Review

Probiotics and prebiotics in animal feeding for safe food production

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Abstract

Recent outbreaks of food-borne diseases highlight the need for reducing bacterial pathogens in foods of animal origin. Animal enteric pathogens are a direct source for food contamination. The ban of antibiotics as growth promoters (AGPs) has been a challenge for animal nutrition increasing the need to find alternative methods to control and prevent pathogenic bacterial colonization. The modulation of the gut microbiota with new feed additives, such as probiotics and prebiotics, towards host-protecting functions to support animal health, is a topical issue in animal breeding and creates fascinating possibilities. Although the knowledge on the effects of such feed additives has increased, essential information concerning their impact on the host are, to date, incomplete. For the future, the most important target, within probiotic and prebiotic research, is a demonstrated health-promoting benefit supported by knowledge on the mechanistic actions. Genomic-based knowledge on the composition and functions of the gut microbiota, as well as its deviations, will advance the selection of new and specific probiotics. Potential combinations of suitable probiotics and prebiotics may prove to be the next step to reduce the risk of intestinal diseases and remove specific microbial disorders. In this review we discuss the current knowledge on the contribution of the gut microbiota to host well-being. Moreover, we review available information on probiotics and prebiotics and their application in animal feeding.

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doi:10.1016/j.ijfoodmicro.2010.02.031
1. Introduction

The first goal of the livestock production is the delivery of safe foods for human consumption taking into account the welfare of the animal and respect for the environment. An important field of zootechnical research is the improvement of the quality and safety of the meat. It is well recognized that pathogens, such as Campylobacter and Salmonella can be transmitted along the food chain and can be the source of human illness. In the past, antibiotics have been included in animal feed at sub-therapeutic levels, acting as growth promoters (Antibiotic Growth Promoters, AGPs), (Dibner and Richards, 2005).

However, worldwide concern about development of antimicrobial resistance and about transference of antibiotic resistance genes from animal to human microbiota (Mathur and Singh, 2005; Salyers et al., 2004) led to banning the use of antibiotics as growth promoters in the European Union since January 1, 2006 (EC 2001, 2003a).

The removal of these compounds from animal diets has put tremendous pressure on the livestock and poultry farms, one of the main consequences being a substantial increase in the use of therapeutic antibiotics (Casewell et al., 2003). There is evidence that AGPs have long been effective in prevention of necrotic enteritis (NE) in poultry flocks and that the incidence of NE has increased in countries where AGPs have been stopped (Van Immerseel et al., 2004). There is evidence of a correlation between the composition of the colonizing bacterial communities in 60 species of mammals based on 16S rRNA-analysis showed that diet, host phylogeny and gut morphology influence the microbial ecology of the gastrointestinal tract (Ley et al., 2008, 2009). If mammals are classified as monogastric and polygastric, their microbiota clusters into groups that correspond to these categories. However, the composition of the fecal microbiota is also a strong predictor of the host physiology status. The major microbial groups in monogastric animals (such as pig, chicken, rabbit and man) are Bacteroides, Clostridium, Bifidobacterium, Eubacterium, Lactobacillus, Enterobacteriaceae, Streptococcus, Fusobacterium, Peptostreptococcus and Propionibacterium. In polygastric animals, (such as cow, sheep and lamb), the rumen is the most important microbial ecosystem with the predominance of fiber-degrading groups belonging to Fibrobacter, Ruminococcus, Butyrivibrio and Bacteroides together with major groups such as Prevotella, Selenomonas, Streptococcus, Lactobacillus and Megasphaera. Some anaerobic fungi and ciliate protozoa and a large number of methanogens are also present in the rumen (Mackie et al., 2000). In mammals, the percentage of the different microbial groups varies between individuals, depending on age (Mueller et al., 2006) and on the health/pathological status (Abt and Artis, 2009). Diet is an additional factor influencing the gut microbiota; herbivores contain a higher number of bacterial phyla, while carnivores, sheep and omnivores are at an intermediate level (Ley et al., 2008).

1.1. Gut microbiota

The microbiota within the GIT tract of mammals can be considered a metabolically active organ with its wide biodiversity in terms of species and the high number of cells that can reach 10^{14} (Macfarlane and Macfarlane, 2004, Backhed et al., 2005, Murphy et al., 2009). Under normal circumstances, commensal bacteria are an essential health asset with a nutritional function and a protective influence on the intestinal structure and homeostasis. The intestinal microbiota protects against infections, and actively exchanges developmental and regulatory signals with the host that primes and instructs mucosal immunity. Although the intestinal microbiota is complex and the role of most of the bacteria in providing benefit to the host is not clear, bacterial species of the genera Lactobacillus and Bifidobacterium have been shown to supply protection against enteric infections. By enhancing the beneficial components of the gut microbiota is possible to treat various intestinal disorders and maintain host well-being (O'Hara and Shanahan, 2007). Moreover, access to beneficial microorganisms has been suggested to be one of the selective advantages of social behaviour in animals (Ley et al., 2008). In particular, the close proximity of individuals in livestock or poultry farms could facilitate the host–host transmission of microbiota. Therefore, in high population density, it is important to maintain a “healthy” microbiota as a barrier against pathogen infection.

A key issue is to identify and know the species present in the gut microbiota of the different animals. Studies on the survey of the gut bacterial communities in 60 species of mammals based on 16S rRNA-analysis showed that diet, host phylogeny and gut morphology influence the microbial ecology of the gastrointestinal tract (Ley et al., 2008, 2009). If mammals are classified as monogastric and polygastric, their microbiota clusters into groups that correspond to these categories. However, the composition of the fecal microbiota is also a strong predictor of the host physiology status. The major microbial groups in monogastric animals (such as pig, chicken, rabbit and man) are Bacteroides, Clostridium, Bifidobacterium, Eubacterium, Lactobacillus, Enterobacteriaceae, Streptococcus, Fusobacterium, Peptostreptococcus and Propionibacterium. In polygastric animals, (such as cow, sheep and lamb), the rumen is the most important microbial ecosystem with the predominance of fiber-degrading groups belonging to Fibrobacter, Ruminococcus, Butyrivibrio and Bacteroides together with major groups such as Prevotella, Selenomonas, Streptococcus, Lactobacillus and Megasphaera. Some anaerobic fungi and ciliate protozoa and a large number of methanogens are also present in the rumen (Mackie et al., 2000). In mammals, the percentage of the different microbial groups varies between individuals, depending on age (Mueller et al., 2006) and on the health/pathological status (Abt and Artis, 2009). Diet is an additional factor influencing the gut microbiota; herbivores contain a higher number of bacterial phyla, while carnivores, sheep and omnivores are at an intermediate level (Ley et al., 2008).

1.2. Balance and unbalance of the gut microbiota

In the gastrointestinal tract (GIT) of humans and animals, the mucosal barrier separates the internal milieu from the luminal environment; the mucus layer is formed by the interaction of various mucosal secretions, including mucin glycoproteins, trefoil peptides, and surfactant phospholipids (Guarner and Malagelada, 2003). The intestinal epithelium, together with the mucus, provides the first sensory line of defence mediating the active sampling of resident bacteria, pathogens and other antigens; three main types of immunosensory cells are involved: surface entherocytes, M cells and intestinal dendritic cells. Resident bacteria may exert a dual function, the stimulation of mucosal mechanisms of defence and the maintenance of the homeostasis of the immune response. Thus there is evidence of a correlation between the composition of the colonizing microbiota and variations in immunity (O'Hara and Shanahan, 2006). The gut microbiota, with its metabolic, trophic and protective functions, is able to affect positively the integrity of the intestinal barrier. Loss of the integrity (i.e., intestinal barrier dysfunction) leads to a progressive increase of intestinal permeability, inducing a switch from “physiological” to “pathological” inflammation that is characteristic of diseases such as intestinal bowel disease (IBD) (Frank et al., 2007; Lambert, 2009). Intestinal pathogens produce toxins and other classes of substances i.e. mucinases, adhesins and invasins, which interfere with epithelial metabolism. All together, the pathogenic phenotype is likely to directly trigger uncontrolled pathological inflammation. Increasing evidence indicates that changes in gut microbiota, with an increase of pathogenic bacteria and a decrease of health-promoting bacteria, such as bifidobacteria and lactobacilli, play an important role in promoting and maintaining intestinal inflammation in IBD (Andoh and Fujiyama, 2006).

