Assessment of the feed additive consisting of endo-1,4-β-xylanase produced by *Trichoderma reesei* CBS 143953 and endo-1,3(4)-β-glucanase produced by *T. reesei* CBS 143945 (Axtra® XB 201 TPT/L) for poultry and pigs for renewal of its authorisation (Danisco (UK) Ltd)

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 Fasmon 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, Jaume Galobart, Elisa Pettenati, Fabiola Pizzo, Joana Revez and Montserrat Anguita

**Abstract**

Following a request from the European Commission, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the renewal of the authorisation of endo-1,4-β-xylanase produced by *Trichoderma reesei* CBS 143953 and endo-1,3(4)-β-glucanase produced by *T. reesei* CBS 143945 (Axtra® XB 201 TPT/L) as a zootechnical feed additive (digestibility enhancers) for poultry and pigs. The endo-1,4-β-xylanase and endo-1,3(4)-β-glucanase are produced by a genetically modified strain of *T. reesei* and a non-genetically modified strain of *T. reesei*, respectively. The applicant also requested a reduction of the minimum recommended level for turkeys for fattening and the extension of use to other species/categories. The Panel concluded that the additive complies with the conditions of the current authorisation. There is no new evidence that would lead the FEEDAP Panel to reconsider previous conclusions on the safety of the additive. These conclusions apply also to the new target species/categories for which a request of use was made, suckling piglets. The Panel concluded that the additive should be considered irritant to eyes and a respiratory sensitiser. However, no conclusions could be drawn on the skin irritancy/sensitisation potential of the additive. No change in the authorisation conditions were requested for poultry species (other than turkeys), weaned piglets, pigs for fattening, sows and minor porcine species; therefore there was no need for an assessment on the efficacy of the additive for those species/categories. The Panel concluded that the additive has the potential to be efficacious in turkeys for fattening/reared for breeding and in suckling piglets (for the period in which solid feed is administered) at an intended level of 610 xylanase U/kg and 76 glucanase U/kg feed. However, the Panel noted that the actual effective level used in the studies supporting this conclusion was approximately 50% higher than the intended level.

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**Keywords:** zootechnical additives, digestibility enhancers, xylanase, glucanase, Axtra® XB 201 TPT and Axtra® XB 201 L, poultry, pigs

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003 establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 14(1) of that Regulation lays down that an application for renewal shall be sent to the Commission at the latest one year before the expiry date of the authorisation. Also, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7. Finally, Article 13(3) of that Regulation lays down that if the holder of an authorisation proposes changing the terms of the authorisation by submitting an application to the Commission, accompanied by the relevant data supporting the request for the change, the Authority shall transmit its opinion on the proposal to the Commission and the Member States.

The European Commission received a request from Danisco (UK) Ltd for a renewal of the authorisation of the product containing endo-1,4-β-xylanase produced by *Trichoderma reesei* CBS 143953 and endo-1,3(4)-β-glucanase produced by *T. reesei* CBS 143945 (Axtra® XB 201 TPT/L), when used as a feed additive for poultry and swine species (category: zootechnical additives; functional group: digestibility enhancers) as well as a modification in the terms of the authorisation and an extension of use to suckling piglets.

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 14(1) (renewal of the authorisation), under Article 13(3) (modification of the authorisation of a feed additive) and under Article 4(1) (authorisation of a feed additive or new use of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 8 September 2020.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the product containing endo-1,4-β-xylanase produced by *Trichoderma reesei* CBS 143953 and endo-1,3(4)-β-glucanase produced by *T. reesei* CBS 143945 (Axtra® XB 201 TPT/L), when used under the proposed conditions of use (see Section 3.1.2).

1.2. Additional information

The additive Axtra® XB 201 is a feed additive that contains endo-1,4-β-xylanase produced by a genetically modified strain of *T. reesei* (ATCC PTA 5588) and endo-1,3(4)-β-glucanase produced by a non-genetically modified strain of *T. reesei* (ATCC SD 2106). The additive is available in two forms, solid (TPT) and liquid (L).

The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) adopted a scientific opinion on the safety and efficacy of this product as a feed additive for poultry, piglets and pigs for fattening (EFSA FEEDAP Panel, 2010) and another scientific opinion on the safety and efficacy of the product as feed additive for lactating sows and minor porcine species (EFSA FEEDAP Panel, 2016).

