beta-xylanase produced by *T. reesei* ATCC PTA-5588, protease produced by *B. subtilis* CBS 148232, and alpha-amylase produced by *B. licheniformis* ATCC SD-6525 (Axtra® XAP 104 TPT) is in line with the

[1] Dossier reference: FAD-2020-0065.
[2] Dossier reference: FAD-2017-0053.

principles laid down in Regulation (EC) No 429/2008[3] 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 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 additive Axtra® XAP 104 TPT is a preparation containing endo-1,4-beta-xylanase, protease and alpha-amylase intended to be used as a zootechnical additive (functional group: digestibility enhancers) in feed for chickens for fattening, laying hens and minor poultry species.

The FEEDAP Panel adopted a scientific opinion on the safety and efficacy of the additive (EFSA FEEDAP Panel, 2020). The product assessed at that time contained endo-1,4-beta-xylanase produced by a genetically modified strain of *T. reesei* (ATCC PTA-5588); protease produced by a genetically modified strain of *B. subtilis* (ATCC SD-2107); and alpha-amylase produced by a genetically modified strain of *B. licheniformis* (ATCC SD-6525). In that assessment, the FEEDAP Panel identified a series of shortcomings which did not allow for conclusions regarding the safety of the product, including (i) uncertainty on the presence of viable cells of the genetically modified strain *T. reesei* ATCC PTA-5588; (ii) uncertainty about the identity and presence in the final additive of the production strains of the [redacted] used in the manufacturing process of the protease, and (iii) uncertainty about the test item used for the toxicological testing of the xylanase. The Panel also could not conclude on the efficacy of the additive in laying hens (EFSA FEEDAP Panel, 2020).

In the current submission, the applicant has provided supplementary information to address the uncertainties above mentioned. The applicant has also declared a change in the manufacturing process of the protease as well as the change in the production strain, substituting *B. subtilis* strain ATCC SD-2107 for *B. subtilis* strain CBS 148232. No new data were provided to support the efficacy of the additive for laying hens. The current assessment will consider all the data submitted to address previously identified limitations as well as the modifications notified by the applicant.

3.1. Characterisation

3.1.1. Characterisation of the production microorganisms

The applicant did not declare any modification on the production strain of the endo-1,4-beta-xylanase (*T. reesei* ATCC PTA-5588) or on the one producing the alpha-amylase (*B. licheniformis* ATCC SD-6525).

For the protease, the applicant declared the change in the production strain. The *B. subtilis* ATCC SD-2107 described in the previous opinion (EFSA FEEDAP Panel, 2020) has been replace by *B. subtilis* CBS 148232. The newly declared strain requires full characterisation.

3.1.1.1. *Bacillus subtilis* CBS 148232 – new production strain of the protease

The production strain of the protease is a genetically modified strain of *B. subtilis* that is deposited in the Westerdijk Fungal Biodiversity Institute with the accession number CBS 148232.[4]

The taxonomic identification of an intermediate strain [redacted][5] confirms its identification as *B. subtilis*. The Panel notes that the guidance recommends the taxonomic identification of the actual production strain (*B. subtilis* CBS 148232). To

[3] 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.
[4] Technical dossier FAD-2020-0065/Supplementary information October 2021/Annex 6.
[5] Technical dossier FAD-2020-0065/Supplementary information October 2021/Annex 8.

comply with this requirement, the applicant submitted [redacted][6] which supports the identification of the production strain as *B. subtilis*. Genome analysis of the production strain was also used to characterise the intended genetic modification.

The production strain was tested by broth microdilution for its susceptibility to the antimicrobials listed for *Bacillus* in the Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018a).[7] [redacted][8] [redacted][9] The FEEDAP Panel concludes that the production strain is resistant [redacted], but WGS analysis revealed no genes of concern.

The toxigenic potential of the production strain *B. subtilis* CBS 148232 was assessed according to the Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018a).[10] No lysis of Vero cells was detected and thus *B. subtilis* CBS 148232 is considered to be non-toxigenic.

