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Safety and efficacy of a feed additive consisting of endo-1,4-β-xylanase (ECONASE® XT) produced by *Trichoderma reesei* CBS 140027 as a feed additive for piglets (weaned), pigs for fattening, chickens for fattening, chickens reared for laying, laying hens, turkeys for fattening, turkeys reared for breeding and minor poultry species (Roal Oy)

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, Pier Sandro Cocconcelli, Boet Glandorf, Jűrgen Gropp, Miguel Prieto Maradona, Guido Rychen, Maria Saarela, Montserrat Anguita, Jaume Galobart, Elisa Pettenati and Jordi Tarrés-Call

Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of ECONASE® XT (endo-1,4-β-xylanase) produced by a genetically modified strain of *Trichoderma reesei* (CBS 140027) as a zootechnical feed additive for piglets (weaned), pigs for fattening, chickens for fattening, chickens reared for laying, laying hens, turkeys for fattening, turkeys reared for breeding and minor poultry species. The recipient strain and the production strain *T. reesei* CBS 140027 are considered safe. The additive is safe for chickens for fattening and weaned piglets at the maximum recommended doses (16,000 and 24,000 BXU/kg feed, respectively) with a wide margin of safety (100-fold and 50-fold, respectively). These conclusions are extended to chickens reared for laying and to pigs for fattening at 16,000 and 24,000 BXU/kg feed, respectively. The additive is safe for turkeys for fattening or reared for breeding at 16,000 BXU/kg feed. The FEEDAP Panel cannot conclude on the safety of the additive for laying hens and for minor poultry species for laying at the proposed conditions of use. The information provided does not allow to conclude on the safety of the use of ECONASE XT® P/L produced by *T. reesei* CBS 140027 in animal nutrition for the consumers. The use of the additive under assessment in animal nutrition does not raise safety concerns for the environment. ECONASE® XT L is non-irritant to the skin or to the eyes. In absence of data, the FEEDAP Panel cannot conclude on the potential of the solid product to be irritant to skin and eyes and on the potential of the additive in all forms to be a dermal sensitiser. All forms of the additive should be considered as respiratory sensitisers. All forms of the additive are considered efficacious at the minimum recommended levels for the target species.

Keywords: zootechnical additives, digestibility enhancers, ECONASE® XT, xylanase, safety, *Trichoderma reesei*

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Question number: EFSA-Q-2019-00330

Correspondence: feedap@efsa.europa.eu

Panel members: Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Mojca Fašmon Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa and Ruud Woutersen.

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Summary

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of ECONASE® XT (endo-1,4-β-xylanase) produced by a genetically modified strain of *Trichoderma reesei* (CBS 140027) as a zootechnical feed additive for piglets (weaned), pigs for fattening, chickens for fattening, chickens reared for laying, laying hens, turkeys for fattening, turkeys reared for breeding and minor poultry species. The additive produced by a different *T. reesei* strain (CBS 114044) is currently authorised in the above-mentioned species.

The recipient strain is considered safe. As no genes of concern were introduced into the recipient strain, the production strain *T. reesei* CBS 140027 is considered to be safe regarding the genetic modifications. No viable cells or recombinant DNA of the production strain could be found in the final products. Nevertheless, the nature of the 'final liquid concentrate' or the 'end fermentation concentrate' (tested for the presence of viable cells of the production strain), or the 'liquid concentrate' (tested for the presence of recombinant DNA) was not described and their relationship with the additive formulations was unclear. Uncertainties remain on the presence of viable cells of the production strain and/or its recombinant DNA in the final products.

Based on the results obtained in the tolerance studies provided in chickens and weaned piglets the FEEDAP Panel concludes that the additive is safe for chickens for fattening and weaned piglets at the maximum recommended doses (16,000 and 24,000 BXU/kg feed, respectively) with a wide margin of safety (100-fold and 50-fold, respectively). These conclusions are extended to chickens reared for laying and to pigs for fattening at 16,000 and 24,000 BXU/kg feed.

The Panel considers that the data in chickens for fattening allows to conclude that the additive is safe for turkeys for fattening or reared for breeding at the same dose (16,000 BXU/kg feed), the results obtained in the tolerance trial in turkeys for fattening would support this conclusion.

The FEEDAP Panel cannot conclude on the safety of the additive for laying hens and for minor poultry species for laying at the proposed conditions of use. The Panel considers the effects on egg weight and egg mass observed in the group treated at 2,021,000 BXU/kg as adverse. Regarding the adverse effects in the group treated at 24,000 BXU/kg, a significant reduction in egg weight was observed that did not result in a significant reduction in egg mass. Nevertheless, it casts doubts on the safety of the additive at the maximum recommended level.

The information provided does not allow to conclude on the safety of the use of ECONASE® XT P/L produced by *T. reesei* CBS 140027 in animal nutrition for the consumers. The development of *T. reesei* CBS 140027 was done in different steps including one step of mutagenesis. The latter would not allow to use surrogate toxicological data from the *T. reesei* strain CBS 114044, because the impact of the mutagenesis step on the toxicological profile of the resulting strain cannot be known.

ECONASE® XT L is non-irritant to the skin or to the eyes. In the absence of data, the FEEDAP Panel cannot conclude on the potential of the solid product to be irritant to skin and eyes and on the potential of the additive in all forms to be a dermal sensitiser. The additive ECONASE® XT in all forms should be considered a respiratory sensitiser.

The use of ECONASE® XT P/L produced by *T. reesei* CBS 140027 in animal nutrition does not raise safety concerns for the environment.

All forms of ECONASE® XT produced by *T. reesei* CBS 140027 are considered efficacious in piglets (weaned), pigs for fattening, chickens for fattening, chickens reared for laying, laying hens, turkeys for fattening, turkeys reared for breeding and minor poultry species at the minimum recommended levels.

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Roal Oy[2] for authorisation of the product ECONASE® XT(endo-1,4-beta-xylanase) produced by fermentation using *Trichoderma reesei* CBS 140027, when used as a feed additive for piglets (weaned), pigs for fattening, chickens for fattening, chickens reared for laying, laying hens, turkeys for fattening, turkeys reared for breeding and minor poultry species (category: zootechnical additive; functional group: digestibility enhancers).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). The particulars and documents in support of the application were considered valid by EFSA as of 02/08/2019.

