SCIENTIFIC OPINION

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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

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Abstract

ECONASE® XT is an enzyme based on endo-1,4-β-xylanase produced by a genetically modified strain of *Trichoderma reesei* [redacted]. 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. This scientific opinion concerns the renewal of the authorisation of the additive when used in weaned piglets, chickens for fattening, chickens reared for laying, turkeys for fattening and turkeys reared for breeding. ECONASE® XT is currently authorised in two forms, a solid and a liquid form with activities of 4,000,000 and 400,000 BXU/g, respectively. The current application also comprises three new less concentrated formulations. The applicant has provided evidence that the additive currently in the market complies with the existing conditions of authorisation. The Panel could not exclude the potential presence of recombinant DNA derived from the production organism in recent batches. However, since no sequences of concern have been introduced in the production strain, the potential presence of recombinant DNA in the final product does not raise any safety concern. No new evidence has been produced that would lead the Panel to reconsider its previous conclusions on the safety of the product for target species, consumers, users and the environment under the authorised conditions of use. Therefore, the FEEDAP Panel confirms that ECONASE® XT is safe for the target species, consumers of products from animals fed the additive and the environment. ECONASE® XT is non-irritant to the skin, the liquid form is non-irritant to the eyes and is not a dermal sensitiser, but the additive in all forms should be considered a potential respiratory sensitiser. There is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

Keywords: zootechnical additives, digestibility enhancers, ECONASE® XT, xylanase, safety, recombinant DNA

Requestor: European Commission

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1. Introduction

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

Regulation (EC) No 1831/2003[1] 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.

The European Commission received a request from Royal Oy[2] for renewal of the authorisation of the product ECONASE® XT (endo-1,4-β-xylanase), when used as a feed additive for piglets (weaned), chickens for fattening, chickens reared for laying, turkeys for fattening and turkeys reared for breeding (category: zootechnical additives; functional group: digestibility enhancers).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 14(1) (renewal of the authorisation). The particulars and documents in support of the application were considered valid by EFSA as of 25 January 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-β-xylanase), when used under the proposed conditions of use (see Section 3.1.2).

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) 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), which included the assessment of the safety for the consumer, the user and the environment as well as the safety aspects of the genetic modification of the production strain. The FEEDAP Panel adopted a further 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) and a further opinion on the modification of the authorisation for laying hens (EFSA FEEDAP Panel, 2018a).

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 now seeking the renewal of the authorisation of the additive when used in piglets (weaned), chickens for fattening, chickens reared for laying, turkeys for fattening and turkeys reared for breeding.

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 as a feed additive.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active agent in animal feed. The Executive Summary of the EURL report can be found in Appendix A.[6]

[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 of endo-1,4-β-xylanase produced by *Trichoderma reesei* ([redacted]) 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. OJ L 256, 29.9.2009. p. 23.
[4] Commission Implementing Regulation (EU) No 1110/2011 of 3 November 2011 concerning the authorisation of an enzyme preparation of endo-1,4-β-xylanase produced by *Trichoderma reesei* ([redacted]) as a feed additive for laying hens, minor poultry species and pigs for fattening (holder of authorisation Roal Oy). OJ L 287, 4.11.2011, p.27.
[5] FEED dossier reference: FAD-2018-0071.
[6] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2018-0071-econasext.pdf

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety of 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 renewal of the authorisation of feed additives (EFSA FEEDAP Panel, 2013) and Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b).

3. Assessment

This assessment regards the renewal of the authorisation of ECONASE® XT (endo-1,4-β-xylanase) when used as a zootechnical additive (functional group of digestibility enhancers) in piglets (weaned), chickens for fattening, chickens reared for laying, turkeys for fattening and turkeys reared for breeding.

The application covers five formulations, two already evaluated by the FEEDAP Panel (EFSA, 2008, 2009) and other three not previously assessed.