Physiological and psychological stressors leading to dysfunction of the intestinal barrier and to increase of intestinal permeability, have an impact on gut microbial composition and susceptibility to enteric pathogens (Gareau et al., 2009). Beneficial bacteria, such as lactobacilli and bifidobacteria, have been shown to decrease when stressing factors occur (Si et al., 2004).
Moreover, stress situations generally result in a poor growth rate and productivity in livestock and poultry. As an example, in piglets, weaning is a critical period that involves stressful factors, such as withdrawal from the mother, lack of antibodies originating from the sow’s milk and dietary changes. In poultry production systems, birds are routinely subjected to stresses such as feed withdrawal, temperature fluctuations, and confinement during transportation (Spreeuwenberg et al., 2001; St-Pierre et al., 2003; Humphrey, 2006).

2. Probiotics, prebiotics and synbiotics

2.1. Probiotics

Many definitions have been proposed for the term “probiotic”. The more widely accepted one is “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host” (FAO/WHO, 2002). This definition implies that a health effect must be demonstrated for the probiotic. The beneficial modes of action include: regulation of intestinal microbial homeostasis, stabilization of the gastrointestinal barrier function (Salminen et al., 1996), expression of bacteriocins (Mazmanian et al., 2008), enzymatic activity inducing absorption and nutrition (Hooper et al., 2002; Timmerman et al., 2005), immunomodulatory effects (Salzman et al., 2003), inhibition of procarcinogenic enzymes and interference with the ability of pathogens to colonize and infect the mucosa (Gill, 2003).

The expected health-promoting characteristics and safety criteria of probiotics are shown in Table 1.

<table>
<thead>
<tr>
<th>Non-toxic and non-pathogenic</th>
<th>Accurate taxonomic identification</th>
<th>Normal inhabitant of the targeted species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival, colonization and being metabolically active in the targeted site, which implies:</td>
<td>Resistance to gastric juice and bile</td>
<td>Persistence in the GIT</td>
</tr>
<tr>
<td>Adhesion to epithelium or mucus</td>
<td>Competition with the resident microbiota</td>
<td>Production of antimicrobial substances</td>
</tr>
<tr>
<td>Antagonism towards pathogenic bacteria</td>
<td>Modulation of immune responses</td>
<td>Ability to exert at least one scientifically-supported health-promoting properties</td>
</tr>
<tr>
<td>Genetically stability</td>
<td>Amenability of the strain and stability of the desired characteristics during processing, storage and delivery</td>
<td>Viability at high populations</td>
</tr>
<tr>
<td>Desirable organoleptic and technological properties when included in industrial processes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.1.1. Regulatory considerations

Significant progress in legislation for the safety evaluation of probiotics, has been made in USA, Canada, and Europe (EFSA, 2005a; HC, 2006; FAO/WHO, 2002); however, no unique standard is available. In the USA, specific utilization of microorganisms for human consumption should possess “GRAS” status (“Generally Regarded As Safe”) regulated by the Food and Drug Administration. In Europe, the European Food Safety Authority (EFSA) has introduced the concept of Qualified Presumption of Safety (QPS) similar in purpose to the GRAS approach. The QPS concept provides a generic assessment system for use within EFSA that in principle can be applied to all requests received for the safety assessments of microorganisms deliberately introduced into the food chain (EFSA, 2005b). According to recent evaluation (Wassenaar and Klein, 2008), QPS system appears more flexible because it takes into account additional criteria to evaluate the safety of bacterial additives such as a history of safe use in the food industry and the acquisition of antibiotic resistance or virulence determinants. EFSA has published a list of microorganisms, which possess a known historical safety, proposed for QPS status (Table 2) (EFSA, 2007a). Table 2 does not include Enterococcus species, even if E. faecium shows a long history of apparent safe use in food or feed. The main reason is due to the possibility of carrying transmissible resistance to antibiotics by Enterococcus spp. (EFSA, 2007a).

A list of the probiotic species for studies or application in animal feed is shown in Table 3; these data were derived from extensive literature and internet search of commercial products. Often not valid taxonomic designations are used in scientific publications or in commercial preparations (Table 3). Lactobacillus, Enterococcus, Bacillus and Saccharomyces are actually the most used probiotics in livestock and poultry. Many studies indicate that the organisms cited on the labels of certain probiotic products are not actually contained within the product and often the products contain other species than those claimed on the label (Huff, 2004; Mattarelli et al., 2002; Wannaprasat et al., 2009). It is necessary to indicate clearly on the label of the products the name of the exact taxonomic species of probiotics utilized in order to avoid confusion and misidentification. Regulatory bodies should carefully monitor and control these indications. Another important point is the viability and consequently derived concentrations of viable bacteria of probiotic preparations at the moment of administration to the animals. The claim made by the producer about the preparation should reflect the actual composition of the food until the “best-before” date of the product at the recommended storage conditions with a decrease of one or two logarithmic units at maximum (Czinn and Blanchard, 2009); often the number of probiotic bacteria found in the products were below the one declared or they were absent (Wannaprasat et al., 2009). It is fundamental to study proper formulations which will allow the maximum viability of the bacteria species utilized.

2.1.2. Efficacy of probiotics

The use of probiotics in animal feeding could be enhanced by a preliminary in vitro screening: antimicrobial activity, survival in the GIT, adhesion studies and antibiotic susceptibility are among the main probiotic properties that should be analysed to assess functionality and safety. The advanced molecular methods, such as microarrays, will improve the detection of these multiple characteristics, also allowing the analysis of phenotypic and genetic properties useful for industrial production.

Probiotic activity could be related to genera, species, or strains. An approach in probiotic application could be the use of mixtures of strains belonging to different genera or species (Timmerman et al., 2004).

Dose, timing and duration of the administration of probiotics may be a factor affecting efficacy: in acute infectious diarrhea, higher dose of probiotic given for short period of time seems to be more effective than lower doses (Sazawal et al., 2006); in atopic dermatitis, early treatment and long period of administration (2 years) induce better and long-lasting improvement in newborn than in children and/or short-course therapy with Lactobacillus species (Rosenfeldt et al., 2003). Another determinant may be the age of the animals; during early life, colonization patterns are instable and newborn animals are then susceptible to environmental pathogens. Initial colonization is of great importance to the host because the bacteria can modulate expression of genes in epithelial cells thus creating a favorable habitat for themselves (Siggers et al., 2007).

2.1.3. Most used probiotic genera

2.1.3.1. Lactobacillus. The genus Lactobacillus is a wide and heterogeneous taxonomic unit, comprising more than 100 different species, belonging to the group of Lactic Acid-producing Bacteria (LAB). Many of the species are significant constituents of the normal gut microbiota of humans and animals, and their occurrence and number are host dependent. Several species of the genus are intentionally introduced
in the food chain, being involved in a range of food and feed fermentations, and applied as probiotics in humans and animals (Hammes and Hertel, 2007). However, some reports stated that these microorganisms might rarely be involved in human diseases, where L. casei and L. rhamnosus are the most common (Vesterlund et al., 2007). No report can be found on safety concerns related to lactobacilli in animals. Due to the long history of safe use, a list of species has been proposed for QPS status (Table 2) (EFSA, 2007a).

