European Food Safety Authority

The EFSA Journal (2008) 712, 1-19

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)[1]

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)

(Questions No EFSA-Q-2007-120a and EFSA-Q-2007-120b)

Adopted on 21 May 2008 by the FEEDAP Panel and on 16 April 2008 by the GMO Panel

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SUMMARY

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) and the Panel on Genetically Modified Organisms (GMO) were asked to issue a scientific opinion 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).

Econase XT is an enzyme feed additive with endo-1,4-beta-xylanase as its declared activity. The production strain for Econase XT is a genetically modified strain of *Trichoderma reesei*.

[1] For citation purposes: Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed and of the Panel on Genetically Modified Organisms 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). *The EFSA Journal* (2008) 712, 1-19.

* One member of the Panel did not participate in the discussion on the subject referred to above.

No DNA sequences causing concern have been introduced in the production organism. The final enzyme preparations contain no cultivable producer organisms and no antimicrobial activity or mycotoxins in concentrations sufficient to cause concern. The absence of DNA from either the fermentation product or the final formulations was not examined. Since no sequences which cause concern are introduced in the final production strain, the potential presence of low concentrations of recombinant DNA in the final product does not raise any particular safety concern.

There is evidence to support the efficacy of Econase XT in improving zootechnical parameters in chickens for fattening at 8000 BXU kg^{-1}, turkeys for fattening at 16000 BXU kg^{-1} and piglets at 24000 BXU kg^{-1}. This evidence is extended to chickens reared for laying and turkeys reared for breeding at the corresponding dose.

Econase XT was shown to be tolerated at 10X (turkeys) or 20X (piglets) the maximum recommended dose (24000 BXU kg^{-1}). It is concluded that Econase XT is safe for these target species at the proposed conditions of use. It is also concluded that the additive is safe for chickens for fattening/reared for laying. However, the results on bone mineralisation raise some doubts on the margin of safety for chickens, which may be lower than the ten-fold tested.

Based on the absence of any adverse effect in two mutagenicity/clastogenicity tests and a subchronic oral toxicity study, it can be concluded that Econase XT is of no concern regarding consumer safety when used as an additive in animal feed.

Econase XT P/L is non-irritant to the skin, and the liquid form is non-irritant to the eyes and is not a dermal sensitiser. The data suggest no additional precautions beyond those required by the labelling of Econase XT as a respiratory sensitiser.

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 risk for the environment is expected and no further environmental risk assessment is required.

Key words: zootechnical additive, digestibility enhancer, enzyme, xylanase, chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding, piglets (weaned), safety, efficacy, Econase XT, genetically modified micro-organisms

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BACKGROUND

Regulation (EC) No 1831/2003[2] 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 an 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 the company ROAL Oy[3] for authorisation of the product Econase XT P/L to be used as a feed additive for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding and piglets (weaned) (category: zootechnical additives; functional group: digestibility enhancers) under the conditions described in Table 1.

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). EFSA received directly from the applicant the technical dossier in support of this application. According to Article 8 of that Regulation, 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. The particulars and documents in support of the application were considered valid by EFSA as of 16 November 2007.

The additive Econase XT P/L is a preparation of beta-1,4-xylanase produced by the genetically modified micro-organism *Trichoderma reesei* (CBS 114044). This product has not been previously authorised in the Community.

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall determine whether the feed additive complies with the conditions laid down in Article 5. Therefore, EFSA shall deliver an opinion on the efficacy and the safety for the target animals, the consumer, user and the environment of the product Econase XT P/L which is a preparation of beta-1,4-xylanase produced by the genetically modified micro-organism *Trichoderma reesei* (CBS 114044) when used under the conditions described in Table 1.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Group on Enzymes of the FEEDAP Panel and the Working Group on Genetically Modified Microorganisms of the GMO Panel as well as Friedrich Schöne for the preparation of this opinion.

[2] OJ L 268, 18.10.2003, p.29
[3] ROAL Oy, Tykkimäentie 15, 05200 Rajamäki, Finland

Table 1. Register entry as proposed by the applicant

Additive	Beta-1,4-xylanase
Registration number/EC No/No (if appropriate)	Pending
Category of additive	Zootechnical additives
Functional group of additive	Digestibility enhancer

Description			
Composition, description	**Chemical formula**	**Purity criteria (if appropriate)**	**Method of analysis (if appropriate)**
Xylanase activity: 4 000 000 BXU/g minimum Dry formulation: Enzyme component 93-100%, wheat flour ad 100% (moisture 5%) Liquid formulation: Enzyme component 9-10%, sorbitol 30%, sodium benzoate 0.35%, water ad 100%	Beta-1,4-xylanase, EC 3.2.1.8	Meets chemical and microbiological purity criteria set by JECFA and FCC.	Spectrophotometric assays: from feed additive and vitamin mineral premixtures by DNS method (reducing sugars); in-feed assay is based on release of dyed fragments.

