

## Probiotics in animal nutrition

# Probiotics in animal nutrition

Editors:

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## Preface

FEFANA is the EU association of feed additives producers. It groups together the EU operators that are putting on the EU market the authorised additives and the premixtures that are made thereof. The association, which was set up in 1963, evolved considerably since its creation, adapting constantly to the evolution of feeding practices and to the functioning of the European Union. It is meant to be the interface between the feed additive industry and the European authorities, including the authorities of the EU Member States. Its network of regional representations, which covers the entire Union, reflects the strong wish of the industry to contribute efficiently to the European integration in the food chain area. Furthermore, FEFANA is a pivotal element of feed additive industry co-ordination with food chain stakeholders, not the less with compound and complementary feed industry.

Since Pasteur showed the implications of micro-organisms in bacterial diseases development, the importance of micro-organisms in industrial fermentation process has been steadily increasing. Food chain largely integrated such processes so that they nowadays, occupy a critical place in food manufacturing. Even before being micro-factories in industrial and traditional food manufacturing processes, science leaves no doubt today about the critical importance of a balanced gut microflora in animal and human physiology. Gnotoxenic animals have reduced growth performances and viability in comparison with xenobiotic animals.

Different solutions in order to maintain the equilibrium of the gut flora have been developed in the past and still are for some, the use of therapeutic dosage antibiotic growth promoters, the use of acidifiers to control the pH, the use of nutritional substances as specific sugars (FOS or MOS), and, more recently, essential oils and herbal extracts. All of these solutions, though working in different ways, pursue the same target: maintain the balance of the gut flora.

The probiotic concept is quite unique in this gut flora perspective. Probiotics act by reducing the feed conversion, resulting in an increase of the daily live weight gain. The improvement of the growth of the animal is achieved through a natural,

physiological way, improving digestion by balancing the gut flora. They help the animal to fulfil its' genetic potential.

In Europe, the feed additive registration process requires a dossier to be submitted to the European Food Safety Authority (EFSA) in order to obtain the right to place and use feed additive on the European market. This dossier contains three main parts: identification of the substance, safety and efficiency of the product. The dossier essentially provides a guarantee to the customer that the food using the additive is safe for the target animal and the final consumer.

FEFANA is pleased to support this booklet which helps to have a better understanding on probiotic mechanisms and EU situation.

Didier JANS

A handwritten signature in black ink, appearing to read 'Didier JANS', with a long horizontal flourish extending to the right.

## 1. 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 stabilising 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, a Russian biologist who worked around 1900 at the Institut Pasteur in Paris, 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.

## 2. 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 colonise 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 autochthonous 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.

### 2.1. Functions, structure and mode of function of the gastrointestinal tract

To demonstrate the essential role of the microbia in the digestive process in polygastric and in monogastric animals, let us take a brief look at the structure and the functions of the gastrointestinal tract.

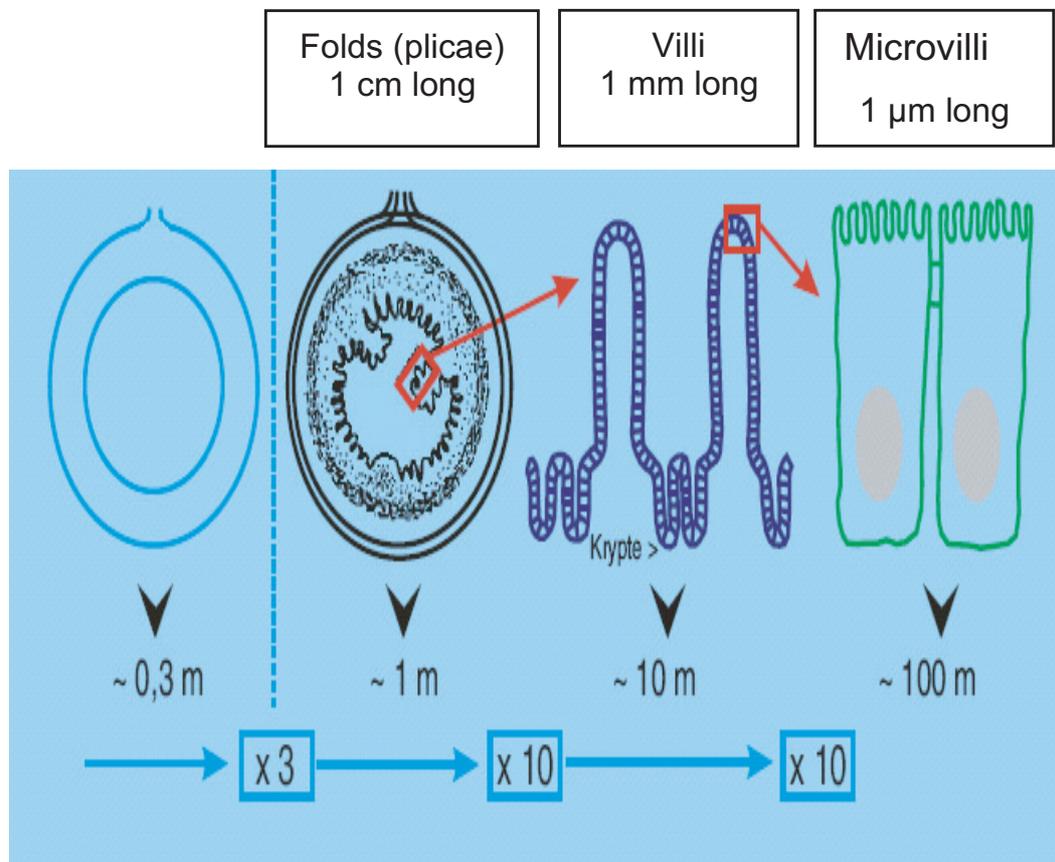
#### Monogastric animals

The gastrointestinal tract is divided into stomach, small intestine (duodenum, jejunum and ileum) and large intestine (caecum, colon and rectum).

The ingested feed is strongly acidified in the stomach by the acid excreted by the mucosa, and pepsin causes protein digestion to begin. In the small intestine, various digestive enzymes separate the nutrients into absorbable constituents. For efficient absorption, the gastrointestinal tract possesses a very large mucous surface which is about 100 times the surface of the skin. This extremely large absorption surface arises mainly from the finger-shaped protrusions, the villi, of the small intestine wall (see Figure 1).

## 2. What are probiotics used for?

Figure 1: Increasing the surface area of the intestinal wall through folds, villi and microvilli (from Aspekte)



The small intestine thus becomes the main site of absorption of nutrients which pass into the blood stream. Non-absorbed constituents of the diet reach the large intestine where they are broken down and digested, mainly by the intestinal microflora. Finally, the undigested remains, together with the bacteria, are excreted in the faeces.

Besides nutrient absorption, the intestine plays an important role as the biggest immune organ of the body. It is hence part of the body's defence system and represents an important barrier against invading pathogens. In addition to general defence mechanisms, the immune system, with its unspecific and specific reactions, helps to protect against pathogenic microorganisms. The stomach acid, bile salts and specific antibodies inhibit the growth of pathogens, potential pathogens and other microorganisms and, to a large extent, prevent them from invading the body, being

## 2. What are probiotics used for?

effectively assisted by the intestinal microflora which decreases pathogens.

### Polygastric animals (ruminants)

The digestive system comprises several different prestomachal compartments : in ruminants they are represented by the reticulo-rumen and the omasum. The abomasum is the “real” stomach of the animal. In ruminants, the foregut does not exhibit any enzymatic activity from the host animal; in fact, the main biological activity in the rumen is from microbial origin. In this large compartment the ecological conditions are well adapted to microbial life (pH relatively stable 6-6.8, temperature 39°C). The low redox potential (-400mV) is characteristic for a strictly anaerobic biotope. In this way the rumen can be assimilated to the monogastric colon. The main role of the rumen microorganisms is related to the degradation of plant polymers (cellulose, hemicellulose, starch, proteins) ingested by the animal. By this process the microbes provide nutrients which are essential for the host nutrition (volatile fatty acids, amino acids, vitamins etc...) [Jouany et al 1995].