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1 Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
2 Danisco (UK) Ltd, trading as Danisco Animal Nutrition and represented by Genencor International B.V., Archimedesweg 30, 2333 CN Leiden, The Netherlands.
3 In the authorisation of the additive 4a15 the production strain of endo-1,4-β-xylanase was deposited with the deposit number ATCC PTA-5588, while in the herein assessment it is CBS 143953 due to a change in the deposit.
4 In the authorisation of the additive 4a15, the strain of endo-1,3(4)-β-glucanase was deposited with the deposit number ATCC SD-2106, while in the herein assessment it is CBS 143945 due to a change in the deposit.
The additive (4a15) is authorised for its use in poultry, weaned piglets and pigs for fattening and for lactating sows and minor porcine species, minor porcine species weaned and for fattening, as a zootechnical additive, functional group of digestibility enhancers.

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 in support of the authorisation request for the use of Axtra® XB 201 TPT/L, endo-1,4-β-xylanase and endo-1,3(4)-β-glucanase, as a feed additive.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers and other scientific reports, to deliver the present output.

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

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Axtra® XB 201 TPT/L, endo-1,4-β-xylanase and endo-1,3(4)-β-glucanase, is in line with the principles laid down in Regulation (EC) No 429/2008 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 renewal of the authorisation of feed additives (EFSA FEEDAP Panel, 2013), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018a), 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) and Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018b).

3. Assessment

The current assessment regards the renewal of the authorisation of the feed additive that contains endo-1,4-β-xylanase (Enzyme Commission (EC) Number 3.2.1.8; xylanase) produced by T. reesei CBS 143953 (formerly ATCC PTA-5588) and endo-1,3(4)-β-glucanase (EC Number 3.2.1.6; glucanase) produced by T. reesei CBS 143945 (formerly ATCC SD-2106) as a zootechnical additive (functional group of digestibility enhancers) for poultry and pig species. The assessment also regards the request to modify the terms of the authorisation for turkeys for fattening and reared for breeding (reduction of the minimum recommended dose) and the request for an extension of use to suckling piglets.

5 Commission Regulation (EU) No 337/2011 of 7 April 2011 concerning the authorisation of an enzyme preparation of endo-1,4-β-xylanase and endo-1,3(4)-β-glucanase as a feed additive for poultry, weaned piglets and pigs for fattening (holder of the authorisation Danisco Animal Nutrition) (OJ L 94, 8.4.2011. p. 19) and amending regulation Commission Implementing Regulation (EU) 2019/221 of 6 February 2019 amending Regulations (EC) No 785/2007, (EC) No 379/2009, (EC) No 1087/2009, (EU) No 9/2010, (EU) No 337/2011 and Implementing Regulations (EU) No 389/2011, (EU) No 528/2011, (EU) No 840/2012, (EU) No 1021/2012, (EU) 2016/899, (EU) 2016/997, (EU) 2017/440 and (EU) 2017/896 as regards the name of the holder of the authorisation and the representative of the holder of the authorisation for certain feed additives (OJ L 35, 7.2.2019. p. 28).

6 Commission Implementing Regulation (EU) 2016/997 of 21 June 2016 concerning the authorisation of endo-1,4-β-xylanase EC 3.2.1.8 produced by Trichoderma reesei (ATCC PTA 5588) and endo-1,3(4)-β-glucanase EC 3.2.1.6 produced by Trichoderma reesei (ATCC SD 2106) as a feed additive for lactating sows and minor porcine species (holder of authorisation Danisco (UK) Ltd) (OJ L 164, 22.06.2016. p. 4) and amending regulation Commission Implementing Regulation (EU) 2019/221 of 6 February 2019 amending Regulations (EC) No 785/2007, (EC) No 379/2009, (EC) No 1087/2009, (EU) No 9/2010, (EU) No 337/2011 and Implementing Regulations (EU) No 389/2011, (EU) No 528/2011, (EU) No 840/2012, (EU) No 1021/2012, (EU) 2016/899, (EU) 2016/997, (EU) 2017/440 and (EU) 2017/896 as regards the name of the holder of the authorisation and the representative of the holder of the authorisation for certain feed additives (OJ L 35, 7.2.2019. p. 28).

7 FEED dossier reference: FAD-2020-0030.

8 The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/publications/fad-2010-0007_en

9 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.
The additive, herein and after referred to as Axtra® XB 201, is available in solid (Axtra® XB 201 TPT) and liquid forms (Axtra® XB 201 L).