Trade name (if appropriate)	ECONASE XT P (dry formulation) and ECONASE XT L (liquid formulation)
Name of the holder of authorisation (if appropriate)	Roal Oy

Conditions of use				
Species or category of animal	**Maximum Age**	**Minimum content**	**Maximum content**	**Withdrawal period (if appropriate)**
		Units of activity kg^{-1} of complete feedingstuffs		
Chickens for fattening/chickens reared for laying	-	8000	-	-
Turkeys for fattening/turkeys reared for breeding	-	16000	-	-
Piglets (weaned)	-	24000	-	-

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use (if appropriate)	Labelled according to the requirements laid down in Article 16 of the EC regulation 1831/2003
Specific conditions or restrictions for handling (if appropriate)	Avoid inhalation of aerosol and contact with skin or eyes.
Post-market monitoring (if appropriate)	No specific requirements for a post-market monitoring plan are needed

Specific conditions for use in complementary feedingstuffs (if appropriate)	ECONASE XT P: Applied directly or through vitamin/mineral premixtures into complete feeds; can be diluted with feed ingredients such as wheat flour prior to use to meet accuracy of the mixer. ECONASE XT L: Applied through liquid dosing systems post pelleting; can be diluted with tap water to meet accuracy of the mixer.

Maximum Residue Limit (MRL) (if appropriate)			
Marker residue	**Species or category of animal**	**Target tissue(s) or food products**	**Maximum content in tissues**
-	-	-	-

ASSESSMENT

1. Introduction

Econase XT is an enzyme preparation with endo-1,4-beta-xylanase as it the main activity, intended for incorporation in cereal-based feed for chickens, turkeys and piglets. Its main function as a digestibility enhancer is breaking down the fiber fraction from cereals, mainly arabinoxylan. It is marketed in powdered (P) or liquid (L) form. The production organism is a genetically modified strain of *Trichoderma reesei*.

2. Characterisation of the product

The active ingredient in Econase XT, endo-1,4-β-xylanase is produced by a genetically modified production strain of the fungus *Trichoderma reesei*.

2.1. Qualitative and quantitative composition

Econase XT L is a liquid preparation containing at least 4 x 10^5 BXU[4] g^{-1} (9-10 % enzyme concentrate), with sorbitol (30 %) as excipient and sodium benzoate (0.35 %) as preservative.

Econase XT P is a solid preparation containing at least 4 x 10^6 BXU g^{-1} (93-100 % enzyme concentrate) with wheat flour as excipients.

Particle size analysis of Econase XT P indicates that median size is 291 µm, and 1.7 % is below 63 µm[5] and has a bulk density of 0.4 kg L^{-1}. The L form is a dark brown liquid and has a density of 1.1-1.2 kg L^{-1}.

The product is manufactured in accordance with chemical (heavy metals as Pb and As) and microbiological (total coliforms, *E. coli*, *Salmonella*) purity criteria as established by JECFA (2006) and FCC (2003). The absence of mycotoxins (aflatoxins B1, B2, G1 & G2, ochratoxin A, sterigmatocystin, zearalenone and T-2 toxin) was demonstrated in five batches of the product. Levels of deoxynivalenol were between 0.25 and 1.18 mg kg^{-1}, well below the EU recommended levels for feed and feed materials.[6, 7]

2.2. Characterisation of the production organism[8]

The dossier contains detailed information on the recipient micro-organism (including data on its pathogenicity/toxicity), the donor organism and the genetic modification process. Details on the production and purification processes are also described in detail. No cultivable producer organisms could be detected in a test volume of 20 ml (liquid samples) or in 0.2 g (enzyme powders) of the final formulations.[7, 9] The absence of DNA from either the fermentation product or the final formulations was not examined.[10]

[4] 1 BXU is the amount of endo-1,4-β-xylanase that liberates 1 nmol xylose from birch xylan per second at pH 5.3 and 50°C.
[5] Technical Dossier/Enclosure 6
[6] Commission Recommendation of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding (2006/576/EC). OJ L 229,23.8.2006, p. 7
[7] Technical Dossier/Enclosure 1
[8] This section has been edited following the provisions of Article 8.6 and 18 of Regulation (EC) No 1831/2003
[9] Technical Dossier/Enclosure 4
[10] Supplementary information, January 2008/Enclosure 10

2.3. Stability and homogeneity

The stability of Econase XT P and L was tested as the final product for up to one year at a relevant temperature range (5-35 ºC, or up to 37 ºC (L form)). Results showed limited loss of activity over this period, typically less than 20 % but occasionally up to 40 %, which were not clearly related to the environmental temperature. Stability tests in broiler, turkey and piglet mineral premixes for four months at room temperature (six months for turkeys) showed more than 80 % residual activity at the end of the storage period. Stability was also measured after pelleting at temperatures of up to 95 ºC, without appreciable loss of activity.

Complete broiler feeds (starter, grower, finisher) with Econase XT P were stored for up to four months at room temperature, and residual activity was between 80 and 125 % of that in the initial feed. A similar study for pelleted turkey feeds showed recoveries between 80 and 110 % and for piglet feeds (pre-starter and starter, mash and pelleted) those figures stood between approximately 85 and 120 %. The stability of Econase XT L in broiler, turkey and piglet mash feed for four months at room temperature showed no loss of activity.

In tests of homogeneity, Econase XT P was mixed in broiler feed. Based on analysis of 20 subsamples the CV was 9.2.[11] The liquid form sprayed onto turkey, broiler and piglet feeds gave CVs of 8.4, 10.1 and 12.4, respectively (20 subsamples per feed).[12]

2.4. Conditions of use

Econase XT is to be used in feed for chickens for fattening/reared for laying, turkeys for fattening/reared for breeding and piglets at a minimum dose of 8000, 16000 or 24000 BXU kg^{-1} complete feed, respectively. The maximum recommended dose for all the animal categories is 24000 BXU kg^{-1} complete feed.

