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 MICRO-ORGANISM PRODUCT PROVITA E® FOR USE AS FEED ADDITIVE

(Adopted on 23 January 2003)

1. BACKGROUND

The product Provita E®, *Enterococcus faecium* DSM 7134, is intended for the use as feed additive in accordance with Council Directive 70/524/EEC. The strain is already authorised in the product "Bonvital LE", being part of a mixture with *Lactobacillus rhamnosus* DSM 7133. The Commission received a request for provisional Community authorisation of this product under the conditions set out in the following table:

Additive	Chemical formula, description	Species or category of animal	Maximum age	Minimum content	Maximum content
				CFU/kg of complete feedingstuff	
MICRO-ORGANISMS					
Enterococcus faecium DSM 7134	Prepared from *Enterococcus faecium* Minimum content: Powder: 1×10^{10} CFU/g of additive Granules: (micro-encapsulated) 1×10^{10} CFU/g of additive	Piglets	2 months	0.5×10^9	4×10^9
		Fattening pigs		0.5×10^9	1×10^9
		Sows		0.5×10^9	1×10^9

The company producing Provita E® prepared a dossier that has been submitted through the national rapporteur (Austria) 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 7th of June 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 7th of June 2001.

2. TERMS OF REFERENCE

The Scientific Committee for Animal Nutrition (SCAN) is requested to give an opinion on the safety of *Enterococcus faecium* DSM 7134:

(1) for the target animal categories:

  (a) Piglets

  (b) Fattening pigs

  (c) Sows

(2) for the user

(3) for the consumer

(4) for the environment

under the conditions of use identified in the above table.

3. OPINION OF SCAN

3.1. Product description

The active component of Provita E is a single strain of *Enterococcus faecium* isolated from "plant material" and deposited in the German Culture collection as DSM 7134. It has not been the subject of genetic modification.

The product is produced in two forms, as a powder and in a granulated (microencapsulated) form. The powder form is considered by the company as optimal for premixes in feed formulations of approximately similar particle size in order to obtain homogenous mixtures. It is produced by mixing a concentrated culture solution (3% by weight) with sweet whey powder (96%) and variety of other minor components (antioxidants etc) representing a total of 1% and spray drying the mixture. Granulation is achieved by spraying the same concentrated culture (3%) into a fluidised bed of saccharose (70% product weight), maltodextran (20%) and the same minor components used in the powder form (7%). Both powder and granules are formulated to contain 1x 10^{10} cfu/g product. The product is said to be stable at 4^{o} and 20^{o}C with no significant loss of activity after 12 months storage. The granular form is also said to resist conventional pelleting, also with no significant loss of activity.

Both formulations are routinely monitored for heavy metals and common mycotoxins (aflatoxins B_1 and M_1, deoxynivalenol, ochratoxin and zearalenone) The product is also monitored for microbial contamination (coliforms, < 10 cfu/g, *Clostridium* spp., < 10 cfu/g, *Salmonella* spp., none detectable in 25g product and fungi < 100 cfu/g).

Strain identification is based both on biochemical characteristics and RAPD-PCR. Genetic stability was demonstrated using protein and DNA methods

applied to the master strains, working strain bank and fermentations. No plasmids have been detected in this strain

3.2. Intended use

The product is intended for use as a feed additive with growing pigs of all ages to slaughter and with sows. The recommended dose is 5 x 10^8cfu/kg complete feed for piglets to two months of age (maximum 4 x 10^9cfu/kg), growers to four months of age (maximum 1 x 10^9cfu/kg) and with sows (maximum 1 x 10^9cfu/kg). A lower dose (5 x 10^8cfu/kg complete feed) is recommended for finishing pigs above four months of age with a maximum dose of 1 x 10^9cfu/kg.

3.3. Effects on target animal

3.3.1. Piglets

A four week tolerance test was performed on 28 days old piglets, which were divided into four groups of 6 animals. One group served as control, while three other groups received increasing doses of Provita E (10^9, 10^{10} and 10^{11} cfu/kg feed, respectively). The highest dose is 25 times higher than the maximum recommended dose ($4x10^9$).

During the test period the weight development, feed intake, faecal consistency and general wellbeing of the piglets in control and test groups were observed. At the end of the test the animals were sacrificed and autopsied, and the intestinal organs macroscopically examined. Haematological examination was done on blood samples obtained immediately before the sacrifice.