3.1.1.1.1. Description of the genetic modification[11]

[redacted]

[6] Technical dossier FAD-2020-0065/Supplementary information September 2022/Annex S1.
[7] Technical dossier FAD-2020-0065/Supplementary information October 2021/Annex 10.
[8] Technical dossier FAD-2020-0065/Supplementary information October 2021/Annexes 7 and 11.
[9] Technical dossier FAD-2020-0065/Supplementary information October 2021/Annexex 7 and 11.
[10] Technical dossier FAD-2020-0065/Supplementary information October 2021/Annex 13.
[11] Technical dossier FAD-2020-0065/Supplementary information October 2021/Annex 5 and Supplementary information September 2022/Annex S1 and S2.

[redacted][12]

3.1.2. Manufacturing process

The applicant did not declare any modification on the manufacturing process of the endo-1,4-beta-xylanase or on the alpha-amylase. The applicant described a modification on the manufacturing process of the protease and provided information on the mixture of [redacted] used in the same manufacturing.

3.1.2.1. Manufacturing process of the protease

The applicant declared, in the previous assessment, the use of a mixture of [redacted] in the manufacturing process of the protease (EFSA FEEDAP Panel, 2020). Uncertainty remained about the identity of the strains used for the production of the [redacted]. In the current submission, the applicant indicated the identification of the strains [redacted] and the fact that they are not genetically modified. Moreover, the amount used in the manufacturing is very low and consequently of little relevance.

In the context of the current submission, and further to the change in the production strain to *B. subtilis* CBS 148232, the applicant declared a change in the manufacturing process of the protease compared to the previous assessment (EFSA FEEDAP Panel, 2020). [redacted] According to the applicant, this step was added to ensure a better [redacted] from the fermentation product. No other modifications were declared.

3.1.3. Characterisation of the additive

No changes have been declared on the composition/characterisation of the final formulation of the additive.

In view of the changes proposed in the production strain of the protease, the applicant submitted new data on the final additive and on the intermediate protease product. Unless otherwise stated, most of the data presented below were obtained with the new production strain and prior to the use of an additional [redacted]. The Panel does not expect a major impact of the last [redacted] on any of the parameters reported below and therefore the data are still considered relevant.

The additive is available in solid form and the minimum guaranteed activity per gram of product is 20,000 xylanase units[13] (U), 2,000 amylase U[14] and 40,000 protease U.[15] The analysis in three batches showed values between 28,139 and 29,619 xylanase U, 2,540 and 2,892 amylase U, and 45,881 and 54,015 protease U.[16] Data on the purity were provided for the same three batches and included arsenic (< 1.6 μg/kg), cadmium (< 0.5 μg/kg), lead (< 3.5 μg/kg), mercury (< 2.3 μg/kg) total aflatoxins (< 5 μg/kg), ochratoxin (< 5 μg/kg), zearalenone (< 25 μg/kg), deoxynivalenol (< 50 μ/kg) and fumonisin (< 100 μg/kg).[17] Microbiological analysis included total viable counts (< 50 colony forming units (CFU)/g), coliforms (< 10 CFU/g), and *Salmonella* spp. (not detected in 25 g). These results are in line with the batch-to-batch variation, and the microbiological and chemical purity of the final additive reported in the previous assessment (EFSA FEEDAP Panel, 2020).

[12] [redacted]

[13] One unit is the amount of enzyme that releases 0.48 μmol of reducing sugar equivalents from wheat arabinoxylan per min at pH 4.2 and 50°C.

[14] One unit is the amount of enzyme required to release 0.20 μmol of glucosidic linkages from a maltoheptasoide substrate per minute at pH 8.0 and 40°C.

[15] One unit is the amount of enzyme that releases 2.3 μg phenolic compound from a casein substrate per minute at pH 10.0 and 50°C.

[16] Technical dossier/FAD-2020-0065/Supplementary information October 2021/Annex 4 and Supplementary information September 2022.

[17] All the values reported correspond to the respective limits of detection.

Three batches of an intermediate product of the protease ( ) were tested for the presence of antimicrobial activity.[18] No antimicrobial activity was detected.