### 2.2. The microflora

The alimentary tract is a rich environment for the development and maintenance of a dense and diverse microbial community. Culture bacterial counts give an estimate of  $10^{10}$  to  $10^{11}$  of predominantly anaerobic bacteria per gram of digesta in rumen and in human colon as well as in hindgut of monogastric herbivores. The mammalian gut ecosystem contains representatives of the three domains of life : Eubacteria, Archaea and Eucaryote. Four to five hundred different bacterial species have been identified up to date in the digestive tract. It is generally assumed that Humans have in their intestinal tract 10 times more microbial cells than whole body cells numbers [Fonty et al 1995].

## 2. What are probiotics used for?

Soon after birth, the initially sterile digestive tract is colonised by microorganisms, the concentrations of which vary widely along its entire length.

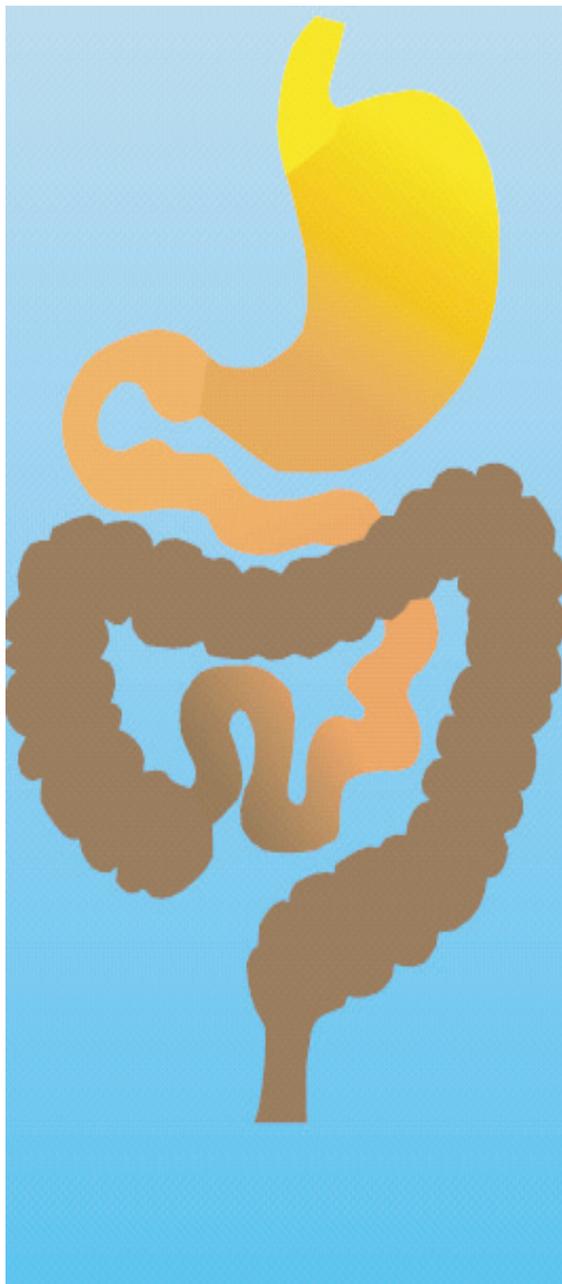
The microflora in the stomach contents of monogastric animals (e.g. pigs and poultry) is relatively small in numbers ( $10^1$ – $10^3$  bacteria per millilitre) and is, according to our current knowledge, less important for the digestive processes.

In contrast to the microflora of the stomach, the function and composition of the intestinal microflora of monogastric animals and ruminants hardly differs. Both the total number and the diversity of the microorganisms increase from the small intestine to the caecum (Figure 2). Living conditions for the microorganisms (oxygen content, pH, and nutrient and water content of the digesta) change considerably from the duodenum to the colon and with them the volume and the composition of the intestinal microflora. Since oxygen is already becoming scarce in the middle segments of the small intestine, the proportion of anaerobic species, i.e. those which live in the absence of oxygen, grows from the duodenum to the large intestine at the expense of the aerobic species (which require oxygen). Besides those bacteria which inhabit the gastrointestinal tract permanently (resident or autochthonous flora), there are species which occur there only temporarily (transient flora). Many representatives of the resident flora (e.g. the lactic acid-producing species) are capable of colonising the mucous membrane or the mucous layer of the intestine. The transient flora does not colonise the intestine but is constantly moved forward with the digesta along the intestinal tract, and is finally excreted with the faeces.

In the rumen, three different categories of living organisms can be identified, the bacteria, the protozoa and the fungi which respectively have a concentration of  $10^{11}$  cells/ml,  $10^6$  cells/ml and  $10^3$  –  $10^4$  zoospores/ml of rumen fluid. The rumen is the key compartment for the ruminant to breakdown the plant structure to produce energy available for the host

## 2. What are probiotics used for?

Figure 2: Colonisation of various intestinal segments by microorganisms (modified from Gedek 1991)



### OESOPHAGUS

Feed microorganisms

### Stomach $10^1-10^3$ per millilitre

Lactobacilli

Streptococci

Enterobacteria

Bacteroides

### Duodenum $10^1-10^4$ per millilitre

### Small intestine $10^5-10^8$ per gram

Bacteroides  $10^4-10^7$

Streptococci

Lactobacilli

Enterobacteria

### Large intestine $10^9-10^{12}$ per gram

Bifidobacteria

Bacteroides

Enterobacteria  $10^5-10^7$

Enterococci  $10^2-10^5$

Lactobacilli

Clostridia

Fusobacteria

Veillonella

Staphylococci

Yeasts

Proteus

Pseudomonas

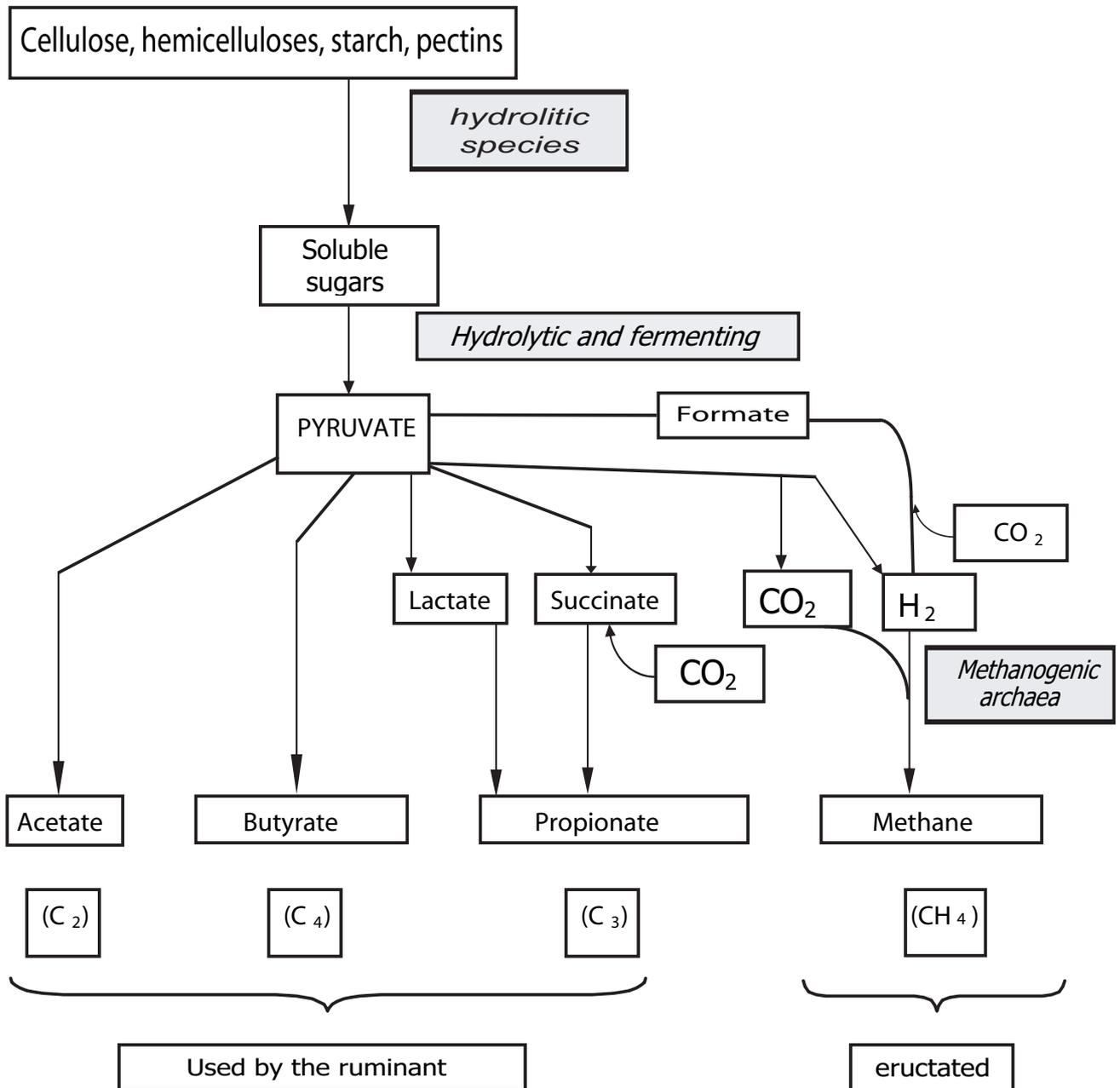
## 2. What are probiotics used for?

