EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

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

OPINION OF THE SCIENTIFIC COMMITTEE ON ANIMAL NUTRITION ON THE SAFETY OF THE USE OF PRODUCT ORALIN® IN TURKEYS

(Adopted on 17 October 2002)

1. BACKGROUND

The product "Oralin" *Enteroccocus faecium* -DSM 10 663/ NCIMB, is already provisionally authorised for the use as feed additive for the animal categories piglets, calves and chicken for fattening. The Commission received a request for a provisional Community authorisation for the animal category "Turkeys for fattening" under the conditions set out in the following table:

No.	Additive (trade name)	Chemical formula, description	Species or category of animal	Maximum age	Minimum content	Maximum content	Other provisions
					CFU/kg of complete feedingstuff		
Micro-organisms							
13	*Enteroccocus faecium* DSM 10 663/ NCIMB 10 415 Oralin	Preparation of *Enteroccocus faecium* containing a minimum of : Powder and granulated forms : 3.5×10^{10} CFU/g additive Coated form : 2.0×10^{10} CFU/g additive Liquid form : 1×10^{10} CFU/ml additive	Turkeys for fattening	-	1×10^{7}	1×10^{10}	May be used in compound feed containing the permitted antibiotics, coccidiostats and other medicinal products: flavophospholipol, amprolium-ethopabat, diclazuril, halofuginone, lasalocid sodium, maduramicin ammonium, meticlorpindol/ methylbenzoquate, monensin sodium, robenidine, nifursol.

The company Chevita GmbH, Germany producing Oralin prepared a dossier that has been submitted through the national rapporteur (Germany) to the Commission. The dossier was checked by the Member States for its compliance with the requirements of Council Directive 87/153/EEC fixing guidelines for the assessment of additives in animal nutrition. The Member States concluded in the Standing Committee of Animal Nutrition on 27 April 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 of Animal Nutrition started the evaluation of the product on 27 April 2001.

2. TERMS OF REFERENCE

The Scientific Committee for Animal Nutrition (SCAN) is requested to give an opinion on the following questions:

2.1. Is the use of *Enteroccocus faecium* - DSM 10 663/ NCIMB 10 415 safe for the target species: turkeys?

2.2. Is the product compatible with the following products: flavophospholipol, amprolium, ethopabat, diclazuril, halofuginone, lasalocid sodium, maduramicin ammonium, meticlorpindol/ methylbenzoquate, monensin sodium, robenidine, nifursol?

2.3. Considering the recent scientific data published on the possible virulence factors associated with *Enterococcus* strains, does the *Enterococcus faecium* strain involved in product Oralin® harbor any virulence factor?

3. OPINION OF THE COMMITTEE

3.1. Product description and intended use

Oralin is a microbial feed additive based on a single strain of *Enterococcus faecium* (DSM 10663, NCIMB 10415) and is already included in the Annex to 70/524/EEC for use with calves, piglets and chickens for fattening. The general aspects of the safety assessment were made at that time. The manufacturer is now seeking an extension of approval to include turkeys for fattening, which requires additional data specific to the target species.

The product is supplied in three forms, a granulated product containing 3.5 x 10^{10} c.f.u./kg additive, a coated form containing 2 x 10^{10} c.f.u./kg additive and a liquid form containing 1 x 10^{10} c.f.u./l additive and is stable. No loss of viability could be detected when the product was mixed with turkey feed and stored at ambient temperatures for a period of 12 weeks.

The proposed dose range for turkeys (1 x 10^{7} to 1 x 10^{10} c.f.u./kg complete feed) covers a three log range but no reasons were given in justification for this range. However, the maximum value is the only one of concern to a

safety assessment. The minimum value is of relevance to the demonstration of efficacy, which is not considered here.

3.2. Effects on turkeys

A tolerance test was made with 32 birds divided into control and test groups of equal numbers. The test group received the liquid form of Oralin sprayed onto a commercial turkey finisher feed at x10 the maximum dose level claimed (1×10^{11} c.f.u./kg complete feed) for a total of 22 days. No mortalities were observed and all birds developed normally. The mean group values for weight gain and feed intake were depressed in the test group (by approximately 8% and 20% respectively) compared to the control birds but the feed to gain ratio was improved. However, as only a small number of birds were used none of these changes reached significance (Mann-Whitney-U Test, with control for sex). The Company and the independent laboratory responsible for this study were unable to offer an explanation for this reduced intake. Haemoglobin content, haemocrit and erythrocyte, leukocyte and thrombocyte counts were not significantly ($P>0.05$) influenced by treatment. No adverse pathological findings (organ weight and tissue histology) were found on dissection at the end of the study.