[19]

The presence of viable cells and DNA of the newly declared protease production strain, *B. subtilis* CBS 148232, was investigated in three batches of the protease fermentation product the that is used to formulate the final additive [20] [21] The limit of detection was 10 ng/mL. No amplification was observed in the samples of the intermediate product, whereas positive controls performed as expected.[22] The applicant also provided data on the absence of DNA in the intermediate product obtained after the additional . The limit of detection was 1 ng/mL. No amplification was observed in the samples of the intermediate product, while positive controls performed as expected.

In the previous assessment, uncertainty remained as regards to the presence of viable cells of the production strain of the endo-1,4-beta-xylanase, *T. reesei* PTA-5588, as well as for the production strains of the used in the manufacturing process of the protease (EFSA FEEDAP Panel, 2020). The applicant submitted data regarding the presence of viable cells of the three strains. Regarding the presence of *T. reesei* PTA-5588, [23] . No growth was observed in the samples of the intermediate product. New data were also submitted to exclude the presence the production strains of the used in the manufacturing process of the protease. . No growth was observed in the samples of the

3.1.3.1. Conditions of use

The additive is to be used as a zootechnical additive (functional group: digestibility enhancers) in feed for chickens for fattening, chickens reared for laying, laying hens and all minor poultry species at a minimum level of 1,000 xylanase U, 100 amylase U and 2,000 protease U per kg feed. The applicant has not modified the conditions of use compared to the previous submission.

[18] Technical dossier FAD-2020-0065/Supplementary information October 2021/Annex 12 and Supplementary information January 2022/Annex S2.
[19] Technical dossier FAD-2020-0065/Supplementary information October 2021/Annex 3.
[20] Technical dossier FAD-2020-0065/ Supplementary information October 2021/Annex 14 and 15
[21] Technical dossier FAD-2020-0065/Supplementary information October 2021/Annex 14.
[22] Technical dossier FAD-2020-0065/Supplementary information October 2021/Annex 15.
[23] Technical dossier FAD-2020-0065/Annex 2 and Supplementary information January 2022/Annex S1.

3.2. Safety

3.2.1. Safety of the production strains

No changes in the production strain of the endo-1,4-beta-xylanase (*T. reesei* ATCC PTA-5588) and the one of the alpha-amylase (*B. licheniformis* ATCC SD-6525) have been declared by the applicant compared to the previous assessment (EFSA FEEDAP Panel, 2020).

In the previous assessment, the Panel concluded that the genetic modification of *T. reesei* ATCC PTA-5588 does not raise any safety concern. Uncertainty remained on the presence of viable cells in the product due to the limited data submitted. The data made available in the current assessment (see Section 3.1.3) showed that no viable cells of *T. reesei* ATCC PTA-5588 were detected in the intermediate product used to formulate the additive. For *B. licheniformis* ATCC SD-6525, the Panel concluded in the previous assessment (EFSA FEEDAP Panel, 2020) that its use as a production strain does not raise safety concerns. The Panel sees no need to reconsider the previous conclusions regarding the safety of *T. reesei* ATCC PTA-5588 and *B. licheniformis* ATCC SD-6525 when used as production strains.

The applicant has declared the use of a new strain to produce the protease, *B. subtilis* CBS 148232. The production strain belongs to a species suitable for the qualified presumption of safety (QPS) approach to safety assessment (EFSA, 2007; EFSA BIOHAZ Panel, 2020). This approach requires the identity of the strain to be conclusively established and evidence that the strain lacks toxigenic potential and does not show acquired resistance to antibiotics of human and veterinary importance. Further, for genetically modified strains, the safety of the genetic modification needs to be established. The identification of the strain has been conclusively established at the species level and the lack of toxigenic potential has been confirmed. The strain is resistant [redacted] but the analysis of the WGS data did not indicate any gene of concern. The genetic modification does not raise concerns, and viable cells and recombinant DNA of the strain were not detected in the intermediate product containing the protease. Therefore, the use of *B. subtilis* CBS 148232 as a production strain of the protease present in the final product does not raise safety concerns.