2.5. Evaluation of the analytical methods by the Community Reference Laboratory (CRL)

EFSA has verified the CRL report as it relates to the methods used for the control of the active substance in animal feeds. The Executive Summary of the CRL report can be found in the Appendix.

3. Efficacy

3.1. Efficacy for chickens for fattening/reared for laying

Four trials in three different locations have been provided by the applicant to support the efficacy of Econase XT P/L for chickens for fattening.

In the first three trials, male and female chickens (50 % each) were used, while in the fourth trial only males were used.

In the first trial,[13] chickens for fattening received wheat-rye-triticale diets with low (11.3/11.72 MJ kg^{-1}) energy content which were supplemented with Econase XT P at 0, 8000, 16000 or 24000 BXU kg^{-1} complete diets. For comparison, an unsupplemented diet with high energy content (12.06/12.48 MJ kg^{-1}, positive control) was introduced. Starting at 12 days of age, ten replicates in each treatment were taken for a four-day balance test to estimate nitrogen and

[11] Technical Dossier/Enclosure 26
[12] Technical Dossier/Enclosure 27
[13] Technical Dossier/Enclosure 49

gross energy retention. At the end of the trial, ten chickens from each group (five per sex) were sacrificed and carcass weight, breast meat and abdominal fat were measured.

The second[14] and the third trial[15] were done at the same location and had the same experimental design. The chickens for fattening received a basal diet based on wheat, soy beans and soybean meal, which was supplemented with Econase XT P at 0, 16000, 24000 and 32000 (Trial 2) or 0, 8000, 16000 and 24000 (Trial 3) BXU kg^{-1} complete feed.

In the fourth combined performance and balance trial,[16] one-day-old chickens for fattening were distributed to four experimental treatments. The treatments resulted from the supplementation of two maize, soybean meal basal diets with Econase XT P at 0 or 8000 BXU kg^{-1} complete feed. The content of metabolisable energy of the basal diets differed by 4 % (12.69/13.19 *vs.* 12.18/12.66 MJ kg^{-1}, for starter/grower). Starting at 31 days of age, eight replicates of four birds per cage in each treatment were taken for a five-day balance test to estimate nitrogen and gross energy retention. Chromic oxide was added to the diet at the level of 0.5 % as an indigestible marker. At the end of the balance trial, the animals were sacrificed and ileal contents and excreta were collected.

In all trials, enzyme activity was confirmed by analysis and body weight gain, feed intake and feed to gain ratio were measured and calculated. In trials 1 and 4, retention of nitrogen and energy were measured.

The design and main results of the four trials are summarised in Table 2.

Table 2. **Summary of the design and results of the four efficacy trials with Econase XT P in chickens for fattening**

Trial number	Total number of animals (replicates/ treatment) birds/replicate	Trial duration (days)	Econase XT (BXU kg^{-1})	Body weight gain (kg $bird^{-1}$)	Feed intake[3] (g day^{-1})	Feed/gain (kg kg^{-1})
1	600 (15) 8	42	0	1.88[a]	90.5	2.02[a]
			8000	2.05[bc]	91.9	1.88[bc]
			16000	2.04[b]	90.7	1.87[bc]
			24000	2.09[c]	92.1	1.85[c]
			Positive control[1]	2.04[b]	92.4	1.91[b]
2	4000 (8) 125	41	0	2.09[b]	92.0[a]	1.82[a]
			16000	2.16[a]	91.1[ab]	1.75[bc]
			24000	2.15[a]	89.9[bc]	1.73[c]
			32000	2.09[b]	88.5[c]	1.77[b]
3	4000 (8) 125	42	0	1.93[c]	89.8	1.99[a]
			8000	2.05[ab]	89.0	1.84[c]
			16000	2.00[b]	90.1	1.90[b]
			24000	2.07[a]	90.8	1.88[b]
4[2]	400 (8) 25/20 (starter/grower)	41	0	2.56[b]	112.0	1.77[b]
			8000	2.67[a]	112.4	1.72[a]

[1] Positive control: diet with high energy content (12.06/12.48 MJ kg^{-1})
[2] No interactions were found between the level of energy and the supplementation with Econase XT. Therefore, the results refer to the means of the two energy levels
[3] For trials 1 and 4 feed intake (g day^{-1}) was calculated from total feed intake for the whole period
[a, b] Means in a column within a given trial with different superscripts are statistically different (P <0.05)

[14] Technical Dossier/Enclosure 50
[15] Technical Dossier/Enclosure 51
[16] Technical Dossier/Enclosure 52

Body weight gain and feed conversion ratio were significantly improved by the supplementation with Econase XT P at 8000 BXU kg^{-1} complete feed in trials 1, 3 and 4. In trial 2 this dose was not tested. Mortality was in the normal range for chickens for fattening for all groups.

Retention of ME was improved by supplementation with Econase XT at 16000 and 24000 BXU kg^{-1} (68 vs. 74 and 73%, $P < 0.05$) in trial 1. In trial 4, supplementation with 8000 BXU kg^{-1} increased the ME of the diets from 14.07 to 14.72 MJ kg^{-1} ($P < 0.001$).