3.1. Characterisation

3.1.1. Characterisation of the additive

The information submitted regarding the manufacturing process of the additive lists modifications applied during the last years to the fermentation process. These changes regard only the production of xylanase and include data showing consistent protein:enzyme activity ratio in the fermentation product throughout the years. The Panel considers that these modifications would not significantly modify the final product. The applicant declared that no antimicrobials of clinical relevance are used in the production process.

The solid formulation of the additive contains the enzymes xylanase and glucanase. The liquid formulation of the additive contains the enzymes xylanase and glucanase. Indications on the amount of total organic solids in the formulations were provided.

The two formulations ensure a minimum activity of 12,200 xylanase U/g and 1,520 glucanase U/g of additive. Eight batches of the solid formulation (Axtra® XB 201 TPT) were analysed for the enzyme activities which showed mean values of 23,598 xylanase U/g additive (ranging from 22,841 to 24,901) and 2,069 glucanase U/g additive (ranging from 1,951 to 2,182). Eight batches of the liquid formulation (Axtra® XB 201 L) were analysed and showed mean values of 18,313 xylanase U/g (ranging from 17,543 to 20,189 U/g) and 1,875 glucanase U/g (ranging from 1,778 to 2,091 U/g).

Three batches of the solid formulation were tested for chemical and microbial contamination. The following chemical contaminants were tested: arsenic (range 0.1–0.2 mg/kg for TPT, < 0.1 for L), cadmium (0.04 ± 0.01 mg/kg for TPT, < 0.01 mg/kg for L), lead (range 0.32–0.38 mg/kg for TPT and < 0.05 mg/kg for L) and mercury (below limit of detection (LOD) of 0.005 mg/kg for both formulations), acrylamide (< 10 μg/kg for TPT, range 6.3 to 17 μg/kg for L), boron (< 1 mg/kg for both formulations) and fluoride (< LOD 20 mg/kg for both formulations). In addition, the following mycotoxins were tested: aflatoxin B1, B2, G1, G2 (< 0.5 μg/kg for TPT, < 1 μg/kg for L), deoxynivalenol (< LOD 20 μg/kg for both formulations), ochratoxin A (< 1 μg/kg for TPT and < 2 μg/kg for L), HT-2 Toxin and T-2 (< 10 μg/kg for TPT and < 2 μg/kg for L), fumonisin B1 + B2 (< LOD 40 μg/kg for both formulations), zearalenone (< 10 μg/kg for both formulations). Regarding the microbial contamination, the solid formulation was tested for the presence of Salmonella spp. (not detected in 25 g), E. coli (not detected in 25 g), total coliforms (< 10 CFU/g for TPT and < 1 CFU/g for L), Enterobacteriaceae (< 200 CFU/g for TPT and from < 1 to 6,500 CFU/g for L), yeasts and moulds (< 10 CFU/g for TPT or < 1 CFU/g for L). No concerns arise from the results of the impurities measured.

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10 Technical dossier/Section II/Annex II.19 and Supplementary information December 2020.
11 Technical dossier/Supplementary information December 2020.
12 Technical dossier/Section II/Annex II.21.
13 Technical dossier/Supplementary information December 2020.
14 One U of endo-1,4-β-xylanase is the amount of enzyme that liberates 0.48 μmol of reducing sugar (xylose equivalents) per minute from a wheat arabinoxylan at pH 4.2 and 50°C.
15 One U of endo-1,4-β-glucanase is the amount of enzyme that liberates 2.4 μmol of reducing sugar (glucose equivalents) per minute from a barley glucan at pH 5.0 and 50°C.
16 Technical dossier/Section II/Annex II.2 and Annex II.4.
17 Technical dossier/Section II/Annex II.3 and Annex II.5.
18 Technical dossier/Section II/Annex II.4 and Annex II.5 /Supplementary information October 2021/Annex 3 and Supplementary information December 2021
19 For L formulation only aflatoxin B1 was measured.
No antimicrobial activity was detected.20 The production strain for xylanase is a genetically modified strain of *T. reesei* deposited,21 with the accession number CBS 143953 (formerly deposited at the American Type Culture Collection, ATCC PTA-5588). This strain22 was developed from. The genetic modification of the production strain was fully described and assessed in a previous evaluation (EFSA, 2007) and it was concluded that the genetic modification does not raise any safety concern. The production strain has not been subject to any further genetic modification.

