Egypt. J. Chem. Environ. Health, 1 (1):38-51 (2015)

Propbiotics in Animal Nutrition

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Abstract

Probiotics are living microorganisms which influence the digestive microflora of the host animal in a beneficial way. They develop their activity exclusively in the digestive tract. In animal nutrition, probiotics used as feed additives belong to one of three different groups: lactic acid bacteria, yeasts and Bacillus spores. They differ from one another in their properties, origin and mode of action. The main activity of probiotics is the maintenance and reconstitution of the equilibrium (eubiosis) of the intestinal microflora which is achieved by various modes of action. The prerequisite for their probiotic action is reaching the gastrointestinal tract alive. Once there, the probiotics support the intestinal microflora by means of specific metabolic activities and/or stimulation of the host's immune system. Undesirable microorganisms are thus reduced and protection is given against colonisation or attachment of harmful microorganisms. Probiotics therefore contribute to averting any disruption of the intestinal microflora (dysbiosis) as may occur during specific growing periods and situations of specific stress for the animals (for instance dietary changes, weaning, regrouping of animals etc.).

A safeguard of performance and health is thus achieved. Registration of probiotics follows a uniform EU procedure. Here, probiotics are evaluated especially regarding their quality, efficacy and safety for humans, animals and the environment. Therefore, only well-defined and safe microorganisms are used, for which the bioregulative properties have been validated under conditions of common feeding practice.

Introduction

Probiotics are live microbial feed supplements which beneficially affect the host by improving its intestinal microbial balance (Fuller, 1992). Correspondingly, in feed regulation, probiotics are included in the group of feed additives for stabilizing the microbial communities of the digestive tract in monogastric animals and ruminants. They are also known as digestive bioregulators or direct-fed microbials (DFMs). In a narrower sense, the term probiotics is confined to products which consist of one, or a few, well-defined strains of microorganisms (**WHO, 1994**).

Historically, bacteria and yeasts have served man very well in agriculture and nutrition. Well-known examples are the use of bacteria (mainly lactic acid bacteria) for production of silage, fermented cabbage (sauerkraut) and sour milk products such as yoghurt, cottage cheese and kefir and the use of yeasts (mainly *Saccharomyces cerevisiae*) for production of bread, beer and wine. Systematic research into probiotics for human use began at the beginning of the 20th century.

Elie Metchnikoff, studied the mystery of the high life expectancy of Cossacks in Bulgaria. He related their extraordinarily high life expectancy of 115 years and more to their very high consumption of fermented milk products. He named the microorganism relevant for the fermentation *Bacillus bulgaricus*, later classified as *Lactobacillus bulgaricus*, which was used against scours and gastrointestinal diseases in humans as early as the 1920s.

There was little interest in probiotics during the following decades until the 1960s and 1970s when they were rediscovered for human and animal nutrition. The first potent products for animal nutrition to fulfil the specific requirements for feed additives did not appear on the European market until the mid-1980s.

Today, modern animal nutrition has at its disposal a whole range of defined strains of probiotics belonging to the groups of lactic acid bacteria, Bacillus spores and yeasts.

What are probiotics used for ?

Man and animals are born with a sterile digestive tract, but very soon after birth a wide diversity of microorganisms begins to colonize the digestive tract [**Fonty et al 1995**]. The digestive compartments which are the most rich in microbes are the large intestine in monogastrics and the foregut in polygastric animals. An open and complex ecosystem is created which has an essential role for the host. On one hand, the digestive microflora is involved in digestion, on the other it has a local impact on the immune system, thus offering the possibility to exert a positive and completely natural effect on health, well-being and performance of the animal through its autochtonous microflora. There has been experience in this area for a long time and recently scientific work has been intensified. The main target of probiotics is therefore the digestive microflora and its functions as its stability are essential for the health and the nutrition of the host.

