REGULATED PRODUCTS SAFETY ASSESSMENT

Assessment 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 for All Growing Poultry Species (Genencor International B.V.) (RP2115)

Food Standards Agency[1], Food Standards Scotland[2]

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FSA Research and Evidence

An application was submitted to the Food Standards Agency and Food Standards Scotland in August 2023 from Genencor International B.V. ("the applicant") for the new authorisation of an 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), under the category 'zootechnical feed additive', functional group 'digestibility enhancer', for its use in all growing poultry species.

In 2020, the European Food Safety Authority (EFSA) could not conclude on the safety of the additive due to: uncertainty on the presence of viable cells of the genetically modified strain *Trichoderma reesei* ATCC PTA-5588; uncertainty regarding the manufacturing process of the protease; and uncertainty regarding the test item used in the toxicological studies of the xylanase, which prevented the FEEDAP Panel reaching a conclusion on the safety, including the endpoint of genotoxicity of the xylanase component of the additive. Additionally, the Panel could not conclude on the efficacy of the additive as a digestibility enhancer in laying hens due to an insufficient number of studies.

The EFSA 2023 opinion states that new data was submitted by the applicant to address some of the limitations previously identified. In addition, a change of the production strain *B. subtilis* ATCC SD-2107 to *B.*

subtilis CBS 148232 was declared by the applicant. The EFSA concluded that at the recommended use level (1,000 xylanase units (U), 100 amylase U and 2,000 protease U per kg feed), the additive is safe for the target species. The additive is also safe for the consumers and environment. However, no conclusions were reached on the eye and skin irritancy potential of the additive and its skin sensitisation potential. The additive is considered a respiratory sensitiser due to the proteinaceous nature of the active substances. 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), the additive is efficacious in chickens for fattening, chickens reared for laying and minor poultry species up to the point of lay. However, the Panel could not conclude on the efficacy of the additive for laying hens due to lack of data.

The FSA and FSS noted that EFSA 2020 and 2023 opinion assessed the safety and the efficacy of the additive for chickens for fattening, laying hens and minor poultry species. However, in the application submitted to FSA and FSS, the applicant requested an authorisation of the additive for its use in all growing poultry species. The FSA and FSS considers sufficient evidence has been provided to extrapolate conclusions to all growing poultry species.

FSA/FSS has reviewed the applicant's authorisation application, supporting documentation, and other regulators' risk assessments, most notably the EFSA risk assessment opinion, and considers sufficient evidence has been demonstrated to conclude without the need for further questions or risk assessment.

This is a joint FSA and FSS publication.

1. Introduction

The FSA and FSS have undertaken an assessment of a feed additive submitted by Genencor International B.V. (Willem Einthovenstraat 4, Oegstgeest, 2342 BH, Netherlands) 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) under Assimilated Regulation (EC) No 1831/2003 (EC, 2003) in each nation of Great Britain (GB). The applicant seeks new authorisation under the category 'zootechnical feed additive', functional group 'digestibility enhancer' for its use in all growing poultry species.

In line with Article 8 of Assimilated Regulation No 1831/2003 (EC, 2003), the assessment has considered and concluded whether the feed additive complies with the conditions laid down in Article 5, including: safety considerations for human, animal and environmental health; efficacy of the additive for its intended effect; potential impairment of the distinctive features of animal products. This, and the guidance put in place by the European Food Safety Authority (EFSA) for the evaluation of feed additive applications, has formed the basis and structure for the assessment.

To ensure the regulatory systems of FSA/FSS are risk proportionate, and resources are used effectively, FSA and FSS have used the evidence submitted by the applicant and other information in the public domain, including the EFSA risk assessment opinion, to provide a summary assessment of the evidence of safety presented in this report.

In 2020, the EFSA (EFSA, 2020) could not conclude on the safety of the additive of Axtra® XAP 104 TPT consisting of endo-1,4-beta-xylanase produced by *Trichoderma reesei* ATCC PTA-5588, protease produced by *Bacillus subtilis* ATCC SD-2107 and alpha-amylase produced by *Bacillus licheniformis* ATCC SD-6525. Additionally, the Panel could not conclude on the efficacy in laying hens due to lack of data. In 2023, EFSA published a risk assessment opinion (EFSA, 2023) 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, addressing some of the previous limitations.

Both EFSA opinions (EFSA, 2020, EFSA, 2023) have been reviewed by FSA/ FSS risk assessors. It has been verified that the standard approach taken, when compared to the relevant guidance applied in GB, has been followed and the conclusions made are consistent with the data summarised in the opinion. The FSA and FSS noted that EFSA 2020 and 2023 opinions assessed the safety and the efficacy of the additive for chickens for fattening, laying hens and minor poultry species. However, in the application submitted to FSA and FSS, the applicant requested an authorisation of the additive for its use in all growing poultry species. The FSA and FSS considers sufficient evidence have been provided to extrapolate conclusions to all growing poultry species.

The result of the assessment is that there is sufficient evidence of safety and efficacy for the UK to conclude this assessment at this time. This assessment represents the opinion of the FSA and FSS.

Table 1. Table showing products included in this assessment.