The taxonomic identity was determined with a phylogenomic approach.23 The Panel notes that the taxonomic identification would be preferred to be done in the production strain.

The production strain for the glucanase is a non-genetically modified strain of *T. reesei* deposited,24 with the accession number CBS 143945 (formerly deposited as ATCC SD-2106). According to the applicant the strain (had been wrongly indicated in the previous assessment EFSA FEEDAP Panel, 2010).25 confirming it belongs to the species *T. reesei*.26

No data were provided regarding the capacity of the production strains to produce secondary metabolites. Some *Trichoderma* species are known to be capable of producing various mycotoxins and antifungal metabolites. *T. reesei* seems to be unable to produce mycotoxins (EFSA, 2007; Frisvad et al., 2018; EFSA BIOHAZ Panel, 2020) but it is known to produce peptaibols (e.g. paracelsin A, C and D (Frisvad et al., 2018)) and its genome has been shown to harbour genes for two peptaibol synthases (Kubicek et al., 2019). Those peptaibols are peptides with antimicrobial activity and are mostly produced under stress conditions (Frisvad et al., 2018), the production predominantly occurs in solid cultivation and correlates with conidiation (Kubicek et al., 2007; Tisch and Schmoll, 2010). The lack of antimicrobial activity in the additive under assessment (described above) would indicate that if peptaibols are produced under the fermentation conditions, their concentration would be of no concern.

The presence of viable cells was investigated.27
No growth was detected. The presence of DNA from the genetically modified strain was thus, no recombinant DNA was detected.

The applicant provided data on the dusting potential in three batches of the TPT formulation which showed that the product is nearly dust free (5-15 mg/m³) and has a mean particle size of 478 µm.

3.1.2. Conditions of use

Axtra® XB 201 (TPT/L) is currently authorised as a zootechnical additive (functional group: digestibility enhancers) in poultry species (other than turkeys and laying hens), weaned piglets and pigs for fattening (including minor porcine species at the same physiological stage) at 610 xylanase U/kg and 76 glucanase U/kg of feed and at 1,220 xylanase U/kg and 152 glucanase U/kg of feed in feed for turkeys for fattening and reared for breeding, laying hens and lactating sows (including minor porcine species). The applicant has asked to modify the conditions of use for turkeys for fattening and reared for breeding, reducing the minimum recommended level to 610 xylanase U/kg and 76 glucanase U/kg of feed. Moreover, the applicant proposes the extension of use in feed for suckling piglets at a minimum level of 610 xylanase U/kg and 76 glucanase U/kg feed.

In the authorisations, the following other provisions are indicated:

- In the directions for use of the additive and premixture, indicate the storage temperature, storage life and stability to pelleting,
- For use in feed rich in non-starch polysaccharides (mainly β-glucans and arabinoxylans), e.g. containing more than 30% wheat, barley, rye and/or triticale,
- For safety reasons: breathing protection glasses and gloves shall be used during handling,
- For piglets (weaned) up to 35 kg.

And,

- For users of the additive and premixtures, feed business operators shall establish operational procedures and appropriate organisational measures to address the potential risks by inhalation, dermal contact or eyes contact. Where risks cannot be reduced to an acceptable level by these procedures and measures, the additive and premixtures shall be used with appropriate personal protective equipment.