animal, in the form of volatile fatty acids (VFA: acetate, propionate, butyrate) [Russell 2002]. The protein degradation will produce  $\text{NH}_3$  which corresponds to the main source of nitrogen for microbial proteosynthesis. In particular cellulolytic bacteria use ammonia as their principal source of N; these bacteria allow ruminants, in contrast to monogastric animals, to use feed components (plant cell wall polymers) which could not be digested by the endogenous enzymes of the body. These microbes are subsequently digested in the small intestine and thereby improve the protein supply of the ruminants.

In the human colon (or colon of the monogastric animal) the microflora is also highly abundant and diversified, and responsible for digestion of dietary components which have not been previously degraded in the upper part of the digestive tract. These components are mainly plant cell wall polymers, starch and some proteins. The degradative and fermentative processes occurring in the colon are then comparable to those described in the rumen.

## 2. What are probiotics used for?

Polyholosides degradative process of plant cell wall polymers :  
a trophic chain



## 2. What are probiotics used for?

### 2.3. The dynamic equilibrium of the microflora (eubiosis)

Ideally, the coexistence of the various bacterial species in the open ecosystem of the gastrointestinal tract is finely regulated. Depending on the conditions in the digestive tract, a dynamic equilibrium among the various species is established.

There are numerous abiotic and biotic factors which influence the microbial balance in the digestive tract (Russell and Rychlik 2001). Some are determined by the physiology of the host and the nature of the feed, other by characteristics of microorganisms themselves or by their relationships. The typical microflora of each intestinal segment only varies within well-defined ranges (Table 1) and, under physiological conditions, always constitutes a dynamic equilibrium.

**Table 1:** Composition of the intestinal microflora in young pigs (modified after Gedek et al. 1992)

Intestinal segment	Lactobacillae/ Bifidobacteria	Eubacteria	Bacteroidaceae	Total main flora	E. coli	Enterococci	Total satellite flora
Duodenum	6,3 ± 1,4	6,3 ± 1,3	4,1 ± 1,3	2,9 ± 0,7	2,3 ± 0,7	6,6 ± 1,3	3,0 ± 0,4
Jejunum	7,7 ± 0,6	7,8 ± 0,6	5,4 ± 1,1	4,8 ± 1,5	3,9 ± 1,5	8,1 ± 0,6	4,9 ± 1,4
Ileum	8,0 ± 1,1	8,3 ± 0,7	6,7 ± 0,8	6,6 ± 1,2	5,7 ± 0,7	8,6 ± 0,7	6,8 ± 1,0
Caecum	8,5 ± 0,2	8,9 ± 0,3	7,9 ± 0,6	7,0 ± 0,9	5,8 ± 0,2	9,2 ± 0,2	7,0 ± 0,9
Colon	8,7 ± 0,4	9,0 ± 0,5	7,9 ± 0,6	7,2 ± 1,0	5,8 ± 0,9	9,2 ± 0,4	7,3 ± 0,9

(n=9)

Bacterial counts given as average with standard deviation in logarithms per gram of sample,  $10^{6.3}$  corresponds to  $10^{6.3} = 1,995,262$  microorganisms per gram

We distinguish between the main, the satellite and the residual flora. However, only those microorganisms capable of being cultured can be examined in such studies. When the microflora is in equilibrium, proportion of the main flora is over 90%. In monogastrics, it is composed mainly of

## 2. What are probiotics used for?

anaerobic species (*Clostridia*, *Bifidobacteria*, *Lactobacilli*, *Bacteroides*, *Eubacteria*) which produce lactic acid and other short-chain fatty acids [Marteau et al 2001, Harmsen et al 2002]. The satellite flora represents approximately 1% and consists mainly of Enterococci and *E. coli*. The residual flora is below 0.01% and is composed mainly of harmful microorganisms such as *Proteus*, Staphylococci and *Pseudomonas*. Methanogens are present in many monogastric animals (pigs, horses...), and in 50% of the human population.

In ruminants, the main bacterial species are fibre-degrading species (*Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefaciens*, *Butyrivibrio fibrisolvens*); *Prevotella ruminicola* can also be a predominant species as these bacteria are able to utilise a broad range of substrates (sugars, proteins, starch) [Fonty et al 1995]. *Selenomonas ruminantium*, *Streptococcus bovis*, and *Megasphaera elsdenii* are also generally present at high numbers when high levels of concentrate are fed [Mackie and Gilchrist 1979]. Other species are found in the rumen and occupy more specialised niches (*Anaerovibrio lipolytica*, *Veillonella alcalescens*, *Wolinella succinogenes*, etc...). Methanogens are present in the rumen and are essential to ensure the good functioning of the ecosystem; they use hydrogen and carbon dioxide which are formed during the fermentation process to produce methane which is eructated by the animal. In the rumen are also found some anaerobic fungi (6 different genera) which are mainly implicated into cellulose breakdown [Fonty and Joblin 1991] ; ciliate protozoa (approx. 20 different genera) play several roles within the ecosystem : they are able to degrade numerous substrates (plant polymers, proteins, soluble compounds) and they engulf bacteria and fungi, that contributes to regulate the microbial balance [Jouany et al 1995]

The state when the main, the satellite and the residual flora form a ratio of > 90:1:0.001 is called "eubiosis" which, freely translated from Greek, means "good coexistence" of the microorganisms amongst themselves as well as with the host organism. In this situation, the host and the microflora live

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together in symbiosis, meaning, with mutual benefit. If this relationship is severely disrupted, the condition is called dysbiosis (“bad” coexistence).

The host provides ideal living conditions such as constant temperature, regulated pH, supply of nutrients and removal of metabolites. In exchange, the digestive microflora, when in the state of eubiosis, supports the host with essential activities such as:

- Digestion of nutrients and detoxification of toxic molecules
- Vitamin synthesis (B and K)
- Antagonistic action against non desirable microorganisms (barrier effect)
- Protection of the intestinal mucous membrane against invading microorganisms
- Contribution to maturation and stimulation of the host’s immune system.