3.2. Efficacy for turkeys for fattening/reared for breeding

Four trials performed at four different locations have been provided by the applicant to support the efficacy of Econase XT P for turkeys for fattening.

The trials had a similar experimental design, the only difference being that trial 3 included a balance trial involving separate animals. Each trial consisted of a negative control with one or more treatment groups supplemented with Econase XT P. Enzyme activity in feed was confirmed by analysis in all trials. In trials 1 to 3, one-day-old male turkeys were used, and in trial 4 females were used. In all cases, feed intake and body weight were measured and feed to gain ratio was calculated. The animals were daily monitored for health.

In trial 3, two turkeys per pen (12 turkeys per treatment) were selected at day 84 for a digestibility trial. Excreta were collected from day 91 to day 95 to calculate apparent metabolisable energy (AMEn), dry matter and organic matter digestibility.

The design and main results of the four trials are summarised in Table 3.

Table 3. **Summary of the design and results of the four efficacy trials with Econase XT P in turkeys for fattening**

Trial number	Total number of animals (replicates/treatment) birds/replicate	Trial duration (weeks)	Econase XT (BXU kg^{-1})	Body weight (kg $bird^{-1}$)	Feed intake (kg or g $bird^{-1}$ day^{-1})[1]	Feed/gain (kg kg^{-1})
1[17]	320 (16) 10	17	0 16000	15.4[b] 15.9[a]	37.1 37.6	2.43 2.38
2[18]	450 (6) 25/13[2]	18	0 16000 240000	17.5 17.8 17.7	52.5 51.7 52.2	3.06[a] 2.96[b] 2.99[ab]
3[19]	552 (6) 23	16	0 6000 16000 24000	14.7 14.6 14.4 14.7	33.6 32.5 32.1 33.1	2.26[a] 2.19[b] 2.22[ab] 2.24[ab]
4[20]	340 (17) 10/8[2]	16	0 24000	11.1[b] 11.2[a]	273[b] 266[a]	2.74[a] 2.64[b]

[1] Feed intake expressed in kg for trials 1-3 and g $bird^{-1}$ day^{-1} for trial 4.
[2] Animals per replicate were reduced at week 15 in trial 2 and at week 10 in trial 4.
[a, b]: Means in a column within a trial with different superscripts are statistically different ($P < 0.05$)

Mortality was in the normal range for turkeys and not affected by treatment. Body weight at the end of the trials was significantly improved by the supplementation with Econase XT P at

[17] Technical Dossier/Enclosure 53
[18] Technical Dossier/Enclosure 41
[19] Technical Dossier/Enclosure 54
[20] Supplementary information, January 2008/Enclosure 14

16000 BXU kg^{-1} complete feed in trial 1 and feed to gain ratio by supplementation at 16000 BXU kg^{-1} in trial 2 and at 6000 BXU kg^{-1} in trial 3.

In the balance trial, part of trial 3, the AMEn was significantly increased after supplementation of 6000 and 16000 BXU kg^{-1} (15.20 vs. 15.64 and 15.64 MJ kg^{-1}, $P < 0.05$). This positive effect was connected with a positive and significant effect on digestibility of dry matter and organic matter of the diet.

3.3. Efficacy for weaned piglets

Four efficacy trials were carried out at three different locations.

Trial 1

An experiment was carried out with 25 litters (250 piglets) from day 7 to day 84 of life.[21] From day 7 of age, piglets were divided in three treatments (control, eight litters; Econase XT P 12000 BXU kg^{-1}, nine litters; and Econase XT P 24000 BXU kg^{-1}, eight litters). The feed was based on wheat, barley, triticale and soybean meal. At weaning (day 35; mean weight 7.8 kg) piglets were kept in separate pens and fed restricted until the end of the experiment (day 84). Faecal apparent digestibility was calculated between days 56 and 70, with Cr_2O_3 in feed as inert marker. Piglets were weighed at days 35, 56, 70 and 84 and feed consumption measured accordingly. Enzyme concentration in the feed was confirmed by analysis.

The effect of Econase XT P on performance and nutrient digestibility are presented in Table 4.

Table 4. **Effect of Econase XT P on performance (days 35-84) and faecal digestibility of some nutrients in piglets**

Econase XT (BXU kg^{-1})	Daily weight gain (g day^{-1})	Feed intake (g day^{-1})	Feed/gain (kg kg^{-1})	Digestibility (%)		
				Crude protein	Crude fat	Crude fibre
0	266[a]	387	1.45	74.9[a]	40.3[a]	30.4[a]
12000	274[ab]	388	1.42	75.2[a]	45.5[ab]	33.1[ab]
24000	286[b]	383	1.35	77.4[b]	49.9[b]	42.1[b]

[a,b]: Means in a column not sharing a common superscript are statistically different ($P < 0.05$)

Mortality during the test period amounted to 6/79, 8/92 and 4/79 for the different diets. Supplementation with Econase XT at 24000 BXU kg^{-1} significantly improved daily weight gain and the faecal digestibility of crude protein, crude fat and crude fibre.