Title	Product type	Intended use/s	Intended dose/intake
Axtra® XAP 104 TPT	Feed additive	Zootechnical additive / digestibility enhancer	A minimum level of 1,000 xylanase U, 100 amylase U and 2,000 protease U per kg feed for all growing poultry species

2. Assessment

2.1. Details of other regulators opinions

2.1.1. Current authorisation

Under Commission Implementing Regulation (EU) No 2023/1713 (EC, 2023), following the EFSA 2023 opinion, the additive is authorised in the European Union (EU) for use in feed for chickens for fattening, chickens reared for laying, minor poultry species for fattening and reared for laying. The additive has not been authorised as feed additive in Great Britain (GB).

The use of a preparation of endo-1,4-beta-xylanase produced by *Trichoderma reesei* ATCC PTA-5588 was authorised as a feed additive for use in chickens for fattening, laying hens, ducks and turkeys for fattening by Assimilated Regulation (EU) No 9/2010 (EC, 2010), for weaned piglets and pigs for fattening by Assimilated Regulation (EU) No 528/2011 (EC, 2011) and for minor poultry species other than ducks by Assimilated Regulation (EU) No 1021/2012 (EC, 2012a). The minimum content of a

preparation of endo-1,4-beta-xylanase produced by *Trichoderma reesei* ATCC PTA-5588 as a feed additive for laying hens was amended by Assimilated Regulation (EU) No 1196/2012 (EC, 2012b).

2.1.2. Other regulators opinions

In 2020, the FEEDAP Panel assessed the safety and efficacy of Axtra® XAP 104 TPT consisting of endo-1,4-beta-xylanase, protease and alpha-amylase produced by three different genetically modified strains as a zootechnical feed additive (digestibility enhancers) for chickens for fattening, laying hens and minor poultry species. Neither recombinant DNA nor viable cells of the strains *Bacillus subtilis* ATCC SD-2107 and *Bacillus licheniformis* ATCC SD-6525 were detected in the final product. Uncertainty remained on the presence of viable cells of *Trichoderma reesei* ATCC PTA-5588 in the additive due to insufficient data.

No safety concerns resulting from the fermentation products used in the formulation/manufacturing were indicated from genotoxicity and sub-chronic oral toxicity studies performed with the three fermentation products. The FEEDAP Panel could not conclude on the toxicological potential of AXTRA® XAP 104 TPT due to uncertainties on the suitability of the test item used in the studies conducted with the xylanase. Consequently, no conclusions were reached on the safety of the additive for the target species, consumers and users. Additionally, due to an uncertainty on the presence of viable cells of *Trichoderma reesei* ATCC PTA-5588 in the additive, the FEEDAP Panel could not conclude on the safety for the environment.

The FEEDAP 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 following levels: 200 units (U) of amylase; 4,000 U of protease and 2,000 U of xylanase per kg of feed. The Panel could not conclude on the efficacy of the additive for laying hens due to lack of data.

2.1.3. Methodology applied in the EFSA opinion

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) assessed the safety and the efficacy of active substances, in accordance with guidance documents:

- Guidance on the identity, characterisation and conditions of use of feed additives (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);
- Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017a);
- Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b);
- 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 environment (EFSA FEEDAP Panel, 2019);

and principles in Assimilated Regulation (EC) No 429/2008.

These guidance documents were developed and implemented prior to the UK's exit from the EU and were also adopted by the FSA and FSS on exit.

2.2. Section II: Identity, characterisation and condition of use

2.2.1. Characterisation of the production microorganism

No modification of the production strains *T. reesei* ATCC PTA-5588 (endo-1,4-beta-xylanase) or *B. licheniformis* ATCC SD-6525 (alpha-amylase) were declared by the applicant. However, the change in the protease production strain from *B. subtilis* ATCC SD-2107, described in the previous opinion (EFSA, 2020), to *B. subtilis* C BS 148232 was declared. Therefore, the new production strain requires full characterisation.

2.2.1.1. *Bacillus subtilis* CBS 148232 – protease production strain

The *Bacillus subtilis* strain is a genetically modified strain, deposited with the accession number CBS 148232 in the Westerdijk Fungal Biodiversity Institute. The taxonomic identification of an intermediate strain was performed confirming its identification as *B. subtilis.* However, the guidance recommends providing taxonomic identification of the actual production strain. To comply with this requirement, the applicant submitted an analysis which supports the identification of the production strain as *B. subtilis.* In addition, to characterise the intended genetic modification, genome analysis of the production strain was also used.

A broth microdilution method was used to test the production strain for its susceptibility to the antimicrobials listed in the Guidance on the characterisation of microorganisms used as feed additives or as production organisms for *Bacillus* (EFSA FEEDAP Panel, 2018b). The FEEDAP Panel concluded that the production strain is resistant to one of the tested antimicrobials, but WGS analysis revealed no genes of concern.

The toxigenic potential of *B. subtilis* CBS 148232 was assessed in accordance with the guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b). *B. subtilis* CBS 148232 was considered to be non-toxigenic as no lysis of Vero cells was detected.

