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Probiotics for animal nutrition in the European Union. Regulation and safety assessment

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Abstract

Probiotics are alive micro-organisms, generally bacteria but also yeasts than, when ingested alive in sufficient amount, they have a positive effect on the health going beyond the nutritional ones commonly known. Probiotics may operate through a nutritional and/or health or sanitary effect. Micro-organisms used in animal feed in the EU are mainly bacterial strains of Gram-positive bacteria belonging to the types *Bacillus*, *Enterococcus*, *Lactobacillus*, *Pediococcus*, *Streptococcus* and strains of yeast belonging to the *Saccharomyces cerevisiae* species and kluyveromyces. While most of the species and genera are apparently safe, certain micro-organisms may be problematic, particularly the enterococci, which may harbour transmissible antibiotic resistance determinants and bacilli, specially those belonging to the *Bacillus cereus* group that are known to produce enterotoxins and an emetic toxin. The history and the current legislation in the European Union on probiotics feed additives including the requirements for the safety assessment for the target animal species, consumers, workers, and environment are presented.

Keywords: Probiotics; Micro-organism; Feed additives; European Union regulation; Safety assessment

1. Introduction

Probiotics are alive micro-organisms, generally bacteria but also yeasts than, when ingested alive in sufficient amount, they have a positive effect on the health going beyond the nutritional ones commonly known. Their use was linked with a proven efficacy on the gut microflora resulted in improved health status. Two main mechanism of action have been suggested and are summarised as follows: (a) *nutritional effect*, characterised by reduction of metabolic reactions that produce toxic substances, stimulation of indigenous enzymes and production of vitamins and antimicrobial substances; and (b) *health or sanitary effect*, distinguished by increase in colonisation resistance, competition for gut surface adhesion and stimulation of the immune response (Guillot, 2003); the last effect acting as 'bio-regulators of the gut microflora' and reinforcing the host natural defences. The probiotics would have therefore a role on the balance of gut microflora increasing the resistance to pathogenic agents, both through a strengthening of the intestinal barrier and stimulating directly the immune system. Micro-organisms used in animal feed in the European Union (EU) are mainly bacterial strains of Gram-positive bacteria belonging to the types *Bacillus* (*B. cereus* var. *toyoi, B. licheniformis*, *B. subtilis*), *Enterococcus* (*E. faecium*), *Lactobacillus* (*L. acidophilus*, *L. casei*, *L. farciminis*, *L. plantarum*, *L. rhamnosus*), *Pediococcus* (*P. acidilactici*), *Streptococcus* (*S. infantarius*); some others probiotics are microscopic fungi such as strains of yeast belonging to the *Saccharomyces cerevisiae* species and kluyveromyces. The bacterial families *Lactobacillus* and *Enterococcus* are present in great quantities, $10^7/10^8$ and $10^5/10^6$ colony forming units (cfu) per gram, respectively, in the digestive microflora of animals. *Bacillus* and *Lactobacillus* bacteria differ in many characteristics and that the *Bacillus* and the yeasts are not usual components of the gut microflora. While most of the species and genera are apparently

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safe, certain micro-organisms may be problematic, particularly the enterococci, which may harbour transmissible antibiotic resistance determinants and bacilli, especially those belonging to the *B. cereus* group that are known to produce enterotoxins and an emetic toxin.

2. History and legal basis in the EU on probiotics in feed

The microbial feed additives were covered by the Council Directive 70/524/EEC of 23 November 1970 concerning additives in feedingstuffs (OJ No. L 270, 14.12.1970). The Directive 70/524/EEC was amended five times; the last amendment was by the Council Directive 96/51/EC of 23 July 1996 (OJ No. L 235, 17.9.1996). In 2003, these Directives were repealed by the new Regulation (EC) No. 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition (OJ No. L 268, 18.10.2003) which sets out the rules for its authorisation, use, monitoring, labelling and packaging. In the Regulation (EC) No. 1831/2003, the micro-organisms are included in the category 'zootechnical additives' and as functional group within the 'gut flora stabilisers' defined as micro-organisms or other chemically defined substances, which, when fed to animals, have a positive effect on the gut flora. Under this Regulation, specific labelling requirements are needed for micro-organisms such as the expire data of the guarantee or storage life from the data of manufacture, the directions for use, the strain identification number, and the number of cfu per gram.