3.1. Characterisation

3.1.1. 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 activity. It is currently authorised in two forms, solid (P) and liquid (L). In the current dossier the applicant described five formulations, the two currently authorised are:

- ECONASE® XT L: is a liquid preparation containing a minimum xylanase activity of 400,000 BXU/g (46% of enzyme concentrate), with sorbitol (30%), sodium benzoate (0.35%) as a preservative and water up to 100%,
- ECONASE® XT P: is a solid preparation containing a minimum xylanase activity of 4,000,000 BXU/g (93% of enzyme concentrate) and wheat flour up to 100%.

and three new formulations:

- ECONASE® XT 25 L: is a liquid preparation containing a minimum xylanase activity of 160,000 BXU/g (18% of enzyme concentrate), with sorbitol (30%) and sodium benzoate (0.35%) as a preservative and water up to 100%,
- ECONASE® XT 25: is a solid preparation containing a minimum xylanase activity of 160,000 BXU/g (4% of enzyme concentrate), sunflower oil (0.4%) 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 enzyme concentrate), sunflower oil (0.4%) and wheat flour up to 100%.

ECONASE® XT 25 L and ECONASE® XT L are brown liquids with a pH of 4.1–4.5 and a density of 1.12 and 1.14 kg/L, respectively. ECONASE® XT 25, ECONASE® XT 5 P and ECONASE® XT P are light brown powders with a bulk density of 6,600, 6,100 and 3,700 kg/m^3, respectively.

The information submitted regarding the manufacturing process of ECONASE® XT L/P confirms that it is the same as the one described in the first assessment of the product (EFSA, 2008, 2009), [redacted]

The manufacturing process instead of the new formulations is the same plus a dilution step. The Panel is of the view that these modifications do not introduce concerns not already identified.

The analysis of batch-to-batch variation of five recent batches of each formulation showed that the enzymatic activity was in compliance with the specifications: ECONASE® XT 25 L (range: 191,794–234,953 BXU/g, mean: 205,498 BXU/g),[9] ECONASE® XT L (range: 441,000–452,000 BXU/g, mean: 444,800 BXU/g),[10] ECONASE® XT 25 (range: 174,524–201,958 BXU/g, mean: 188,969 BXU/g),[11]

[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/Supplementary information May 2019/Annex 01.
[9] Technical dossier/Section II/Annex II-3.
[10] Technical dossier/Section II/Annex II-4.
[11] Technical dossier/Section II/Annex II-5.

ECONASE® XT 5 P (range: 814,374–894,579 BXU/g, mean: 860,669 BXU/g)[12] and ECONASE® XT P (range: 4,254,867–4,462,079 BXU/g, mean: 4,360,215 BXU/g).[13] The Panel notes that the minimum enzymatic activity was exceeded in all the samples tested.

Five recent batches (manufactured in 2018) of each formulation were analysed for chemical contaminants: heavy metals (lead, mercury, cadmium), arsenic and mycotoxins (aflatoxins B1, B2, G1 and G2 ochratoxin A, sterigmatocystin, zearalenone, T-2 toxin and deoxynivalenol).[13] The results of the analysis of chemical impurities showed values below the respective limits of quantification (LOQ),[14] with the exception of deoxynivalenol in which values were below the LOQ (< 20 μg/kg additive) for the liquid forms and in the range 50–250 μg/kg for the solid forms. None of these values raises safety concerns. Analyses of the same batches showed compliance with microbiological specifications (total coliforms < 1 CFU/g, *Salmonella and Escherichia coli* absent in 25 g, yeasts and filamentous fungi < 10^2 CFU/g).

Four recent batches (manufactured in 2018) of each solid form of the additive were analysed for the dusting potential, measured according to the Stauber–Heubach method. Results for ECONASE® XT 25[15] and ECONASE® XT 5 P[16] showed that the products are dust-free (0–0.003 g/m^3 of air). Those for ECONASE® XT P[17] were 0.05–0.37 g/m^3.