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28 Technical dossier/Section II/Annex II.8 and supplementary information October 2021 Annex 13 and 14 and Supplementary information December 2021 Annex 2.
29 Technical dossier/Section II/Annex II.8.Conf
30 Technical dossier/Section II/Annex II.9.
31 Commission Regulation (EU) No 337/2011 of 7 April 2011 concerning the authorisation of an enzyme preparation of endo-1,4-β-xylanase and endo-1,3(4)-β-glucanase as a feed additive for poultry, weaned piglets and pigs for fattening (holder of the authorisation Danisco Animal Nutrition) (OJ L 94, 08.04.2011. p. 19) and amending regulation Commission Implementing Regulation (EU) 2019/221 of 6 February 2019 amending Regulations (EC) No 785/2007, (EC) No 379/2009, (EC) No 1087/2009, (EU) No 9/2010, (EU) No 337/2011 and Implementing Regulations (EU) No 389/2011, (EU) No 528/2011, (EU) No 840/2012, (EU) No 1021/2012, (EU) 2016/899, (EU) 2016/997, (EU) 2017/440 and (EU) 2017/896 as regards the name of the holder of the authorisation and the representative of the holder of the authorisation for certain feed additives (OJ L35, 7.2.2019, p.28).
32 Commission Implementing Regulation (EU) 2016/997 of 21 June 2016 concerning the authorisation of endo-1,4-β-xylanase EC 3.2.1.8 produced by Trichoderma reesei (ATCC PTA 5588) and endo-1,3(4)-β-glucanase EC 3.2.1.6 produced by Trichoderma reesei (ATCC SD 2106) as a feed additive for lactating sows and minor porcine species (holder of authorisation Danisco (UK) Ltd) (OJ L 164, 22.06.2016. p. 4) and amending regulation Commission Implementing Regulation (EU) 2019/221 of 6 February 2019 amending Regulations (EC) No 785/2007, (EC) No 379/2009, (EC) No 1087/2009, (EU) No 9/2010, (EU) No 337/2011 and Implementing Regulations (EU) No 389/2011, (EU) No 528/2011, (EU) No 840/2012, (EU) No 1021/2012, (EU) 2016/899, (EU) 2016/997, (EU) 2017/440 and (EU) 2017/896 as regards the name of the holder of the authorisation and the representative of the holder of the authorisation for certain feed additives (OJ L35, 7.2.2019. p. 28).
3.2. Safety

The safety aspects regarding the use of this additive in feed were previously assessed, including the safety of the genetic modification of the production strain of the xylanase, the safety for the target species, consumers, users and the environment (EFSA FEEDAP Panel, 2010, 2016; EFSA, 2017a).

In those assessments, the Panel concluded that the additive is safe for poultry species, weaned piglets and pigs for fattening, sows and minor porcine species at the recommended use levels. The applicant has requested for an extension of use to suckling piglets. Based on the results of a tolerance trial in piglets, in 2010 the Panel that the additive was safe for weaned piglets administered at levels 15-fold the maximum recommended dose (at the time of the assessment) of 2,449 xylanase and 304 glucanase units/kg feed. The FEEDAP Panel considers that the conclusions reached in weaned piglets can be extended to pigs in the suckling period and therefore that the additive is safe for suckling piglets at the recommended level of 610 xylanase U/kg and 76 glucanase U/kg of feed.

Similarly in the assessments conducted in 2010 and 2016, the Panel concluded that the use of the additive in poultry and pig species would raise no concerns for the consumers. In the current application, the applicant described modifications in the fermentation process used to obtain the xylanase. However, the Panel considers that these modifications would have little impact on the fermentation product (see Section 3.1.1).

The toxicological data set for the fermentation product containing the xylanase present in the additive and assessed in 2007 by the FEEDAP Panel included an Ames test, and an in vitro chromosomal aberration test (covering structural and numerical aberrations) and a sub-chronic oral toxicity study. The results of the studies showed no genotoxicity potential and no systemic oral toxicity. Therefore, the Panel considers that there is no need for further toxicological data in the context of the renewal of the authorisation.

For the glucanase present in the additive, the toxicological data set assessed in 2010 included a bacterial reverse mutation assay, a chromosomal aberration test, an in vivo micronucleous test (with no demonstration of exposure at target tissue) and a sub-chronic oral toxicity study which showed no concerns. The applicant complemented the data set for the glucanase with an in vitro micronucleus test.\(^{33}\)

The fermentation product did not induce structural and numerical chromosome aberrations under the experimental conditions applied in this study.

In previous assessments (EFSA, 2007; EFSA FEEDAP Panel, 2010), the irritancy and skin sensitisation potential of the two fermentation products was evaluated when tested independently. The data set included skin and eye irritation studies for the two enzymes and a skin sensitisation study for the xylanase. The Panel concluded that ‘the additive should be considered as a potential eye irritant and a skin and respiratory sensitisrer. However, the risk resulting from inhalation exposure for the solid formulation is low’. In order to complement, the data previously assessed in relation to the user safety, the applicant submitted skin sensitisation studies conducted in compliance with the OECD TG 429 using the fermentation products present in the additive which showed no sensitisation potential.\(^{34}\) However, no data was made available with the two forms of the additive. The studies previously assessed were conducted with the individual enzymes and showed a potential for eye irritancy. Consequently, the FEEDAP Panel concludes that the additive is a potential eye irritant and a respiratory sensitiser but cannot conclude on the skin irritation/sensitisation potential of the additive.