2.1.3.2. Enterococcus. The genus Enterococcus belongs to the LAB group. Enterococci are found naturally in food products. These microorganisms are used as starter cultures in food products, such as cheese, as probiotic cultures for humans and animals and as silage additives (Foulque Moreno et al., 2006). Enterococci are sometime associated with human infections. The Enterococcus genus is of particular medical relevance because of increased incidence as a cause of disease in hospital-acquired (nosocomial) infections, and acquired antibiotic resistance towards the available antibiotic therapies. Several virulence factors have been described and the number of vancomycin-resistant enterococci is increasing (Leavis et al., 2006). Strains belonging to E. faecium have a long history of apparent safe use in industrial and agricultural applications; however other species, such as E. durans and E. hirae, have been associated with infections in chickens (Abe et al., 2006; Chadfield et al., 2005). The use of enterococci as probiotics remains a controversial issue. While the probiotic benefits of some strains are well established, the emergence and the increased association of enterococci with human diseases and multiple antibiotic resistances have raised concern regarding their use as probiotics. The concern that antimicrobial resistance genes or genes encoding virulence factors could be

<table>
<thead>
<tr>
<th>Species</th>
<th>Qualifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bifidobacterium adolescentis</td>
<td>Bifidobacterium bifidum</td>
</tr>
<tr>
<td>Bifidobacterium animalis</td>
<td>Bifidobacterium breve</td>
</tr>
<tr>
<td>Corynebacterium glutamicum</td>
<td></td>
</tr>
<tr>
<td>Lactobacillus acidophilus</td>
<td>Lactobacillus farciniminis</td>
</tr>
<tr>
<td>Lactobacillus amylyticus</td>
<td>Lactobacillus fermentum</td>
</tr>
<tr>
<td>Lactobacillus amylavorus</td>
<td>Lactobacillus gallinarum</td>
</tr>
<tr>
<td>Lactobacillus alimentarius</td>
<td>Lactobacillus gasseri</td>
</tr>
<tr>
<td>Lactobacillus aviaries</td>
<td>Lactobacillus helveticus</td>
</tr>
<tr>
<td>Lactobacillus brevis</td>
<td>Lactobacillus hilgardii</td>
</tr>
<tr>
<td>Lactobacillus buchneri</td>
<td>Lactobacillus johnsonii</td>
</tr>
<tr>
<td>Lactobacillus casei</td>
<td>Lactobacillus kefiranofaciens</td>
</tr>
<tr>
<td>Lactobacillus crispatus</td>
<td>Lactobacillus kefiri</td>
</tr>
<tr>
<td>Lactobacillus curvatus</td>
<td>Lactobacillus mucosae</td>
</tr>
<tr>
<td>Lactobacillus delbrueckii</td>
<td>Lactobacillus panis</td>
</tr>
<tr>
<td>Lactococcus lactis</td>
<td></td>
</tr>
<tr>
<td>Leuconostoc citreum</td>
<td>Leuconostoc lactis</td>
</tr>
<tr>
<td>Pediococcus acidilactici</td>
<td>Pediococcus dextrinicus</td>
</tr>
<tr>
<td>Propionibacterium freudenreichii</td>
<td></td>
</tr>
<tr>
<td>Streptococcus thermophillus</td>
<td></td>
</tr>
<tr>
<td>Bacillus</td>
<td>Bacillus lentus</td>
</tr>
<tr>
<td>Bacillus amyloliquefaciens</td>
<td>Bacillus licheniformis</td>
</tr>
<tr>
<td>Bacillus atrophaeus</td>
<td>Bacillus megaterium</td>
</tr>
<tr>
<td>Bacillus clausii</td>
<td>Bacillus mojavensis</td>
</tr>
<tr>
<td>Bacillus coagulans</td>
<td></td>
</tr>
<tr>
<td>Bacillus fusiformis</td>
<td></td>
</tr>
<tr>
<td>Yeasts</td>
<td></td>
</tr>
<tr>
<td>Debaryomyces hansenii</td>
<td></td>
</tr>
<tr>
<td>Hanseniaspora uvarum</td>
<td></td>
</tr>
<tr>
<td>Kluyveromyces lactis</td>
<td>Kluyveromyces marxianus</td>
</tr>
<tr>
<td>Pichia angusta</td>
<td>Pichia anomala</td>
</tr>
<tr>
<td>Saccharomyces bayanus</td>
<td>Saccharomyces cerevisiae</td>
</tr>
<tr>
<td>Pichia fermentum</td>
<td>Geobacillus stearothermophilus</td>
</tr>
<tr>
<td>Xanthophyllomyces dendrorhous</td>
<td></td>
</tr>
</tbody>
</table>

* Absence of acquired antibiotic resistance should be systematically demonstrated unless cells are not present in the final product.

b When strains of these QPS units are to be used as seed coating agents, testing for toxic activity is not necessary, provided that the risk of transfer to the edible part of the crop at harvest is very low.
transferred to other bacteria in the gastrointestinal tract contributes to this controversy (Foulquie Moreno et al., 2006; Kayer, 2003). Due to safety concerns, no members of the genus Enterococcus have been proposed for QPS status (EFSA, 2007a).

2.1.3.3. **Bacillus.** Bacillus species are Gram-positive, spores-forming microorganisms, commonly associated with soil, water and air. Bacillus species are normally allochthonous microbes to the intestinal tract as a result of an involuntary ingestion of contaminated feed. The use of viable spores of Bacillus as probiotic supplement raised a number of questions, including their safety: several Bacillus species used as animal feed supplements, probiotics, plant protection products or seed coating agents are also known as agents of food poisoning (Sanders et al., 2003). The knowledge gained from their use, as animal feed supplement, suggests that, for some species at least, their safety could be assured by the QPS approach (EFSA, 2007a) (Table 2). Since most Bacillus species potentially possess toxigenic traits, absence of toxigenic activity needs to be verified for qualification.

2.1.3.4. **Saccharomyces.** Saccharomyces is a genus of budding yeast. Yeasts are also part of the residual microbial system of the intestinal microbiota. Saccharomyces cerevisiae is widespread in nature and can be found in plants, fruit and soil. S. cerevisiae is included in foods and beverages for its key role in fermentation processes and in health foods. Strain known as S. boulardii was isolated from the skin of lychees grown in Indochina. This species does not have a taxonomic status and it is considered a biotype of

S. cerevisiae (van der Aa Kühle and Jespersen, 2003). S. boulardii is used as probiotic especially in ruminants and pig feeding.

2.1.3.5. **Bifidobacterium.** In the intestinal tracts of animals and humans, bifidobacteria are considered one of the key genera. Their presence in high numbers is associated with good health status of the host. There is a general belief that bifidobacteria are helpful in maintaining appropriate balance of the microbiota in the GIT, reducing the risk of pathogen infection. Several species are host specific (Biavati and Mattarelli, 2006). Bifidobacteria are very promising probiotics even if it is to be considered that probiotic properties are species and/or strain specific. They are frequently used in food and pharmaceutical preparations and their application in animal feeding is increasing. Due to the long history of safe use of bifidobacteria, many species are proposed for QPS status (Table 2).

2.1.4. **Undeﬁned microbial preparations used as probiotics: competitive exclusion**

Competitive exclusion (CE), also indicated as the “Nurmi concept”, originate from the finding that newly hatched chicks could be protected against Salmonella colonization of the gut by dosing them with a suspension of gut content prepared from healthy adult chickens (Nurmi and Rantala, 1973). The introduction of CE bacteria from the gut content should occur early in life, such that the CE bacteria are preferentially established in the gastrointestinal system to become competitive or antagonistic to opportunistic pathogens.