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 ECONASE® XT(endo-1,4-beta-xylanase) produced by fermentation using a genetically modified strain of *Trichoderma reesei* (CBS 140027), when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

The EFSA Panel on Additives and Products or Substances used in Animal feed (FEEDAP) delivered two opinions on the safety and efficacy of ECONASE® XT P (solid)/L (liquid) produced by *T. reesei* CBS 114044 as a feed additive for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding and piglets(weaned) (EFSA, 2008, 2009). The FEEDAP Panel adopted another opinion on the safety and efficacy of ECONASE®XT when used as a feed additive for laying hens, minor poultry species and pigs for fattening (EFSA FEEDAP Panel, 2011), an opinion on the modification of the authorisation for laying hens (EFSA FEEDAP Panel, 2018a), and an opinion on the renewal of authorisation of ECONASE® XT (endo-1,4-beta-xylanase) as a feed additive for piglets (weaned), chickens for fattening, chickens reared for laying, turkeys for fattening and turkeys reared for breeding (EFSA FEEDAP Panel, 2019a).

The additive is currently authorised for use in chickens for fattening or reared for laying, turkeys for fattening or reared for breeding, laying hens, weaned piglets, pigs for fattening and minor poultry species.[3,4] The applicant is changing the production strain (substituting strain CBS 114044 of *T. reesei* for strain CBS 140027 of the same species of filamentous fungi) and seeks authorisation for the same target species that are currently authorised (piglets (weaned), pigs for fattening, chickens for fattening, chickens reared for laying, laying hens, turkeys for fattening, turkeys reared for breeding and minor poultry species).

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[5] in support of the authorisation request for the use of ECONASE® XT (solid and liquid forms)

[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] Roal Oy, Tykkimäentie 15b, 05200, Rajamäki, Finland.
[3] Commission Regulation (EC) No 902/2009 of 28 September 2009 concerning the authorisation of an enzyme preparation ofendo-1,4-beta-xylanase produced by *Trichoderma reesei* (CBS 114044) as a feed additive for weaned piglets, chickens for fattening, chickens reared for laying, turkeys for fattening and turkeys reared for breeding (holder of authorisation Roal Oy). OJL 256, 29.9.2009. p. 23.
[4] Commission Implementing Regulation (EU) No 2018/1569 of 18October 2018amending Implementation Regulation (EU) No 1110/2011 concerning the authorisation of an enzyme preparation of endo-1,4-beta-xylanase produced by *Trichoderma reesei* (CBS 114044) as a feed additive for laying hens, minor poultry species and pigs for fattening (holder of authorisation Roal Oy). OJ L 262, 19.10.2018, p. 37.
[5] FEED dossier reference: FAD-2019-0029.

produced by fermentation using a genetically modified strain of *T. reesei* (CBS 140027) as a feed additive.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the endo-1,4-β-xylanase in animal feed. The Executive Summary of the EURL report can be found in Annex A.[6]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the ECONASE® XT is in line with the principles laid down in Regulation (EC) No 429/2008[7] and the relevant guidance documents: Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), 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 target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012a,b), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019b) and Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018c).

3. Assessment

This assessment regards a change in the production strain for an already authorised product: ECONASE®XT (endo-1,4-β-xylanase) when used as a zootechnical additive (functional group of digestibility enhancers) in piglets (weaned), pigs for fattening, chickens for fattening, chickens reared for laying, laying hens, turkeys for fattening, turkeys reared for breeding and minor poultry species.

The application covers five formulations, two liquid and three solid forms, similar in composition to the ones assessed in previous opinions (EFSA, 2008, 2009; EFSA FEEDAP Panel, 2019a) and already authorised.

3.1. Characterisation

3.1.1. Characterisation of the production organism

The endo-1,4-β-xylanase present in the additive is obtained by fermentation with a genetically modified strain of the filamentous fungus *T. reesei*, [redacted], which is deposited in the Centraalbureau voor Schimmelcultures (CBS) with the deposition number CBS 140027.[8]

3.1.1.1. Information relating to the genetic modification

Characteristics of the recipient or parental organism

[redacted]

Characteristics of the donor organism

[redacted]

[6] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2019-0029_econase-xt.pdf

[7] 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.

[8] Technical dossier/Section II/Annexes/Annex II-010_ Appendix09_[redacted]

[9] Technical dossier/Section II/Annexes/Annex II-010_ Appendix03_[redacted]

Description of the genetic modification process

[redacted][10][11][12]

3.1.2. Manufacturing process

[redacted]

3.1.3. Characterisation of the additive

ECONASE® XT is an enzyme preparation with endo-1,4-β-xylanase (xylanase, EC 3.2.1.8) as the main active ingredient. It is currently authorised in two forms, solid (P) and liquid (L), ensuring activities of 4,000,000 and 400,000 BXU/g,[13] respectively. In the current dossier, the applicant described five formulations (two liquid and three solid):

- ECONASE® XT L is a liquid preparation containing a minimum xylanase activity of 400,000 BXU/g (42% of liquid enzyme concentrate), sorbitol (35%), sodium benzoate (0.35%), sodium citrate, dehydrate (1.5%), citric acid (1.1%) and water up to 100%.
- ECONASE® XT 25 L is a liquid preparation containing a minimum xylanase activity of 160,000 BXU/g (17% of liquid enzyme concentrate), sorbitol (35%), sodium benzoate (0.35%), sodium citrate, dehydrate (1.5%), citric acid (1.1%) and water up to 100%.
- ECONASE® XT P is a solid preparation containing a minimum xylanase activity of 4,000,000 BXU/g (97% of solid enzyme concentrate) and wheat flour up to 100%.
- ECONASE® XT 5 P is a solid preparation containing a minimum xylanase activity of 800,000 BXU/g (21% of solid enzyme concentrate), sunflower oil (0.4%) and wheat flour up to 100%.
- ECONASE® XT 25 is a solid preparation containing a minimum xylanase activity of 160,000 BXU/g (4% of solid enzyme concentrate), sunflower oil (0.4%) and wheat flour up to 100%.

[10] Technical dossier/Section II/Annexes/Annex II-010_ Appendix10_[redacted]
[11] Technical dossier/Section II/Annexes/Annex II-010_ Appendix06_[redacted]
[12] Technical dossier/Section II/Annexes/Annex II-010_Appendix06_[redacted]
[13] One BXU is the amount of enzyme which liberates 1 nmol reducing sugars as xylose from birch xylan per second at pH 5.3 and 50°C.