The various types of probiotics and their modes of action

The probiotics used in animal nutrition can be divided into three main groups: lactic acid bacteria, Bacillus spores and yeasts. Microbial strains used as probiotics differ from wild strains of the same species in some specific characteristics, especially with regard to their greater safety of use and their mode of action in the gastrointestinal tract. There are marked differences between the various probiotic groups regarding their properties, origin and mode of action

Lactic acid bacteria

Lactic acid bacteria have been used for millennia in the production of fermented milk products and silage. Some form the main intestinal microflora and are therefore an indispensable part of the resident microflora in man and animals.

Lactic acid bacteria convert certain types of sugars by fermentation, mainly into lactic acid. Some appropriate strains were chosen from a broad range of known species and developed as probiotic feed additives. Important lactic acid bacteria in probiotics belong to the genera Lactobacilli, Pediococci, Bifidobacteria and Enterococci. *Enterococcus faecium* (previously known as *Streptococcus faecium*) is the most important species used in animal nutrition.

According to current knowledge, the characteristic feature of probiotics producing lactic acid is mainly their metabolic activity in the intestine with the release of antimicrobial substances and the formation of a biofilm to protect the intestinal mucous membrane.

Several mechanisms of action have been identified in lactic acid bacteria, mostly from *in vitro* experiments [**Servin 2004**] :

- Production of inhibitory substances such as short-chain fatty acids and other antimicrobial substances providing a selection advantage, e.g by lowering the pH value, without suppressing the desirable intestinal microflora; this is the case for lactic acid and hydrogen peroxide.
- Exclusion of potentially pathogenic microorganisms and/or preventing them from adhering to the intestinal mucous membrane: by rapid proliferation the probiotic lactic acid bacteria form a barrier against other microorganisms in the intestine. Included in this line of defence are the mucopolysaccharides and other mucous substances produced by some of the lactic acid bacteria.
- Suppression of toxin production.
- Stimulation of the local immune system in the intestine : the mucous layer contains mainly bacteria of the main resident flora and immunoglobulins.

Another beneficial effect of the lactic acid bacteria on the host animal is the strengthening of non specific immunity.

- Influence on the physico-chemical conditions in the intestine, for instance on pH and redox potential, thereby limiting the growth conditions of undesirable microorganisms,
- Influence on the metabolism of bile acids and thus promoting the absorption of fat.
- Improved absorption capacity.

Bacillus spores

The genus Bacillus comprises a multiplicity of rod-shaped gram-positive microorganisms naturally found in soil. Some strains of this heterogeneous group have been chosen for the use in animal nutrition because of their beneficial effects (**Alexopoulos *et al*., 2004, Adami & Cavazzoni, 1998, Duc *et al*., 2004; Hoa *et al*., 2001; Jadamus *et al.*, 2002; Jørgensen & Kürti, 2004**). The natural ability of Bacillus probiotics to form spores offers a good protection against external influences. The viability of the microorganisms is thus preserved, even under strong challenges, which is essential for their activity. The optimisation of the sporulation process during manufacturing is therefore essential for good product quality.

When Bacillus spores are ingested with the feed, they germinate in the digestive tract and grow as vegetative cells but do not proliferate to a larger extent. Bacillus species do not colonize the intestine and are therefore, by definition, included in the transient flora.

As exogenous microorganisms, Bacillus probiotics have a high potential for stimulating local intestinal immunity **(Sanders et al., 2003**). The germination process, a typical feature of the Bacillus species, only takes place in the presence of nutrients, water and under warm conditions. To what extent this process is influenced by other factors such as pH has not yet been clarified.

Bacillus spores used as probiotics must germinate in the upper digestive tract in order to display their activity in those sections of the intestine which are relevant for nutrient absorption. Metabolism rises dramatically in the germinating spore, comparable to a sprouting grain. Metabolites are excreted into the environment and may be responsible for deteriorating the conditions for the development of pathogens.

Yeasts

Selected strains of the yeast *Saccharomyces cerevisiae* have been used by man for centuries for producing foods, for instance as bakery yeast and in the production of alcoholic beverages. Some of the numerous *Saccharomyces cerevisiae* strains that occur in nature were tested for their efficacy in the digestive tract and propagated in pure culture. Products consisting of live yeast cells and their dried culture media were then developed from them.