Because of the use of undefined preparations from the cecal or fecal material could result in the transmission of pathogens, regulatory restrictions for probiotic microorganisms (SCAN, 2000) made authorization difﬁcult for this kind of products. However, CE products with deﬁned and identiﬁed microorganisms have been developed and applied in animal breeding (Schneitz, 2005).

2.2. **Prebiotics**

Prebiotics are “nondigestible food ingredients that beneﬁcially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon” (Gibson and Roberfroid, 1995). For a dietary substrate to be classed as a prebiotic, at least three criteria are required: (1) the substrate must not be hydrolysed or absorbed in the stomach or small intestine, (2) it must affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and (3) fermentation of the substrate should induce beneﬁcial luminal/systemic effects within the host. (Scantlebury-Manning and Gibson, 2004). The effects of dietary ﬁber on upper and lower gastrointestinal tract are shown in Table 4.

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**Table 3**

List of probiotics studied for application in animal feed.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bifidobacterium</td>
<td>B. animalis subsp. animalis (B. animalis)*</td>
</tr>
<tr>
<td></td>
<td>B. lactis subsp. lactis (B. lactis)</td>
</tr>
<tr>
<td></td>
<td>B. longum subsp. longum (B. longum)</td>
</tr>
<tr>
<td></td>
<td>B. pseudolongum subsp. pseudolongum (B. pseudolongum)</td>
</tr>
<tr>
<td></td>
<td>B. thermophilum</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>E. faecalis (Streptococcus faecalis)</td>
</tr>
<tr>
<td></td>
<td>E. faecium (Streptococcus faecium)</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>L. acidophilus</td>
</tr>
<tr>
<td></td>
<td>L. amylovorus</td>
</tr>
<tr>
<td></td>
<td>L. brevis</td>
</tr>
<tr>
<td></td>
<td>L. casei subsp. casei (L. casei)</td>
</tr>
<tr>
<td></td>
<td>L. crispatus</td>
</tr>
<tr>
<td></td>
<td>L. fermentum</td>
</tr>
<tr>
<td></td>
<td>L. murinus</td>
</tr>
<tr>
<td></td>
<td>L. plantarum subsp. plantarum (L. plantarum)</td>
</tr>
<tr>
<td></td>
<td>L. reuter</td>
</tr>
<tr>
<td></td>
<td>L. rhamnosus</td>
</tr>
<tr>
<td></td>
<td>L. salivarius</td>
</tr>
<tr>
<td></td>
<td>L. amylovorus (L. sobrius)</td>
</tr>
<tr>
<td>Lactococcus</td>
<td>L. lactis subsp. cremoris (Streptococcus cremoris)</td>
</tr>
<tr>
<td></td>
<td>L. lactis subsp. lactis</td>
</tr>
<tr>
<td>Leuconostoc</td>
<td>L. citreum</td>
</tr>
<tr>
<td></td>
<td>L. lactis</td>
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<tr>
<td></td>
<td>L. mesenteroides</td>
</tr>
<tr>
<td>Pediococcus</td>
<td>P. acidilactici</td>
</tr>
<tr>
<td></td>
<td>P. pentosaceus subsp. pentosaceous</td>
</tr>
<tr>
<td>Propionibacterium</td>
<td>P. freudenreichii</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>S. infantarius</td>
</tr>
<tr>
<td></td>
<td>S. salivarius subsp. salivarius</td>
</tr>
<tr>
<td></td>
<td>S. thermophilus (S. salivarius subsp. thermophilus)</td>
</tr>
<tr>
<td>Bacillus</td>
<td>B. cereus (B. cereus var. toysii)</td>
</tr>
<tr>
<td></td>
<td>B. licheniformis</td>
</tr>
<tr>
<td></td>
<td>B. subtilis</td>
</tr>
<tr>
<td>Saccharomyces</td>
<td>S. cerevisiae (S. boulardii)</td>
</tr>
<tr>
<td></td>
<td>S. pastorianus (S. carlibergensis)</td>
</tr>
<tr>
<td>Kluyveromyces</td>
<td>K. fragilis</td>
</tr>
<tr>
<td></td>
<td>K. marxianus</td>
</tr>
<tr>
<td>Aspergillus</td>
<td>A. orizae</td>
</tr>
<tr>
<td></td>
<td>A. niger</td>
</tr>
</tbody>
</table>

* In bracket not valid taxonomic designations used in commercial preparations or in scientific publications.

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**Table 4**

Intestinal functions assigned to prebiotics.

<table>
<thead>
<tr>
<th>Dietary fibers and gastrointestinal functions</th>
<th>Effects on upper GI tract</th>
<th>Effects on lower GI tract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resistance to digestion</td>
<td>Acting as food for colonic microbiota</td>
</tr>
<tr>
<td></td>
<td>Retarded gastric emptying</td>
<td>Acting as substrates for colonic fermentation</td>
</tr>
<tr>
<td></td>
<td>Increased oro-caecal transit time</td>
<td>Production of fermentation end products (mainly SCFAs)</td>
</tr>
<tr>
<td></td>
<td>Reduced glucose absorption and low glycaemic index</td>
<td>Stimulation of saccharolytic fermentation</td>
</tr>
<tr>
<td></td>
<td>Hyperplasia of the small intestinal epithelium</td>
<td>Acidification of the colonic content</td>
</tr>
<tr>
<td></td>
<td>Stimulation of secretion of intestinal hormonal peptides</td>
<td>Hyperplasia of the colonic epithelium</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stimulation of secretion of colonic hormonal peptides</td>
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<tr>
<td></td>
<td></td>
<td>Bulking effect on stool production</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Regularization of stool production (frequency and consistence)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acceleration of ceco-anal transit</td>
</tr>
</tbody>
</table>
Most identified probiotics are carbohydrates and oligosaccharides with different molecular structures normally occurring in the human and animal diet; dietary carbohydrates such as fibers, are candidate probiotics, but most promising are nondigestible oligosaccharides (NDOs).

NDOs which meet the critical point of the definition are fructooligosaccharides (FOS, oligofructose and inulin), galactooligosaccharides (GOS), transgalacto-oligosaccharides (TOS), and lactulose. However a large number of other NDOs, to which less rigorous studies have been so far applied are glucooligosaccharides, glycooligosaccharides, lactitol, isomaltooligosaccharides, maltoligosaccharides xylo-oligosaccharides, stachyose, raffinose, and sucrose thermal oligosaccharides have also been investigated (Patterson and Burkholder, 2003). Although mannanoligosaccharides (MOS) have been used in the same manner as the prebiotics listed above, they do not selectively enrich for beneficial bacterial populations. Investigation on the mode of action of mannanoligosaccharide pointed out that these compounds are able to bind to mannose-specific lectin of gram-negative pathogens that express Type-1 fimbriae such as Salmonella and E. coli, resulting in their excretion from the intestine (Baurhoo et al., 2007; Thomas et al., 2004).

Diets modulation of the human gut flora has been carried out for many years. In humans, probiotic addition to the diet has brought positive aspects to the gut microbial balance. The use of probiotics in animal production, as a possible alternative to antimicrobial growth promoters, has given contradictory results, while their use in the modulation of the gut microbial equilibrium is worthwhile. They contribute to the establishment of a ‘healthier’ microbiota where bifidobacteria and/or lactobacilli become predominant and exert possible health-promoting effects at the expense of more harmful species.

2.3. Symbiotics

Symbiotics may be defined as a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract (Gibson and Roberfroid, 1995). The acquisition of data on the efficacy of symbiotic products as feed additives in livestock and poultry needs further investigation. However, results on in vivo trials are promising, showing a synergistic effect coupling probiotics and prebiotics in the reduction of food-borne pathogenic bacterial populations (Bombà et al., 2002).