Trial 2

The experiment was carried out with 252 weaned piglets (barrows and gilts) with a mean live weight of 8.1 kg (age 30 days).[22] Piglets were assigned to three dietary treatments (84 piglets per diet, divided in five blocks of four pens with three to five piglets each): control or supplementation with Econase XT P at 16000 or 24000 BXU kg^{-1}. The meal diets were fed *ad libitum* and were based on wheat, barley and soybean meal. Individual growth data and feed intake and feed/gain ratio per pen were calculated at day 14 and at the end of the trial at day 39. The enzyme concentration in feed was confirmed by analysis.

The effect of Econase XT P on performance is presented in Table 5.

[21] Technical Dossier/Enclosure 55
[22] Technical Dossier/Enclosure 56

Table 5. **Effect of Econase XT P on performance in piglets**

Econase XT (BXU kg^{-1})	Daily weight gain (g day^{-1})	Feed intake (g day^{-1})	Feed/gain (kg kg^{-1})
0	303^{a}	521	1.73^{a}
16000	323^{ab}	503	1.57^{b}
24000	325^{b}	527	1.63^{ab}

$^{a, b}$: Means in a column not sharing a common superscript are statistically different (P <0.05)

Mortality was low and not related to treatments (1/84, 5/84 and 0/84). Supplementation with Econase XT at 16000 BXU kg^{-1} significantly improved feed/gain ratio. Weight gain was improved with supplementation of 24000 BXU kg^{-1} feed.

Trial 3

The experiment[23] consisted of 56 weaned piglets (25 days of age; mean weight 7.5 kg; barrows and females), allocated equally according to body weight, litters and gender to two dietary treatments (28 flat deck pens with two piglets each): control and Econase XT P 24000 BXU kg^{-1}. The pelleted feed consisted of wheat, wheat bran and soybean meal and was given *ad libitum*. Faecal digestibility was calculated during days 47 and 50 with Cr_2O_3 in feed as inert marker. Every week, feed intake and growth were measured per pen and feed to gain ratio calculated. The enzyme concentration in feed was confirmed by analysis.

The effects of Econase XT P on performance and nutrient digestibility are presented in Table 6.

Mortality rate was very low (1 in control diet). Supplementation with Econase XT P at 24000 BXU kg^{-1} did not significantly improve performance but significantly improved faecal digestibility of organic matter, protein and fat.

Table 6. **Effect of Econase XT P on performance and faecal digestibility of some nutrients in piglets**

Econase XT (BXU kg^{-1})	Weight gain (kg per pen)	Feed intake (kg per pen)	Feed/gain (kg kg^{-1})	Digestibility (%)		
				Organic matter	Protein	Fat
0	20.7	32.2	1.57	74.3^{a}	74.8^{a}	81.6^{a}
24000	20.7	31.0	1.51	79.2^{b}	76.7^{b}	84.7^{b}

$^{a, b}$: Means in a column not sharing a common superscript are statistically different (P <0.05)

Trial 4

A performance/digestibility trial was carried out with weaned piglets (Duroc x Landrace) from day 35 till day 63 of age.[24] A total of 24 individually housed piglets were divided into three treatments, with eight piglets per treatment. The pelleted diet used was mainly based on wheat and soybean meal supplemented with Econase® XT P at a level of 0 (NC), 12000 or 24000 BXU kg^{-1} feed (confirmed by analysis) and fed in two phases (0–14 and 14–28 days). Feed and water were *ad libitum*. The measured parameters included growth performance (body weight and daily weight gain) as well as feed intake and feed efficiency. In addition, apparent nutrient digestibly was measured from day 25 to day 28 of the experiment, with TiO_2 as inert marker.

There were no deaths nor any case of dietary scour observed over the course of the study. There were no significant differences in body weight and feed intake between all treatments. However, daily weight gain and feed/gain ratio, measured over the whole period, were significantly improved in piglets fed diets containing 24000 BXU kg^{-1} feed compared to the

[23] Technical Dossier/Enclosure 57
[24] Supplementary information, February 2008/Enclosure 3

negative control (Table 7). There were no significant effects of treatment for the faecal digestibility of organic matter, nitrogen and fat.

Table 7. **Effect of Econase XT P on performance of piglets (0–28 days)**

Econase XT (BXU kg^{-1})	Daily weight gain (g day^{-1})	Feed intake (g day^{-1})	Feed/gain (kg kg^{-1})
0	448[a]	640	1.44[a]
12000	431[a]	626	1.46[a]
24000	499[b]	655	1.32[b]

[a, b]: Means in a column not sharing a common superscripts are statistically different (P <0.05)

3.4. Conclusions on efficacy

Based on the data from three trials in which the body weight gain and the efficiency of feed conversion of chickens for fattening was positively affected by the supplementation with Econase XT P, it is concluded that there is evidence of efficacy of this product at a dose of 8000 BXU kg^{-1} complete feed.

For turkeys, two trials provided evidence of efficacy of the product at the minimum recommended dose of 16000 BXU kg^{-1}, the first showing an effect on body weight gain and the second on the efficiency of feed conversion. In one other study, efficacy was demonstrated at 24000 BXU kg^{-1}, the only dose tested and in a fourth study efficacy was demonstrated at 6000 but not at 16000 or 24000 BXU kg^{-1}. On balance, it is concluded that there is evidence of potential efficacy of Econase XT at the recommended dose (16000 BXU kg^{-1}) in turkeys for fattening.