The analysis of batch-to-batch variation of five batches of each formulation showed that the xylanase enzymatic activity met the specifications: ECONASE® XT 25 L had an average of 192,400 BXU/g (range 180,000–203,000 BXU/g),[14] ECONASE® XT L an average of 480,400 BXU/g (range 453,000–511,000 BXU/g),[15] ECONASE® XT P an average of 4,261,200 BXU/g (range from 4,050,000 to 4,450,000 BXU/g),[16] ECONASE® XT 5 P an average of 946,200 BXU/g (range 860,000–984,000 BXU/g)[17] and ECONASE® XT 25 an average of 197,200 BXU/g (range 192,000–201,000 BXU/g).[18]

3.1.3.1. Impurities

Five batches of each form of the additive were analysed for impurities.[19] The analysis of heavy metals (cadmium, lead, mercury) and arsenic resulted in values below the limit of quantification (LOQ) in all the different forms of the additive.[20] Regarding mycotoxins (aflatoxins B1, B2, G1, G2; fumonisins B1, B2, B3; sterigmatocystin, ochratoxin A, deoxynivalenol (DON), T2 toxin, HT-2 toxin and zearalenone) they were below the LOQ in all forms of the additive except for DON in the solid forms (in ECONASE® XT P ranged from < 20 to 35 μg/kg; in ECONASE® XT 5 P from 99 to 110 μg/kg and in ECONASE® XT 25 from 87 to 260 μg/kg).[21] Trichodermin was not detected in three batches of ECONASE® XT, with a limit of detection (LOD) of 1.2 μg/kg.[22] None of these values raises safety concerns. Microbiological contamination complied with the specifications in all batches of all forms of the additive except for total viable count in one batch of the ECONASE® XT P in which it was 1.5×10^3 colony forming units (CFU)/g.[23]

The absence of antimicrobial activity was demonstrated in five batches of the liquid concentrate used to produce the different forms of the additive according to FAO/WHO/JEFCA specifications. This has also been confirmed in five batches of each ECONASE® XT formulation.[24]

The presence of the production strain was tested in [redacted] of three batches of the final liquid concentrate [redacted] The production strain was not found in any of the batches tested.[25] In addition, the applicant reports absence of the production strain in all formulations and the reports state the analyses were done with the 'end fermentation concentrate'.[26] Nevertheless, the nature of the final liquid concentrate or the 'end fermentation concentrate' and its relationship with the formulations is not described and uncertainties remain on the potential presence of viable cells of the production strain in the final products.

The presence of recombinant DNA in the final product was assessed in three batches in triplicate of the liquid concentrate before final formulation [redacted] Recombinant DNA was not detected in any of the samples.[27] The identity of the liquid concentrated tested, however, is unclear and uncertainties remain on the potential presence of recombinant DNA of the production strain in the final product.

3.1.3.2. Physico-chemical characteristics

ECONASE® XT L and ECONASE® XT 25 L are brown liquids. Five batches of each form of the additive were analysed. ECONASE® XT L has a pH of 4.1–4.3 and a density of 1.19 kg/L. ECONASE® XT 25 L has a pH of 4.3 and a density of 1.16–1.18 kg/L.[28]

[14] Technical dossier/Section II/Annexes/Annex II-004.
[15] Technical dossier/Section II/Annexes/Annex II-005.
[16] Technical dossier/Section II/Annexes/Annex II-008.
[17] Technical dossier/Section II/Annexes/Annex II-007.
[18] Technical dossier/Section II/Annexes/Annex II-006.
[19] Technical dossier/Section II/Annexes/Annex II-004 to Annex II-008.
[20] LOQ in mg/kg were 0.5 for arsenic and 0.05 for cadmium, lead and mercury.
[21] LOQ in μg/kg were 0.1 for aflatoxins B1, B2, G1, G2; 2 for ochratoxin A; 10 for sterigmatocystin, T2 toxin, HT-2 toxin, and zearalenone; and 20 for fumonisins B1, B2, B3, and DON (in the liquid products).
[22] Technical dossier/Supplementary information March 2020/Annex 2 Trichodermin analysis results.
[23] Microbiological contamination specifications were 10 CFU/g for coliforms at 37°C; 10^3 CFU/g for total viable count, yeasts and filamentous fungi; and absence in 25 g sample for *Salmonella* spp. and *Escherichia coli*.
[24] Technical dossier/Supplementary information March 2020/Annex 3 M002 analysis of microbial activity, and Section II/Annexes II.4 to II.8.
[25] Technical dossier/Supplementary information March 2020/[redacted]
[26] Technical dossier/Section II/Annexes II.4 to II.8.
[27] Technical dossier/Supplementary information March 2020/[redacted]
[28] Technical dossier/Section II/Annexes/Annex II.004 and II.005.

ECONASE® XT P, ECONASE® XT 5 P and ECONASE® XT 25 are light brown powders. Five batches of each form of the additive were analysed and ECONASE® XT P has a bulk density of 260–280 kg/m^3; ECONASE® XT 5 P 0.67 of 560–570 kg/m^3 and ECONASE® XT 25 of 660–690 kg/m^3.[29]

Four batches of each solid form of the additive were analysed (Stauber–Heubach method) for the dusting potential.[30] Results for ECONASE® XT P were 50–371 mg/m^3 air.[31] Those for ECONASE® XT 5 P showed 0 mg/m^3 air except one batch that contained 3 mg/m^3 air.[32] Those for ECONASE® XT 25 showed 0 mg/m^3 air.[33]

3.1.3.3. Stability and homogeneity

The shelf-life of the liquid forms (three batches each) was studied at four different conditions: at 6, 25 or 30°C for 24 months and at 40°C for 12 months. The samples were kept in closed bottles during storage. The xylanase activity after 24 months at 6°C was 100%. The xylanase activity after 24 months at 25°C was 100% for ECONASE® XT 25L and 95–100% for ECONASE® XT L. The xylanase activity after 24 months at 30°C was 91–100% and 86–100%, respectively. The xylanase activity after 12 months at 40°C was 76–99% and 67–92% for for ECONASE® XT 25L and 95–100% for ECONASE® XT L, respectively.[34,35]

The shelf-life of the solid forms (three batches each) was studied when stored in sealed plastic bags at 25°C or at 30°C for 24 months; or at 40°C for 6 months. The xylanase activity after 24 months at 25°C ranged 94–98% for ECONASE® XT 25, 99–100% for ECONASE® XT 5P and 84–89% for ECONASE® XT P. The xylanase activity after 24 months at 30°C was above 71–85%, 83–86% and 73–36%, respectively. The xylanase activity after 9 months at 40°C was 83–91%, 87–96% and 75–81%.[36,37,38]

The stability of the solid products (three batches/product) in a vitamin/mineral premixture (without choline chloride) for breeder hens was tested when stored in a packaging that was not described, at room temperature for 6 months.[39] The targeted concentration of xylanase activity was 10,000 BXU/kg feed. At the end of the storage period, the xylanase activity of ECONASE® XT 25 ranged 80–83%; that of ECONASE® XT 5 P ranged 95–100%; and that of ECONASE® XT P ranged 86–88%.