2.2.1.1.1. Description of the genetic modification

The applicant provided a description of the genetic modification, which was assessed by the FEEDAP Panel in 2023. This description is sufficient for the FSA and FSS.

2.2.1.2. *Trichoderma reesei* ATCC PTA-5588 – xylanase production strain

Note: The following information was described in the 2020 EFSA opinion (EFSA, 2020).

The taxonomic identification of the recipient strain was confirmed as *T. reesei* by analysis (EFSA, 2020). It was noted by the FEEDAP Panel that for the purposes of the assessment, the taxonomic identification of the *T. reesei* ATCC PTA-5588 strain would be preferred. The production stain was previously assessed, and its genetic modification was fully described (EFSA, 2007). Since the original assessment, no further genetic modification was carried out.

The recipient strain was tested for its ability to produce mycotoxins, with no trichothecenes (trichodermin, trichodermol and harzianum A) or gliotoxin being detected in the supernatant of culture of the recipient strain. However, it was noted that the test with the production strain would have been preferred.

2.2.1.3. *Bacillus licheniformis* ATCC SD-6525 – alpha-amylase production strain

Note: The following information was described in the 2020 EFSA opinion (EFSA, 2020).

The taxonomic identification of the recipient strain as *B. licheniformis* was confirmed by analysis. However, the analysis was not conducted on the production strain (ATCC SD-6525). Taxonomic identification performed with the production strain *B. licheniformis* ATCC SD-6525 would be preferred for the purpose of the assessment.

The resistance of the production strain to a relevant antimicrobial was reported and its susceptibility to other antimicrobials was not tested. Therefore, uncertainty remains on the susceptibility of the production strain to relevant antimicrobials. The search of the whole genome sequence (WGS) of the production strain *B. licheniformis* ATCC SD-6525 for the presence of antimicrobial resistance (AMR) genes was briefly described by the applicant. Matches to proteins known to be associated with any antimicrobial resistance were not identified apart from one enzyme gene introduced in ATCC SD-6525.

The assessment of toxigenic potential of the *B. licheniformis* ATCC SD-6525 was carried out in accordance with the Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b). *B. licheniformis* ATCC SD-6525 was not considered to be toxigenic.

2.2.1.3.1. Characteristics of the recipient microorganism and the introduced sequences

The applicant provided characterisation of the recipient microorganism, characteristics of the introduced sequence and description of the genetic modification, which were assessed by the FEEDAP Panel in 2020. The submitted information was considered sufficient by the FSA and FSS.

2.2.2. Manufacturing process

The manufacturing process of the endo-1,4-beta-xylanase and the alpha-amylase was previously assessed, and no modification was declared (EFSA, 2020). A modification of the protease manufacturing process was described and submitted information was considered sufficient by the FSA and FSS.

2.2.2.1. Manufacturing process of the enzymes

Note: The following information was described in the 2020 EFSA opinion (EFSA, 2020).

The xylanase, amylase and protease used in the additive Axtra® XAP 104 TPT are produced separately with the corresponding production strain by a fermentation process. The applicant provided a description of the manufacturing process, which was assessed by the FEEDAP Panel in 2020.

2.2.2.2. Manufacturing process of the protease

The applicant provided a description of the manufacturing process of the protease, which was assessed by the FEEDAP Panel in 2023. The submitted information was considered sufficient by the FSA and FSS.

The applicant declared a change in the production strain to *B. subtilis* CBS 148232 and manufacturing process of the protease compared to the previous assessment (EFSA FEEDAP Panel, 2020). No other modifications were declared.

2.2.3. Characterisation of the additive

The composition of the final formulation of the additive has remained unchanged since the previous evaluation (EFSA, 2020).

New data on the final additive and on the intermediate protease product were submitted by the applicant. Most of the data evaluated in the current assessment were obtained with the new production strain, but prior to the change in manufacturing process. However, a major impact of this change on any of the parameters reported below was not expected and therefore the provided data was still considered relevant.

The additive is solid with the minimum guaranteed activity of 20,000 xylanase units (U), 2,000 amylase U and 40,000 protease U per gram of product. Analysis of three batches confirmed compliance with the predefined specification, showing values from 28,139 to 29,619 U of xylanase, 2,540 to 2,892 U of amylase, and 45,881 to 54,015 U of protease. In the previous EFSA opinion, the analysis of 5 batches showed compliance with predefined specifications (EFSA, 2020). Analysis of the same three batches showed levels of arsenic < 1.6 µg/kg, cadmium < 0.5 µg/kg, lead < 3.5 µg/kg, mercury < 2.3 µg/kg. In addition, levels of total aflatoxins were < 5 µg/kg, deoxynivalenol was < 50 µg/kg, fumonisin was < 100 µg/kg, ochratoxin was < 5 µg/kg and zearalenone was < 25 µg/kg. Furthermore, microbiological analysis was carried out showing total viable counts < 50 colony forming units (CFU)/g), coliforms < 10 CFU/g and *Salmonella* spp. was not detected in 25 g.

To test presence of antimicrobial activity, three batches of an intermediate product of the protease were tested. No antimicrobial activity was detected.