At the start of the 20th century, Indochinese used a native Indonesian cure for diarrhea by drinking tea made with tropical fruits (lychee and mango). It has since been discovered that the agent in the tea responsible for stopping diarrhea was a live yeast (*Saccharomyces cerevisiae* var *boulardii*).

Probiotic yeasts differ from brewery yeasts by their metabolic activity, the latter being fed in an inactivated form for their nutrient content.

Monogastrics

The genus Saccharomyces has 4 differents species. *Saccharomyces cerevisiae* has thousands of strains. Only a few *S. cerevisiae* strains are used for animal nutrition.
Some of these strains intestinal action result by:

- neutralization of certain bacterial toxins [**Castagliuolo et al 1998**]
- adherence of flagellate bacteria, due to the presence of mannose receptors. These pathogens are eliminated by feces [**Czerucka and Rampal 2002**]. Moreover, lactic bacteria, a beneficial flora, are increased.
- reinforcement of mucosal integrity and intestinal cells. Live yeasts have a documented efficacy on villi height and crypt depth, enhancing the assimilation of nutrients.
- modulation of the immune system by stimulation of IgA response to pathogens [**Qamar et al 2001**].

Finally, these intestinal beneficial effects optimize the growth potential of the monogastric animal.

<u>Ruminants and monogastric herbivores (equines):</u>

Studies have shown that probiotic yeasts (*Saccharomyces cerevisiae*) are metabolically active in the rumen and the small intestine after ingestion but their number is decreasing in the lower sections of the intestine [**Chaucheyras-Durand et al 1998, Dawson, K.A. et al 1990**]. Several mechanisms of action of these yeasts on the ruminal microbial growth and activity have been identified up to now. A very important activity, is the ability of live yeasts to consume oxygen, which is especially important in the rumen ecosystem [**Newbold 1995, Dawson et al 1991**]. Indeed, oxygen scavenging by the yeasts creates more favorable conditions for growth and activity of rumen anaerobic microorganisms [**Chaucheyras-Durand and Fonty 2002, El Hassan et al 1993**]. Oxygen is entering the rumen during feed intake, water intake, rumination, salivation. This action is particularly relevant for cellulolytic bacteria which are very sensitive to oxygen [**Fonty et al 1995, Girard 1996**]. The colonisation of the rumen of newborn ruminants by these bacterial community has been shown to be accelerated and its cellulolytic activity was stimulated in the presence of a probiotic yeast strain [**Chaucheyras-Durand and Fonty, 2001, 2002**]. Increased cellulolytical activity in the rumen increases nutrient digestibility, especially for diets rich in fibre. In horses, yeast probiotics also contribute to increase the digestibility of crude fibre in the caecum [**Medina et al. 2002**]. Yeasts have also been shown to regulate the ruminal pH and limit acidosis risks via interactions with lactate producing and lactate utilising bacteria [**Chaucheyras et al 1996, Michalet-Doreau and Morand 1996, Girard et al 1993, Girard Dawson 1994**]. Certain *Saccharomyces cerevisiae* strains are able to supply nutrients i.e. peptides, vitamins, organic acids and cofactors which may be required by the lactate utilising bacteria [**Chaucheyras et al 1996, Nisbet and Martin 1994, Rossi et al 1995, Girard 1996**] and can utilise soluble sugars more efficiently than lactate producing bacteria such as *Streptococcus bovis* [**Chaucheyras et al 1996**].

Activity in the digestive tract

It is possible to evaluate to what extent the probiotic remains stable during feed production and storage by detecting live microorganisms in the feed. However, this alone is not an indication of vitality and activity in the gastrointestinal tract. Probiotics must reach the site of their main activity in the digestive tract unharmed to be efficacious. This implies, for the groups of probiotics under discussion, that the growth of the yeasts and of the lactic acid bacteria and the germination of the spores must take place in the upper parts of the gastrointestinal tract. In the main target species this is the small intestine for monogastric animals, the crop for poultry and the rumen for ruminants. Since factors such as pH, the transit time of the digesta and the concentration of active substances in the feed can influence the growth of probiotics, their growth or germination in the digestive tract must be evaluated in feeding trials using diets which are relevant under practical conditions. This can be measured indirectly via performance parameters but, better, directly by counting the living probiotic microorganisms in the various intestinal segments.