In the EU, there is a positive list of the micro-organisms to be included in market products; this list contains nine columns which describe the following specifications: (1) EU number, (2) additive, (3) chemical formula, description, (4) species or category of animal, (5) maximum age, (6) minimum content (colony forming units per kilogram, cfu/kg) in the complete feedingstuffs, (7) maximum content (cfu/kg) in the complete feedingstuffs, (8) other provisions, and (9) period of authorisation.

With respect to the guidelines for the assessment of micro-organisms, the Council Directive 87/153/EEC of 16 February 1987 established the composition of the submission dossier for all feed additives (OJ No. L 64, 7.3.1987). The Commission has updated these guidelines in 1994 introducing specific requirements for enzymes and micro-organisms (Commission Directive 94/40/EC, OJ No. L 208, 11.8.1994). In 2001, the Directive 87/153/EEC was amended by the Commission Directive 2001/79/EC of 17 September 2001 fixing guidelines for the assessment of additives in animal nutrition (OJ No. L 267, 6.10.2001). Currently, is in preparation a Commission Regulation on implementing rules concerning applications for authorisation of feed additives in accordance with Regulation (EC) No. 1831/2003; this new Regulation shall contain specific guidelines for the authorisation of feed additives (Article 7 of the Regulation). For that, the scientific panel on additives and products or substances used in animal feed (FEEDAP Panel) of the European Food Safety Authority (EFSA) has worked in the last time on specific guidelines regarding the additive categories including the micro-organisms using as background the adopted previous guidelines provide by Council Directive 87/153/EEC. These guidelines will be submitted to the Health and Consumer Protection Directorate-General of the European Commission for its consideration and establishment.

In the meantime, Scientific Committee on Animal Nutrition (SCAN) (European Commission, 2001a,b) has published its opinion concerning guidelines for the assessment of additives in feedingstuffs, Part II: enzymes and micro-organisms. The guidelines impose the layout of the submission dossiers based on six sections: (1) summary of the data in the dossier; (2) identity, characterisation, and conditions of use of the additive. Methods of control; (3) studies concerning the efficacy of the additive, (4) studies concerning the safety of the use of the additive; (5) form of monograph; (6) form of identification note.

3. Requirements for the assessment of microbial feed additives

3.1. Identity, characterisation, and conditions of use, methods of control

This section relates the *identity of the additive* (proposed proprietary name, type of additive according to its main function, qualitative and quantitative composition, qualitative and quantitative composition of any impurities, physical state of each form of the product and manufacturing process); *characterisation of the active agent(s)* (nomenclature, biological origin, genetic modification, compliance with release Directive for genetically modified micro-organisms (GMOs), toxin production and virulence factors, antibiotic production and antibiotic resistance, other relevant properties); *characterisation of the additive*: *physico-chemical and technological* properties (stability of the additive, other physico-chemical or biological properties, incompatibilities with other feed ingredients); *conditions of use of the additive* (technological and zootechnical additives, safety data sheet); *control methods* (general methods and description of the qualitative and quantitative methods for routine control of the active agent in premixtures and feedingstuffs).

3.2. Efficacy

Studies on efficacy of probiotics strains must be performed in target species/animal categories. The claims for microbial products are: improved performance and feed conversion for the target species; reduced morbidity or mortality which improves the welfare of the target species; benefits for the consumer through improved product quality and benefits for the wider environment. According to the current guidelines, the demonstration of these effects should be based on a minimum of three trials demonstrating a statistically significance ($P < 0.05$) on the specific