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 ALLZYME PT ® FOR USE AS FEED ADDITIVE IN TURKEYS FOR FATTENING

(Adopted on 27 March 2003)

1. BACKGROUND

The product "Allzyme PT " preparation of endo-1,4-beta-xylanase EC 3.2.1.8 produced by *Aspergillus niger* (CBS 520.94) (E.C. No. 14) is already provisionally authorised for the use as feed additive for the animal category chickens for fattening, either in the solid and liquid form. The Commission received a request for a provisional Community authorisation for the animal category turkeys for fattening under the conditions set out in the table 1 hereafter.

Table 1: Conditions of use of the product proposed by the company

No	Additive	Chemical formula, description	Species or category of animal	Minimum Content Units of activity per Kg of complete feedingstuff	Other provisions
ENZYMES					
14	Endo-1,4-beta-xylanase EC 3.2.1.8	Preparation of endo-1,4-beta-xylanase produced by *Aspergillus niger* (CBS 520.94) having a minimum acitvity of: Solid form: 600 U/g[1] Liquid form: 300 U/ml	Chickens for fattening	300 U	Recommended dose per kg of complete feedingstuff: 300-600 U For use in compound feed rich in non-starch polysaccharides, (mainly arabinoxylans), e.g. containing more then 50% wheat
			Turkeys for fattening	300 U	Recommended dose per kg of complete feedingstuff: 300-1200 U For use in compound feed rich in non-starch polysaccharides, (mainly arabinoxylans), e.g. containing more then 50% wheat

[1] One U is the amount of enzyme which liberates one micromole of xylose from birchwood xylan per minute at pH 5.3 and 50°C

The company producing "Allzyme PT" prepared a dossier that has been submitted through the national rapporteur (Ireland) 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 in the Standing Committee for Animal Nutrition on 9th of July 2001 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 Animal Nutrition started the evaluation of the product on 9th of July 2001.

2. TERMS OF REFERENCE

The Scientific Committee for Animal Nutrition (SCAN) is requested to give an opinion on the safety of the use of "Allzyme PT" preparation of endo-1,4-beta-xylanase EC 3.2.1.8. Produced by *Aspergillus niger* (CBS 520.94) for the turkeys for fattening.

3. INTRODUCTION

This enzyme preparation is produced by ALLTECH by fermentation of a selected strain of *Aspergillus niger* (CBS 520.94). The strain used has not been genetically modified. The active substance is an Endo- 1,4 –beta-xylanase (1,4-β-D-Xylan xylanohydrolase). Formerly, this main enzymatic activity was identified as EC 3.2.1.37. In supplementary information given by the company of January 1998, the EC classification was revised and is now identified as EC 3.2.1.8. The enzyme preparation also contains a variable amount of other side activities such as cellulase, betaglucanase and amylases.

The active substance represents 5 % of the final powder preparation and 2.5% of the final liquid preparation. The final powder preparation contains a minimum specified activity of 600 xylanase units per gram. The liquid preparation contains a minimum specified activity of 300 xylanase units per milliliter.

There are two forms of presentation: powder and liquid. 5 % of final powder preparation and 95 % of dried fermentation media composes the powder form. The liquid preparation is derived from the powder form by dilution. The diluents (97,5%) are glycerol, water and sodium chloride at the following rate 50:50 of water and glycerol with 1 % of NaCl. *E. coli*, *Salmonella* and heavy metals are analyzed and monitored routinely, since the final product is a dilution of the powder into a dried fermentation media.

The additive was previously assessed by SCAN for use in chickens for fattening (broilers) and the assessment of the safety included: acute inhalation toxicity, skin and eye irritation, Ames test, chromosomal aberration and sub chronic toxicity such as a 90-day oral toxicity in rats. The 90 days oral toxicity (test in rats) was carried out with the active substance at dietary concentrations of 750, 8000 and 22 000 ppm, which represent a daily intake of 22.5, 240 and 600 Units of Endo-1,4-beta-xylanase (Pentosanase) per day. Therefore the present assessment, being an extension of use to turkeys for fattening, is limited to tolerance tests on this target animal. The company proposed to extend the use of Allzyme PT in turkeys and recommends to use that enzyme preparation in a range between 300 – 1200 units per kg of complete feed.