3. Pathogens and food-borne disease

Zoonotic bacteria can cause clinical disease, morbidity and mortality in animals and are a major source of economic loss to the livestock and poultry industry worldwide. Moreover these enteric pathogens could be carried in the animal intestinal tract asymptomatically and can be transmitted through the food chain to humans becoming a risk for the health as food-borne disease. Contamination of food can happen at any stage of the production chain: raw materials used in animal nutrition, feed manufacturing, farm level, slaughter plant, meat processing, retail and preparation of meat at home. To improve food safety, the industry is requested to decrease the level of contamination to zero or at least to acceptable levels depending on the pathogen (EFSA, 2007b).

In the EFSA-ECDC (2009) report, the analysis, which was conducted in 2007, of the occurrence of infectious diseases transmitted from animals to humans shows the following figures: Campylobacter was the most frequently reported zoonotic disease in humans across the European Union with 200,507 cases compared to 175,561 in the previous year, with an increase of 14.2%. Regarding Salmonella, although the number of cases showed a decrease for a fourth successive year, 151,995 people were affected by the bacterium in 2007 compared to 164,011 in 2006. Other food-pathogens, such as Verocytotoxigenic-producing Escherichia coli (VTEC), Listeria, Echinococcus, Trichinella and Lyssavirus (rabies), despite the low relative number of cases (Fig. 1), can cause infections considered important due to the severity of the illness and higher case fatality rate.

3.1. Most frequent food-borne disease agents

3.1.1. Campylobacter

Campylobacter together with Salmonella are currently considered the most frequent bacterial causes of human gastroenteritis in developed countries worldwide (Fig. 1). Wildlife reservoirs play an important role in the ecology of Campylobacter. A wide variety of warm-blooded animals may be colonized by more than one Campylobacter species or strain without showing any obvious symptoms (Humphrey et al., 2007). Intestinal tracts of farm animals, such as poultry, cattle, and pigs, are frequently colonized. The wide diffusion of Campylobacter species in animal gut populations cause the risk of contamination of food products such as raw meat and milk as well as water, having major consequences for human health in terms of food-borne diseases (Wysok and Uradziński, 2009).

3.1.2. Salmonella

Salmonella spp. infection remains a major cause of food-borne illnesses in humans (Fig. 1). The Salmonella genus contains over 2500 serotypes, all of which are potentially pathogenic. In the EU, among the food-borne cases of human salmonellosis, eggs and egg products are still the most frequently implicated sources. Meat is also an important source of food-borne salmonellosis, with poultry and pork implicated more often than beef and lamb (Young et al., 2009). Salmonella can infect humans and animals. The nomenclature and taxonomy of Salmonella spp. are still a matter of discussion. In the article of Tindall et al. (2005), only two species S. bongori and S. enterica are considered or proposed to be valid. The last one is divided into 6 subspecies. Serovar Typhimurium and serovar Enteritidis, belonging to S. enterica subsp. enterica, evenly present in pig and chicken respectively, are the serovars most commonly implicated in human food-borne illnesses, and often enters the human food supply chain via contamination of poultry, pork, beef and dairy products (Hanning et al., 2009).

4. Application of probiotics, prebiotics and synbiotics in livestock

4.1. Pigs

Farm animals are often subjected to environmental stresses (management methods, diet, etc.) which can cause imbalance in the intestinal ecosystem and could be a risk factor for pathogen infections. Each species of livestock has its critical point in the production chain. In commercial swine production, for example, the most stresses are related to the weaning and post-weaning (PW) periods (separation from the sow, end of the lactation immunity, early and critical transition from milk onto a diet based on plant polysaccharides, transport to a production
farm). These periods are characterized by an immediate but transient drop in feed intake impairing growth performance of the animals. All these factors can negatively disturb the immune function and the intestinal microbiota equilibrium of the pigs (Modesto et al., 2009), leading to increased susceptibility to gut disorders, infections and diarrhoea. In the past, the management of weaning and PW has involved the preventive use of antibiotics and metals (copper and zinc) in weaner diets. However, after the full ban on in-feed antibiotics and the drastic reduction in the levels of incorporation of copper and zinc by the European Union (EC, 2003b), alternative substances to control PW disorders with growth-promoting characteristics are continuously explored.

There is increasing research to the use of probiotics to intervene in decreasing pathogen load and in ameliorating gastrointestinal disease symptoms in pigs. Beside the in vitro test to identify the best potential probiotics, several studies are conducted in vivo utilizing different probiotic microorganisms.

In contrast to poultry, CE culture has not been studied intensively in pig. Genovese et al. (2000) found that porcine-derived CE culture of known bacterial composition reduced the mortality and shedding of enterotoxigenic E. coli in neonatal pigs. Neonatal pigs treated with the same CE culture shed significantly lower pathogen numbers after challenge with Salmonella enterica serovar choleraesuis and also exhibited reduced counts in the lower intestine (Genovese et al., 2003). Alleviation of infection symptoms was not described. Selected colicin-producing E. coli targeting pathogenic E. coli K-88 isolated from environmental sources (cattle and swine feces and soil) showed a significant beneficial effect on performance diarrhoea in weaning piglets infected with E. coli K88 (Setia et al., 2005). Enterococcus faecium and E. faecalis, on the contrary, are subject of numerous clinical trials even if they have not been proposed for QPS status by the European Union (EFSA, 2007a). Daily oral supplementation of E. faecium to piglets, from birth to weaning, reduced the portion of subjects suffering from diarrhoea, improving performance as indicated by the higher daily weight gain (Zeyner and Boldt, 2006). In contrast, no obvious benefits resulted from an additional supply of E. faecium via electrolyte solution when diarrhoea was still present. E. faecium was also found to reduce in the colon of weaned pig population of Enterococcus faecalis which is responsible for the onset of some cases of post-weaning diarrhoea (Valjien et al., 2007). Szabó et al. (2009), on the other hand, suggests that E. faecium enhanced the course of infection in weaning piglets challenged with Salmonella enterica serovar typhimurium DT104, although the probiotic treatment resulted in greater production of specific antibodies against Salmonella enterica serovar typhimurium DT104. Addition of E. faecium to the feed of pregnant sows and piglets showed no obvious immune-stimulatory effects. However, the supplementation influenced the early intestinal bacterial colonization of suckling piglets with a reduction of the enteropathogenic bacterial load (Scharek et al., 2005). An interdisciplinary research study on the modes of action of probiotics in swine, showed that E. faecium NCIMB 10415 reduced the pathogenic bacterial load of healthy piglets and sows (Lodemann et al., 2006; Taras et al., 2006): the precise mode of action of probiotics is not currently known but the authors showed a correlation between their administration and the decline of virulence gene expression of the resident E. coli microbiota and of the inflammatory response of the host.

The clinical trials utilizing Lactobacillus strains are increasing accordingly to their recognized importance as frequent component of pig microbiota. Supplementation of the diet of neonatal pigs with a strain of Lactobacillus plantarum resulted in an increase in total gut populations of lactobacilli in weaned pigs (Takahashi et al., 2007). A synbiotic product containing L. plantarum, maltodextrin and/or fructooligosaccharides (FOS) reduced counts of E. coli O8:K88 in the jejunum and colon of piglets, and it was associated with increased acetate concentrations in the ileum and colon (Nemcova et al., 2007). L. sobrius significantly reduced the levels of ETEC in the ileum when fed directly to piglets after weaning. In contrast, the number of days when the piglets had increased fecal water content was significantly higher in the probiotic group. Nevertheless, an improved daily weight gain was also observed in the animals that received probiotic L. sobrius compared to the control fed group (Konstantinov et al., 2008). L. rhamnosus GG (LGG) was effective in ameliorating diarrhoea in post-weaning piglets induced by E. coli K88, possibly via modulation of intestinal microflora, enhancement of intestinal antibody defences, and regulation of production of systemic inflammatory cytokines (Zhang et al., 2010).