The stability of ECONASE® XT 25 (three batches) in a vitamin/mineral premixture (containing 38 g/kg choline chloride) for pigs for fattening was tested when stored in a packaging that was not described, at room temperature for 6 months.[40] The additive was supplemented at 5%. At the end of the storage period the xylanase activity ranged 82–98%.

The stability of ECONASE® XT 25 L (three batches) was tested in a pelleted feed for chickens for fattening consisting of wheat, soybean meal and barley that was sprayed to a target concentration of 16,000 BXU/kg feed.[41] The pelleted feed was stored at room temperature in a packaging that was not described for 3 months. At the end of the storage period, the xylanase activity ranged from 93% to 100%.

The stability of the solid products (three batches/product) was tested in a feed for chickens for fattening (meal and pelleted feed) consisting of wheat, soybean meal and barley that had been supplemented at 0.07% ECONASE® XT 25 or 0.03% ECONASE® XT 5 P or 0.016% ECONASE® XT P and stored at room temperature in a packaging that was not described, for 6 months.[42] Pelleting was performed at 80°C. At the end of the storage period, the xylanase activity of ECONASE® XT 25 and ECONASE® XT 5 P was 100% in both meal and pelleted feed. ECONASE® XT P showed 100% xylanase activity in meal feed but that of pelleted feed ranged 96–100%.

The stability of ECONASE® XT 25 (three batches) in meal and pelleted feed (pelleting at 80°C) for weaned pigs (basal diet and packaging during storage were not described) was tested when stored at

[29] Technical dossier/Section II/Annexes/Annex II-006 to Annex II-008.
[30] Technical dossier/Section II/Annexes/Annex II.054.
[31] Technical dossier/Section II/Annexes/Annex II-053a, 053b, 053c and Annex II-054.
[32] Technical dossier/Section II/Annexes/Annex II-052a, 052b, 052c and Annex II-054.
[33] Technical dossier/Section II/Annexes/Annex II-051a, 051b, 051c and Annex II-054.
[34] Technical dossier/Section II/Annexes/Annex II-29 and Annex II-30.
[35] Technical dossier/Section II/Annexes/Annex II-30.
[36] Technical dossier/Section II/Annexes/Annex II-31.
[37] Technical dossier/Section II/Annexes/Annex II-32.
[38] Technical dossier/Section II/Annexes/Annex II-33.
[39] Technical dossier/Section II/Annexes/Annexes II.37a to II.37c.
[40] Technical dossier/Section II/Annexes/Annex II-38.
[41] Technical dossier/Section II/Annexes/Annex II.39.
[42] Technical dossier/Section II/Annexes/Annex II.40a to II.40c.

room temperature for 3 months.[43] Target concentration was 16,000 BXU/kg feed. Xylanase activity in meal at the end of the storage period ranged 92–100%; that of pelleted feed ranged 78–90%. The stability of ECONASE® XT 25 L (three batches) in a sprayed pelleted feed for weaned pigs consisting of wheat, soybean meal and maize was tested when stored in a packaging that was not described at room temperature for 3 months. Target concentration was 16,000 BXU/kg feed. At the end of the storage period, the xylanase activity was 100%.[44]

Stability of the solid forms of the additive (three batches/product) during pelleting at different temperatures (70, 75, 80, 85, 90 and 95°C) was tested in a feed containing wheat and soybean meal that had been supplemented at 0.07% with ECONASE® XT 25 or at 0.03% with ECONASE® XT5 P or at 0.02% with ECONASE® XT P.[45] Taking the lower and the higher pelleting temperatures used as reference, ECONASE® XT 25 showed recoveries ranging from 93–99% at 70 C to 77–89% at 95° C; ECONASE® XT 5 P showed recoveries ranging from 96–100% at 70°C to 87–89 at 95°C; and ECONASE® XT P showed recoveries ranging from 99–100% at 70°C to 88–92% at 95°C.

The capacity of the solid products to homogeneously distribute in feed was studied in meal and pelleted feed for chickens for fattening at a nominal dose of 24,000 BXU/kg feed.[46] 20 subsamples were analysed for each product and feed form. The coefficient of variation (CV) in meal was 6% in ECONASE® XT 25, 7% in ECONASE® XT 5 P and 8% in ECONASE® XTP; the CV in pelleted feed was 5, 6 and 10%, respectively.

The capacity of the liquid form ECONASE® XT 25 L to distribute homogeneously in feed was studied in a pelleted feed for chickens for fattening when sprayed at a nominal concentration of 16,000 BXU/kg feed. 20 subsamples were analysed and the CV was 10%.[47] The same form of the additive was used to spray a pelleted feed for weaned pigs to achieve a nominal concentration of 16,000 BXU/kg feed. The 20 subsamples analysed yielded a CV of 13%.[48]

3.1.4. Conditions of use

ECONASE® XT produced by *T. reesei* (CBS 140027) is proposed to be used in feed containing cereals. The solid forms can be added to feed via premixture or directly to compound feed. The liquid form is applied to pelleted feed by spraying. The recommended levels (all liquid and solid forms of the additive) for the target species are as follows:

- chickens for fattening, chickens reared for laying and minor poultry species other than laying birds: minimum 8,000 BXU/kg complete feed, recommended 8,000 to 16,000 BXU/kg complete feed.
- laying hens: minimum 12,000 BXU/kg complete feed and recommended content 12,000 to 24,000 BXU/kg compete feed.
- laying birds of minor poultry species: minimum and recommended content 24,000 BXU/kg complete feed.
- turkeys for fattening and reared for breeding: minimum and recommended content 16,000 BXU/kg complete feed.
- weaned piglets: minimum and recommended dose of 24,000 BXU/kg complete feed.
- pigs for fattening: minimum 20,000 BXU/kg complete feed, and recommended content of 20,000 to 24,000 BXU/kg complete feed.

The minimum concentrations proposed in these conditions of use are the same as for the product currently authorised produced using strain CBS 114044 of *T. reesei*.[49]

[43] Technical dossier/Section II/Annexes/Annex II.42.
[44] Technical dossier/Section II/Annexes/Annex II.41.
[45] Technical dossier/Section II/Annexes/Annex II-34a to II34c, Annex II-35a to II-35c and Annex II-36a to II-36c.
[46] Technical dossier/Section II/Annexes/Annexes II.45a and b, II.46a and b, II.47a and b.
[47] Technical dossier/Section II/Annexes/Annex II.48.
[48] Technical dossier/Section II/Annexes/Annex II.49.
[49] Commission implementing Regulation (EU) No 2018/1569, OJ L 262, 19.10.2018. p 37–39; and Commission Regulation (EC) No 902/2009.