Three batches of the protease fermentation product that is used to formulate the final additive were investigated to determine the presence of viable cells and DNA of the newly declared protease production strain, *B. subtilis* CBS 148232. The limit of detection was 10 ng/mL. The samples of intermediate product showed no amplification, with positive controls performing as expected. Absence of DNA in the intermediate product

obtained after the change in manufacturing process was also confirmed by the data provided by the applicant. The limit of detection was 1 ng/mL. The samples of intermediate product showed no amplification, with positive controls performing as expected.

The FEEDAP Panel noted in the previous assessment an uncertainty regarding the presence of viable cells of *T. reesei* PTA-5588 (production strain of the endo-1,4-beta-xylanase), as well as an uncertainty regarding the manufacturing process of the protease (EFSA, 2020). Data addressing these uncertainties was submitted by the applicant. Regarding the presence of *T. reesei* PTA-5588, the samples of intermediate product showed no growth. New data submitted to address the uncertainty regarding the manufacturing process of the protease was considered sufficient.

The physio-chemical properties were described in the previous EFSA opinion (EFSA, 2020). Particle size distribution was determined in three batches by laser diffraction showing that 90% of particles were below 650 µm, 10% particles were below 400 µm and no particles were detected below 282 µm (mean particle size of 509 µm). The dusting potential determined in three batches by Stauber Heubach method ranged from 5 to 15 mg/m^3. A bulk density of product was 1,400 kg/m^3.

The stability and homogeneity of the feed additive were described in the previous EFSA opinion (EFSA, 2020). Mean recovery of xylanase, amylase and protease activity was of 99%, 83% and 95%, respectively, after 9 months at 25°C and 78%, 57% and 86%, respectively, after 3 months at 40°C. Mean recovery of xylanase, amylase and protease activity in a vitamin-mineral complete premixture for poultry (including choline chloride) was 99%, 102% and 97%, respectively, after 6 months at 25°C. Mean enzyme activity recovery of xylanase, amylase, protease in a complete feed (mash form) was 77%, 48% and 104%, respectively, after 3 months at 25°C. Mean enzyme activity recovery of xylanase, amylase and protease after pelleting at 95°C was 84%, 101% and 102%, respectively and was 85%, 73% and 95%, respectively, after 3 months of storage at 25°C. The coefficient of variation based on analysis of 10 samples in mash feed was 7% for protease and 8% for both amylase and xylanase.

The FSA and FSS agree with the conclusions reached for the characterisation of the additive and active agent. Part of the data was reviewed by EFSA in 2020, prior to the UK's exit from the EU; thus, this opinion is applicable to GB. The certificates of analysis were reviewed by the FSA and FSS and confirmed compliance with the specifications.

2.2.2. Conditions of use

In the EU, Axtra® XAP 104 TPT is proposed to be used 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 conditions of use have not been modified since the previous submission (EFSA, 2020).

In the application submitted to the FSA and FSS, the applicant proposed use of the additive in all growing poultry species at the same minimum levels as EFSA evaluated in their latest opinion (EFSA, 2023).

2.2.3. Conclusion on Section II: Identity, characterisation and conditions of use

The FEEDAP Panel concluded in the 2023 EFSA opinion that the change of protease production strain to *B. subtilis* CBS 148232 does not raise safety concerns. No viable cells and no DNA of *B. subtilis* CBS 148232 were detected in the analysed samples of protease fermentation product that is used to formulate the final additive. No viable cells of the endo-1,4-beta-xylanase production strain *T. reesei* ATCC PTA-5588 were detected in the analysed samples of the liquid intermediate product. The FEEDAP Panel previously concluded that no viable cells and no DNA of the *B. licheniformis* ATCC SD 6525 were detected in the final additive (EFSA, 2020).

The FEEDAP Panel concluded that the amounts of the detected impurities, as well as microbial contamination, do not raise safety concerns in both 2020 and 2023, opinions.

FSA and FSS noted the extensive information on identity and characterisation and agree with the conclusions reached for the characterisation of the additive and active agent, and that the change of protease production strain to *B. subtilis* CBS 148232 does not raise safety concerns. The FSA/FSS agree with the conditions of use proposed by the applicant.

2.3. Section III: Safety

2.3.3. Safety of the production strain

The applicant declared that the production strains *T. reesei* ATCC PTA-5588 (endo-1,4-beta-xylanase) and *B. licheniformis* ATCC SD-6525 (alpha-amylase) remained same as in the previous assessment (EFSA, 2020).

The FEEDAP Panel previously concluded that the genetic modification of *T. reesei* ATCC PTA-5588 does not raise any safety concerns (EFSA, 2020). The data provided in the current assessment addressed uncertainty regarding the presence of viable cells in the product showing that no viable cells of

T. reesei ATCC PTA-5588 were detected in the intermediate product used to formulate the additive. The FEEDAP Panel previously concluded that use of *B. licheniformis* ATCC SD-6525 as a production strain does not raise safety concerns (EFSA, 2020). Consequently, the FEEDAP Panel considered the previously reached conclusions regarding the safety of *T. reesei* ATCC PTA-5588 and *B. licheniformis* ATCC SD-6525 when used as production strains remain applicable for this assessment.