A first assessment by the Scientific Committee for Animal Nutrition (SCAN) on the basis of the initial dossier submitted by the Company to support the extension of use of its product to turkeys for fattening led the SCAN to ask for further information before reaching a final conclusion. The following questions were addressed to the company in the course of 2002 through the Member State rapporteur:

(1) There are two forms of presentation of the product Allzyme PT: powder and liquid. The powder form includes 5 % of the final powder preparations and 95 % of dried fermentation media. Information should be given on the origin of the fermentation media used to dilute the active substance and the quality control applied to that carrier

(2) It appears from tolerance test that females (fed Allzyme PT) weight was significantly different from weight of the control animals. This was considered not relevant in the dossier because of the weak experimental design of the study. This is not convincing for SCAN and new tolerance test should be carried out. The new tolerance test should comply with the requirements (duration of study, levels of inclusion…) of the guidelines for assessment of additives in feedingstuffs, part II: enzymes and micro-organisms adopted by SCAN in 2001 and available on its internet site.

4. COMPOSITION OF THE PRODUCT

The company stated that 95% of the final enzyme preparation (carrier) is produced on-site as of a yeast production process. The company also indicates that the yeast produced is Yea-Sacc ^{1026}, a product already assessed for calf, cattle for fattening and dairy cows.

A detailed description of quality control procedures used during the fermentation process of Yea-Sacc^{1026} is given. The limits have been identified for microbial analysis, mycotoxins and heavy metals. The safety toxicological studies are based on acute inhalation, dermal irritation, eye irritation, contact sensitisation, gene mutations in bacteria (Ames test) and chromosomal aberration *in vitro*. The absence of antimicrobial activity following WHO screening method is also reported.

In conclusion, the safety of the dried fermentation media used as carrier appears studied in accordance with the guidelines.

5. TOLERANCE TEST IN TURKEYS FOR FATTENING.

The company carried out a new tolerance test in order to assess the tolerance of turkeys fed with ten times the maximum recommended dose. This new test was in compliance with the SCAN recommendations.

Table 2 Experimental design. (M: male¸ F: female)

No	Treatment description	Route	Pens per treatment	Birds per pen	Birds per treatment
T-1	Control	Feed	12	10 F	120
T-2	Control	Feed	12	10 M	120
T-3	Tolerance 13673 Xu/Kg	Feed	12	10 F	120
T-4	Tolerance 13673 Xu/Kg	Feed	12	10 M	120

Experimental conditions: 480 (BUT 9), 240 males and 240 females were used with and initial age of one day. The experiment lasted 56 days (8 weeks). The birds were divided in two groups: control and tolerance group. The experimental design was a factorial two feed groups and two sex (2x2).

The analysis of enzyme and feed were conducted. The average of xylanase enzyme present in crumbs feed (0-4 weeks) and pelleted fed (4-8 weeks) was 13673 XU/kg of feed a little bit superior as expected 12000 XU/kg.

Parameters recorded: Animal performance (Feed intake, Body weight, feed conversion and mortality), blood chemistry, hematology and post mortem evaluation were conducted. Data were statistically analysed by ANOVA as a randomized block experiment with a factorial treatment (dietary treatment X sex).

As it can be seen in table 3, the feed intake and body weight of turkeys were not affected by dietary treatment. In the case of feed conversion rate the effect of supplementation of Allzyme PT was positive. The effect of sex was significantly demonstrated. Dead birds were sent to post mortem inspection and veterinary inspection was conducted every week. No effect on mortality was found due to dietary treatment.

At the end of the experiment two birds from each pen were randomly selected in order to sample blood. Carcass evaluation and post mortem examination including an anatomical gross examination was produced with the same group of birds. No negative effect was found in any of the analytical parameters examined.

Table 3. Feed intake, body weight, feed conversion rate and mortality of turkeys fed with control and ten times the maximum recommended dose of Allzyme PT.

Dietary treatment	Female	Male
Feed intake 7-56 days, Kg feed / bird		
Control	7.257 a	8.513 b
Tolerance Allzyme PT	7.198 a	8549 b
Body weight at 56 days, Kg		
Control	4.252 a	5.211 b
Tolerance Allzyme PT	4.289 a	5.369 b
Feed conversion		
Control	1.73 a	1.65 c
Tolerance Allzyme PT	1.71 b	1.61 d
Mortality %		
Control	0.83	0.83
Tolerance Allzyme PT	0.83	3.33

Number in rows and columns with different letters differ significantly ($P<0.001$)

6. CONCLUSION

Data provided by the company both on the fermentation media used in the product and on the tolerance test allow SCAN to conclude that the product does not harm the turkey for fattening when used under the conditions proposed and summarised in Table 1.