3.2. Safety

3.2.1. Safety aspects of the genetic modification

The production strain differs from the recipient strain [redacted] As no genes of concern remain in the recipient strain, the production strain CBS 140027 is considered to be safe regarding the genetic modifications.

3.2.2. Safety for the target species

The applicant provided four tolerance trials (one in chickens for fattening, another one in turkeys for fattening, one in laying hens and one in weaned piglets) to support the safety for the target species. The ECONASE® XT under assessment was used in all trials, in different formulations, and the analytical confirmation of the enzyme activities were provided for the four batches used.

3.2.2.1. Safety for chickens for fattening

A total of 320 one-day-old male chickens (Ross 308) were allocated to 32 pens in groups of ten chickens each and allocated to four dietary treatments (8 replicates of ten birds per treatment).[50] Chickens were fed a starter diet (pelleted feed) from day 1 until day 21 and a grower diet (pelleted feed) from day 22 until day 42 of the experiment. The basal diets consisted of wheat and soybean meal and were either not supplemented (control) or supplemented with ECONASE® XT 25 to provide endo-1,4-β-xylanase at the minimum recommended dose 8,000, 16,000 (1× maximum recommended dose) and 1,600,000 (100× maximum recommended dose) BXU/kg of feed. Enzyme activities were confirmed by analysis. Feed and water were available *ad libitum* over the experimental period of 42 days. General health status and mortality were monitored throughout the study. Feed intake was recorded daily and body weight at days 1, 21 and 42. Average feed intake, average body weight gain and feed to gain ratio were calculated for the starter (1–21 day), the grower (22–42 day), and the overall period. Data were statistically analysed by analysis of variance (ANOVA) and group means were compared with the least significant difference (LSD) test. The pen was the experimental unit and the significance level was set at 0.05.

Overall mortality was 4.37% (2.5, 3.75, 3.75, and 7.5%, for control, 8,000, 16,000, and 1,600,000 BXU/kg feed, respectively) and not different between treatments. No significant differences were observed in the total feed intake per bird (mean 5,202 g) and the final body weight (mean 3,525 g). The average feed to gain ratio was 1.50 and showed significant improvements in 1× and 100× (1.48) groups compared to control (1.52). The supplementation of the experimental diets with ECONASE® XT at up to 100× the maximum recommended level did not have any negative effect on the performance of chickens for fattening.

The FEEDAP Panel concludes that the additive is safe for chickens for fattening at the recommended inclusion level of 16,000 BXU/kg of feed. The conclusions of the safety of the additive for chickens for fattening can be extrapolated to turkeys for fattening and reared for breeding as well as to minor poultry species at the same physiological stage.

3.2.2.2. Safety for turkeys for fattening

A total of 720 one-day-old female Hybrid Converter turkeys were allocated to 45 pens in groups of 16 turkeys each and allocated to three dietary treatments (15 replicates per treatment of 16 turkeys each).[51] Turkeys were fed a starter 1 diet (pelleted feed) from day 1 until day 21, a starter 2 diet (pelleted feed) from day 22 until day 42. The basal diet consisted of wheat, barley, rye and soybean, and were either not supplemented (control) or supplemented with ECONASE® XT P/25 to provide endo-1,4-β-xylanase at 16,000 (1× maximum recommended level) or 1,600,000 (100× maximum recommended level) BXU/kg of feed. The enzyme activities were analysed and confirmed 1× the maximum recommended level but showed a 70% of the intended activity for 100× maximum recommended level. Feed and water were available *ad libitum* over an experimental period of 42 days. General health status and mortality were monitored throughout the study. Feed intake and body weight were registered throughout the study. Data were statistically analysed by ANOVA and group

[50] Technical dossier/Section III/Annexes 1, 2 and 3; and supplementary information March 2020/Annexes 9 and 12a.
[51] Technical dossier/Section III/Annexes III.7 and III.7a; and supplementary information March 2020/Annex 10.

means were compared with the Duncan's test. The pen was the experimental unit and the significance level was set at 0.05.

Overall mortality/culling were 8.2% (7.5, 7.5 and 9.6%, for control, 1× and 100× respectively) and not different between treatments. No significant differences were observed in the average daily feed intake (99 g/bird per day) and the average body weight (2,411 g). The feed to gain ratio was significantly better in the 100× treatment compared with the control and use level group (1.65, 1.62 and 1.59, for control, 1× and 100×, respectively). The supplementation of the experimental diets with the xylanase contained in ECONASE® XT at up to 100× the maximum recommended level did not have any negative effect on the performance of turkeys for fattening.

The Panel notes that the study is not fully acceptable because the supplemental dose 100× was not confirmed analytically (only 70% of the intended dose was reached); and blood biochemistry and haematology should have been performed. The results obtained in the study conducted in chickens for fattening allows to conclude that the additive is safe for turkeys for fattening (at 16,000 BXU/kg feed) and the study performed in turkeys would support this conclusion.

3.2.2.3. Safety for laying hens

A total of 420 twenty-week-old Isa Brown laying hens (initial body weight 1,520 g) were allocated to 42 cages in groups of ten hens each and allocated to three dietary treatments (14 replicate cages per treatment).[52] A basal diet consisting of wheat, barley, rye and soybean was either not supplemented (control) or supplemented with ECONASE® XT P/25 to provide endo-1,4-β-xylanase at 24,000 (1× maximum recommended level, confirmed analytically) or 2,400,000 (100× maximum recommended level, although the analysis detected only 84× [2,021,000]) BXU/kg of feed. Feed, in mash form, and water were available *ad libitum* over an experimental period of 56 days. General health status and mortality were monitored throughout the study. Body weight was measured at 20 and 28 weeks of age. Feed intake was recorded daily and body weight at days 1 and 56. Feed to egg mass ratio was calculated per cage on days 28 and 56. Number of eggs laid was monitored continually, egg weight was measured at days 28 and 56 (in all eggs laid in a 24 h period) and total egg mass was calculated per cage. Egg quality traits (yolk colour, albumen height, shell-, albumen- and yolk weight, and egg-shell thickness) were also measured at days 28 and 56 (in all eggs laid in a 24-h period). Data were statistically analysed by ANOVA and group means were compared with the Tukey's test. The cage was the experimental unit and the significance level was set at 0.05.