No evidence of reduced feed intake or any other adverse effects were seen during a second study made with a larger number of birds (768) for a longer period (104 days) when bird were dose to a maximum of 1×10^9 cfu /kg feed.

An extensive microbiological study of the faecal flora and the gut flora of 30 poults between 4 and 18 days of age administered 1×10^9 c.f.u./kg feed between days 4 and 11 did not show any significant changes in numbers of enterobacteriaceae, *E. coli* or lactobacilli when compared to birds in the control population. *Ent. faecalis, Ent. durans* and *Ent. gallinarum* were the predominant enterococci in the faeces of untreated birds. *Ent. faecium* was only found in treated birds in addition to the other species. No differences were noted in the jejunum/ileum or caecum other than the presence of *Ent. faecium* in the jejunum/ileum of treated birds at the end of the study with numbers between 10^5 and 5×10^5 cfu/g and in the caeca of some treated birds after the feed administration period.

3.3. Virulence factors

The active strain was screened for virulence determinants using PCR. Known positive control strains were included and, in each case, amplification occurred demonstrating that appropriate primers had been selected. *Enterococcus faecium* DSM 10663 was free of genes encoding the known virulence determinate with the exception of *efaAfm* coding for a cell wall adhesin. However this gene appears commonly distributed amongst strains of *Ent. faecium* having been found in 82% of starter culture, food and clinical isolates (n=49) by Eaton and Glasson (2001) and all 18 *Ent. faecium* strains examined by the company. Although possibly a contributory factor in virulent strains, adhesion to mucosal surfaces brings other ecological benefits for organisms of gut origin and, in the absence of other virulence determinants, is most probably not a cause for concern.

3.4 Compatibility with antibiotics, coccidiostats and other medicinal products permitted at the time of application

The production strain was shown to be resistant *in vitro* to:

The antibiotic/coccidiostats:

Amprolium (100 µg/ml), ethopabat (100 µg/ml), aprinocid (100 µg/ml), decoquinat (50 µg/ml), diclazuril (100 µg/ml), dinitolmide (100 µg/ml), halofuginone (100 µg/ml), lasalocid (100 µg/ml), maduramycin (10 µg/ml), meticlorpindol (100 µg/ml), methylbenzoquat (100 µg/ml), monensin (100 µg/ml), narasin (10 µg/ml), nicarbazin (100 µg/ml), robenidine (100 µg/ml), salinomycin (10 µg/ml).

Other medicinal substance:

Nifursol (10 µg/ml).

A statement by the Company that the product strain was also resistant to flavophospholipol was not supported by any documented evidence.

4. CONCLUSION AND RECOMMENDATION

The unexplained reduction in feed intake and consequent effect on weight gain when turkey poults were dosed with 1 x 10^{11}cfu/kg feed is a possible cause for concern. However, the absence of adverse post mortem findings suggests that any effect may be marginal. Nonetheless it would seem prudent to reduce the maximum permitted application level for turkeys to 1 x10^{9}. This is the maximum application rate used in studies on the efficacy of the product with turkeys and the maximum permitted for use with chickens for fattening. Use at this level produced no observable adverse effects in turkey poults. Should the Company wish to retain the maximum rate of 1 x 10^{10}, then this should be supported with further studies. Otherwise, SCAN concluded that the product was safe for use as a feed additive for turkeys for fattening under the conditions described by the company.

The product is compatible with permitted coccidiostats and with nifursol and could be used in conjunction with these additives. Compatibility with flavophospholipol has not been demonstrated.

5. REFERENCE

Eaton, T.J. and Glasson, M.J. 2001. Molecular screening of *Enterococcus* virulence determinants and potential for genetic exchange between food and medical isolates. *Applied and Environmental Microbiology* **67**, 1628-1635.