It is also concluded that the evidence extends to chickens reared for laying and turkeys reared for breeding at the corresponding dose.

Three trials showed positive significant effects on body weight gain of piglets and one of the three also on feed to gain ratio with Econase XT P supplemented at the recommended dose (24000 BXU kg^{-1} feed). Therefore, it is concluded that there is evidence of efficacy of Econase XT P at this dose for weaned piglets.

Although all the studies were made with the solid preparation of the enzyme, it is considered that the same effects would be produced by the liquid preparation at the same level of activity.

4. Safety

4.1. Safety aspects of the genetic modification[25]

The presence of recombinant DNA in the final formulations was not examined[10] and, therefore, the presence of recombinant DNA cannot be excluded.

The inserted genes are well known and fully sequenced. No sequences which cause concern are introduced. Evaluation of the possible occurrence of unintended effects is covered by the studies of mutagenicity and animal feeding trials and studies on mycotoxins and antimicrobials

4.2. Safety for the target species

In all tolerance studies, the solid form of the enzyme preparation was used.

[25] This section has been edited following the provisions of Article 8.6 and 18 of Regulation (EC) No 1831/2003

4.2.1. Safety for chickens for fattening/reared for laying

A tolerance trial[26] was preformed with a total of 1680 male and 1680 female one-day-old chickens for fattening housed in 24 pens (eight replicates of 70 male and 70 females per treatment). The treatments resulted from the supplementation of a basal diet based on wheat and soybean, with Econase XT P at 0, 24000 or 240000 BXU kg^{-1} (10X maximum recommended dose) (confirmed by analysis). The measurements recorded comprised weight gain, feed consumption, carcass yield and abdominal fat proportion and density and chemical analysis of tibia (n = 30/29/26 birds). The trial lasted 35 days.

No negative effects on body weight, feed consumption or feed conversion were observed as a result of the overdose with the enzyme preparation. No negative effects on health were reported and mortality was not affected by treatment. Carcass yield was significantly higher for all enzyme treatments than in control.

No adverse effects on bone density, bone volume and tibia weight were observed as a result of the overdose with the enzyme preparation. Contents of tibia ash (37.6^{a} %; 38^{a}; 35.9^{b}), Ca (12.6^{a} %; 12.3^{ab}; 11.9^{b}) and P (6.4^{a} %; 6.3^{ab}; 6.1^{b}) showed that the enzyme supplementation resulted in a lower mineral deposition than in the control.

4.2.2. Safety for turkeys for fattening/reared for breeding

The tolerance trial with turkeys for fattening was integrated in the second efficacy trial (*q.v.*).[27] At the end of the trial, ten turkeys per treatment were slaughtered. During slaughtering blood samples were obtained from each animal for haematology and clinical chemistry parameters. The weights of meat (breast, thigh) and abdominal fat, liver and spleen were recorded and morphological examination of liver, spleen and kidneys were performed.

No adverse effects on body weight, feed consumption, feed conversion or carcass composition were observed as a result of the overdose with the enzyme preparation. No significant effects were found in the results of haematology and clinical chemistry (erythrocytes, haemoglobin, PCV, MCV, MCH, MCHC, AST and ALT). No significant differences in organ weight and morphology were observed.

4.2.3. Safety for piglets

A tolerance test was performed during 42 days with 144 weaned piglets (males and females; initial weight 8.2 kg).[28] The animals were assigned to three dietary treatments (36 pens with four animals per pen): control or supplemented with Econase XT P at 24000 BXU kg^{-1} (1X) or 480000 BXU kg^{-1} (20X maximum recommended dose) (confirmed by analysis). Weight and feed intake were measured at day 14 and at the end of the experiment (day 42). On day 42, blood samples were taken from six animals per treatment for haematology and clinical chemistry measurements followed by necropsy. Due to abnormal feed spill (see intake data on Table 8) and high mortality, the experiment was repeated but without the blood measurements.

The effect of Econase XT at 20X overdose on performance is presented in Table 8.

[26] Technical Dossier/Enclosure 40
[27] Technical Dossier/Enclosure 41
[28] Technical Dossier/Enclosure 42

Table 8. **Overall effect of Econase XT on performance of piglets**

Econase XT (BXU kg^{-1})	Daily weight gain (g day^{-1})		Feed intake (g day^{-1})		Feed/gain (kg kg^{-1})	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
0	385	394	719[a]	635	1.87[a]	1.62
24000	369	362	849[b]	582	2.34[b]	1.62
480000	365	384	835[b]	612	2.34[b]	1.60

[1] Values for the first and second trial respectively

[a, b] Means in a column within a given trial not sharing a common superscripts are statistically different (P <0.05)

Mortality was not related to treatment (2/48, 7/48 and 7/48 (first trial) and 2/48, 1/48 and 3/48 (second trial)). In the first trial, feed intake was significantly higher in the Econase XT groups, resulting in increased feed/gain ratios. In the second trial, growth performance, feed intake and feed to gain ratio in the overdosed treatment did not differ from the control. Econase XT did not result in any adverse effect in blood chemistry and haematological parameters nor in gross pathology.

4.2.4. Conclusions on the safety for target species

Based on the zootechnical parameters measured, it is concluded that the additive under the recommended conditions of use is safe for chickens for fattening. However, the results on mineral deposition raise some doubts on the margin of safety, which may be lower than the ten-fold tested.