3.2.2. Toxicological studies

In the previous opinion, the FEEDAP Panel concluded that *B. licheniformis* ATCC SD-6525 can be presumed safe and the toxicological data submitted supported this conclusion. Since no modifications have been declared for the fermentation product produced by *B. licheniformis* ATCC SD-6525, the Panel considers that the same conclusion still applies.

Similarly, and considering the conclusions in Section 3.2.1, the strain *B. subtilis* CBS 148232 can be presumed safe from the toxicological point of view and no toxicological data are required.

3.2.2.1. Toxicological studies for the strain producing the xylanase - *T. reesei* (ATCC PTA-5588)

Toxicological data is required to support the safety of the fermentation product produced by *T. reesei* (ATCC PTA-5588). The applicant submitted in the previous assessment a bacterial reverse mutation assay, an *in vitro* chromosomal aberration test (which addressed structural and numerical aberrations) and a subchronic oral toxicity study. The Panel concluded that the results of the studies would raise no safety concerns (EFSA FEEDAP Panel, 2020). The test item used in the toxicological studies showed differences with the fermentation product of the xylanase described in characterisation section. Therefore, the Panel noted that 'owing to the lack of information on the difference between the two fermentation products, the Panel cannot conclude on the suitability of the test item used in the toxicological tests'. [redacted]

[redacted]

The applicant addressed, in the current submission, the discrepancy [redacted][24]

Thus, the FEEDAP Panel concludes that the newly submitted information allows to consider the test item used in the toxicological tests as suitable for the assessment of the xylanase fermentation product currently used for the preparation of the additive. Therefore, the conclusions from the toxicological studies would be valid for the additive under assessment. The product shows no genotoxic potential and no toxicological concerns were identified in the subchronic oral toxicity study.

3.2.3. Safety for the target species

In the previous opinion, the FEEDAP Panel concluded from the tolerance trials that: 'Axtra® XAP 104 TPT is safe for chickens for fattening and laying hens under the proposed conditions of use. This conclusion can be extended to chickens reared for laying. Considering the wide margin of safety shown, the FEEDAP Panel extrapolates the conclusion to minor poultry species for fattening or laying'. However, owing to the uncertainty on the test item used in the toxicological studies for the xylanase produced by *T. reesei* ATCC PTA-5588, the Panel could not conclude on the safety of the additive for the target species.

With the newly submitted data regarding the test item used in the toxicological studies for the xylanase from *T. reesei* ATCC PTA-5588, no uncertainty remains on the toxicological data previously submitted (see Section 3.2.2). Moreover, the Panel considers that the use of the new strain *B. subtilis* CBS 148232 would not have an impact on the safety of the additive for the target species.

In view of the above, the FEEDAP Panel reiterates the conclusion from the tolerance trials and concludes that AXTRA® XAP 104 TPT is safe for chickens for fattening and laying hens under the proposed conditions of use. The conclusion can be extended to chickens reared for laying and extrapolated to minor poultry species for fattening or laying.

3.2.4. Safety for the consumers

In 2020, the FEEDAP Panel could not conclude on the safety for the consumer of the additive due to the uncertainties on the test item used in the toxicological studies for the xylanase produced by *T. reesei* ATCC PTA-5588. With the newly submitted data regarding the test item used in the toxicological studies for the xylanase from *T. reesei* ATCC PTA-5588, no uncertainty remains on the toxicological data previously submitted. Therefore, the FEEDAP Panel concludes that the fermentation product from *T. reesei* ATCC PTA-5588 showed no genotoxic potential, and no toxicological concerns were identified in the sub-chronic oral toxicity study.

The amylase and the protease are produced by microorganisms (*B. licheniformis* ATCC SD-6525 and *B. subtilis* CBS 148232) which qualify for the QPS approach to safety assessment and therefore would raise no safety concerns for the consumers.

Considering all the above, the FEEDAP Panel concludes that the additive AXTRA® XAP 104 TPT used as a feed additive for poultry is safe for the consumers.