The applicant has declared a change of protease production strain to *B. subtilis* CBS 148232. *B. subtilis* CBS 148232 is a species suitable for the Qualified Presumption of Safety (QPS) approach to safety assessment. The use of QPS requires conclusively establishing the identity of the strain and safety of the genetic modification, as well as evidence that the strain is lacking toxigenic potential and acquired resistance to antibiotics of human and veterinary importance (EFSA BIOHAZ Panel, 2020; EFSA, 2007). The identity of *B. subtilis* CBS 148232 strain has been conclusively established at the species level, and it was confirmed that the strain lacks the potential to be toxigenic. The strain is resistant to one assessed antimicrobial. However, no genes of concern were indicated by the analysis of the WGS. No viable cells and recombinant DNA of the strain were detected in the intermediate product containing the protease; the genetic modification also did not raise any concerns. Therefore, the use of *B. subtilis* CBS 148232 as a production strain of the protease does not raise safety concerns.

The FSA and FSS agree with the conclusions reached for the safety of the production strains and that change of protease production strain to *B. subtilis* CBS 148232 does not raise any safety concerns. Part of the data was reviewed by EFSA in 2020, prior to the UK's exit from the EU; thus, this opinion is applicable to GB. The FSA and FSS agree that the additional information address the uncertainties identified in the 2020 EFSA opinion.

2.3.4. Toxicological studies

No modifications have been declared for the fermentation product produced by *B. licheniformis* ATCC SD-6525. The FEEDAP Panel considered the previous conclusion (EFSA, 2020) regarding the safety of the strain, which was supported by the submitted toxicological data, applicable to current assessment, and therefore the strain is presumed safe.

The FEEDAP Panel did not require any toxicological data for *B. subtilis* CBS 148232 as they concluded that the strain met the criteria for QPS and can be presumed safe from the toxicological point of view (EFSA, 2023).

To support the safety of the fermentation product produced by *T. reesei* (ATCC PTA-5588), toxicological data were required. A bacterial reverse mutation assay, an *in vitro* chromosomal aberration test (which addressed

structural and numerical aberrations) and a subchronic oral toxicity study were evaluated in the previous assessment and raised no safety concerns (EFSA, 2020). However, differences were noted between the test item used in these studies and the xylanase fermentation product described in characterisation section. As a result, 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".

The newly submitted information regarding the discrepancy between the test item used in these studies and the xylanase fermentation product described in the characterisation section allowed the FEEDAP panel to consider the test item used in the toxicological studies as suitable for the safety assessment of the of the additive. The xylanase fermentation product showed no genotoxic potential, and the subchronic oral toxicity study identified no toxicological concerns.

The FSA and FSS agree with the conclusions reached for toxicological studies. Part of the data was reviewed by EFSA in 2020, prior to the UK's exit from the EU; thus, this opinion is applicable to GB. The FSA and FSS agree that the additional information addresses the uncertainties identified in the 2020 EFSA opinion.

2.3.5. Safety for the target species

The tolerance studies in chickens for fattening and laying hens showed that feeding the animals with the additive up to 30 times the minimum recommended dose (1,000 xylanase U, 100 amylase U and 2,000 protease U per kg feed), did not result in any adverse effects (EFSA, 2020). Based on the results of these studies, the FEEDAP Panel concluded that Axtra® XAP 104 TPT was safe for chickens for fattening and laying hens under the proposed conditions of use, and this conclusion can be extended to chickens reared for laying. In addition, the FEEDAP Panel extrapolated the conclusion to minor poultry species for fattening or laying, considering the wide margin of safety shown. However, the Panel could not conclude on the safety of the additive for the target species due to the uncertainty regarding the test item used in the toxicological studies for the xylanase produced by *T. reesei* ATCC PTA-5588 (EFSA, 2020).

In the current assessment, no uncertainty remains on the toxicological data previously submitted as it was addressed by newly submitted data. In addition, 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 according to the FEEDAP Panel.

Therefore, the FEEDAP Panel reiterated the previous conclusion regarding the safety for target species trials, concluding that the additive is safe for chickens for fattening and laying hens under the proposed conditions of use. Furthermore, the FEEDAP Panel concluded that these conclusions can be extended to chickens reared for laying and extrapolated to minor poultry species for fattening or laying.

The FSA and FSS agree with the conclusions on the safety for chickens for fattening and the use of the extrapolation for minor poultry species for fattening or laying, which is supported by the guidance that is also applicable in GB. In addition, the FSA and FSS considers sufficient evidence have been provided to extrapolate conclusions to all growing poultry species. Furthermore, the use of the new strain *B. subtilis* CBS 148232 would not have an impact on the safety of the additive for target species.

2.3.6. Safety for the consumer

Due to the uncertainties regarding the test item used in the toxicological studies for the xylanase produced by *T. reesei* ATCC PTA-5588, the FEEDAP Panel could not previously conclude on the safety for the consumer of the additive in 2020 (EFSA, 2020). These uncertainties were addressed in the latest assessment with the newly submitted data, and therefore the FEEDAP Panel concluded 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 (EFSA, 2023). In addition, the amylase and the protease used in the additive raised no safety concerns for the consumers as they are produced by strains *B. licheniformis* ATCC SD-6525 and *B. subtilis* CBS 148232, which qualify for the QPS approach to safety assessment. Therefore, the FEEDAP Panel concluded that AXTRA® XAP 104 TPT used as a feed additive for poultry is safe for the consumer.