Probiotics as feed additives

Feed regulation

The probiotics used in animal nutrition in the European Union must be registered as microbial feed additives. The manufacturers demonstrate the safety, efficacy and stability of their products by appropriate trials. Studies conducted in the laboratory and under practical conditions follow the requirements of the European Community for registration (Regulation 1831/2003 on additives in animal nutrition and the relevant guidelines to be published on the basis of Directive 87/153/EC on the establishment of guidelines for the evaluation of additives). These documents contain detailed information on the data required for identity, compatibility with other additives, and efficacy. In addition, comprehensive studies are requested to ensure that toxicity and transfer of resistance, which could endanger the effective treatment of diseases with antibiotic substances, are excluded. Registration comes into effect only after the European Food Safety Authority Panel on Feed Additives have positively assessed the quality and efficacy of the probiotic as well as its safety in humans, animals and the environment. The experts from the Member States authorize the use of the feed additive, on a proposal from the Commission, by adopting a Regulation authorizing the product. When the probiotic is registered, the microorganism contained is included in the register of feed additives of the above-mentioned Regulation. This register also includes the dosage range and the approved target species.

Recommendations for use

The stabilization of the digestive or microflora in ruminants and in monogastric animals can only be effectively achieved by continuous supplementation of the feed with probiotics because the microorganisms used in animal nutrition do not permanently colonize the intestine. Increased short-term supplementation of probiotics may be useful under certain conditions but should be followed by continuous supplementation thereafter. General guidelines on the optimal dosage and the period of supplementation are not possible because factors such as stability of the probiotic in the feed and in the digestive tract, the specific mode of action of the microbes contained in the product and the status of the intestinal microflora in the host all modulate the effect of the corresponding product. It is hence not possible to deduce the inclusion rate for feed from the content of the colony forming units (CFU) alone.

Consequently, the efficacies of different products cannot be compared on the basis of the declared CFU content. On the contrary, the optimal dose must be determined individually for each product and each target species in feeding trials. The rate of inclusion given by the manufacturer, therefore, is based on information gained from efficacy studies.

In general, however, it is accepted that the inclusion rate of all probiotics should be higher when the intestinal microflora is unstable and particularly when for ruminant the diet composition contain high rapid-fermentescible sugar which can entail sub-acidosis. In addition, the overall consumption of probiotics by older animals will be higher because of a higher feed intake compared to younger animals. Therefore, with continuous supplementation, the inclusion rate may be reduced during the growth of the animals without the concentration of the probiotic microorganisms in the intestine dropping below the level of efficacy.

In general, higher concentrations of probiotics in feed are recommended when:

.- the intestinal microflora is not yet established, e.g. in young animals .

- the intestinal microflora is disturbed by stress factors such as change of feed, transportation and climate .
- an increased infection pressure is expected (mixing animals of different origin, climatic influences) .
- the feed composition encourages the proliferation of pathogenic microorganisms in the digestive tract (increased content of buffer ingredients such as proteins, phosphorus and calcium, .low crude fibre content) .
- the intestinal microflora is compromised by the use of therapeutics, especially antibiotics ;

For ruminant animals, at the weaning period and for the transition feed period, when the diet is composed of high rapid-fermentescible carbohydrates .

The dosage of probiotics is defined as weight units per tonne of compound feed. The content of the microorganisms (given in CFU per gram) varies between the different product formulations which may frequently cause confusion over the corresponding inclusion rates in premixes and compound feed. Also, the declaration of activity units (CFU/g) differs from the weight-based units (mg/kg) used for most other additives.

Compatibility with other active ingredients

Premixes of active substances and compound feeds contain many substances which must be checked with one another for compatibility. The stability of the probiotics used and their availability and efficacy in the animal must be ensured. Since active antibiotic substances in particular inhibit microorganisms, the question arises whether they reduce the activity of probiotics. At first glance it therefore may seem contradictory to put probiotics and antibiotics into a feed together. However, studies indicate that positive combinatory effects can be achieved by suppressing the pathogens with antibiotics and at the same time supporting the intestinal microflora by probiotics.

