EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

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

Opinion on the use of certain micro-organisms as additives in feedingstuffs

Expressed, 26 September 1997
Updated 25 April 2003

TERMS OF REFERENCE (September 1996)

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

1. Is the use of the micro-organisms shown in the annexed list[1] safe to corresponding animal species under the conditions proposed?

2. Can their use result in development of resistance in bacteria to prophylactic or therapeutic preparations or exert an effect on the persistence of bacteria in the digestive tract of corresponding animal? Is or can the micro-organism become resistant to antibiotics?

3. Do the products indicated in the annexed list contain or consist of genetically modified organisms within the meaning of Article 2-1 and 2-2 of Council Directive 90/220/EEC[2]? If it is the case, was a specific environmental risk assessment carried out, similar to that laid down in the above-mentioned Directive, is the outcome satisfactory in view of the requirements of this Directive?

4. Do the toxicology studies allow to conclude that the proposed use does not present risks to the consumers, to the users ?

5. In the light of the answer to the above-mentioned questions, are the proposed conditions of use acceptable?

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1 The List – not attached to this opinion - contained a list of those micro organisms submitted at the time of the original question.

2 On the deliberate release into the environment of genetically modified organisms O.J. No. L 117, 08/05/90, p.15

BACKGROUND

1. Advances in scientific and technological knowledge permit the use of certain micro-organisms and their preparations in animal nutrition in order to improve the digestibility of nutrients or to stabilise the flora of the digestive system of animals and to reduce the quantity of certain environmentally undesirable substances.

2. The Council, by adopting Directive 93/114/EC[3] requested the inclusion of micro-organisms (also called probiotics) in Directive 70/524/EEC[4]. Consequently, the same requirements which apply to the authorisation of additives and to manufacturers in general also apply to them. According to Article 2 (1) of Council Directive 93/113/EC[5] and by derogation to Directive 70/524/EEC, Member States are allowed to temporarily use and market micro-organisms and their preparations in animal nutrition within their territory, provided that on the basis of the information available, the products do not present a danger to human or animal health, and that they are included in a national list.

   The Member States agreed to forward to the Commission and to other Member States the national list of authorised products and the information provided by the persons responsible for putting these products into circulation. Before 1 January 1997, according to the provisions of Article 5 of Directive 93/113/EC, a ruling should be given on the micro-organisms and preparations manufactured by them and which are part of the national lists.

3. The micro-organisms examined are part of the national list of products provided to the Commission by the Member States, pursuant to Article 3 (b), of Council Directive 93/113/EC. These products are currently marketed in the Member States. Safety is the major concern of the Commission.

4. The micro-organism issues were discussed at the 101st, 102nd and 103rd SCAN meetings. It was agreed to form an ad hoc working group to rapidly review the registration files which are considered "admissible" in regard of the prerequisite of Directive 93/113/EC. The objective is to rule out any potential safety concerns before a possible entry into annex II.

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3 Concerning the use and marketing of enzymes, micro-organisms and their preparations in animal nutrition (O.J. No. L 334, 31/12/93, p. 24)

4 O.J. No. L 270, 14/12/70, p.1., as amended by Council Directive 84/587/EEC (O.J. No. L319, 08/12/84, p.13)

5 Concerning the use and marketing of enzymes, micro-organisms and their preparations in animal nutrition (O..J. No. L 334, 31/12/93 p.17)

OPINION OF THE COMMITTEE

Introduction

Dossiers concerning the micro-organisms submitted to the Committee were evaluated . Not all dossiers were complete and for some of the micro-organisms listed in the annex to Question 85, a dossier has not yet been submitted. Therefore, to give an opinion which includes all micro-organisms is only possible on the basis of basic knowledge on the general characteristics and the pathogenicity of the species to which the probiotic strain belongs according to the information given in the submitted dossiers .

1 ***Is the use of the micro-organisms shown in the annexed list safe to corresponding animal species under the conditions proposed?***

The micro-organisms used as additives in feedingstuffs belong to the microflora of humans or animals (natural component of endogenous animal flora) and/or occur in normal soil and plant flora of the animal environment . The main habitat of most of the probiotic micro-organisms (except *Saccharomyces cerevisae*) is the intestinal tract and all micro-organisms are considered to be non pathogenic .

Potential harmful effects of most of the strains were tested in target animals. Application of multiple doses (100 to 10.000 fold) of probiotic micro organisms or spores had no negative influence on the weight gain or on the health status of animals . Investigations of organs and tissues demonstrated no negative effect, when performed .