In line with the requirements established in the EFSA guidance on the renewal (EFSA FEEDAP Panel, 2013), the applicant performed literature searches to provide evidence that in the light of the current knowledge the additive remains safe under the approved conditions for target species.

\(^{33}\) Technical dossier/Supplementary information June 2022/Annex 3.

\(^{34}\) Technical dossier/Supplementary information October 2021/Annex 15 and 16.
consumers, users and the environment. The literature search covered the period from the first authorisation of the product to the year in which the request for renewal was made and was limited to publications in English language. The searches were conducted separately for the two enzymes present in the additive, including at least two databases (among CAB Abstracts, Veterinary Science Database and Medline), and searching for terms relevant for the target species safety and toxicological aspects. The publications identified did not relate to safety aspects or were not relevant to the additive under assessment. The applicant stated that no adverse events have been detected under its global monitoring plan since its first approval.35

3.2.1. Conclusions on the safety

In view of the above and the fact that the manufacturing and composition of the additive have not been significantly modified since the original authorisation, the FEEDAP Panel concludes that the additive remains safe for the target species, consumers and the environment. These conclusions also apply for the new use requested in suckling piglets. Finally, based on the data submitted in previous applications and the newly submitted one, the Panel concludes that the additive should be considered a potential eye irritant and a respiratory sensitiser (but exposure via inhalation is considered unlikely). However, no conclusions can be drawn on the skin irritation/sensitisation potential of the additive.

3.3. Efficacy

The efficacy of the additive was assessed in previous opinions for poultry and pig species (EFSA FEEDAP Panel, 2010; EFSA FEEDAP Panel, 2016). The efficacy was demonstrated in trials for chickens for fattening, weaned piglets and pigs for fattening at the intended use level of 610 xylanase U/kg and 76 glucanase U/kg feed and in laying hens, turkeys for fattening and lactating sows at the intended level of 1220 xylanase U/kg and 152 glucanase U/kg feed. The FEEDAP Panel noted that the analysed enzyme levels in the diets were approximately 50% higher than the intended (with the exception of sows). The present application for renewal of the authorisation does not include a proposal for amending the conditions of the authorisation for poultry species (other than turkeys), weaned piglets, pigs for fattening, sows and minor porcine species. Therefore, there is no need for further efficacy assessment.

The applicant requested to lower the minimum recommended dose in turkeys for fattening and reared for breeding and an extension of use to suckling piglets at a level of 610 xylanase U/kg and 76 glucanase U/kg feed. No new data has been submitted. The Panel considers that the conclusions reached in chickens for fattening can be extrapolated to turkeys for fattening and reared for breeding and those in weaned piglets can be extended to suckling piglets. Therefore, the additive is considered efficacious as a zootechnical additive in turkeys for fattening/reared for breeding and in suckling piglets (for the period in which solid feed is administered) at an intended level of 610 xylanase U/kg and 76 glucanase U/kg feed. However, the Panel notes that the actual effective dose in the studies supporting this conclusion was approximately 50% higher than the intended dose.

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 Regulation36 and Good Manufacturing Practice.

4. Conclusions

The additive currently in the market complies with the conditions of authorisation.

There is no new evidence that would lead the FEEDAP Panel to reconsider its previous conclusions that the additive is safe for poultry species, weaned piglets, pigs for fattening, lactating sows and minor porcine species, and that is safe for consumers and the environment. The Panel considers that the conclusion also applies to the use of the additive for suckling piglets (for the period in which solid feed is administered).

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35 Technical dossier/Section III/Annex III.1.
36 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 concludes that the additive should be considered as a potential eye irritant and respiratory sensitiser. However, no conclusions can be drawn on the skin irritation/sensitisation potential of the additive.

There is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation for poultry species (other than turkeys), weaned piglets, pigs for fattening, lactating sows and minor porcine species. The Panel concludes that the additive has the potential to be efficacious as a zootechnical additive in turkeys for fattening/reared for breeding and in suckling piglets (for the period in which solid feed is administered) at an intended level of 610 xylanase U/kg and 76 glucanase U/kg feed.