During the trial the piglets were in a good state of health. Feed intake was higher in all Provita E-groups than in the controls, especially at the two lowest dose levels. This was also reflected in the weight gain development.

No adverse effects were detected at autopsy in any of the test groups. Nor were there any treatment-related changes in the measured blood parameters.

3.3.2. Sows

In the tolerance test with pregnant sows the number of animals was 32 divided into four groups of 8 animals each. The Provita E doses in the test groups were 7.5 x 10^8 9.6 x 10^9 and 3.6 x 10^{10} cfu/kg feed, the highest dose corresponding to 36 times the highest recommended level.

The duration of the test was from the 90^{th} day of pregnancy to the next insemination. Besides the observations on sows (weight development, body temperature, feed consumption) also the numbers

of piglets in the litters and their development until the withdrawal were recorded.

No adverse health effects were observed in sows. In Provita E-treated groups, weight losses during lactation were smaller than in the control group. There was a tendency to higher numbers in litters and improvement in the weight development of the piglets in the test groups.

3.3.3. Effect on gastrointestinal flora

The gastrointestinal flora of the treated piglets showed, as expected, an increase in the number of enterococci in the intestinal tract and a tendency for reduced numbers of enterobacteriaceae. At the doses of 10^9 and 10^{10} cfu/kg feed this decrease was statistically significant ($P < 0.05$).

In a separate experiment with 30 pregnant sows, 14 of which served as controls, the rest receiving the recommended dose of Provita E from the 90^{th} day of pregnancy until the 28^{th} day of lactation, there was no difference in the faecal *E. coli* counts of the sows. Nor were the numbers of potentially pathogenic adhesive or haemolytic variants affected. The results indicate that the Provita E supplementation of the sow feed does not expose piglets to increased shedding of maternal enterobacteriaceae.

3.4. Antibiotic resistance and virulence determinants

The strain is sensitive to ampicillin, chloramphenicol, tetracycline, vancomycin, trimethoprim, ciprofloxacin, gentamicin, streptomycin and linezolid. It is resistant to macrolides (lincosamid and erythromycin, the latter described as intermediate at 6 mg/l). The results with rifampicin were variable (<0.5 at 16 hours-sensitive and 8 mg/l at 20 hours–resistant against the SCAN breakpoint of 4). This resistance is borderline and may result from slight variations due to methodology.

PCR methods demonstrated the absence of *ermA, ermB* and *ermC* Conjugation experiments made with vancomycin resistant strains with the production strain as recipient showed a low rate of uptake of 1.2×10^{-6}.

A full study was made of ten virulence factors by PCR in the production strain and compared to known positive controls. The positive controls all gave amplification products confirming the suitability of the primer pairs selected. *Enterococcus faecium* DSM 7134 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)[1]. 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.

3.5. Worker safety

A full laser-based particle size analysis was made with both formulations. The powder had a mean particle size of 80.1 μm with approximately 10% having a particle size of 10 μm or less. This represents the potentially respirable fraction. As would be expected the dusting potential as measured by the Stauber-Heubach test of this formulation was substantially higher than the granulated form with a value of 1.146 g/m^3. The powder formulation should be regarded as a potential respiratory sensitiser, and appropriate protective measures should be adopted. The mean particle size for the granulated form was 920 μm with no particles below approximately 250 μm being detectable. The dust content as measured by the Stauber-Heubach test is 0.019 g/m^3. The granulated form is neither inhalable or respirable and consequently would not be expected to cause problems of respiratory sensitisation.

3.6. Conclusions

The strain appears to be safe for the target animals. Tolerance test was not performed on fattening pigs, but this can in this case be accepted because the tolerance was tested on a more sensitive target animal category (piglets) and also on pregnant and lactating sows. Regarding the latter animal category, the safety has been demonstrated only for the period immediately before parturition and six weeks thereafter. SCAN considers that the authorisation should be restricted to this period. This would still meet the primary purpose of providing an alternative route for exposure of the new born piglets to the product.

The strain is not resistant to clinically important antibiotics to a degree to cause concern. Because of the lack of virulence factors it should not present any danger either to the worker handling the feedingstuff or the consumer incidentally exposed to the strain *via* animal products. Since enterococci are common commensals in the intestinal tract, the use of the strain does not have any foreseeable environmental consequences.

---

[1] Eaton, T.J. and Glasson, M.J. (2001). Molecular screening of *Enterococcus* virulence determinants and potential for genetic exchange between food and medical isolates. Appl. Environ. Microbiol. **67**:1628-1635