As for microorganisms, the efficacy of which depends to a large extent on their metabolic activity in the intestine (multiplication, germination of spores), it must be ensured that this activity is maintained despite the presence of antibiotics. According to feed regulation, the use of probiotics together with performance enhancers and coccidiostats is legal. Those substances which can be combined are included in the approval of each probiotic.

Production and quality control

Selection of production strains

Microorganisms chosen for the production of probiotics are subject to a careful selection process. They are isolated from their natural environment and subjected to specific studies. First, microbiological tests and selection procedures are carried out to evaluate their suitability for fermented to which metabolites (example: API assay for the fermentation of sugar to lactic acid).

Comprehensive and accurate characterisation of the microorganism is also necessary. Amongst others, the genetic fingerprint, which is determined by molecular biological tests such as DNA analysis, is used for this purpose. In addition, the behaviour of the microorganism in the animal is studied, i.e. whether it survives the intestinal passage, how long it remains in the intestine and how it regulates the intestinal ecosystem. All this is the basis for an additional selection criterion – the efficacy in the animal. In addition, safety aspects also play a decisive role.

For production purposes it is important that the microorganism is capable of effective large-scale proliferation and that it remains genetically stable.

Production

Probiotics are manufactured by fermentation which is a biological procedure under the controlled supply of nutrients. All raw materials used are subject to strict quality controls. The sterile fermentation vessel is inoculated with the master seed culture either directly or indirectly after a pre-culture stage with all important parameters of production being monitored continuously. This is followed by concentration, also called cell harvesting. Special drying stages and, if necessary, the addition of specific stabilisers, complete the manufacturing process. In some products, the microorganisms are protected by microcapsules or microspheres for better stability.

Product quality and environment

Quality control

Quality control is performed both during the production process and on the final product. It comprises a check for genetic purity, microorganism count and analysis for undesirable substances (for example mycotoxins and heavy metals). The microorganism content is determined by decimal dilution chains in specific culture media. Final formulation and standardisation are usually achieved by mixing with a carrier to ensure a homogeneous distribution of the probiotic in a certain feed type.

Safety for use

Only microorganisms characterized by modern techniques and evaluated according to registration requirements are used in animal nutrition. All probiotic strains used are deposited in officially approved culture collections and it is ensured that the specific properties of the probiotic strains remain stable and in line with the highest purity requirements.

Safety for humans

People come into contact with probiotics used in animal nutrition in two ways, either as workers in the production of premixes and compound feeds, or as farmers during feeding. In both cases there are no hazards for the users. Comprehensive studies have shown that direct contact of registered probiotic products with skin, mouth and nose do not compromise human health. In model trials it has been established that even long-term or increased exposure do not constitute a risk to health. As a food consumer, however, man does not come into contact with the probiotics fed to the animal. Probiotics are administered exclusively via the feed, and their action is restricted to the gastro-intestinal tract. Since they are not absorbed, they cannot be transferred into foodstuffs of animal origin and hence do not lead to residues.

Safety for animals

In general, the microorganisms approved for animal nutrition have a very good safety record. Even in cases of overdoses of more than a thousand times the recommended levels in feed, there are no signs of dysbiosis in the gastrointestinal tract. Therefore, probiotics do not constitute any health hazard for the animal. Since they are not transferred from the intestine into the body of the animal, they do not affect any metabolic processes, nor do they have any negative impact on the animal.

Safety for environment

Having exerted their effect in the digestive tract, the probiotic reaches the exit of the intestine in the digesta, together with other intestinal microorganisms. On their way along the digestive tract the majority of the probiotic bacteria die off, since their growth and proliferation is severely restricted by competition from other microorganisms present in the large intestine. The development of yeasts is also suppressed by a lack of oxygen. The probiotics are already partly broken down and digested like other organic nutrients in the intestine so that only a small proportion is excreted viable in the faeces and survives in the manure to reach fields and grassland. Evidence of the harmlessness of the probiotic to the environment is one important subject for its registration. In general, any negative impact is highly unlikely since all these

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