Overall, mortality (including culling) was low (0.71, 1.43 and 2.86% for control, 1× and 100×, respectively) and not affected by treatments. The performance was poor, and the body weight was not reported. Laying rate was very low in all treatments (76.4, 74.0 and 71.7%, respectively, instead of the 96% expected for the breed at that age (ISA Brown management guide, 2015)), but no significant differences were found among treatments. Significant differences were identified in the average feed intake, which was lower in the 100× treatment compared to the control (96.9 vs 101.9 g/hen per day); in egg weight that was significantly lower in the 1× and 100× treatments compared to the control (54.6, 53.4 and 53.5 g for control, 1× and 100× treatment groups, respectively (it should have been 62.1 g according to ISA Brown expected performance at 28 weeks of age); and total egg mass was lower in the 100× treatment compared to control (21.3 vs 23.4 kg/cage). The feed to egg mass ratio was not significantly different between the groups (2.48). FI was in general low (it should have been 114 g/hen/day for ISA brown layers of 28 weeks of age) probably due to the low crude protein content of the diet (analytical values around 15% instead of 17%).

No differences were observed in the other parameters measured.

The Panel considers the effects on egg weight and egg mass observed in the group treated at 2,021,000 BXU/kg as adverse. Regarding the adverse effects in the group treated at 24,000 BXU/kg, a significant reduction in egg weight was observed that did not result in a significant reduction in egg mass. Nevertheless, it casts doubts on the safety of the additive at the maximum recommended level. Consequently, the Panel cannot conclude on the safety of the additive for laying hens and for minor poultry species for laying at the proposed conditions of use.

3.2.2.4. Safety for piglets (weaned)

A total of 252 weaned male and female 21-day-old piglets ([Landrace × Large White] × Pietrain and initial body weight 5.9 kg) were allocated to 18 pens in groups of 14 piglets each and allocated to

[52] Technical dossier/Section III/Annexes III.4, III.5 and III.6. Supplementary information March 2020/Annex 12c layer power analysis.

three dietary treatments (6 replicate pens/treatment).[53] Piglets were fed a pre-starter diet from day 1 until day 14 and a starter diet from day 15 until day 42 of the experiment. The basal diets consisted of wheat, soybean and whey and were either not supplemented (Control) or supplemented with ECONASE® XT P to provide endo-1,4-β-xylanase at 24,000 (1× maximum recommended level) and 2,400,000 (100× maximum recommended level) BXU/kg of feed. The enzyme activities were measured and showed that the supplemented diets contained lower enzyme activities than the intended, as only 50% and 70% of the intended dosages were reached in pre-starter and starter feeds, respectively. Feed, in pelleted form, and water were available *ad libitum* over an experimental period of 42 days. General health status and mortality were monitored throughout the study. Feed intake was recorded daily and body weight on days 1 and 42. Average feed intake, average body weight gain and feed to gain ratio were calculated for the overall period. On day 42 of the experiment, blood samples were obtained from 12 piglets per treatment (2 piglets per replicate having a BW in the 75th percentile) for routine blood haematology (only from 8 piglets, due to haemolysis of other samples)[54] and biochemistry (from 12 piglets).[55] Data were statistically analysed by ANOVA and treatment means were compared with the Tukey's test. The pen was the experimental unit and the significance level was set at 0.05.

Overall mortality/culling were low (3.2%) and not affected by treatments.[56] Significant differences were found in average final body weight (18,523 g in control group vs 19,640 g in 1× treatment group) and in average body weight gain (302 g/day for control vs 328 g/day in the 1× treatment group).[57] No significant differences were observed between the groups in the average daily feed intake (mean value 428 g) and the feed to gain ratio (1.356). No significant differences were identified in the blood parameters measured with the exception of the percentage of monocytes which in the 1× group showed higher values compared to control and the 100x group (6.23 vs 4.26 and 3.45%, respectively), and therefore, not treatment related.[58]

The supplementation of the experimental diets with the xylanase present in ECONASE® XT at up to 70× the maximum recommended level did not have any negative effect on the performance and blood biochemistry and haematology in weaned piglets. The FEEDAP Panel concludes that the additive is safe for weaned piglets at the recommended inclusion level of 24,000 BXU/kg of feed, with a wide margin of safety. The Panel considers that the conclusion can be extended to pigs for fattening at the same level.

3.2.2.5. Conclusions on safety for the target species

Based on the results obtained in the tolerance studies provided in chickens and weaned piglets, the FEEDAP Panel concludes that the additive is safe for chickens for fattening and weaned piglets at the highest recommended doses (16,000 and 24,000 BXU/kg feed, respectively) with a wide margin of safety (100-fold and 50-fold, respectively). These conclusions are extended to chickens reared for laying and to pigs for fattening at 16,000 and 24,000 BXU/kg feed, respectively.

The Panel considers that the data in chickens for fattening allows to conclude that the additive is safe for turkeys for fattening or reared for breeding at the same dose (16,000 BXU/kg feed), the results obtained in the tolerance trial in turkeys for fattening would support this conclusion.

The Panel considers the effects on egg weight and egg mass observed in the group treated at 2,021,000 BXU/kg as adverse. Regarding the adverse effects of the group treated at 24,000 BXU/kg, a significant reduction in egg weight was observed that did not result in a significant reduction in egg mass. Nevertheless, it casts doubts on the safety of the additive at the maximum recommended level. Consequently, the Panel cannot conclude on the safety of the additive for laying hens and for minor poultry species for laying at the proposed conditions of use.

[53] Technical dossier/Section III/Annexes III.8, III.9, III.10; and supplementary information March 2020/Annex 11a_Pig performance_SIn, Annex 11b_Pigs mortality_SIn, Annex 11c_Pigs blood_SIn, Annex 11d_Pigs haemo_SIn, Annex 12d_Piglets Power analysis_SIn.

[54] Including: red blood cells, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, white blood cells (including differential counts), and platelets.

[55] Including: glucose, total proteins, albumin, urea, cholesterol, creatinine, total bilirubin, amylase, alanine aminotransferase activity, aspartate aminotransferase activity, lactate dehydrogenase, gamma glutamyl trans-peptidase activity, alkaline phosphatase activity, creatinine kinase, calcium, phosphorus, magnesium, sodium, potassium and chloride.

[56] Technical dossier/Supplementary information March 2020/Supplement 1/Annex 11b.

[57] Technical dossier/Supplementary information March 2020/Supplement 1/Annex 11a.

[58] Technical dossier/Supplementary information March 2020/Supplement 1/Annex 11d.

3.2.3. Safety for the consumer

No studies were provided with the enzyme concentrate obtained from the production strain subject to the current application (*T. reesei* CBS 140027). The applicant submitted a bacterial reverse mutation assay performed in compliance with OECD TG 471,[59] a chromosome aberration test done with the fermentation product from strain CBS 114044 in compliance with OECD TG 473[60] and a sub-chronic oral toxicity study performed according to OECD Guideline 408[61] conducted with the enzyme concentrate from *T. reesei* CBS 114044. All these studies have been previously assessed by the FEEDAP Panel (EFSA, 2008).

