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 BELFEED B1100 MP/ML® FOR USE AS FEED ADDITIVE FOR TURKEYS FOR FATTENING

Adopted on 18 April 2002

1. BACKGROUND

The product "Belfeed B1100 MP/ML" preparation of endo-1,4-beta-xylanase EC 3.2.1.8 produced by *Bacillus subtilis* (LMG S-15136) is already provisionally authorised for the use as feed additive for the animal category chickens for fattening under the name of "Belfeed B1100 MP for chickens for fattening - solid form". The Commission received a request for a provisional Community authorisation for the animal category turkeys for fattening both in the solid and in liquid forms, under the conditions set out in the following table:

No	Additive	Chemical formula, description	Species or category of animal	Minimum Content	Maximum Content	Other provisions
				IU/kg of complete feedingstuff		
ENZYMES						
51	Endo-1,4-beta-xylanase EC 3.2.1.8	Preparation of endo-1,4-beta-xylanase EC 3.2.1.8 produced by *Bacillus subtilis* (LMG S-15136) having a minimum activity of: Solid and liquid: Endo-1,4-beta-xylanase: 100 IU/g[1]	Turkeys for fattenning	10 IU		1. In the directions for use of the additive and premixture, indicate the storage temperature, storage life, and stability to pelleting. 2. Recommended dose per kilogram of complete feedingstuff: Endo-1,4-beta-xylanase: 10 IU/Kg 3. For use in compound feed rich in arabinoxylans e.g. minimum 40% wheat or barley.

[1] IU is the amount of enzyme which liberates 1 micromole of reducing sugars (expressed in equivalent of xylose) from xylan per minute at pH 4.5 and 30°C

The company producing "Belfeed B1100 MP/ML", prepared a dossier that has been submitted through the national rapporteur (Belgium) 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 18th of September 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 18th of September 2001.

2. TERMS OF REFERENCE

The Scientific Committee for Animal Nutrition (SCAN) is requested to give an opinion on the safety of "Belfeed B1100 MP/ML®" preparation of endo-1,4-beta-xylanase EC 3.2.1.8 produced by *Bacillus subtilis* (LMG S-15136) for Turkeys for fattening, when used as feed additive under the conditions presented in the above table.

3. SCAN OPINION

3.1. History

Belfeed 1100 MP is an enzymatic preparation already assessed for feed additive use in broilers and piglets (January 25, 2001).

Such assessment implies compliance with safety assessment requirements; these requirements include absence of toxic effects in a bacterial mutagenicity assay and an in vitro test for chromosome aberrations, absence of skin and eye irritation and acute inhalation toxicity, and absence of adverse effects in a 90day rodent repeated dose toxicity study.

For the specific target species a tolerance study is required.

3.2. Target animal category safety

The tolerance study in fattening turkeys was carried out in young birds (51 g) during 4 weeks, 50 males per group (negative control and Belfeed ML 10 x overdose, *i.e.* 100 IU kg/feed). Only the granular form (MP) was tested. Parameters were weight gain, feed intake and feed conversion.

Mortality was daily registered. Gross pathological examination was performed when mortality appeared Statistical analysis of variance (Tukey test) was performed with individual turkey weights.

No peculiar findings were observed in behaviour. The production parameters were comparable with the recommended dose, and were improved compared to the negative controls. Thus a tolerance margin of 10 x can be established.

The Company did not present any tolerance study for the liquid form. It justifies the non provision of a dedicated tolerance test by the fact that the activity of Belfeed 1100MP and Belfeed B1100ML are the same (min 100 IU/g) and that the carrier (wheat flour or glycerol) is food grade.

It seems reasonable to assume that the general toxicological aspects as studied in laboratory studies and which are mainly aimed at possible toxic contaminants or irritation, are not influenced by this different formulation. For the target species, the only difference is the carrier glycerol instead of flour. As glycerol is approved unconditionally as additive, a tolerance test for this formulation is indeed not considered necessary.

3.3. Toxin production by the enzyme producing strain

For enzymes and micro-organisms from the *Bacillus* family additional safety requirements apply with respect to the toxin production potential.

The strain used for the production of Belfeed 1100M, *B. subtilis* 168 has undergone transformation with multicopy xylanase gene from another *B. subtilis* strain. No other extra DNA in addition to Bacillus sequences is added in the construct.

The host strain has been tested for the production of enterotoxin-like and bacillar toxins and emetic toxins. The assays were the Vero cell test (as recommended in the SCAN opinion on the safety of bacillus species) for enterotoxin detection and the boar sperm motility test for the detection of emetic toxins. Both tests were negative.

On the basis of these data the SCAN considers that the absence of toxin production potential of the bacillus strain used for the production of Belfeed 1100 M has been satisfactorily demonstrated.

3.4. Conclusion

On the basis of the above, the data presented by the company and the previous SCAN opinion, Belfeed MP and ML can be considered safe for turkeys for fattening under the proposed conditions of use.