The FSA and FSS agree with the conclusions reached for the safety for the consumer that AXTRA® XAP 104 TPT used as a feed additive for poultry is safe for the consumer. Part of the data was reviewed by EFSA in 2020, prior to the UK's exit from the EU; thus, this opinion remains applicable to GB. The FSA and FSS agree that the additional information address the uncertainties identified in the 2020 EFSA opinion.

2.3.7. Safety for the user

The uncertainties regarding the test item used in the toxicological studies for the xylanase produced by *T. reesei* ATCC PTA-5588 were addressed in the latest assessment with the newly submitted data, and therefore no uncertainty remained on the toxicological data previously submitted for the xylanase product.

Due to lack of specific studies submitted in 2020 to address the safety for the user, the FEEDAP Panel could not conclude on the potential of the additive to be irritant to eyes or skin, or its potential to be a skin sensitiser. In addition, the Panel concluded that the additive is considered a respiratory sensitiser. As no new studies have been submitted regarding the safety for the user, the FEEDAP Panel reiterated the previous conclusions that the Panel cannot conclude on the potential of the additive to be eye or skin irritant, or its potential to be a skin sensitiser. The additive is considered a respiratory sensitiser due to the proteinaceous nature of active substances.

FSA and FSS agree with the conclusions reached on the safety for the user as no specific studies have been submitted, and therefore no conclusion can be reached on the potential of the additive to be an irritant to eyes or skin, or on its skin sensitising properties. The additive is considered to be a respiratory sensitiser.

2.3.2. Safety for the environment

In the 2020 opinion, it was stated that no viable cells and no recombinant DNA of the genetically modified strains of *B. licheniformis* ATCC SD 6525 and *B. subtilis* ATCC SD 2107 were detected in the final product (EFSA, 2020). However, The FEEDAP Panel could not conclude on the safety for the environment, due to an uncertainty regarding the presence of viable cells of the genetically modified *T. reesei* ATCC PTA-5588 in the final additive. Data indicating that no viable cells were detected in the intermediate product used to formulate the additive were provided by the applicant in the current assessment. In addition, data was provided by the applicant showing that no DNA or viable cells of *B. subtilis* CBS 148232 were detected. Therefore, the FEEDAP Panel concluded that no risks for the environment are expected from the use of the additive.

The FSA and FSS agree with the conclusions reached for the safety of the environment. Some of the data was reviewed by EFSA in 2020, prior to the UK's exit from the EU; thus, this opinion remains applicable to GB. The additional data provided addressed the uncertainties from the previous assessment.

2.3.3. Conclusion on Section III: Safety

The FEEDAP 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). In addition, the Panel concluded that the additive is safe for the consumers of food products obtained from animals receiving the additive at recommended levels, and is safe for the environment. The FEEDAP Panel could not conclude on the potential of the additive being eye

or skin irritant, or on its potential to be a skin sensitiser. The additive is considered a respiratory sensitiser due to the proteinaceous nature of the active substances.

The FSA and FSS agree with the conclusions reached on the safety of the additive for target species, the consumer and the environment. In addition, the FSA and FSS considers sufficient evidence has been provided to extrapolate conclusions to all growing poultry species. The FSA and FSS agree with the conclusions reached on the safety for the user as no specific studies have been submitted and therefore no conclusion can be reached on the potential of the additive to be an irritant to eyes or skin, or on its skin sensitising properties. The additive is considered to be a respiratory sensitiser.

2.4. Section IV: Efficacy as a digestibility enhancer

2.4.1. Efficacy for chickens for fattening

Five efficacy trials were submitted and evaluated during the previous assessment in 2020 (EFSA, 2020). However, two of those studies were not further considered due to a low performance of the birds in one case (30% below the performance objectives) and the high mortality registered in the other case.

One-day-old male Ross 308 birds, kept under study for at least 35 days, were used in the 3 remaining trials considered for the assessment. The birds received either a diet containing the additive providing the minimum recommended level of 1,000 U xylanase, 100 U amylase and 2,000 U protease per kg feed or a non-supplemented diet (control). Study 2 and 3 included additional treatment with double the recommended dose. Throughout the study, health and mortality were monitored, and body weight and feed intake were recorded. The data was analysed using an analysis of variance (ANOVA) (pen basis) and group means were compared with Tukey (trial 2) or Duncan (trial 3) tests. The details of the study designs are summarised in Table 2 and the results in Table 3.

Table 2. Experimental design of the efficacy trials performed in chickens for fattening.