### 2.4. Disruptions of the dynamic equilibrium of the microflora (dysbiosis)

The ecosystem of the gastrointestinal microflora is subject to several external factors. We can differentiate dietary factors from those originating within the host.

Feed is the nutritional basis of the microorganisms. Feeding errors and substantial dietary changes, low-quality feed components and inadequate feed hygiene all compromise eubiosis. Similarly, digestive secretions (bile, enzymes, buffer and mucous substances) and immune substances of the host animal affect the microorganisms directly. The release of digestive secretions and the type and frequency of the intestinal movements (peristaltis) are influenced to a large extent by stress. Important causes of stress in animal husbandry are, for example, gestation, birth, weaning, regrouping of herds, transport, overcrowding, incorrect housing climate and disease. Long before scours become evident, a microbial imbalance in the digestive tract may exist and feed conversion may be clearly decreased.

## 2. What are probiotics used for?

One of the most common examples of microbial imbalance due to feed management is ruminal acidosis; when high amounts of rapidly fermentable carbohydrates (starch, sugars) are fed to dairy cows or finishing beef cattle, significant alterations in the structure of the rumen microbial communities occur [Tajima et al 2000], with a sharp increase in certain bacterial species (amylolytic, fermentative and lactate-utilising species) and a decrease in the level of fibrolytic bacteria. This leads to changes in the fermentation pattern which may in turn lead to lactic acid accumulation and increase the risk of acidosis [Russell and Hino 1985].

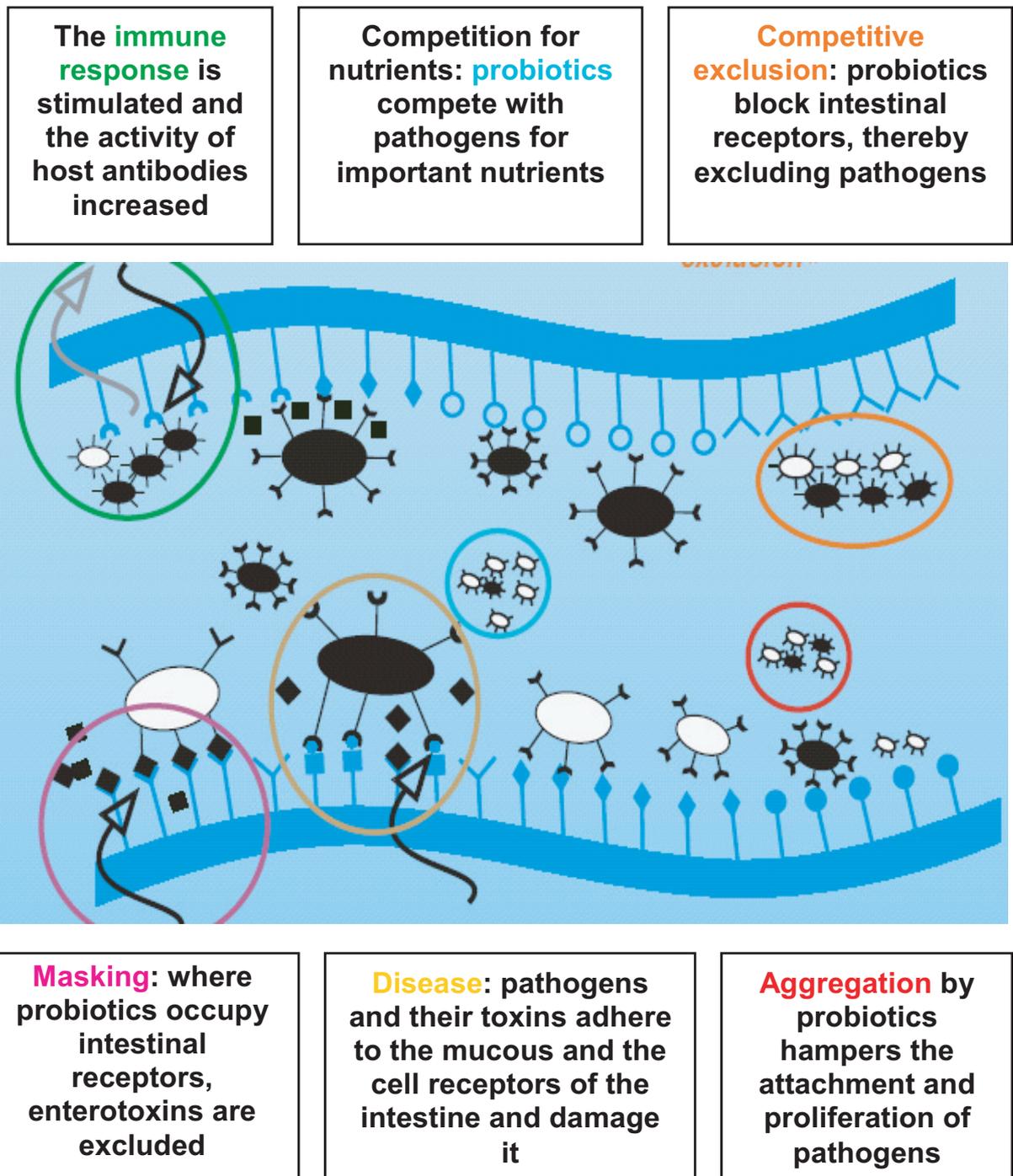
The negative impact of dysbiosis on the host animal can be significant and may be expressed, for instance, by poor body condition and insufficient growth or even digestive symptoms such as, diarrhoea. These are caused mainly by bacterial toxins which may harm the host by:

- Damage to the intestinal epithelium and thickening of the intestinal wall, thus leading to reduced nutrient absorption ;
- Weakening of the immune system ;
- Metabolic burden on the host owing to their detoxification.

### 3.The various types of probiotics and their modes of action

#### 3.1 Mode of action of probiotics

Figure 3: Microbiological interactions in the intestine (modified after Stewart et al. 1995)



Certain microorganisms which are intentionally added to the feed (probiotics) counteract possible disruptions of the equilibrium and lead to

### 3.The various types of probiotics and their modes of action

eubiosis. Thus the colonisation of the intestine by undesirable microorganisms can be suppressed. As yet, not all actions of probiotics have been satisfactorily explained by science. Their overall positive effects, based on developing metabolic activity, comprise both direct and especially indirect effects (Figure 3). In general, the modes of action of probiotics described in Figure 3 are assumed.

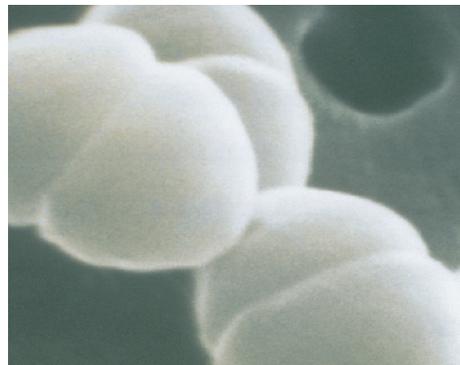
The probiotics used in animal nutrition can be divided into three main groups: lactic acid bacteria, Bacillus spores and yeasts. Microbial strains used as probiotics differ from wild strains of the same species in some specific characteristics, especially with regard to their greater safety of use and 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.

#### 3.2. 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 (figure 4) convert certain types of sugars by fermentation, mainly into lactic acid.

Figure 4 : Lactic acid bacteria under the electron microscope



### 3.The various types of probiotics and their modes of action

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,

### 3.The various types of probiotics and their modes of action

- Effect on the intestinal epithelium,
- Improved absorption capacity.

#### 3.3. 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 (figure 5) offers a good protection against external influences (see chapter 5.3.). 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.