The endo-1,4-β-xylanase present in the additive is produced by a genetically modified strain of *Trichoderma reesei* deposited in the Centraalbureau voor Schimmelcultures (CBS) with the deposition number [redacted] The production strain was assessed by EFSA (2008, 2009); it was concluded that the genetic modification does not raise safety concerns. The FEEDAP Panel is not aware of any new information that would lead to reconsider the safety conclusions drawn previously with regard to the genetic modification of the production strain.

The absence of the production strain was demonstrated in duplicate samples of 20 mL and 2 g of product for the liquid and solid formulations (5 batches each), respectively.[19] Samples from end fermentation concentrates were diluted with sterile saline and mixed with tempered molten potato dextrose agar (PDA) medium. Positive controls were included. The production strain could not be recovered from any of the batches analysed.

[redacted] Therefore, the presence of recombinant DNA in the final product cannot be excluded. However, since no sequences of concern have been introduced in the final production strain, the potential presence of recombinant DNA in the final product does not raise any safety concern.

3.1.2. Conditions of use

ECONASE® XT is currently authorised for use in feed for the target species as follows:

- weaned piglets at a minimum recommended dose of 24,000 BXU/kg complete feed,
- chickens for fattening and reared for laying at a minimum recommended dose of 8,000 BXU/kg, and
- turkeys for fattening and reared for breeding at a minimum recommended dose of 16,000 BXU/kg complete feed.

The authorisation, under other provisions, foresees:

[12] Technical dossier/Section II/Annex II-6.
[13] Technical dossier/Section II/Annex II-7.
[14] LOQs. Lead mercury and cadmium: <0.05 mg/kg; arsenic: < 0.5 mg/kg; aflatoxins B1, B2, G1 and G2: < 0.1 μg/kg; ochratoxin A: < 2 μg/kg; sterigmatocystin: <10 μg/kg; zearalenone: < 10 μg/kg; T-2 toxin: < 10 μg/kg; deoxynivalenol: < 20 μg/kg.
[15] Technical dossier/Section II/Annexes II-44 and II-47.
[16] Technical dossier/Section II/Annexes II-45 and II-47.
[17] Technical dossier/Section II/Annexes II-46 and II-47.
[18] Technical dossier/Section II/Annex II-13.
[19] Technical dossier/Section II/Annexes II-3 to II-7 and Supplementary information May 2019/Annex 02.
[20] Technical dossier/Section II/Annexes II-22a, 22b and 22c and Supplementary information May 2019/Annex 03.

- For use in compound feed rich in non-starch polysaccharides (mainly arabinoxylans), e.g. containing more than 20% wheat.
- For safety: breathing protection, glasses and gloves shall be used during handling.

The applicant proposes to keep the same conditions of use as authorised.

3.2. Safety

The FEEDAP Panel evaluated in 2008 the safety of the genetic modification of the production strain, for the consumer, users and environment and concluded that the genetic modification is of no concern and that the use of the product as a feed additive would be of no concern for the consumers of products derived from animals fed with the additive, or for the environment (EFSA, 2008).

In the same opinion, the safety of the additive (ECONASE® XT) for the target species was established based on tolerance trials: 'Econase XT was shown to be tolerated at 10x (turkeys) or 20x (piglets) the maximum recommended dose (24,000 BXU/kg feed). It is concluded that Econase XT is safe for these target species at the maximum recommended level. It was also concluded that the additive under the recommended conditions of use is safe for chickens for fattening/reared for laying (min 8,000 and max 24,000 BXU/kg feed). However, the results on mineral deposition raise some doubts on the margin of safety for chickens, which may be lower than the ten-fold tested'.

In the same opinion, Econase XT P/L was found to be non-irritant to the skin and the liquid form non-irritant to the eyes and not a dermal sensitiser. Both forms were considered to be respiratory sensitisers. This was translated in the authorising legislation in provisions to protect of users during the handling of the additive.

