EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Scientific Opinions
C2 - Management of scientific committees; scientific co-operation and networks

REPORT OF THE SCIENTIFIC COMMITTEE ON ANIMAL NUTRITION ON THE SAFETY OF THE ENZYMATIC PRODUCT ECONASE WHEAT PLUS® FOR USE AS FEED ADDITIVE IN CHICKENS AND TURKEYS FOR FATTENING

(Adopted on 22 January 2003)

1. BACKGROUND

The product "Econase Wheat Plus" includes the following enzymatic activities: endo-1, 4-beta-xylanase (EC 3.2.1.8) and endo-1, 3(4)-beta-glucanase (EC 3.2.1.6). Product "Econase Wheat" is already provisionally authorised for the use as feed additive for the animal categories chickens for fattening and piglets.

"Econase Wheat Plus" differs from "Econase Wheat" having a different xylanase to beta-glucanase activity ratio: respectively 4:1 and 1:1. The Commission received a request for a provisional Community authorisation for the animal categories chickens for fattening and turkeys for fattening, under the conditions set out in the following table:

Table 1. Conditions of use proposed by the company for Econase Wheat Plus®

Additive	Chemical formula, description	Species or category of animal	Maximum age	Minimum content Units of activity/kg of complete feedingstuff	Other provisions
Enzymes					
Endo-1,4-beta-xylanase EC 3.2.1.8 Endo-1, 3(4)-beta-glucanase EC 3.2.1.6	Preparation of Endo-1,4-beta xylanase produced by *Trichoderma reesei* (CBS 529.94), and Endo-1, 3(4)-beta-glucanase produced by *Trichoderma reesei* (CBS 526.94) with a minimum activity of: Solid form: 800 000 BXU/g[1] 200 000 BU/g[2] Liquid form: 120 000 BXU/g[1] 30 000 BU/g[2]	Chickens for fattening	-	6 000 BXU 1 500 BU	1.Recommended dose per kg of complete feedingstuff: 16000 – 24000 BXU 4000 – 8000 BU 2. For use in compound feed rich in non starch-polysaccharides (mainly arabinoxylans and glucans) e.g. containing more than 53.5% wheat
		Turkeys for fattening	16 weeks	20000 BXU 5000 BU	1.Recommended dose per kg of complete feedingstuff: 20000 – 40000 BXU 5000 – 10000 BU 2. For use in compound feed rich in non starch-polysaccharides (mainly arabinoxylans and glucans) e.g. containing more than 53.5% wheat

[1] 1 BXU is the amount of the enzyme which liberates 0.06 micromoles of reducing sugars (xylose equivalents) from birch xylan per minute at pH 5.3 at 50°C.

[2] 1 BU is the amount of the enzyme which liberates 0.06 micromoles of reducing sugars (glucose equivalents) from barley beta-glucan per minute at pH 4.8 at 50°C.

The company producing "Econase Wheat Plus" prepared a dossier that has been submitted through the national rapporteur (Finland) to the Commission. The dossier was checked by the Member States for its compliance with the requirements of Council Directive 87/153/EEC fixing the guidelines for the assessment of additives in animal nutrition. The Member States concluded on the 26th of September 2002 that the dossier fulfilled these requirements.

The authorisation procedure laid down in article 4 of Council Directive 70/524/EEC as last amended by Council Directive 96/51/EC includes a period of 320 days for the evaluation of the dossier submitted to the Commission. The Standing Committee for Food Chain and Animal Health started the evaluation of the product on 26th of September 2002.

2. TERMS OF REFERENCE

The Scientific Committee for Animal Nutrition (SCAN) is requested to assess the safety of "Econase Wheat Plus" – Endo-1, 4-beta-xylanase (IUB 3.2.1.8); Endo-1, 3(4)-beta-glucanase (IUB 3.2.1.8), in liquid and solid form, enzymatic activities produced respectively by *Trichoderma reesei* (CBS 529.94), and by *Trichoderma reesei* (CBS 526.94) for the chickens and the turkeys, for fattening.

3. OPINION OF SCAN

3.1. Introduction

Econase Wheat Plus is a modified version of Econase Wheat. Econase Wheat was already considered by the Scientific Committee on Animal Nutrition and found safe for use in piglets and in chickens for fattening. Product Econase Wheat Plus is characterised by a different ratio xylanase / β-glucanase activity.

In the case of Econase wheat Plus the ratio is 4 xylanase units / 1 β-glucanase unit. The company does not mention any other difference in this enzyme preparation. The strains used for the production are the same.

The company claims a use in chickens for fattening and also in turkeys for fattening, when the feed is based in wheat with some short amount of barley.

This enzyme preparation contains endo-1,4- β-xylanase (IUB 3.2.1.8) and endo 1,3(4)-β-glucanase (IUB 3.2.1.6). Xylanase and β-glucanase are produced from genetically modified (intrageneric) strains of *Trichoderma reesei* ALKO3450 and ALKO2656 respectively. These strains are deposited in The Netherlands CBS (ALKO3450=CBS 529.04 and ALKO2656= 526.94).

The company presented two forms, liquid and powder. The final formulation for each type is presented in Table 2.

Table 2. Formulation of the products.