5. Documentation as provided to EFSA/Chronology

| Date         | Event                                                                 |
|--------------|----------------------------------------------------------------------|
| 30/04/2020   | Dossier received by EFSA. FAD-2020-0030. Submitted by Danisco UK Ltd. |
| 29/06/2020   | Reception mandate from the European Commission                        |
| 08/09/2020   | Application validated by EFSA – Start of the scientific assessment   |
| 03/12/2020   | 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 and safety |
| 09/12/2020   | Comments received from Member States                                  |
| 07/10/2021   | Reception of supplementary information from the applicant - Scientific assessment re-started |
| 22/11/2021   | 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 |
| 10/12/2021   | Reception of supplementary information from the applicant - Scientific assessment re-started |
| 14/12/2021   | 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 |
| 20/07/2022   | Reception of supplementary information from the applicant - Scientific assessment re-started |
| 27/09/2022   | Opinion adopted by the FEEDAP Panel. End of the Scientific assessment |

References

EFSA (European Food Safety Authority), 2007. Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) and of the Scientific Panel on Genetically Modified Organisms (GMO) on a request from the European Commission on the safety and efficacy of Danisco Xylanase G/L (endo-1,4-beta-xylanase) as a feed additive for chickens for fattening, laying hens and ducks for fattening. The EFSA Journal 2007;5(10):548, 18 pp. https://doi.org/10.2903/j.efsa.2007.548

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), 2013. Guidance on the renewal of the authorisation of feed additives. EFSA Journal 2013;11(10):3431, 8 pp. https://doi.org/10.2903/j.efsa.2013.3431

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2016. Scientific opinion on the safety and efficacy of Axtra® XB 201 (endo-1,4beta-xylanase and endo-1,3(4)-beta-glucanase) as a feed additive for lactating sows and minor porcine species. EFSA Journal 2016;14(1):4350, 11 pp. https://doi.org/10.2903/j.efsa.2016.4350

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2017. 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, Koubi 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, 2017b. 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, Koubi 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, 2018a. 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), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Koubi 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 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 Nutrition), 2010. Scientific Opinion of the Panel on Additives and Products or Substances used in Animal feed on the safety and efficacy of Danisco Glycosidase (and endo-1,3(4)-beta-glucanase) as feed additive for poultry, piglets and pigs for fattening. EFSA Journal 2010;8(12):1916. 22 pp. https://doi.org/10.2903/j.efsa.2010.1916

Frisvad JC, Møller LLH, Larsen TO, Kumar R and Arnau J, 2018. Safety of the fungal workhorses of industrial biotechnology: update on the mycotoxin and secondary metabolite potential of Aspergillus niger, Aspergillus oryzae, and Trichoderma reesei. Applied Microbiology and Biotechnology, 102, 9481–9515.

Jourdier E, Baudry L, Poggi-Parodi D, Vicq Y, Koszul R, Margeot A, Marbouty M and Bidard F, 2017. Proximity ligation scaffolding and comparison of two Trichoderma reesei strains. Biotechnol Biofuels, 10, 151. https://doi.org/10.1186/s13068-017-0837-6

Kubicek CP, Komorni-Zelazowska M, Sandor E and Druzhinina IS, 2007. Facts and challenges in the understanding of the biosynthesis of peptaibols by Trichoderma. Chemistry & Biodiversity, 4, 1068–1082. https://doi.org/10.1002/cbdv.200600006

Kubicek CP, Steindorff AS, Chenthamara K, Manganiello G, Henriassat B, Zhang J, Cai F, Kopchinskiy AG, Kubicek EM, Kuo A, Baroncelli R, Sarrocco S, Noronha EF, Vannacci G, Shen Q, Grigoriev IV and Druzhinina IS, 2019. Evolution and comparative genomics of the most common Trichoderma species. BMC Genomics, 20, 485. https://doi.org/10.1186/s12864-019-5680-7

Tisch D and Schmoll M, 2010. Light regulation of metabolic pathways in fungi. Appl Microbiol Biotechnol, 85, 1259–1277. https://doi.org/10.1007/s00253-009-2320-1

Abbreviations

- **ATCC**: American Type Culture Collection
- **CBS**: Centraalbureau voor Schimmelcultures
- **CFU**: colony forming unit
- **EURL**: European Union Reference Laboratory
- **FEEDAP**: EFSA Panel on Additives and Products or Substances used in Animal Feed
- **LOD**: limit of detection
- **TOS**: total organic solids