**Bifidobacterium** species are widely applied as probiotics in humans, but only few studies (and often together with Lactobacillus) are conducted in animals and particularly in pigs. Lactobacilli and bifidobacteria administration, immediately after birth, promotes the colonization of a beneficial commensal microbiota capable of limiting the artificial formula-induced mucosal atrophy, dysfunction, and pathogen load in premature neonatal piglets, thereby reducing the incidence and severity of necrotizing enterocolitis and lowering colonization density of the potential pathogen Clostridium perfringens (Siggers et al., 2008). Probiotic preparations including Bifidobacterium lactis and Lactobacillus rhamnosus individually reduced adherence of Salmonella, E. coli and Clostridium spp. to the intestinal mucosa in swine. Together the two organisms were more effective and reduced each other’s adherence (Collado et al., 2007). Reduced mucosal adhesion by pathogens is presumed to lead to reduced severity of clinical disease. Probiotic treatment using Bifidobacterium lactis HN019 reduced post-weaning diarrhoea associated with rotavirus and E. coli infections in a piglet model (Shu et al., 2001). B. animalis subsp. lactis affected positively the growth performance in weaning piglets and the ratio of bifidobacteria to E. coli in the gut (Modesto et al., 2009).

Probiotics less frequently utilized are Pediococcus and yeast such as Saccharomyces. Pediococcus acidilactici and S. cerevisiae boulardii may have the potential to modulate the establishment of lymphocyte populations and IgA secretion in the gut and to reduce bacterial translocation to mesenteric lymph nodes after E. coli ETEC infection (Lessard et al., 2009). Casey et al. (2007) showed that a five-strain probiotics combination, containing Lactobacillus and Pediococcus, reduces pathogen shedding and alleviates diarrhoea in pigs challenged with Salmonella enteritidis serovar typhimurium both early in the course of infection and over a longer time frame.

Finally, Bacillus species are also used as probiotics in some clinical trials in pigs. Inclusion of a Bacillus subtilis strain in the feed resulted in a reduction in scours 24 h after challenge of weaned pigs with a K88-positive ETEC (Bhandari et al., 2008). Alexopoulos et al. (2004) reported that the administration of spores of Bacillus licheniformis and B. subtilis reduces the morbidity and the mortality in recently weaned piglets, improves the performance parameters of the fattening pigs and improves carcass quality.

Most of the studies above mentioned showed a beneficial role of probiotic administration in piglets, improving the number of beneficial bacteria and decreasing the load of pathogens; moreover, they display a major role in stimulating the immune cell response, showing high IgM and IgA activities towards pathogens in comparison to control, and increasing defensive tools against pathogenic invasion. In contrast, some authors reported an enhancement of the course of infection or a partial alleviation of diarrhoea.

Different types of chemically defined or undefined dietary compounds are added to the diet of pigs to test their influence on gastrointestinal microbiota or on health status improvement during challenge with pathogens. TOS included at 35 g/kg in a diet for growing pigs resulted in a significant increase in fecal bifidobacteria and lactobacilli without growth performance increase (Smiricky-Tjardes et al., 2003). A novel galactooligosaccharide (GOS) mixture, supplied at 40 g/kg diet, resulted in a significant increase of the density of bifidobacteria and acetate concentration, and in a decrease of pH compared with the control diet and a control diet supplemented with inulin. In addition, the oligosaccharide mixture, strongly inhibited the attachment of ETEC E. coli and S. enterica serotype typhimurium to HT29 cells in vitro.
An interesting study was conducted on the effects of barley and oat cultivars, with different carbohydrate compositions, on the intestinal bacterial communities in weaned piglets. Increased levels of $\beta$-glucans and altered amylopectin/amyllose ratio seemed to selectively promote butyrate-producing bacteria, able to hydrolyze complex carbohydrates. Furthermore, bifidobacteria and lactobacilli counts were positively affected by the choice of the cereal variety (Pieper et al., 2008). Oligosaccharides incorporated into swine diets at levels ranging from 5 to 40 g/kg diet have resulted in mixed but generally not significant effects regarding beneficial modulation of microbial populations determined in various intestinal segments and feces of swine (Mikkelsen et al., 2003). Mountzouris et al. (2006) showed that the dietary treatment with fructooligosaccharides (FOS) or trans-galactooligosaccharides (TOS) did not influence the populations of the beneficial bacterial but promoted saccharolytic activities in the porcine colon basing on the value of total volatile fatty acids, acetate concentrations and glycolytic activities. Modesto et al. (2009) reported that GOS from milk whey, and sugar beet fructooligosaccharides (SBFOS) added to the diet of weaned pigs in different amounts had no effect on the hindgut microbiota, except for SBFOS at 40 g/kg which tended to increase the endogenous bifidobacteria, whereas growth performance was not influenced.

Prebiotics, similar to probiotics, give contradictory results in pigs: they sometime modulate the microbiota towards beneficial bacteria, such as bifidobacteria and lactobacilli, enhancing the intestinal defence systems (immunomodulatory action, pathogen displacement, bacteriocin production, etc.) and only rarely influence positively growth performance.

### 4.2. Poultry

The adaptation to the post hatching period and the increased stressors, deriving from practices used in modern broiler production, e.g. feed changes or imbalances, transportation, processing at the hatchery and high stocking densities (Pinchasov and Noy, 1993), may weaken immune functions and thus predispose broilers to colonization of the gastrointestinal tract by bacterial pathogens, posing a threat to birds health and food safety. Among pathogens, Salmonella spp. has been the most studied because of its ability to infect chickens and hens increasing the risk of contamination through the food chain (Humphrey, 2006). In the last years, application studies have been extended to other bacteria such as Campylobacter jejuni and Clostridium perfringens, which could be both considered an emerging and increasing threat for poultry and human health (Humphrey et al., 2007; Van Immerseel et al., 2004).

Probiotics could be a possible strategy to control pathogen shedding and thus maintain a healthy indigenous gut microbiota.

The application of probiotics in poultry is strictly associated with the concept of competitive exclusion (CE). Since the first applications on new hatched chicks, several experiments with undefined and defined probiotic cultures have been developed and successfully applied to control and reduce Salmonella colonization. Moreover, it has been shown experimentally that the CE treatment also protect chicks against C. jejuni, Listeria monocytogenes, pathogenic E. coli, Yersinia enterocolitica and C. perfringens (Nisbet, 2002; Schneitz, 2005).

A variety of well-characterized probiotic strains have been selected to evaluate modulation of the avian gut microbiota and protection against a variety of pathogens; in particular, there has been a recent increase in the investigation of the effect of feeding Lactobacillus spp. to broilers. Studies have focused on strains previously selected in vitro for adherence properties and antimicrobial activity (Patterson and Burkholder, 2003).

Mountzouris et al. (2007) investigated the efficacy of selected probiotic bacteria, isolated from the gut of healthy chickens (Lactobacillus reuteri, L. salivarius, Enterococcus faecium, Bifidobacterium animalis and Pediococcus acidilactici and) on body weight, feed intake and feed conversion ratio of broiler chickens; overall the probiotic formula added to water and feed displayed a growth-promoting effect that was comparable to avilamycin treatment. In addition, the probiotic cultures modulated the composition and the enzymatic activities of the cecal microflora, resulting in a significant probiotic effect.