The applicant states that no adverse effects or incompatibilities with other feed ingredients have been reported for the additive.[21]

The applicant conducted two literature searches on the safety of the additive covering the period 2006–2018.[21] The first included the databases Agris, PubMed and Web of Science and the search terms: 'Econase® XT', 'safety' and 'toxicity'; no hits were identified, except for EFSA opinions. Therefore, a second search was done including the terms: 'xylanase', 'safety' and 'toxicity' and the databases PubMed and Web of Science. The search identified five hits, four of which were considered relevant, although none regarded the additive under assessment (Driss et al., 2014; Ahmad et al., 2013; Dillon et al., 2017; Van Dorna et al., 2018). The four studies investigated the effects of xylanases in animals (e.g. broilers, rats) and did not report any adverse effect.

There is no new evidence that would lead the FEEDAP Panel to reconsider its previous conclusions. Therefore, the Panel concludes that the use of Econase® XT under the authorised conditions of use is safe for the target species consumers, users and the environment.

3.3. Efficacy

The present application for renewal of the authorisation does not include a proposal for amending or supplementing the conditions of the original authorisation that would have an impact on the efficacy of the additive. Therefore, there is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

The Panel notes that three new formulations of the additive (ECONASE® XT 25 L, ECONASE® XT 25 and ECONASE® XT 5 P) are described in the dossier and they are considered equivalent to the authorised ones when used to deliver the same enzyme activity.

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[22] and Good Manufacturing Practice.

4. Conclusions

The applicant has provided evidence that the additive currently in the market complies with the existing conditions of authorisation. The Panel could not exclude the potential presence of recombinant

[21] Technical dossier/Supplementary information May 2019.
[22] 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.

DNA derived from the production organism in recent batches. However, since no sequences of concern have been introduced in the production strain, the potential presence of recombinant DNA in the final product does not raise any safety concern.

No new evidence has been produced that would lead the Panel to reconsider its previous conclusions on the safety of the product for target species, consumers, users and the environment under the authorised conditions of use. Therefore, the FEEDAP Panel confirms its previous conclusion that ECONASE® XT is safe for the target species, consumers of products from animals fed the additive and the environment. Econase XT is non-irritant to the skin, the liquid form is non-irritant to the eyes and is not a dermal sensitiser, but the additive in all forms should be considered a potential respiratory sensitiser.

There is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

5. Documentation as provided to EFSA/Chronology

Date	Event
08/10/2018	Dossier received by EFSA
26/10/2018	Reception mandate from the European Commission
25/01/2019	Application validated by EFSA – Start of the scientific assessment
12/03/2019	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*
10/05/2019	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
10/05/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
04/10/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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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

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Abbreviations

CBS	Centraalbureau voor Schimmelcultures
CFU	colony forming unit
EC	Enzyme Commission
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
LOQ	limit of quantification
PDA	potato dextrose agar

Appendix A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of analysis for ECONASE® XT

Econase® XT is the trade name of a feed additive containing as active substance *endo-1,4-beta-xylanase* (EC 3.2.1.8) produced from *Trichoderma reesei* ([redacted]). This *feed additive* is currently authorised by different Commission Regulations under the category/functional 4(a) "zootechnical additives"/"digestibility enhancers" according to the classification system of Annex I of Regulation (EC) No 1831/2003 (*feed additive* identification number 4a8). In the current application a renewal of the *feed additive* authorisation under Article 14 of the Regulation (EC) No 1831/2003 is requested 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 nanomole 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 5 P* and *Econase XT P*) or as brown liquids (*Econase XT 25L* and *Econase XT L*) with minimum activities ranging from, BXU/g 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) at pH 5.3 and 50°C 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 produced at pH 5.0 and 50°C by the action of endo-1,4-β-xylanase on commercially available azurine cross-linked wheat arabinoxylan substrates from Megazyme. 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 respective validation and verification studies, relative standard deviations for repeatability (RSD_r) and for intermediate precision (RSD_{ip}) ranging from 2.1 to 8.9% and from 4.1 to 7.2%, respectively, were obtained for the quantification of the activity of endo-1,4-beta-xylanase in the *feed additive*, *premixtures* and *feedingstuffs*.