	Trial 1	Trial 2	Trial 3
Total no of animals	480	1,056	1,500
Animals x replicate	20	22	50
Replicates x treatment	12	16	10
Breed and sex	Ross 308, Male	Ross 308, Male	Ross 308, Male
Duration (days)	35	42	42
Composition and form of feed	Maize, wheat, soya bean meal	Maize, soya bean, wheat middling	Maize, soya bean, wheat middling

		Trial 1	Trial 2	Trial 3
		(pelleted)	(mash)	(mash)
Enzyme activity (xylanase/ amylase/ protease, U/kg feed)	Intended	0/0/0 1,000/100/2,000	0/0/0 1,000/100/2,000 2,000/200/4,000	0/0/0 1,000/100/2,000 2,000/200/4,000
	Analysed	-/-/- 1,135/103/2,116	-/-/- 1,105/144/2,436 2,427/216/6,000	-/-/- 1,154/126/2,688 2,092/269/5,163

Low mortality was reported with no significant difference between the groups. An improvement in the final body weight (trial 3) and the feed gain ratio (trial 1) was observed in a group that received the additive at the minimum recommended level compared to control treatment. Improvements in the final body weight and the feed to gain ratio were observed in trial 2 in birds receiving the additive at double the minimum recommended dose. In conclusion, the studies showed that the additive has the potential to be efficacious in chickens for fattening at the level of 2,000 xylanase U, 200 amylase U and 4,000 protease U per kg feed.

Table 3. Effects of Axtra® XAP 104 TPT on the performance and mortality of chickens for fattening.

Trial	Enzyme activities xylanase/ amylase/protease (U/kg feed)	Feed intake (g)(1)	Final body weight (g)	Feed to gain ratio	Mortality and culling (%)
1	0/0/0	3644	2408	1.54^{a}	2.5
	1000/100/2000	3639	2438	1.52^{b}	3.6
2	0/0/0	93.3	2516^{b}	1.58^{a}	3.1
	1000/100/2000	95.1	$2581^{a,b}$	$1.57^{a,b}$	4.5
	2000/200/4000	94.4	2599^{a}	1.55^{b}	3.4
3	0/0/0	101	2748^{b}	1.57	1.4
	1000/100/2000	103	2852^{a}	1.55	1.0
	2000/200/4000	102	2830^{a}	1.56	2.0

^{a,b}: Mean values within a trial and within a column with a different superscript are significantly different $p < 0.05$.
(1): Total feed intake for trial 1 and daily feed intake for trials 2 and 3.

As the composition and the conditions of use of the additive remained unchanged, the FEEDAP Panel reiterated its previous conclusions that the additive is efficacious in chickens for fattening, chickens reared for laying and minor poultry species at the level of 2,000 xylanase U, 200 amylase U and 4,000 protease U per kg feed (double the minimum recommended dose) up to the point of lay. In addition, use of the new protease production strain, *B. subtilis* CBS 148232, has no impact on the conclusions drawn previously for efficacy in growing poultry.

2.4.3. Conclusions on efficacy

The FEEDAP Panel concluded that Axtra® XAP 104 TPT is efficacious in chickens for fattening at the level of 2,000 U xylanase, 200 U amylase and 4,000 U protease per kg feed (double the minimum recommended dose) and this conclusion is extended to chickens reared for laying. The conclusion was extrapolated to minor poultry species for fattening or reared for laying/breeding due to well-known mode of action of the enzymes. Due to lack of independent studies, the FEEDAP Panel cannot conclude on the efficacy of the product in laying hens or in other poultry species for laying.

These conclusions were made by EFSA in 2020 and were applicable to the UK. The FSA and FSS agree with the conclusions reached on the efficacy for chickens for fattening and minor poultry species. In addition, the FSA and FSS considers sufficient evidence has been provided to extrapolate conclusions to all growing poultry species. This demonstrates that Axtra® XAP 104 TPT favourably affects animal production, performance or welfare, particularly by affecting the digestibility of the feedingstuffs.

3. Analytical method evaluation

The FSA/FSS evaluated the EURL analytical method evaluation, noting it was carried out in 2017, when the UK was still part of the EU and would have participated of their approval. No concerns are raised at this stage for the validity of the methods for UK/GB use, and therefore the FSA/FSS accept the EURL analytical method evaluation report (EURL, 2017). The FSA/FSS determined the analytical method as appropriate for official controls for this feed additive.

4. Conclusions

The FEEDAP Panel concluded that the change of the protease production strain to *B. subtilis* CBS 148232 does not raise safety concerns. No viable cells and no DNA of this strain were detected in the analysed samples. In addition, no viable cells of *T. reesei* ATCC PTA-5588 were detected in the analysed samples. 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), as well as at the efficacious dose of 2,000 xylanase U, 200 amylase U and 4,000 protease U per kg feed in chickens for fattening. The additive is safe for the consumers and the environment. The FEEDAP Panel could not conclude on the potential of the additive to be eye or skin irritant, or on its potential to be skin sensitiser. The additive is considered a respiratory sensitiser due to the proteinaceous nature of the active substances.

The FEEDAP Panel concluded that the additive is efficacious in chickens for fattening, chickens reared for laying and minor poultry species for fattening or reared for laying/breeding 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) up to the point of lay. The Panel could not conclude on the efficacy of the additive for laying hens due to lack of data.

5. Caveats and uncertainties

No conclusion can be drawn on potential of the additive to be eye or skin irritant, or on its potential to be skin sensitiser, due to lack of data.