The FEEDAP Panel assessed the differences between the two production strains (CBS 114044 and CBS 140027). The two strains share a common ancestor which was subject to different steps to obtain each of them. The development of CBS 1140044 included [redacted] that has been described previously (EFSA, 2009). The development of CBS 140027 was done in different steps including [redacted] and one step of mutagenesis. The latter would not allow to use surrogate toxicological data from the strain CBS 114044, because the impact of the mutagenesis step on the toxicological profile of the resulting strain cannot be known.

The FEEDAP Panel cannot conclude on the safety of the additive obtained by fermentation using *T. reesei* CBS 140027 for the consumer based on the information provided.

3.2.4. Safety for the user

Two *in vitro* studies were submitted testing the ECONASE® XT L under assessment.

3.2.4.1. Effect on respiratory system

The dusting potential is low in the ECONASE® XT P form of the additive (50–371 mg/m^3) and it is negligible in the other two powder forms ECONASE® XT 5P and in the ECONASE® XT 25 (0–3 mg/m^3).

No specific studies were submitted testing the effects of the additive in the respiratory tract. Because of the proteinaceous nature of the additive, the Panel considered the additive (all forms) as respiratory sensistiser.

3.2.4.2. Effect on eyes and skin

In a valid *in vitro* skin irritation study (human skin model test) performed according to OECD TG 439 and Good laboratory practice (GLP) compliant, ECONASE® XT L was not irritant to skin.[62]

The eye irritation potential of ECONASE® XT L was tested in a valid *in vitro* study (bovine corneal opacity and permeability assay) performed according to OECD TG 437, and GLP compliant.[63] This form of the additive was not irritant to eyes.

3.2.4.3. Conclusions on safety for the user

ECONASE® XT L is non-irritant to the skin or to the eyes. In absence of data, the FEEDAP Panel cannot conclude on the potential of the solid product to be irritant to skin and the eyes and on the potential of the additive in all forms to be a dermal sensitiser. The additive ECONASE® XT in all forms should be considered a respiratory sensitiser.

3.2.5. Safety for the environment

Although no viable cells and/or DNA of the production strain were found in the final products, the items tested ('end fermentation concentrate' and 'liquid concentrate') were not properly identified and uncertainties remain on the potential presence of viable cells and/or DNA of the production strain in the final products. The production strain CBS 140027 of *T. reesei* is considered to be safe regarding the genetic modifications.

The active component of ECONASE® XT is a protein and as such will be degraded/inactivated during the passage through the digestive tract of animals. Therefore, no risks for the environment are expected and no further environmental risk assessment is required.

[59] Technical dossier/Section III/Annexes/Annex III-14.
[60] Technical dossier/Section III/Annexes/Annex III-13.
[61] Technical dossier/Section III/Annexes/Annex III-15.
[62] Technical dossier/Section III/Annexes/Annex III-17_Skin iritation tests_report.
[63] Technical dossier/Section III/Annexes/Annex III-16_Eye irritation test_report.

3.3. Efficacy

The production strain CBS 140027 of the product under evaluation is different from the previous strain CBS 114044 of *T. reesei* (see Section 3.2.3). However, the gene producing 1,4-β-xylanase is the same. Consequently, the efficacy results obtained testing the β-xylanase produced using *T. reesei* CBS 114044 was assessed in previous opinions (EFSA, 2008, 2009; EFSA FEEDAP Panel, 2011, 2018a) are considered applicable to the efficacy of the additive under assessment.

Two new studies were submitted comparing the efficacy of the additive under assessment (derived from *T. reesei* CBS 140027) with the authorised ECONASE® XT derived from *T. reesei* CBS 114044: one in chickens for fattening and another in weaned piglets. The study on chickens for fattening was of short duration (only 21 days) and cannot be considered in the assessment. The study on weaned piglets would have had proper duration (35 days) if the growth rate (average daily gain [ADG]) would have been ≥ 0.5 kg/day. As this was not the case (ADG in control group was 0.315 kg/day and the maximum ADG was 0.338 kg/day in the group treated with 8,000 BXU/g), the study was not considered in the assessment.

3.3.1. Conclusions on efficacy

All forms of ECONASE® XT (liquid and solid) produced by *T. reesei* CBS 140027 are considered efficacious in piglets (weaned), pigs for fattening, chickens for fattening, chickens reared for laying, laying hens, turkeys for fattening, turkeys reared for breeding and minor poultry species at the minimum recommended levels.

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[64] and Good Manufacturing Practice.

4. Conclusions

The recipient strain is considered safe. As no genes of concern were introduced into the recipient strain, the production strain *T. reesei* CBS 140027 is considered to be safe regarding the genetic modifications. Uncertainty remains, however, on the presence of viable cells of the production strain and/or its recombinant DNA in the final products.

Based on the results obtained in the tolerance studies provided in chickens and weaned piglets, the FEEDAP Panel concludes that the additive is safe for chickens for fattening and weaned piglets at the maximum recommended doses (16,000 and 24,000 BXU/kg feed, respectively) with a wide margin of safety (100-fold and 50-fold, respectively). These conclusions are extended to chickens reared for laying and to pigs for fattening at 16,000 and 24,000 BXU/kg feed.

The Panel considers that the data in chickens for fattening allows to conclude that the additive is safe for turkeys for fattening or reared for breeding at the same dose (16,000 BXU/kg feed), the results obtained in the tolerance trial in turkeys for fattening would support this conclusion.

The FEEDAP Panel cannot conclude on the safety of the additive for laying hens and for minor poultry species for laying at the proposed conditions of use.

The information provided does not allow to conclude on the safety of the use of ECONASE® XT P/L produced by *T. reesei* CBS 140027 in animal nutrition for the consumers.

ECONASE® XT L is non-irritant to the skin or to the eyes. In absence of data, the FEEDAP Panel cannot conclude on the potential of the solid product to be irritant to skin and eyes and on the potential of the additive in all forms to be a dermal sensitiser. The additive ECONASE® XT in all forms should be considered a respiratory sensitiser.

The use of ECONASE® XT P/L produced by *T. reesei* CBS 140027 in animal nutrition does not raise safety concerns for the environment.

All forms of ECONASE® XT produced by *T. reesei* CBS 140027 are considered efficacious in piglets (weaned), pigs for fattening, chickens for fattening, chickens reared for laying, laying hens, turkeys for fattening, turkeys reared for breeding and minor poultry species at the minimum recommended levels.