The tolerance trial provided showed that turkeys for fattening tolerated at least ten-fold the maximum recommended dose of Econase XT P. Therefore, it is concluded that the use of the additive at under the recommended conditions of use is safe for growing turkeys.

These conclusions also apply to chickens reared for laying and turkeys reared for breeding.

The combined data from two tolerance trials, each of which had some inadequacies, showed that piglets tolerated a 20X overdose of Econase XT. Therefore it is concluded that Econase XT is safe for weaned piglets at the proposed use level.

4.3. Safety for the consumer

For consumer safety assessment, two mutagenicity/clastogenicity studies were performed *in vitro* and a 13-week repeated dose oral toxicity study in rats. In all cases, a solid enzyme preparation was used, said by the applicant to be equivalent to Econase XT.

4.3.1. Genotoxicity including mutagenicity

4.3.1.1. Bacterial reverse mutation assay

The enzyme preparation was tested in *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* WP2uvrA, both with and without microsomal enzyme activation according to OECD guideline 471.[29] There was no evidence for mutagenicity of the test article in this study, using up to 5000 µg mL^{-1}.

[29] Technical Dossier/Enclosure 43

4.3.1.2. Chromosome aberration assay

The enzyme preparation was tested for chromosome aberrations in Chinese Hamster ovary cell cultures, with and without microsomal enzyme activation according to OECD guideline 473.[30] Exposure lasted for six or 22 hours and cells were harvested 24 or 48 hours later. While there was a clear response in the positive controls, there was no evidence for chromosome aberrations in Chinese Hamster ovary cells exposed to the test article up to 5000 µg mL^{-1}.

4.3.2. Subchronic repeated dose oral toxicity study

A 13-week day oral toxicity study was conducted.[31] Groups of ten Sprague Dawley rats per sex per group were given daily 0, 250, 500 or 1000 mg kg^{-1} day^{-1} by gavage (equivalent to 1, 2 or 4 x 10^6 BXU kg^{-1} day^{-1}). The study was conducted according to OECD guideline 408, and included a full range of clinical, functional, haematological, clinical chemistry, urinalysis and pathological endpoints. There were two non-treatment-related mortalities in the lower dose groups near the end of the study. A slight body weight reduction and initial drop in food intake was observed in all treated female and in the two higher dose male groups, accompanied with softer faeces. No further effects of treatment were observed on other endpoints investigated in the study. The transient food intake and body weight effects are not considered of toxicological significance.

4.3.3. Conclusions on consumer safety

Based on the absence of any compound-related adverse effects in two mutagenicity/clastogenicity tests and a subchronic oral toxicity study, it is concluded that Econase XT is of no concern regarding consumer safety when used as an additive in animal feed.

4.4. Safety for the user

An acute dermal irritation study according to OECD guideline 404 in three female New Zealand White rabbits was conducted with Econase XT (P and L) using application of 0.5 g or 0.5 mL for four hours and observation for up to 72 hours. Only very slight erythema was observed in one rabbit shortly after removal of the patch. Therefore, it is concluded that those preparations were non-irritant.[32]

In an acute eye irritation study in rabbits with Econase XT L, conducted according to OECD guideline 405, the absence of irritation/corrosion potential was demonstrated.[33]

A skin sensitisation test (local lymph-node assay) was performed with Econase XT L in mice according to OECD guideline 429.[34] The results did not indicate a sensitisation potential. The test item is therefore considered as non-sensitising.

An acute inhalation study has not been submitted; however, in the absence of adverse effects by oral, dermal or mucosal route, risk from inhalation toxicity is not expected. In addition, normal precautionary measures which are taken to prevent respiratory sensitisation from enzyme feed additives should prevent exposure by this route, and particle size distribution (see Section 2.1) suggests negligible respirable fraction.

[30] Technical Dossier/Enclosure 44
[31] Technical Dossier/Enclosure 45
[32] Technical Dossier/Enclosure 46
[33] Technical Dossier/Enclosure 47
[34] Technical Dossier/Enclosure 48

4.4.1. Conclusions on safety for the user

Econase XT P/L is non-irritant to the skin, and in the liquid form it is non-irritant to the eyes and is not a dermal sensitiser. The data suggest no additional precautions beyond those required by the labelling of Econase XT as a respiratory sensitiser.

4.5. Safety for the environment

The production micro-organism is removed from the product. The presence of recombinant DNA in the final product was not examined and, therefore, the presence of recombinant DNA cannot be excluded. Since no sequences which cause concern are introduced in the final production strain, the potential presence of low concentrations of recombinant DNA in the final product does not raise any particular safety concern.

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.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

The solid and liquid forms of the product are considered to be equivalent in terms of safety and efficacy.

There is evidence to support the efficacy of Econase XT in chickens for fattening at 8000 BXU kg^{-1}, turkeys for fattening at 16000 BXU kg^{-1} and piglets at 24000 BXU kg^{-1}. It is also concluded that the evidence extends to chickens reared for laying and turkeys reared for breeding at the corresponding dose.

Econase XT was shown to be tolerated at 10X (turkeys) or 20X (piglets) the maximum recommended dose. 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. 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.