Safety problems would only be expected from probiotic *Bacillus* and *Enterococcus* strains which belong to facultatively pathogenic genera. Strains from these genera have been described as opportunistic pathogens of animals and humans. *Bacillus cereus, subtilis*, and *licheniformis* as well as *E. faecium* are sporadically isolated from mastitis, pneumonia, urogenital infections, enteritis and septicemia, but mainly in animals and humans subjected to prolonged therapy, injuries, surgical treatment, poor hygiene or spoiled food . The use of probiotics will not raise any particular risk, because entereococci and bacilli are widely distributed in the natural environment. Data on the pathogenicity of *Enterococcus* or *Bacillus* strains must be given special attention in the dossiers. *Bacillus* strains must not produce enteric or emetic toxins, when assayed in reliable test systems.

2 ***Can their use result in development of resistance in bacteria to prophylactic or therapeutic preparations or exert an effect on the persistence of bacteria in the digestive tract of corresponding animal ? Is or can the micro-organism become resistant to antibiotics?***

Many of the micro-organisms are intrinsically resistant to some antibiotics. A minimum prerequisite for these micro-organisms to be accepted as feed additives is that they do not carry transferable resistance genes. Only in some dossiers is this question sufficiently answered.

All *Enterococcus* strains listed in the annex are sensitive to glycopeptide antibiotics. However, enterococci are developing resistance to glycopeptide antibiotics, a problem which was discussed in connection with the ban of avoparcin .

As the antibiotic sensitivity pattern of the probiotics listed in the annex does not differ from the patterns of wild type strains, new resistance genes are not introduced into animal husbandry with probiotics. Probiotic strains may acquire antibiotic resistance genes . For example, all tested *Enterococcus* strains accepted *vanA* genes from vancomycin resistant donor strains in vitro. However, in comparison with the normal flora, the risk for probiotic strains to acquire new resistance genes after usage is probably low, because probiotic strains probably do not colonize the intestinal tract efficently, and probably disappear within a few days after they are withdrawn from the feed. Therefore, one can reasonably expect that the spread of resistance genes would not be significantly influenced by probiotic strains .

The question of antimicrobial activity of probiotic microorganisms was not investigated in vitro or specifically addressed in the dossiers. Field experiments have shown that oral probiotic treatment does not significantly influence the digestive flora. According to some registration-files, there was only a slight decrease in the excretion of enterobacteriaceae when probiotic bacteria were added to the feed.

It should be recognised that the wide usage of enterococci as probiotics will lead to an increased concentration of enterococci in the animal population. In the future, the impact of this increased level of enterococc*i* on human health should be considered.

3 ***Do the products indicated in the annexed list contain or consist of genetically modified organisms within the meaning of article 2-1 and 2-2 of Council Directive 90/220/EEC ? and If it is the case, was a specific environmental risk assessment carried out, similar to that laid down in the above mentioned Directive, is the outcome satisfactory in view of the requirements of this Directive?***

The probiotics of the annexed list do not contain or consist of genetically modified or engineered microorganisms .

4 ***What are the nature and the persistence of excreted products derived from products indicated in the annexed list ? Can these products be prejudicial to the environment ?***

Orally administered probiotics are excreted as living cells or as non viable digestion products. The latter are of no significance for the environment . The persistence of excreted bacteria or yeasts in the environment depends on the nature of these microorganisms and can range from hours to months for

vegetative cells and up to years for spores. In general, probiotics of gut origin rarely thrive outside their natural environment.

Only in the case of *Bacillus* products, where spores can be excreted or vegetative cells can sporulate after excretion does the organism persist in the environment for years. However, spore forming *Bacillus sps* are natural inhabitants of soil and the wider environment. Consequently, their re-excretion into the environment does not pose a hazard.

Because other strains of *Bacillus cereus* can cause food poisoning, manufacturers should be required to detail practical test methods, suitable for use in food hygiene laboratories to differentiate their products from pathogenic organisms.

5 ***Do the toxicology studies allow to conclude that the proposed use does not present risks to the consumers, to the users?***

Toxicity should not be a problem, because only non pathogenic and non toxigenic microorganisms are allowed for use. The possibility that toxicological problems arise from the excipients in the final product should be excluded

The size of microbial cells makes dusting a hazard when the product is marketed as powder. Manufacturers should provide suitable advice on handling and operator safety based on the measured particle distribution in the final product.

6 ***In the light of the answer to the above questions, are the proposed conditions of use acceptable?***

Under the proposed conditions, the use of *Saccharomyces* strains and *Lactobacillus* strains (provided they do not carry transferable resistance genes) as probiotics is acceptable.

The use of *Enterococcus* and *Bacillus* strains (even when they do not carry transferable resistance genes) may be problematic and should be accepted only for clearly defined strains which have been tested negative for toxicity and pathogenicity *in vitro* and *in vivo*.

Annex

The Scientific Committee on Animal Nutrition concludes on the basis of the information provided and knowing of no adverse reports elsewhere that the following microbial products are safe for use as feed additives when used according to the manufacturers instructions and with the target animal categories specified.