The available body of literature offers a variety of conflicting results concerning the efficacy of probiotics for increasing growth performance in broilers; inconsistent results have been also reported from other authors (Estrada et al., 2001; O’Dea et al., 2006) showing a confusing state of the art. Timmerman et al. (2006) underlined the importance of way and timing in the administration as main factors affecting the efficacy of the probiotic preparations. Administration via the feed, compared to administration in the drinking water, resulted in a higher increase of average daily gain; moreover the supplementation of probiotics during early life is of great importance to the host because the bacteria can modulate expression of genes in intestinal epithelial cells, thus creating a favorable habitat for themselves.
Eggs production has been also investigated in relation to probiotic application; Davis and Anderson (2002) reported that a mixed cultures of Lactobacillus acidophilus, L. casei, Bifidobacterium thermophilus and Enterococcus faecium, improved egg size and lowered feed cost in laying hens. Moreover, probiotics increase egg production and quality (Kurtoglu et al., 2004; Panda et al., 2008).

The probiotic approach has not a long history of use in broiler chickens (Yang et al., 2009). However, application studies have been increasing in the last years to assess their effect on gut health, performance, and reduction of pathogen shedding. Xu et al. (2003) found a dose-dependent effect of fructooligosaccharides (FOS) on average daily gain; whereas Juskiewicz et al. (2006) reported no impact on the performance or productivity of turkeys after feeding for eight weeks with different amounts of FOS.

By feeding chicory fructans to broilers, Yusrizal and Chen (2003a) showed an improvement in weight gain, feed conversion, carcass weight and serum cholesterol decrease; additionally, the supplementation of fructans resulted in increase of lactobacilli counts in the gastrointestinal tract and Campylobacter and Salmonella decrease (Yusrizal and Chen, 2003b). Kleessen et al. (2003) described decreased C. perfringens number and a reduction in bacterial endotoxin levels by adding 0.5% of fructan-rich Jerusalem artichokes syrup in broilers drinking water.

No weight gain was observed in turkeys fed two different concentration of inulin and mannanoligosaccharides (MOS) (Stanczuk et al., 2005), whereas Sims et al. (2004), feeding turkeys a standard diet supplemented with MOS, reported an improvement on live weight.

Yeast cell wall containing MOS reduced intestinal Salmonella concentrations by 26% in broiler chicks compared with chicks fed an unsupplemented diet (Spring et al., 2000).

Thitaram et al. (2005), with different amounts of inulosulooligosaccharide (IMO), showed a significant 2-log reduction in the level of inoculated S. enterica serovar typhimurium present in the ceca of young broiler chickens. Feed consumption, feed conversion and feed efficiency were not significantly changed compared to the control; however, the IMO containing diets significantly increased the number of the intestinal bifidobacteria. Feeding young chicks with five different oligosaccharides (inulin, oligofructose, mannanoligosaccharide, short-chain fructooligosaccharide, and transgalactooligosaccharide), no significant responses in weight gain for any of the oligosaccharides fed have been registered. Moreover the study outlined that a high dosage of prebiotics can have negative effects on the gut system and retard the growth rate of birds (Biggs et al., 2007).

Likewise, a recent study reported no effects in body weight, feed intake and feed conversion ratio in broiler chickens fed with a standard diet and GOS at two different concentrations; however the study clearly showed a significant increase in the intestinal bifidobacteria population (Jung et al., 2008).

Mainly, prebiotics seem to selectively enhance lactobacilli and bifidobacteria populations and reduce colonization by pathogenic bacteria (Baurhoo et al., 2009; Biggs and Parsons, 2008).

Results on animal performance, either with a probiotic or a prebiotic treatment, are often contradictory and mostly affected by the microorganisms or compound chosen, the dietary supplementation level, and duration of use. In many cases, the environmental and the stress status of the animals are not reported or considered, as the experimental settings are often too far from farm conditions.

Recent development and applications of symbiotic products have focused on the assessment of beneficial effects in poultry health and production; however, information available to date is scarce. Mohln et al. (2007) found that a symbiotic product had a comparable potential to improve broiler performance as avilamycin treatment. A Lactobacillus spp.-based probiotic product, in combination with dietary lactose, was successfully assessed, improving body weight and feed conversion in Salmonella-challenged turkeys (Vicente et al., 2007). Li et al. (2008), adding FOS and B. subtilis to the diet, observed that average daily gain and feed conversion ratio were improved; diarrhoea and mortality rate were reduced compared to aureomycin treatment.

A considerable increase in the bifidobacteria, lactobacilli and total anaerobes populations has been shown when feeding a diet containing a combination of a galactooligosaccharide and Bifidobacterium lactis but no effect on body weight, feed intake and feed conversion was observed (Jung et al., 2008).

Awad et al. (2009) investigated the effect of a dietary treatment with a symbiotic product (a combination of E. faecium, a probiotic derived from chicory, and immune modulating substances derived from sea algae) on broiler chickens. Body weight, average daily weight gain, carcass yield percentage, and feed conversion rate were significantly increased compared with the control, whereas no increase in organ weight was found, with exception for the small intestine; a significant increase in the villus height in both duodenum and ileum was also observed.

Overall, all the authors agreed that a symbiotic product displayed a greater effect than individual preparations (Awad et al., 2009; Jung et al., 2008; Revollo et al., 2009; Vandeplass et al., 2009). This coupling could represent an important and synergistic strategy to improve gut health of chickens from the first days of life and control pathogen release in the environment, decreasing the risk of foodborne infections in humans. Thus, future research and applications in field trials are necessary to look for new combinations with the aim to produce standard safe compositions at a high functional level.

4.3. Ruminants

Studies on the application of probiotics in ruminants have been performed in the pre-ruminant’s life and in adult ruminants, considering both the health status of the animals (reduction of incidence/severity of diarrhoea, carriage of pathogenic microorganisms) and the economic parameters. Mostly, applications have been addressed to cows and calves whereas less information is available for lambs, ewes and goats.

Neonatal-calf diarrhoea, most often caused by enterotoxigenic E. coli, is an important cause of morbidity and mortality in young ruminants. Rodriguez-Palacios et al. (2004) have documented inconsistent results for the oral administration of probiotics on the incidence and severity of calf diarrhoea, with around 40% of the clinical trials lacking adequate scientific support on the probiotic strains tested.

Reduction in the incidence of diarrhoea was obtained in calves fed milk fermented with either mixed Lactic Acid Bacteria, or L. acidophilus 15 or S. cerevisiae NCDC49 (Agarwal et al., 2002). Likewise, the administration of viable E. coli bacteria, strain Nissle 1917, had a clear beneficial effect on the prophylaxis and treatment of neonatal-calf diarrhoea. (Von Buenau et al., 2005). In young calves, incorporating live yeasts into the grain, reduced the number of days with diarrhoea (Galvao et al., 2005). Two different probiotic preparations, containing six Lactobacillus spp. of bovine and human origin, were successful in reducing the overall mortality, incidence of diarrhoea and fecal coliforms counts in veal calves (Timmerman et al., 2005).

Among zoonotic pathogens, E. coli O157:H7 is, undoubtedly, the main health threat for ruminants, and cattle are considered the major reservoir (Lejeune and Wetzel, 2007), playing an important role in the epidemiology of human infections (Griffin and Tauxe, 1991).

To reduce carriage and shedding of E. coli O157:H7, selected cultures containing generic E. coli strains have been successfully applied to adult cattle (Schamberger and Diez-Gonzalez, 2002; Tkalic et al., 2003). A daily addition of 10^8 of colicin E7-producing E. coli per gram of feed has been shown to reduce the fecal shedding of serotype O157:H7 in cattle (Schamberger et al., 2004).