Econase Wheat Plus	Liquid	Powder
Xylanase activity (BXU/g) β-glucanase activity (BU/g)	Min. 120 000* Min. 30 000*	Min. 800 000* Min. 200 000*
%	2.9	19
Glycerol (%)	35	-
Sodium Benzoate (%)	0.35	-
Water (%)	61.7	9
Wheat flour (%)	-	72

*: declared activity.

The dosages proposed by the company for the target animals are described in Table 1.

The only difference between Econase Wheat Plus and Econase Wheat is the different enzyme concentration ratio. The safety evaluation done previously by the SCAN for Econase Wheat is applicable to Econase Wheat Plus. Consequently the safety assessment of Econase Wheat Plus is limited to the impact on target animals safety.

3.2. Tolerance test on target species

Table 3. Summary experimental conditions.

Experimental conditions:	Broiler chickens	Turkeys for fattening
Number of birds	40 (20 males, 20 females) Four groups of 10 birds, two groups per dietary treatment.	360 males, Four groups of 30 birds per dietary treatment
Length (days)	35	112
Enzyme presentation	powder	powder
Maximum recommended dose (powder)	30 g/t	50 g/t
Dietary treatments	2 : Control and tolerance test	3 : Control, maximum recommended 50 g/t and tolerance test 500 g/t
Tolerance test level used	300 g/t	500 g/t

3.2.1. Chickens for fattening

The experiment used 40 one–day-old broilers (20 females and 20 males). Four pens were used in total, each pen holding ten birds. The study compared two pens from control (no enzyme) against two groups for tolerance. The enzyme was supplied in powder form at ten times the maximum recommended dose.

A full report in the dossier is given with a description of procedures used and results obtained. The birds were weighed at the beginning and at the end of the experiment at 35 days. Feed intake was measured per pen. Animal heath status was recorded daily. At the end ten birds (five males and five females) were autopsied in order to check the status of the internal organs. Feed samples were collected in order to analyze and record the presence of enzyme in the feed.

Blood chemistry and hematological measurements were not determined.

Results

The animal performance of birds, weight gain, feed conversion and feed intake are presented in table 4. Final body weight of birds fed the ten-times recommended dose was higher than the control group. Differences between females groups were significant (P<0.05). The feed intake was reduced when enzymes such as xylanase were used in wheat basal diets.

Table 4. Broilers performance.

		Control	Econase Wheat Plus 300 g/t	Significance
Final weight	males	1959	2018	NS
	females	1565	1690	*
	mean	1762 (100)	1854 (105)	NS
Feed consumption	g/bird/day	86.6	89.2	-
	FCR	1.792 (100)	1.737 (97)	-
Mortality (number)		0	0	-
Dry matter litter %		43.4	43.0	-

NS : non-significant *: P<0.05 FCR : feed conversion ratio

The autopsy showed a tibial dyschondroplasia in some of the birds from the tolerance level group. In addition an enlargement of duodenum and jejunum was also observed. The tibial dyschondroplasia observation could be related to the genetic instead of nutritional treatment (*i.e.* overweight). The dilatation and enlargement of duodenum and jejunum, according to the author, was a consequence of the action of enzymes and was not related with a pathological sign.

Conclusion

The test conducted in broiler chickens shows some problems such as an enlargement of duodenum, jejunum and tibial dyschondroplasia. This weak experimental design and the small number of birds used do not allow a sound conclusion. Another test of tolerance is therefore necessary in order to assess the safety of this product for this target species.

3.2.2. Turkeys for fattening

Tolerance test was conducted in a dose response study. A full report is given with a description of procedures used and results obtained. The birds were weighed at the beginning and at the end of experiment at 112 days. Feed intake was measured per pen. Animal heath status was recorded daily. Feed samples were collected in order to analyze and record the presence of enzyme in the feed. Blood chemistry and hematological measurements were not determined.

The tolerance test was conducted as part of a dose response study. The inclusion levels of Econase Wheat Plus in powder form were 0, 25, 50, 100, 150, 500 g/t. Among the 720 male turkeys used in the dose response study and distributed in 24 pens (four pens per level), a total of 360 animals were used for the tolerance test. The tolerance test was based on three levels of enzyme supplementation control 0, maximum recommended 50 g/t and ten fold the recommended 500 g/t.

The overall growth of turkeys was affected by the enzyme supplementation, however the feed conversion was not affected by an overdose of Econase Wheat Plus as it is shown in table 5. The final body mass of turkeys fed with enzyme was significantly higher than control. Post mortem examination was done and did not reveal any problem.

Table 5. Body weight and feed conversion of turkeys

Amount Econase g/t	Number of birds at 0 days and 112 days	Final body weight	Feed conversion
0	118/108	11159 [b]	2.185
50	119/113	11645 [a]	2.225
500	118/105	11710 [a]	2.185
Probability		0.0001	0.21

Means with different superscript letters are significantly different.

3.3. Toxicological studies (laboratory animals)

Full toxicological description was provided by the company according to the guidelines. This toxicological package corresponds to the already assessed enzyme preparation approved.

3.4. Conclusion

SCAN is of the opinion that the modification of enzymes concentration produced in Econase Wheat Plus does not harm the turkeys. However, in the case of broiler chickens, some problems were found in the tolerance test conducted. As a consequence, the safety for broilers could not be demonstrated. An other tolerance test in broilers is necessary in order to assess and to demonstrate the safety.