No conclusion can be drawn on the efficacy of the additive for laying hens due to lack of data.

The additive was considered efficacious in chickens for fattening, chickens reared for laying and minor poultry species at the level that is a double of the minimum recommended use level.

The EFSA 2020 and 2023 opinions assessed the safety and the efficacy of the additive for chickens for fattening, laying hens and minor poultry species. However, in the application submitted to FSA and FSS, the applicant requested an authorisation of the additive for its use in all growing poultry species. The FSA and FSS considers sufficient evidence have been provided to extrapolate conclusions to all growing poultry species.

6. FSA/FSS conclusions for GB risk analysis

The application has been assessed in line with the applicable guidance and is partially based on considerations of detailed proprietary information available to the Panel, which were also submitted to the FSA and FSS. The EFSA opinions (EFSA, 2020, EFSA, 2023) identify and characterise the hazards present from the proposed use and conclude there is sufficient information to enable an assessment of exposure, which is also relevant to GB. The risk characterisation is unchanged from the 2020 opinion for most areas, and appropriate evidence was submitted to address the uncertainties from the 2020 assessment. The conclusions of the EFSA opinion have been reviewed in detail by the FSA and FSS, and are considered appropriate and consistent, including the caveats and uncertainties identified in the opinion which are applicable to GB. Sufficient evidence has been demonstrated to conclude without further questions or risk assessment.

Abbreviations

Abbreviation	Definition
AMR	Antimicrobial resistance
ANI	Average nucleotide identity
CFU	Colony forming units
EC	European Commission
EU	European Union
EFSA	European Food Safety Authority
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
FSA	Food Standards Agency
FSS	Food Standards Scotland
GB	Great Britain
MIC	Minimum inhibitory concentration
OECD	Organisation for Economic Co-operation and Development
QPS	Qualified Presumption of Safety
RP	Regulated product
U	Units
UK	United Kingdom
WGS	Whole genome sequence

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References

EC (European Commission). (2003). *Regulation No 1831/2003 of the European Parliament and of the Council on additives for use in animal nutrition*. https://www.legislation.gov.uk/eur/2003/1831/contents

EC (European Commission). (2010). *Commission Implementing Regulation No 9/2010 of the European Parliament and of the Council on additives for use in animal nutrition*. https://www.legislation.gov.uk/eur/2010/9/introduction

EC (European Commission). (2011). *Commission Implementing Regulation No 528/2011 of the European Parliament and of the Council on additives for use in animal nutrition*. https://www.legislation.gov.uk/eur/2011/528/contents

EC (European Commission). (2012a). *Commission Implementing Regulation No 1021/2012. of the European Parliament and of the Council on additives for use in animal nutrition*. https://www.legislation.gov.uk/eur/2012/1021/contents

EC (European Commission). (2012b). *Commission Implementing Regulation No 1196/2012 of the European Parliament and of the Council on additives for use in animal nutrition*. https://www.legislation.gov.uk/eur/2012/1196

EC (European Commission). (2023). *Regulation No 2023/1713 of the European Parliament and of the Council on additives for use in animal nutrition*. https://eur-lex.europa.eu/eli/reg_impl/2023/1713/oj

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards). (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*, *18*(2), 5966. https://doi.org/10.2903/j.efsa.2020.5966

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*, *5*(12), 587. https://doi.org/10.2903/j.efsa.2007.587

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*, *10*(1), 2539. https://doi.org/10.2903/j.efsa.2012.2539

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2017a). Guidance on the assessment of the safety of feed additives for the consumer. *EFSA Journal*, *15*(10), 5022. https://doi.org/10.2903/j.efsa.2017.5022

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2017b). Guidance on the assessment of the safety of feed additives for the target species. *EFSA Journal*, *15*(10), 5021. https://doi.org/10.2903/j.efsa.2017.5021

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2017c). Guidance on the identity, characterisation and conditions of use of feed additives. *EFSA Journal*, *15*(10), 5023. https://doi.org/10.2903/j.efsa.2017.5023

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2018a). Guidance on the assessment of the efficacy of feed additives. *EFSA Journal*, *16*(5), 5274. https://doi.org/10.2903/j.efsa.2018.5274

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2018b). Guidance on the characterisation of microorganisms used as feed additives or as production organisms. *EFSA Journal*, *16*(3), 5206. https://doi.org/10.2903/j.efsa.2018.5206

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2019). Guidance on the assessment of the safety of feed additives for the environment. *EFSA Journal*, *17*(4), 5648. https://doi.org/10.2903/j.efsa.2019.5648

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2020). Safety and efficacy 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*, *18*(6), 6165. https://doi.org/10.2903/j.efsa.2020.6165

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (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*, *21*(2), 7816. https://doi.org/10.2903/j.efsa.2023.7816

EURL-FA (European Reference Laboratory for Feed Additives). (2017). *CRL Evaluation Report on the Analytical Methods submitted in connection with the Application for Authorisation of Feed Additive according to Regulation (EC) No 1831/2003*. https://joint-research-centre.ec.europa.eu/system/files/2018-07/finrep-fad-2017-0053-axtra_xap_104_tpt.pdf