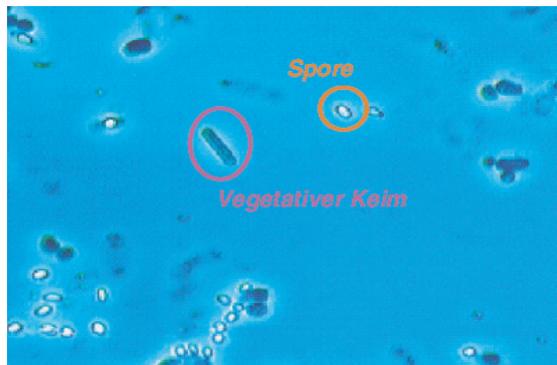


Figure 5 : Bacillus spores and vegetative microorganisms as seen in a light microscope

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 colonise 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.

### 3.The various types of probiotics and their modes of action

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.

#### 3.4. 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.

Humans have historically used yeast products as health aids. Yeast has been used for 5 000 to 8 000 years when Babylonians, Egyptians, and Celts, who also used it for alcohol production, used yeast for benefits on the skin and "color".

Around 370 BC, Hippocrates discovered the diuretic action of yeast and considered it as a drug.

During the middle ages, clergy used yeast against leprosy to prevent contamination. It was also used to cure rubella and scarlet fever.

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.

### 3.The various types of probiotics and their modes of action

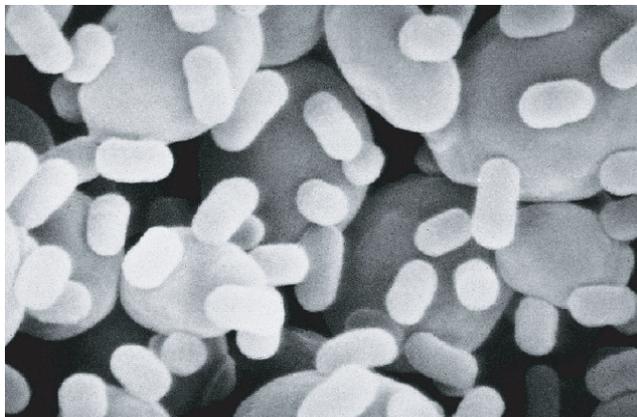
#### Monogastrics :

The genus *Saccharomyces* has 4 different 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 (figure 6). 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.



**Figure 6:** Attachment of *E. coli* to the surface of a yeast

#### Ruminants and monogastric herbivores (equines):

- 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

### 3.The various types of probiotics and their modes of action

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].

## 4. Probiotics as feed additives

### 4.1. 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.

### 4.2. Recommendations for use

The stabilisation 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 colonise 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

#### 4. Probiotics as feed additives

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,

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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.

In table 2, examples of the inclusion rates of the most commonly used probiotic formulations in complete feed, premixes and supplementary feeds are given together with the declared CFU values.

### 4.3. 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.

## 4. Probiotics as feed additives

Other substances such as acids, active agents of plant origin, trace elements and minerals may impair microorganisms and any combination therefore requires appropriate evaluation.

**Table 2:** Calculation of the inclusion of different probiotic formulations feedstuffs

**Example:** A complete feed for fattening pigs should contain  $0.2 \times 10^9$  CFU/kg. Probiotic A is used ( $1 \times 10^{10}$  CFU/g).

If the probiotic is mixed directly into the feed, product A should be used at a rate of 20 mg/kg (Product A contains  $1 \times 10^{10}$  CFU/g; therefore 20 mg contain  $0.02 \times 10^{10}$  CFU/kg =  $0.2 \times 10^9$  CFU).

If the probiotic is included in a 0.5% premix, product A should be used at a rate of 4000 mg/kg premix in order to obtain the target concentration of  $0.2 \times 10^9$  CFU/kg feed (= 20 mg) ( $20 \text{ mg} : 0.5\% = 4000 \text{ mg}$ ).

Mixture	Required content in the complete feed (CFU/kg)	Declaration of the mixture (CFU/kg) <sup>1</sup>	Dosage of the various formulations (g/t)				
			Product A ( $1 \times 10^{10}$ ) <sup>2</sup>	Product B ( $5 \times 10^9$ ) <sup>2</sup>	Product C ( $3.5 \times 10^9$ ) <sup>2</sup>	Product D ( $1 \times 10^9$ ) <sup>2</sup>	Product E ( $0.2 \times 10^9$ ) <sup>2</sup>
Complete feed	$2.5 \times 10^9$	$2.5 \times 10^9$	250	500	715	2,500	-
	$1.0 \times 10^9$	$1.0 \times 10^9$	100	200	286	1,000	-
	$0.7 \times 10^9$	$0.7 \times 10^9$	70	140	200	700	3,500
	$0.2 \times 10^9$	$0.2 \times 10^9$	20	40	57	200	1,000
Supplementary feed 1 (4 %)	$2.5 \times 10^9$	$62.5 \times 10^9$	6,250	12,500	17,875	62,500	-
	$1.0 \times 10^9$	$25.0 \times 10^9$	2,500	5,000	7,143	25,000	-
	$0.7 \times 10^9$	$17.5 \times 10^9$	1,750	3,500	5,000	17,500	87,500
	$0.2 \times 10^9$	$5.0 \times 10^9$	500	1,000	1,429	5,000	25,000
Supplementary feed 2 (3 %)	$2.5 \times 10^9$	$83.3 \times 10^9$	8,333	16,666	23,833	83,333	-
	$1.0 \times 10^9$	$33.3 \times 10^9$	3,333	6,667	9,533	33,333	-
	$0.7 \times 10^9$	$20.0 \times 10^9$	2,000	4,667	6,667	23,333	116,665
	$0.2 \times 10^9$	$6.67 \times 10^9$	667	1,333	1,900	6,667	33,335
Premix (0,5 %)	$2.5 \times 10^9$	$500 \times 10^6$ CFU/g <sup>1</sup>	50,000	100,000	143,000	500,000	-
	$1.0 \times 10^9$	$200 \times 10^6$ CFU/g <sup>1</sup>	20,000	40,000	57,200	200,000	-
	$0.7 \times 10^9$	$140 \times 10^6$ CFU/g <sup>1</sup>	14,000	28,000	40,000	140,000	700,000
	$0.2 \times 10^9$	$40 \times 10^6$ CFU/g <sup>1</sup>	4,000	8,000	11,400	40,000	200,000

1) According to feed regulation, the dosage level must be given in CFU per gram for additives and premixes, and in CFU per kilogram for compound feed.

2) Concentration of microorganisms in the corresponding probiotic formulations expressed in CFU/g.  
CFU: colony-forming units

## 4. Probiotics as feed additives

### Methods for the evaluation of compatibility

Internationally, different test methods are used for evaluating compatibility.

In vitro testing (laboratory assays)

1. Dilution Test
2. Agar Diffusion Test
3. Epsilon Test (E-test)

The Minimal Inhibitory Concentration (MIC) determined by these tests gives a first indication of whether a microorganism can be combined with other substances.

### Discrepancy between in vitro and in vivo results

Some studies showed that the inhibition of a probiotic in feeding trials did not occur as expected from the corresponding MIC values which can be explained by the fact that in a complex intestinal system many factors can reduce inhibitory action of substances.

Therefore, in vitro methods can provide useful hints on possible interactions between probiotics and other substances. However, in case of doubt these should be evaluated in feeding trials.

### 4.4. Economical and ecological benefits

According to current knowledge, all probiotics act by supporting the dynamic equilibrium of the intestinal microflora (see Chapter 2). Thus the vitality and the well-being of the animals can be improved and digestive problems and losses caused by nutrition reduced. Because healthy animals convert the ingested nutrients into consistent growth the direct impact of probiotics on the intestinal microflora also results in ensuring high performance, for instance by improving feed conversion and improving daily weight gain.

Results from numerous scientific trials and practical studies corroborate the positive effect of probiotics (Table 3).

## 4. Probiotics as feed additives

However, as shown in Table 3, in some trials no additional benefits from the use of probiotics were observed. This is understandable since the initial status of the microbial colonisation of the intestine can differ widely between studies.