3.2.5. Safety for the user

The Panel considered that the uncertainties on the test item used in the toxicological studies for the xylanase produced by *T. reesei* ATCC PTA-5588 would have an impact on the conclusions on the safety for the user. With the newly submitted data regarding the test item used in the toxicological studies for the xylanase from *T. reesei* ATCC PTA-5588, no uncertainty remains on the toxicological data

[24] Technical dossier FAD-2020-0065/Supplementary information October 2021.

previously submitted for the xylanase product (see Section 3.2.2; addressing genotoxicity and subchronic oral toxicity).

In the previous opinion, no specific studies were submitted to address the safety for the user. Consequently, the FEEDAP Panel could not conclude on the potential of the additive to be irritant to skin and eyes or on its skin sensitising properties and concluded that the additive is considered a respiratory sensitiser. No new studies have been submitted addressing the safety for the user.

In view of the above, the Panel cannot conclude on the potential of the additive to be irritant to skin and eyes or on its skin sensitising properties. Owing to the proteinaceous nature of the active substances the additive is considered a respiratory sensitiser.

3.2.6. Safety for the environment

In the previous opinion, uncertainty remained on the presence of viable cells of the genetically modified *T. reesei* ATCC PTA-5588 in the final additive and thus the Panel could not conclude on the safety for the environment. The applicant has provided data to address the uncertainty indicating that no viable cells were detected in the intermediate product used to formulate the additive (see Section 3.1.3). Moreover, the applicant also provided data showing that no *B. subtilis* CBS 148232 DNA or viable cells were detected.

The Panel concludes that no risks for the environment are expected from the additive.

3.3. Efficacy

In 2020 and based on the efficacy studies provided by the applicant, the Panel concluded that the additive is efficacious in chickens for fattening, chickens reared for laying and minor poultry species up to the point of lay at the level of 2,000 xylanase U, 200 amylase U and 4,000 protease U per kg feed (double the minimum recommended dose). Owing to the lack of sufficient data, the Panel could not conclude on the efficacy of the additive for laying hens.

No new data were submitted by the applicant. The composition and the conditions of use of the additive have not been modified. The new strain producing the protease, *B. subtilis* CBS 148232, produces the same enzyme as the previous one, and therefore the change in the production strain has no impact on the conclusions drawn previously for efficacy.

Therefore, the Panel concludes that additive is efficacious in chickens for fattening, chickens reared for laying and minor poultry species up to the point of lay at the level of 2,000 xylanase U, 200 amylase U and 4,000 protease U per kg feed (double the minimum recommended dose). Owing to the lack of sufficient data, the Panel cannot conclude on the efficacy of the additive for laying hens.

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[25] and Good Manufacturing Practice.

4. Conclusions

The Panel concludes that the use of *B. subtilis* CBS 148232 as a production strain of the protease present in the final product does not raise safety concerns. No viable cells and no DNA of this strain were detected in the samples analysed. No viable cells of *T. reesei* ATCC PTA-5588 were detected in the samples analysed. No viable cells [redacted] were detected in the [redacted] used in the manufacturing process to obtain the protease.

The additive is safe for the target species at the recommended use level (1,000 xylanase U, 100 amylase U and 2,000 protease U per kg feed), and the data would also allow conclusions at the efficacious dose in chickens for fattening (2,000 xylanase U, 200 amylase U and 4,000 protease U per kg feed).

The additive is safe for the consumers of food products obtained from animals receiving the additive.

[25] 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.

The Panel cannot conclude on the skin/eye irritancy potential of the additive or on its dermal sensitisation potential. Owing to the proteinaceous nature of the active substances the additive is considered a respiratory sensitiser.

The additive does not raise safety concerns for the environment.

The additive is efficacious in chickens for fattening, chickens reared for laying and minor poultry species up to the point of lay at the level of 2,000 xylanase U, 200 amylase U and 4,000 protease U per kg feed (double the minimum recommended use level). Owing to the lack of sufficient data, the Panel cannot conclude on the efficacy of the additive for laying hens.