[64] Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

5. Documentation provided to EFSA/Chronology

Date	Event
16/05/2019	Dossier received by EFSA. Econase® XT (endo-1,4-beta-xylanase) produced using *Trichoderma reesei* CBS 140027. Submitted by Roal Oy.
06/05/2019	Reception mandate from the European Commission
02/08/2019	Application validated by EFSA – Start of the scientific assessment
12/09/2019	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 of the production organism, characterisation of the additive, safety for the target species*
02/11/2019	Comments received from Member States
02/12/2019	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
09/03/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
17/03/2021	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

References

EFSA (European Food Safety Authority), 2008. 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 safety and efficacy of Econase XT P/L as feed additive for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding and piglets (weaned). EFSA Journal 2008;6(6):712, 19 pp. https://doi.org/10.2903/j.efsa.2008.712

EFSA (European Food Safety Authority), 2009. Scientific Opinion of the Panel on Genetically Modified Organisms on a request from the European Commission related to the enzyme preparation of trade name 'Econase XT P/L (endo-1,4 β-xylanase) as a feed additive for chickens and turkeys for fattening, chickens reared for laying, turkeys reared for breeding and piglets (weaned). EFSA Journal 2009;7(4):1058, 6 pp. https://doi.org/10.2903/j.efsa.2009.1058

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011. Scientific Opinion on the safety and efficacy of Econase XT P/L (endo-1,4-β-xylanase) as a feed additive for laying hens, minor poultry species (including ducks, geese, quails, pheasants and pigeons) and pigs for fattening. EFSA Journal 2011;9(6):2277, 15 pp. https://doi.org/10.2903/j.efsa.2011.2277

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012a. Guidance for the preparation of dossiers for additives already authorised for use in food. EFSA Journal 2012;10(1):2538, 4 pp. https://doi.org/10.2903/j.efsa.2012.2538

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012b. 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, Galobart J and Innocenti ML, 2017a. 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, 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, 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, 2017c. 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, Lopez-Alonso M, Lopez Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Brantom P, Dierick NA and Anguita M, 2018a. Scientific Opinion on the safety and efficacy of ECONASE® XT (endo-1,4-β-xylanase) as a feed additive for laying hens.EFSA Journal 2018;16(3):5216, 7 pp. https://doi.org/10.2903/j.efsa.2018.5216

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), 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, 2018c. 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), Bampidis V, Azimonti G, Bastos ML, 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 R, Woutersen R, Cocconcelli PS, Glandorf B, Herman L, Prieto MM, Saarela M, Galobart J, Gregoretti L, Innocenti ML, López Gálvez G, Sofianidis K, Vettori MV and Brozzi R, 2019a. Scientific Opinion on the assessment of the application for renewal of authorisation of ECONASE® XT (endo-1,4-β-xylanase) as a feed additive for piglets (weaned), chickens for fattening, chickens reared for laying, turkeys for fattening and turkeys reared for breeding. EFSA Journal 2019;17(11):5880, 10 pp. https://doi.org/10.2903/j.efsa.2019.5880

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Bastos ML, 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, Knecht J, Kolar B, Beelen P, Padovani L, Tarrés-Call J, Vettori MV and Azimonti G, 2019b. 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

ISA Brown management guide, 2015. Joice and Hill (Hendrix Geneticx Company). Available online: http://www.supremefeeds.com/wp-content/uploads/2015/08/ISA-Brown-Management-Guide.pdf

Abbreviations

ADFI	average daily feed intake
ADG	average daily gain
ADI	average daily intake
ANOVA	analysis of variance
bw	body weight
CBS	Centraalbureau voor Schimmelcultures
CFU	colony forming unit
CV	coefficient of variation
DM	dry matter
DNS	dinitrosalicylic acid
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
LOD	limit of detection
LOQ	limit of quantification
LSD	least significant difference
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MIC	minimum inhibitory concentration
RSDip	relative standard deviations for intermediate precision
RSDr	relative standard deviations for repeatability

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Methods of the Analysis for endo-1,4-β-xylanase (ECONASE® XT) produced by fermentation using *Trichoderma reesei*

Econase® XT is the trade name of a feed additive preparation containing as active substance endo-1,4-beta-xylanase (EC 3.2.1.8) produced by *Trichoderma reesei* (CBS 140027). In the current application authorisation is sought under Article 4 (1) for Econase® XT under the category/functional group 4 (a) "zootechnical additives"/"digestibility enhancers" according to the classification system of Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use of the feed additive for different avian and porcine species.

The endo-1,4-beta-xylanase activity is expressed in BXU units, where "one BXU is the amount of enzyme, which liberates one nanomol per second of reducing sugars, expressed as xylose equivalents, from birch xylan at pH 5.3 and 50 °C". The feed additive is intended to be marketed as light-brown powder formulations (Econase® XT 25; Econase® XT 5P and Econase® XT P) or as brown liquids (Econase® XT 25L and Econase® XT L) with minimum endo-1,4-beta-xylanase activities ranging from 160 000 to 4 000 000 BXU/g.

Endo-1,4-beta-xylanase (4a8) is intended to be incorporated directly or through premixtures at a minimum endo-1,4-beta-xylanase activity in feedingstuffs of 8 000, 16 000 or 24 000 BXU/kg, depending on the target species.

For the quantification of the activity of endo-1,4-beta-xylanase in the feed additive and premixtures the Applicant submitted a single-laboratory validated and further verified spectrophotometric method, based on the formation of reducing sugars reacting with 3,5-dinitrosalicylic acid (DNS), while for the feedingstuffs the Applicant submitted a different single-laboratory validated and further verified spectrophotometric method based on the quantification of water soluble dye fragments, by the action of endo-1,4-β-xylanase on commercially available azurine cross-linked wheat arabinoxylan substrates. For the feed additive and premixtures external calibration is performed using a commercially available xylose standard, while for feedingstuffs external calibration is carried out using a xylanase standard with known enzyme activity and subjected to the same experimental conditions than the feedingstuffs samples. For all matrices the measurements are performed by spectrophotometry at 540 nm.

According to the results provided by the Applicant in the frame of the validation and verification studies, relative standard deviations for repeatability (RSDr) and for intermediate precision (RSDip) ranging from 2.1 to 8.9% and from 4.1 to 7.2%, respectively, were obtained for the quantification of endo-1,4-beta-xylanase in the feed additive, premixtures and feedingstuffs. Additionally, the Applicant estimated a limit of quantification (LOQ) of 4491 BXU/kg feedingstuffs.

Based on the performance characteristics available the EURL recommends for official control of these methods for the quantification of the total endo-1,4-beta-xylanase activity in these three matrices.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761) is not considered necessary.