**Table 3:** Influence of various probiotics on the performance of animals

Production branch	DWG (% of control)	FCR (% of control)
<b>Piglet production</b>	+ 4.8 (-8.1 to +24.3)	-1.5 (+3.1 to -9.3)
<b>Calf production</b>	+ 5.4 (-5.3 to +21.7)	-2.5 (+3.6 to -7.9)
<b>Growing/fattening pigs</b>	+ 3.7 (-0.3 to +6.7)	- 5.1 (-1.4 to -7.1)
<b>Growing/fattening cattle</b>	+ 3.4 (-4.3 to +7.2)	- 2.7 (+7.6 to -4.7)

Furthermore, the extent to which the well-being and the performance are improved or maintained also depends on other factors, especially the composition of the diet, the sanitary conditions and the performance level. An accurate and reliable prediction of the probiotic efficacy is therefore not possible.

In piglets, for instance, probiotics have a positive impact particularly on mortality, daily weight gain and feed conversion. In addition, more uniform growth and consequently better homogeneity of the groups are often observed which may result in additional profit at sales. For example, at a production level of 20 piglets per sow per year, 0.5 kg weight improvement per piglet results in approximately 10 kg additional weight at sale and thus additional turnover. A feed saving of 50 g per kg piglet weight (i.e. approximately 1 kg less feed per piglet) means approximately 20 kg feed saving when 20 piglets per sow per year are reared. In addition, reduced mortality leads to considerable economic benefits.

## 4. Probiotics as feed additives

In grower pigs, an unfavourable intestinal microflora rarely results in apparent scour symptoms or mortality. However, depressed performance is very often linked to intestinal dysbiosis. The stabilisation of the intestinal microflora in growing animals therefore leads to a high performance level. The corresponding economic benefit can be calculated by the increase in daily weight gain and the reduction of both fattening period and feed expenditure. In addition, the use of probiotics may reduce medication and the corresponding costs.

By ensuring high performances and averting problems, probiotics contribute to an environmentally friendly animal husbandry. The burden on the environment is eased by the reduction of slurry and the decrease of its nutrient content. For example, the use of probiotics may lower nitrogen excretion by increasing nitrogen digestibility and deposition (see Table 4).

**Table 4:** Influence of probiotics on protein digestibility and crude protein deposition in piglets

Nitrogen digestibility (%)		Nitrogen deposition (g W <sup>-0.75</sup> per day) <sup>1</sup>		Authors
Control	Probiotic**	Control	Probiotic**	
81.05 <sup>a</sup>	82.86 <sup>b</sup>	1.24 <sup>a</sup>	1.34 <sup>b</sup>	SE Scheuermann, 1993
78.70 <sup>c</sup>	83.20 <sup>d</sup>	1.76	1.81	Tossenberger et al., 1995

1: Relative to metabolic body weight

\*\* : dosage 1 x 10<sup>9</sup> CFU per kg of piglet feed

a , b , c , d significant differences

## 5. Product quality and environment

### 5.1. 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 animal nutrition. Their fermentation profiles are determined revealing which substrates are 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 (see Chapter 5.2.).

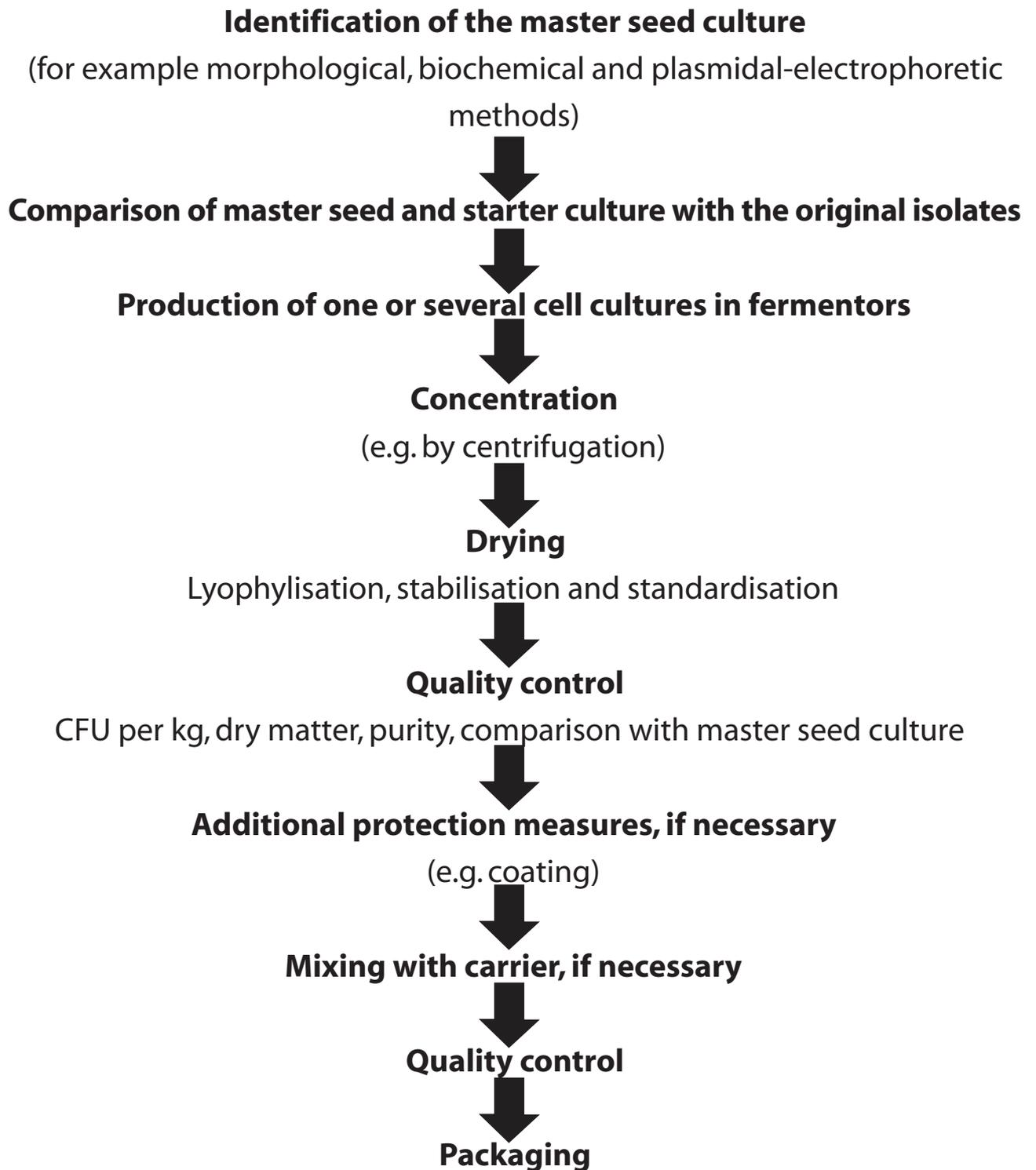
For production purposes it is important that the microorganism is capable of effective large-scale proliferation and that it remains genetically stable.

#### 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.

A schematic illustration of the manufacturing process is shown in Figure 7.