Based on the absence of any adverse effects in two mutagenicity/clastogenicity tests and a subchronic oral toxicity study, it is concluded that Econase XT is of no concern regarding consumer safety when used as an additive in animal feed.

Econase XT P/L is non-irritant to the skin, and the liquid form is non-irritant to the eyes and is not a dermal sensitiser. The data suggest no additional precautions beyond those required by the labelling of Econase XT as a respiratory sensitiser.

Although the production micro-organism is removed from the product, the presence of recombinant DNA in the final product was not examined and, therefore, this possibility cannot be excluded. However, since no sequences which cause concern are introduced in the final production strain, the potential presence of low concentrations of recombinant DNA in the final product does not raise any particular safety concern.

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 risk for the environment is expected and no further environmental risk assessment is required.

RECOMMENDATIONS

In the register entry, the activity of the liquid preparation should be expressed in BXU mL^{-1}.

The register entry should include information on the maximum recommended dose for each target species. These should not exceed 24000 BXU kg^{-1} for chickens and turkeys and 48000 BXU kg^{-1} for weaned piglets.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier on Econase® XT (ß-1,4-xylanase, EC 3.2.1.8) as an additive in feedingstuffs. May 2007. Submitted by Roal Oy.
2. Dossier on Econase® XT (ß-1,4-xylanase, EC 3.2.1.8) as an additive in feedingstuffs in compliance with EC Regulation 1831/2003 Supplement 2. January 2008. Submitted by Roal Oy.
3. Dossier on Econase® XT (ß-1,4-xylanase, EC 3.2.1.8) as an additive in feedingstuffs in compliance with EC Regulation 1831/2003 Supplement 3. February 2008. Submitted by Roal Oy.
4. Evaluation report of the Community Reference Laboratory for Feed Additives on the methods(s) of analysis for Econase® XT P/L for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding and piglets (weaned).
5. Comments from Member States received through the ScienceNet.

REFERENCES

Food Chemical Codex (FCC). 2003. Food Chemicals Codex, 5th Ed. National Academies Press.

Joint FAO//WHO Expert Committee on Food Additives (JECFA). 2006. General Specifications and Considerations for Enzyme Preparations Used in Food Processing. Compendium of food additive specifications. FAO JECFA monographs 3.

APPENDIX

Executive Summary of the Evaluation Report of the Community Reference Laboratory for Feed Additives on the Method(s) of Analysis for Econase XT P/L for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding and piglets (weaned)

The current application authorisation is sought for *Econase XT L & P* under the category 'zootechnical additives', group 4(a), according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought to use *Econase XT L&P* as a digestibility enhancer for chickens for fattening/reared for laying; turkey for fattening/reared for breeding; and for piglets (weaned). The product is intended to be marketed as solid (*Econase XT P*) and as liquid (*Econase XT L*) formulations.

The active agent of *Econase XT L&P* is endo-1,4-β-*xylanase* produced by a strain of *Trichoderma reesei* (CBS 114044). The enzymatic activity is expressed in *xylanase* unit (BXU) where 1 BXU is the amount of endo-1,4-β-*xylanase* that liberates 1 nmol xylose from birch xylan per second at pH 5.3 and 50^{o}C. The solid product (*Econase XT P*) has a target activity of 4 000 000 BXU/g. It is intended to be mixed into *premixtures* and/or *feedingstuffs* to provide an enzyme activity range of 6 000 to 24 000 BXU/kg *feedingstuffs*. The liquid product (*Econase XT L*) has an enzyme activity of 400 000 BXU/g, and is sprayed directly onto the post-pelleted feed to obtain an enzyme activity range of 6 000 to 24 000 BXU/kg *feedingstuffs*.

An absolute colorimetric method based on the formation of reducing sugar reacted with dinitrosalysilic acid (DNS) is in-house validated for the determination of the activity of endo-1,4-β-*xylanase* in the *feed additive* and *premixtures*. The following performance characteristics were obtained for liquid and powder *feed additives* and for turkey and broiler *premixtures*: recovery greater than 85% and relative standard deviations for repeatability and intermediate precision ranging from 2.0 to 6.0% and 4.0 to 11.0%, respectively. However, low recovery rate (60%) and high relative standard deviations for repeatability and intermediate precision (ca. 24%) were reported for the piglet premixture.

An analytical method based on the measurement of the rate of release of water soluble dyed fragments by endo-1,4-ß-*xylanase* from the dye cross-linked wheat arabinoxylan in a form of "Xylazyme AX tablet" is in-house validated for the determination of the activity of endo-1,4-β-*xylanase* in the *feedingstuffs*. The following performance characteristics were obtained for turkey and broiler *feedingstuffs*: recovery of 104% and relative standard deviations for repeatability and intermediate precision ranging from 4 to 7% and 5 to 7%, respectively. Insufficient experimental data was provided to establish the validity of analytical method for the determination of active substance (*xylanase*) in the piglet *feedingstuffs*.

Based on acceptable performance characteristics, the two proposed methods are considered suitable for determination of *xylanase* activity - in *feed additives, premixtures* and *feedingstuffs* for turkeys and broilers (not for piglets) - for official control purposes in the frame of authorisation.

Further testing or validation for the methods determining xylanase activity - in *feed additives, premixtures* and *feedingstuffs* for turkeys and broilers is not considered necessary.