5. Documentation provided to EFSA/chronology

Date	Event
27/08/2020	Dossier received by EFSA. AXTRA XAP for poultry species. Submitted by Danisco UK Ltd.
15/09/2020	Reception mandate from the European Commission
02/10/2020	Application validated by EFSA – Start of the scientific assessment
08/01/2021	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*
08/10/2021	Reception of supplementary information from the applicant – Scientific assessment re-started
21/12/2021	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*
21/01/2022	Reception of supplementary information from the applicant - Scientific assessment re-started
04/03/2022	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*
02/09/2022	Reception of supplementary information from the applicant – Scientific assessment re-started
06/01/2023	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

References

EFSA (European Food Safety Authority), 2007. Opinion of the Scientific Committee on a request from EFSA on the introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. EFSA Journal 2007;5(12):587, 85 pp. https://doi.org/10.2903/j.efsa.2007.587

EFSA (European Food Safety Authority), 2009.Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) and of the Panel on Genetically Modified Organisms (GMO) on the efficacy of Avizyme 1505 (endo-1,4-beta-xylanase, alpha-amylase, protease) as a feed additive for chickens and ducks for fattening. EFSA Journal 2009;7(7):1156, 25 pp. https://doi.org/10.2903/j.efsa.2009.1156

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Hilbert F, Lindqvist R, Nauta M, Peixe L, Ru G, Simmons M, Skandamis P, Suffredini E, Cocconcelli PS, Fernández Escámez PS, Maradona MP, Querol A, Suarez JE, Sundh I, Vlak J, Barizzone F, Correia S and Herman L, 2020. Scientific Opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA (2017–2019). EFSA Journal 2020;18(2):5966, 56 pp. https://doi.org/10.2903/j.efsa.2020.5966

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539, 5 pp. https://doi.org/10.2903/j.efsa.2012.2539

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Dujardin B, Galobart J and Innocenti ML, 2017a. Guidance on the assessment of the safety of feed additives for the consumer. EFSA Journal 2017;15(10):5022, 17 pp. https://doi.org/10.2903/j.efsa.2017.5022

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J and Innocenti ML, 2017b. Guidance on the identity, characterisation and conditions of use of feed additives. EFSA Journal 2017;15(10):5023, 12 pp. https://doi.org/10.2903/j.efsa.2017.5023

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J, Innocenti ML and Martino L, 2017c. Guidance on the assessment of the safety of feed additives for the target species. EFSA Journal 2017;15(10):5021, 19 pp. https://doi.org/10.2903/j.efsa.2017.5021

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J, Innocenti ML and Martino L, 2018a. Guidance on the assessment of the efficacy of feed additives. EFSA Journal 2018;16(5):5274, 25 pp. https://doi.org/10.2903/j.efsa.2018.5274

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Glandorf B, Herman L, Kärenlampi S, Aguilera J, Anguita M, Brozzi R and Galobart J, 2018b. Guidance on the characterisation of microorganisms used as feed additives or as production organisms. EFSA Journal 2018;16(3):5206, 24 pp. https://doi.org/10.2903/j.efsa.2018.5206

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Bastos M, Christensen H, Dusemund B, Kouba M, Kos Durjava M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Brock T, de Knecht J, Kolar B, van Beelen P, Padovani L, Tarres-Call J, Vettori MV and Azimonti G, 2019. Guidance on the assessment of the safety of feed additives for the environment. EFSA Journal 2019;17(4):5648, 78 pp. https://doi.org/10.2903/j.efsa.2019.5648

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Kos Durjava M, Kouba M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechovà A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Cocconcelli PS, Dierick NA, Glandorf B, Herman L, Maradona MP, Martelli G, Tosti L, Saarela M, Svensson K, Galobart J, Pettenati E, Pizzo F and Anguita M, 2020. Scientific Opinion on the safety andefficacy of Axtra®XAP 104 TPT (endo-1,4-xylanase, protease and alpha-amylase) as a feed additive for chickens for fattening, laying hens and minor poultry species. EFSA Journal 2020;18(6):6165, 24 pp. https://doi.org/10.2903/j.efsa.2020.6165

Abbreviations

AMR	antimicrobial resistance
CFU	colony forming unit
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
MIC	minimum inhibitory concentration
QPS	qualified presumption of safety
TOS	total organic solids
WGS	whole genome sequence