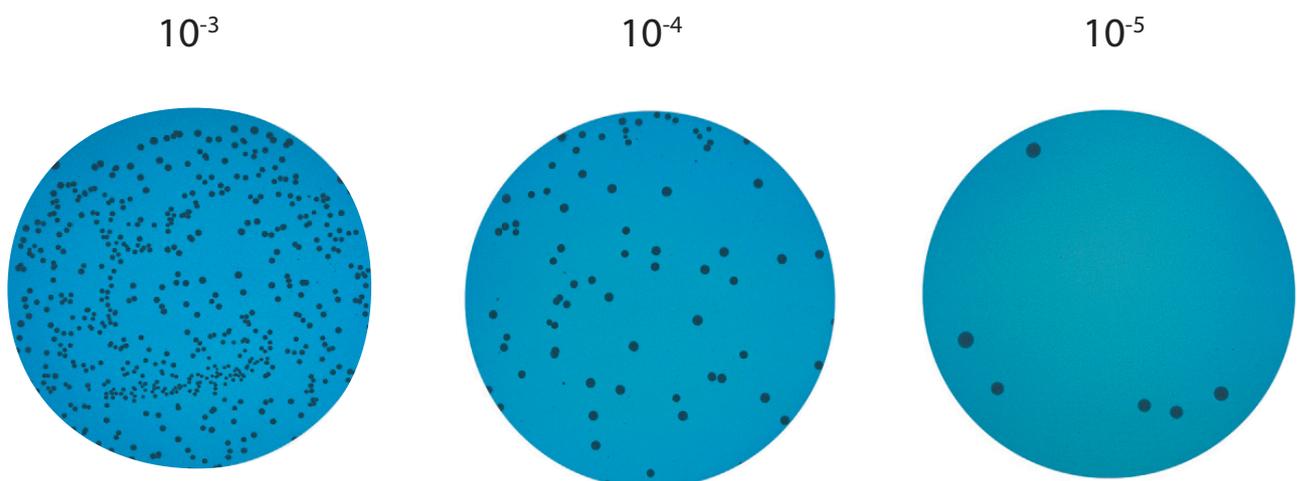
Figure 7: Production of probiotics



### 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 (Figure 8). 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.

**Figure 8:** Decimal dilution series for the determination of the microorganism count



### 5.2. Safety for use

Only microorganisms characterised 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 the 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.

## 5. Product quality and environment

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 microorganisms are derived from nature.

### 5.3. Stability and detectability in feeds

#### Stability

In the production of compound feeds, especially during pelleting and in mineral supplements, probiotics are subjected to various chemical, mechanical and heat stresses. It is essential that the viability (i.e. the stability) of the probiotics is maintained for them to remain efficacious. The probiotics currently used in animal nutrition generally differ in their technical properties.

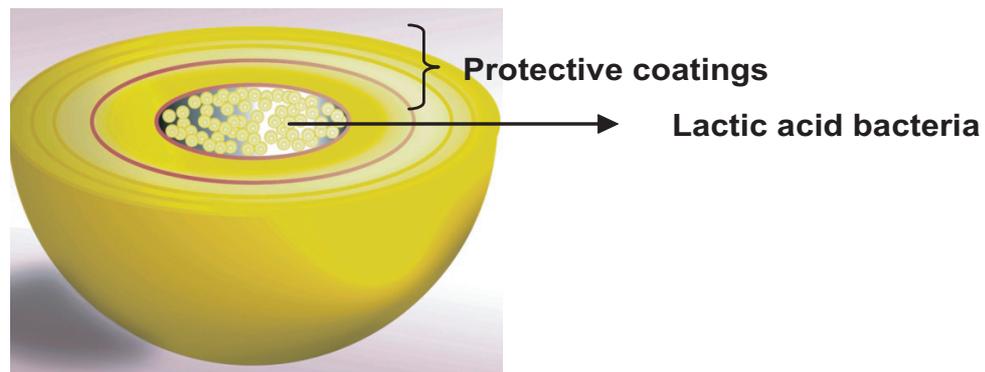
#### Lactic acid bacteria

As natural residents of the intestine, lactic acid bacteria do not form spores. They are therefore, in ordinary dried form, unprotected against the chemical and physical stresses, for example during pelleting. It is hence necessary either to use them only for feed types which place little technical stress on the microorganisms (for example in milk replacers) or to protect them specifically against mechanical and heat impacts during feed manufacturing, transport and storage.

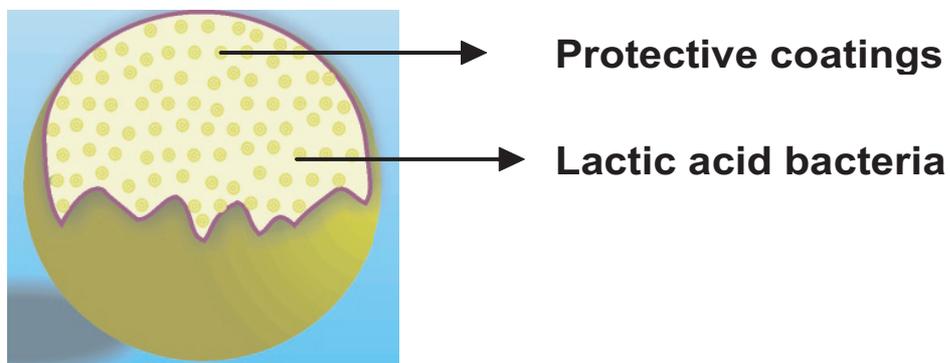
## 5. Product quality and environment

Lactic acid bacteria may be given a protective coating using special technological procedures such as microencapsulation or microsphering (Figures 9 and 10) thus ensuring that these non-spore bacteria are able to reach the site of action intact and become active. The stability of the coating is determined by the quality of the process.

**Figure 9:** Cross-section of a microcapsule



**Figure 10:** Cross-section of a microsphere

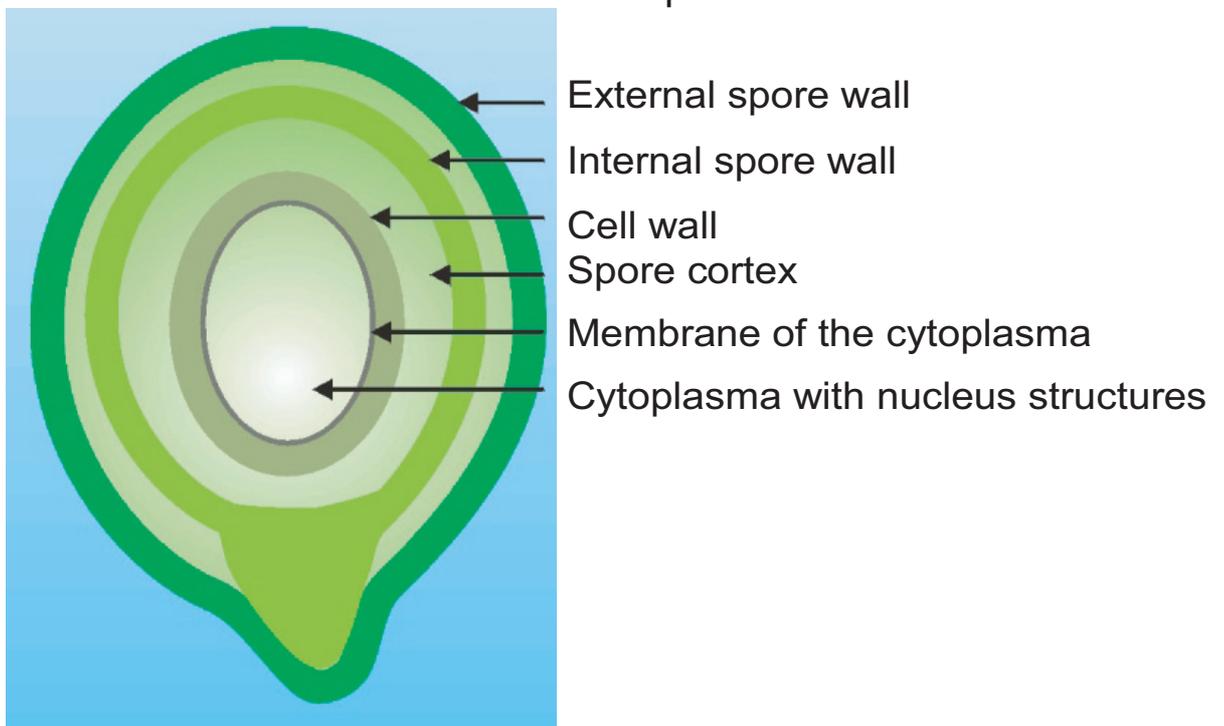


### Bacillus spores

Probiotics of this group are sporulated living microorganisms. Spores are their natural stable form which allows them to survive in their original habitat, the soil, protected from extreme heat, cold and mechanical strain, without any loss in their vital potential. Various cell walls (Figure 11) protect the nucleus from external stresses. This natural protection enables the Bacillus products to withstand massive strains during feed production and

storage, such as high temperatures, pressure, shear forces or oxidation impacts. (Sneath, 1986; Mazza, 1994; Madigan et al., 2003). Therefore, Bacillus spores are suitable for all types of feeds. In addition, their vitality is not compromised by low pH values in the stomachs of monogastric animals. Spore quality and stability of the Bacillus products and their ability to germinate are influenced by the fermentation conditions during production.