Recently, some authors have focused on the use of lactobacilli as probiotic additives in ruminant feeding. In a large-scale trial, steers
fed a standard steam-flaked corn-based finishing diet containing *L. acidophilus* NP51 showed a reduction of *E. coli* O157 fecal shedding by 57% (Younts-Dahl et al., 2004).

A field trial, lasting two years, clearly showed that *E. coli* O157:H7 fecal shedding decreased (35%) in beef cattle, following daily administration of *L. acidophilus* strain NP51 (Peterson et al., 2007). Other authors have found greater reductions (Brashears et al., 2003; Tabe et al., 2008).

With regard to animal performance, improved weight gain and rumen development have been reported in young calves with several bacterial and yeast strains supplementation (Adams et al., 2008). In dairy ruminants, live yeasts have been shown to improve performance, the most consistent effects being an increase in dry matter intake and milk production (Jouan, 2006; Stella et al., 2007). A recent meta-analysis study conducted by Desnoyers et al. (2009) showed that yeast supplementation, in ruminants, increased dry milk intake, milk yield, rumen pH, rumen volatile fatty acids concentration, and organic matter digestibility.

Other benefits have been related to greater total culturable ruminal bacterial population densities and cellulo-lytic microorganisms (Chauheyras-Durand et al., 2008) and increase fiber digestibility (Guedes et al., 2008; Maiden et al., 2008).

In beef cattle, the overall results led Krehbiel et al. (2003) to conclude that probiotics, potentially, increase some growth parameters when fed to finishing cattle; however, effects on performance have not been consistent. Similar findings have been reported, recently, by other authors (Peterson et al. 2007; Younts-Dahl et al., 2005; Vasconcelos et al., 2008).

The use of prebiotics in cattle has been limited due to the ability of ruminants to degrade most prebiotics; however enhancements in rumen-protective technologies may allow these compounds to be used in feedlot and dairy cattle (Callaway et al., 2008), considering also that several classes of nondigestible oligosaccharides are found in plant cell wall in nature including feeds normally used for livestock feeding (Lema et al., 2002).

Addition of MOS to the diet of Holstein calves improved fecal scores just as for antibiotic treatment when compared to control milk replacer; whereas body weight was not affected (Heinrichs et al., 2003). Supplementation of sorbitol, l-arabinose, trehalose, and mannose to cattle rumen medium displaced *E. coli* O157:H7 within 72 h (de Vaux et al., 2002). The overall studies on the effect of forage and concentrate diets on fecal shedding and colonization of the gut by *E. coli* O157:H17 are still unclear and little information is available; however the manipulation of the fiber content could bring new perspectives maintaining the animals on a concentrate diet without sacrificing cattle weight gain as showed by Lema et al. (2002).

The concept of symbiotics in ruminants, to our knowledge, has not been widely applied. Fliege et al. (2007) investigated the effect of lactulose in pre-ruminant calves in combination with *E. faecium* to determine influence and effect on the growth performance and on the intestinal morphology; while Yasuda et al. (2007) evaluated the effect of a new symbiotic product consisting of *Lactobacillus casei* subsp. *casei* and dextran on milk production in Holstein dairy cows.

Both combinations were beneficial for the animals; in particular the second study evidenced the improvement in milk production as a result of a positive change in the intestinal bovine microbiota, a reduction in the incidence of infectious diseases and a decrease in some forms of stress.

5. Concluding remarks

Antibiotics at sub-therapeutic levels as AGPs have been used in the European Union by the member states for the last fifty years. After their complete ban by the European Union in January 2006 for the possibility of generation of resistant pathogen strains, the negative consequences for animal health and welfare and for food safety on a multi-national scale have become particularly evident in the food chain. For example the incidence of *C. jejuni*, one of the most common pathogens in livestock, has increased from 2006 to date (EFSA, 2009). This is in agreement with the idea that the use of antibiotics resulted, besides the improvement of nutrient absorption enhancing feed intake and weight gain, in the inhibition of pathogens, widespread at the primary production level. Therefore the research of a rational development of new alternative strategies for good animal performance together with low or absence of pathogens in the livestock food chain has to be intensified.

The subtle manipulation of gastrointestinal microbiota in maintaining animal gut health, through diversity, stability, metabolites and crosstalk with the epithelium and the underlying immune system by probiotics and prebiotics could be a favorable route. Probiotics can find their main application in the prevention of gastrointestinal infection and disease more than a curative approach. This is because the action of probiotics is not generally aimed, as for antibiotics, to kill pathogen bacteria but they modulate the gastrointestinal environment reducing the risk of gastrointestinal disease synergistically with the immune system of the host.

Most studies dealing with probiotics and prebiotics are conducted in man, but increasing research has been developed in animals. Several studies in farm health or stressed animals demonstrated the effects of these feed additives in pigs, poultry and ruminants, by improving the number of beneficial bacteria and the reduction of the potential pathogen load.

Moreover, the effects of probiotics and prebiotics on the growth performance of livestock are contradictory with the improvements significant only in some feeding trials. Probiotic treatments sometimes positively influenced the growth performance, promoting the feed efficiency of the animal. The low or absent effect on growth performance in animals treated with prebiotic can be due to the fact that there is enough prebiotic compounds in a normal diet, such as oat, barley or wheat, and prebiotic availability is not a limiting factor: therefore feed efficiency is not influenced.

The study of the mechanisms of action of pro and prebiotics is a network where from human studies it is possible to increase knowledge of animal application and vice versa. For example, animal studies suggest that dietary consumption of oligofructose might enhance satiety, thereby resulting in a reduction in energy intake in rats (Daubioul et al., 2002). Oligofructose treatment increases satiety following breakfast and dinner, reduces hunger and prospective food consumption following dinner also in healthy humans stabilizing the weight of an individual, and it can be helpful in overweight individuals (Cani et al., 2006; DiBaise et al., 2008). These results applied in livestock production should be analyzed with care because the administration of prebiotics can cause a satiety effect also in animals and so jeopardizing the attempt to increase weight gain.

The studies on the efficacy of probiotics and prebiotics in animals and man often produced contrasting results: these can derive from the heterogeneity of the experimental protocol utilized. An increased effort could be made in the standardization concerning doses, time and way of administration, animal condition, etc. In this perspective it would be possible to compare data from different experiments and to provide the basis for more refined hypothesis-driven clinical trials.

It has been recognized that the beneficial effects of probiotics are shared among a vast number of genera and species belonging not only to the human or animal gastrointestinal tract. Many of them have been utilized for many years without causing any problem, but when a probiotic has to be chosen one should take in consideration also that some of these genera, such as *Enterococcus* and *Bacillus*, contain species with proven pathogenic effects.

Future research should focus on determining the mechanism of action, evaluating probiotic and prebiotic interaction, and elucidating how the genetic and bacterial profiles of the host can influence treatment responsiveness. The future target is to increase the genomic
information on both probiotic and microbacterium activities to improve the understanding of the interactions with specific intestinal diseases. Then the goal is to apply the knowledge of GIT health normal microbiota composition in comparison with microbiota present during disease to select the right probiotic, prebiotic or symbiotic combinations.

Acknowledgement

This work was performed in the framework of the EU funded project entitled “Control and prevention of emerging and future pathogens at cellular and molecular level throughout the food chain” (PathogenCombat FP6-007081). The topic belongs to tasks of the project.

References

EFSA, 2005a. European Food Safety Authority. Opinion of the scientific committee on a request from EFSA related to a generic approach to the safety assessment by EFSA of microorganisms used in food/feed and the production of food/feed additives. EFSA Journal 226, 1–12.
EFSA, 2005b. Opinion of the scientific panel on additives and substances used in animal feed on the updating of the criteria used in the assessment of bacteria for resistance to antibiotics of human or veterinary importance. EFSA Journal 223, 1–12.