The Committee also concludes that these products pose no risk to the wider environment, to those handling the preparations, or to individuals of any age consuming products derived from animals produced using the microbial feed additive.

Name	Active Constituent(s)	Culture collection and accession number	Target animal categories
Adjulact 2000®	*Streptococcus infantarius* *Lactobacillus plantarum*	CNCM I-841 CNCM I-840	Calves
Bactocell®	*Pediococcus acidilactici*	CNCM MA 18/5 M	Broilers
Biacton®	*Lactobacillus farciminis*	CNCM MA 67/4 R	Piglets
Bioplus 2B®	*Bacillus licheniformis* *Bacillus subtilis* [in a 1/1 ratio]	DSM 5749 DSM 5750	Piglets, sows and pigs for fattening Broilers and turkeys Calves
Biosprint®	*Saccharomyces cerevisiae*	BCCM / MUCL 39885	Beef cattle Piglets and pigs for fattening
Bonvital®	*Enterococcus faecium* *Lactobacillus rhamnosus*	DSM 7134 DSM 7133	Pigs for fattening Calves
Biosaf SC 47®	*Saccharomyces cerevisiae*	NCYC Sc 47	Piglets and sows Beef and dairy cattle
Cylactin LBC®	*Enterococcus faecium*	NCIMB 10415	Piglets and pigs for fattening Calves Broilers
Fecinor plus®	*Enterococcus faecium*	CECT 4515	Piglets and pigs for fattening Calves and beef cattle
Gardion®	*Lactobacillus casei* *Enterococcus faecium*	NCIMB 30096 NCIMB 30098	Calves
Kluyten	*Kluyveromyces marxiamus*	MUCL 39434	Dairy cattle
Lactiferm ®	*Enterococcus faecium*	NCIMB 11181	Calves Piglets

Lactobacillus acidophilus D2/CSL®	*Lactobacillus acidophilus*	CECT 4529	Broilers Laying hens
Levucell SB20®	*Saccharomyces cerevisiae*	CNCM I-1079	Piglets and sows
Levucell SC20®	*Saccharomyces cerevisiae*	CNCM I-1077	Beef and dairy cattle
Microferm®	*Enterococcus faecium*	DSM 5464	Piglets Calves Broilers
Mirimil-Biomin®	*Enterococcus faecium*	DSM 3520	Calves
Oralin®	*Enterococcus faecium*	NCIMB 10415	Pigs for fattening Calves Broilers
Primver Pro ®	*Enterococcus mundtii*	CNCM MA 27/4E	Lambs
Probios PDFM Granular®	*Enterococcus faecium* *Enterococcus faecium*	DSM 4788 / ATCC 53519 DSM 4789 / ATCC 55593	Broilers
Yea-Sacc®	*Saccharomyces cerevisiae*[1026]	CBS 493 94	Calves, beef and dairy cattle

Following the examination of dossiers submitted and clarifications received, the Scientific Committee on Animal Nutrition is of the opinion that the following microbial products **pose a risk** to human or animal health for the claimed animal categories or to the environment.

Name	Active Constituent(s)	Culture collection and accession number
Esporafeed Plus ® *	*Bacillus cereus*	CECT 953
Neoferm BS 10® *	*Bacillus clausii* *Bacillus clausii*	CNCM MA23/3V CNCM MA66/4M
Paciflor C10®	*Bacillus cereus*	CIP 5832 / ATCC 14893
Pronifer MSB® *	*Pediococcus acidilactici* *Lactobacillus plantarum* *Lactobacillus reuteri* *Lactobacillus brevi* *Lactobacillus casei*	CNCM MA 28/6B CNCM MA40/5B-p CNCM MA28/6E-g CNCM MA28/6R-p CNCM MA28/6U-g

*: See the relevant SCAN Executive Summary dedicated to this product

Following the examination of dossiers submitted and due to insufficient data provided by the Company, the SCAN cannot conclude on the safety of the following products.

Name	Active Constituent(s)	Culture collection and accession number
Reuteri ®	*Lactobacillus reuteri*	ATCC 55148
Seb volatili ®	*Lactobacillus acidophilus* *Lactobacillus salivarius* *Lactobacillus fermentum*	BCCM / LMG S-16515 BCCM / LMG S-16516 BCCM / LMG S-1657
Velab bovini ®	*Lactobacillus reuteri* *Lactobacillus reuteri* *Lactobacillus salivarius*	LMG S-16557 LMG S-16559 LMG S-16558
Velab suini ®	*Enterococcus faecium* *Lactobacillus reuteri* *Lactobacillus amylovorus*	LMG S-16555 LMG S-16554 LMG S-16556