**Figure 11:** Cross-section of a Bacillus spore



### Yeasts

Yeasts are living fungi and are made dormant by drying. Since their external surface is more stable and less permeable in this state, yeasts survive many processes of feed production and storage undamaged. Then the presence of sufficient moisture and warmth in the digestive tract allows them to regain their metabolic activity.

### Detectability

A prerequisite for the official approval of probiotics is their reliable detectability. For all product groups, scientifically acknowledged methods of analysis have been established in agricultural research and analytical facilities. The principle of these methods is based upon their cultivation on specific media and subsequent visual counting of the colonies formed (Figure 12). The obtained counts are expressed in Colony Forming Units (CFU).

Figure 12: Counting colonies



Results obtained by biological detection techniques naturally have a higher variation compared to chemical-physical detection techniques. The analytical ranges for probiotics must therefore be relatively wide. The reason for this is mainly because probiotics are not as evenly distributed as chemical substances in the dilution samples and because their vitality may be compromised during extraction.

Another reason for the relatively high variability is the possibility that single colonies may have been formed by more than one microorganism and that some closely situated colonies may be recognised and counted as just one CFU. As a consequence, some of the single microorganisms may be overlooked. However, these microorganisms are present and able to display

their vitality and bioregulative function under suitable conditions in the intestine. Practical experience proves that contents found within the analytical range allow for an appropriate efficacy. The various analytical ranges are set and legally regulated by the respective official laboratories following several ring evaluations.

### 5.4. 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 (see chapter 4.3.) 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.

## 6. Method of analysis

Methods for the official control of probiotic yeast used as feed additives were developed within the European Community project SMT4 CT98-2235 and subsequently validated. For acceptance of standards by the Comité Européen pour la Standardisation (CEN), methods need to be validated by means of a collaborative study.

The method development was based on a fundamental literature review and on already available standard methods [Anonymous 1998, Anonymous 1999a, Bovill R. et al 2001].

The project partnership comprised a coordinator and four contractors :

- Coordinator : Central Science Laboratory (CSL, York, UK),
- Contractors :
  - o Agricultural University of Vienna (Institute für Milchforschung und Bakteriologie, Vienna, Austria),
  - o University of Caen (Laboratoire de Microbiologie Alimentaire, Institut de Biochimie et de Biologie Appliquée, Université de Caen-Basse Normandie, Caen, France),
  - o Dairy Products Research Centre (Teagasc, Fermoy, Ireland),
  - o Gaiker Laboratories (Gaiker, Bilbao, Spain).

Twenty laboratories from 12 European countries were invited to participate in the trial.

Each method was challenged with samples containing single microorganisms or mixtures. Data from the collaborative trial were used to calculate the repeatability ( $r$ ), and reproducibility ( $R$ ) of methods with selective and non-selective media using feed and premixtures as validation samples. The results are intended for submission to CEN in the corresponding CEN methods and subsequently by ISO via the Vienna Agreement.

FEFANA members played an important role in the development of these methods and is continuously involved in their acceptance.

## 6. Method of analysis

List of published methods for probiotics (enumeration, typing) which entered into the procedure of CEN evaluation for harmonisation :

- "A collaborative study of a method for enumeration of probiotic enterococci in animal feed" R.G.K. Leuschner, J. Bew; K.J. Domig and W. Kneifel, *Journal of Applied Microbiology*, 2002, 93, 781-786
- "A collaborative study of a method for the enumeration of probiotic **lactobacilli** in animal feed" R.G.K. Leuschner, J. Bew, V. Coeuret, J.-P. Vernoux, M. Gueguen, *Food Microbiology*, 20 (2003) 57-66
- "Validation of an official control method for enumeration of authorised probiotic **yeast** in animal feed" R.G.K. Leuschner, J. Bew, G. Bertin, *Systematic and Applied Microbiology*, 26, 147-153 (2003)
- "Enumeration of probiotic **bacilli** spores in animal feed : interlaboratory study" R.G.K. Leuschner, J. Bew, *Journal of AOAC International*, Vol. 86, n° 3, 2003, 568-575
- "Enumeration of probiotic **pediococci** in animal feed : interlaboratory study" R.G.K. Leuschner, J. Bew, P.J. Simpson, P.R. Ross, C. Stanton, *Journal of AOAC International*, Vol. 86, n° 4, 2003, 791-801
- "A collaborative study of a method for the enumeration of probiotic **bifidobacteria** in animal feed" R.G.K. Leuschner, J. Bew, P. Simpson, P.R. Ross, C. Stanton, *International Journal of Food Microbiology*, 83 (2003), 161-170
- "Validation of the Official Control Method Based on **Polymerase Chain Reaction (PCR)** for Identification of Authorised Probiotic **Yeast** in Animal Feed" R.G.K. Leuschner, J. Bew, P. Fourcassier, G. Bertin, *System. Appl. Microbiol.* 27, 492-500 (2004)

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.

## 8. Glossary

Aerobic	Living in the presence of oxygen
Anaerobic	Living in the absence of oxygen
Antagonistic	Inhibiting, acting against
API assay	Determination of the biochemical activity of a microorganism and its differentiation
Bacteriostatic	Inhibiting bacteria
Bacteriocidal	Killing bacteria
Digesta	Content of the gastrointestinal tract
DNA analysis (DNA: desoxyribonucleic acid)	Determination of the genetic information
Dysbiosis	State of a disrupted ecological balance of the gastrointestinal microflora
Eubiosis	State of ecological flow equilibrium of the gastrointestinal microflora
CFU	Colony Forming Unit, measure for concentration of living microorganisms
Microflora	Totality of microorganisms, here in the gastrointestinal tract
Mucopolysaccharides	High-molecular mucous-forming carbohydrates
Protozoa	Single-celled animals (singular: protozoon)
Redox potential	Potential of a dissolved substance to accept or donate electrons and thereby to oxidize or reduce other substances
Symbiosis	biological community between organisms with mutual benefits

Qamar A., Aboudola S., Warny M., Michetti P., Pothoulakis C., Lamont J.T. and Kelly C.P. 2001. *Saccharomyces boulardii* Stimulates Intestinal Immunoglobulin A Immune Response to *Clostridium difficile* Toxin A in mice. *Infect. Immun.* (am. society for microbiology ).69: 2762-2765

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Anonymous 1998: BS 5763:1998(ISO 7954:1987), microbiological examination of food and animal feedingstuffs, Part 12, Enumeration of yeasts and moulds, BSI, London, UK

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Bovill R., Bew J., Robinson S. 2001: Comparison of selective media for the recovery and enumeration of probiotic yeasts from animal feed. *Int. J. Food Microbiol.* 67,55-61

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# List of Contributors

AWT Germany

ALLTECH

CHR-HANSEN

DSM Nutritional Products

KEMIN

LALLEMAND

LESAFFRE

VOLAC



**fefana**  
EU Feed Additives &  
Premixtures Association

FEFANA Absl  
120 avenue Louise  
Box 13  
B 1050 Brussels  
Belgium  
Tél. : +32 2 639 66 60  
Fax : +32 2 640 41 11  
Email : [info@fefana.org](mailto:info@fefana.org)  
Website : [www.fefana.org](http://www.fefana.org)

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