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Review

Chicken intestinal microbiota function with a special emphasis on the role of probiotic bacteria

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Abstract

Bacterial colonization of the chicken gut by environmental microbes begins immediately after hatching. Composition of the intestinal microbiota is dependent on the surrounding environment, diet variation, pathological conditions, antibiotic therapy, and others. The genomes of all these intestinal microbes form a microbiome which by far outnumbers the host's genome. As a consequence, the microbiome provides additional metabolic functions to the host, including nutrient utilization and absorption, fermentation of non-digestible dietary fiber, synthesis of some vitamins, biotransformation of bile acids, and the well-being of their chicken host. Microorganisms can also directly interact with the lining of the gastrointestinal tract, which may alter the physiology and immunological status of the bird. Since newly hatched broiler chickens demonstrate delayed commensal colonization and low bacterial diversity, the most effective and harmless method available to control the development and composition of the intestinal microbiota is a competitive exclusion treatment by applying probiotic bacteria. Additionally, recent research has shown that probiotic bacteria have a variety of beneficial effects, including counteraction of dysbiosis, promotion of gut health and homeostasis, enhancement of immune defenses and antagonization of infectious agents.

Key words: chicken intestinal microbiota, probiotics

Introduction

Microbiota is a term defining a set of commensal, autochthonic microorganisms, co-existing with a host without causing any harm (Sekirov et al. 2010). The chicken gastrointestinal tract (GIT) is colonized by a variety of such microorganisms. The number of each microbial group is dictated by the local GIT conditions, such as pH, feed passage rate, composition of nutrients, and oxygen content. Members of the gut microbiota belong to various taxa, including Bacteria, Archaea and Fungi. The GIT is sparsely colonized by viruses, protists and helminths as well; however, because of their abilities to disrupt the microbial balance, most of them are considered pathogens (Permin et al. 2006, Qu et al. 2008, Danzeisen et al. 2011). Interestingly, some bacteriophages, which are commonly found in the GITs of various hosts, including

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chickens, can be used as probiotics in farming (Atterbury et al. 2007).

Among the chicken gut microbiota the Bacteria domain is considered the most important, since the maximum amount of this group reaches 10^{10}-10^{11} cells per gram in caecal intestinal content, and 10^{11} cells in total recovered from the caecal mucosa of a single bird (Gong et al. 2002). Each part of the GIT has a specific set of bacterial species. The small intestine is colonized mainly by lactobacilli followed by streptococci and enterobacteria, whereas the caecum is colonized by anaerobes and only by a small number of facultative anaerobes (Lu et al. 2003). Culture-independent studies reveal that *Firmicutes* is a predominant phylum in the caeca, followed by *Bacteroidetes*, then *Proteobacteria* and *Actinobacteria*. Moreover, some studies show a great number of unclassified bacteria, which reach up to 10% of all caecal bacteria (Qu et al. 2008).

Another group of gut organisms, Archaea, is less abundant (approx. 10^5-10^7 cells per gram in caeca) and is represented predominantly by methanogenic *Methanobrevibacter* (Saengkerdsub et al. 2007a). Archaea DNA provides about 1-2% of all caecal genetic information, whilst bacterial DNA provides about 95-97% (Qu et al. 2008, Danzeisen et al. 2011). However, according to Saengkerdsub et al. (2007b) colonization of the methanogenic Archaea begins very early, within 3-5 days after hatching, and prior to that time, when the GIT is finally formed. Interestingly, some cellulolytic bacteria also found in the GITs of young chicks, such as *Enterococcus* spp. and *Ruminococcus* spp., may play an important role in the development of a methanogenic community in caeca.

Influence of gut microbiota on its host

The gut represents a natural interface between the intestinal microbiota and the host. Bacterial cells by far outnumber the host cells, harbor millions of genes and form a community termed the *microbiome*. Through expression of this amount of genes they can execute numerous enzymatic reactions that the host is not able to catalyze. As a consequence the microbiome has an influence on many aspects of intestinal tract development and provides metabolic contributions well in excess of the host genome. It is well known that the intestinal microbiota affects intestinal maturation and development, possesses an immunomodulatory capacity, and is essential for homeostasis and pathogenic challenges. It also has a great impact on metabolic reactions, such as fermentation of non-digestible energy substrates and energy storage in the host, biotransformation of conjugated bile acids, synthesis of some vitamins, and others. Host-microbe interactions are also relevant in the control of the gut microbiota composition. Bacterial-epithelial cross-talk seems to be fundamental in the regulation of the microbial colonization of the gut immature epithelia. Interaction among microbes, and gut epithelial and immune cells is based on sensing of the microbial signals through host pattern recognition receptor systems (PRRs) which interact with, and identify, microbial associated molecular patterns (MMAMPs) on both commensal and pathogenic bacteria. Innate effectors, such as IgA and defensins, are additionally involved in modification of the intestinal ecology. Other important mechanisms regulating the intestinal colonization are represented by interactions among bacteria, in particular bacterial metabolic activities, such as the production of short-chain fatty acids (SCFA), modification of redox potential, synthesis of bacteriocins, and competition for receptors on the mucus layer and epithelial cells. All these activities create an ecosystem that is appropriate for some bacterial genera and hostile for others. The health of the eukaryotic organisms is sustained by an effective intestinal barrier which protects them against pathogenic prokaryotes, and appropriate housing conditions providing commensal symbionts. Microbial functions are intimately strain-related and even different strains of a single species may differ in the effect they produce (Binek et al. 2011, Binek 2012, Yeoman et al. 2012).

In poultry, absence of the normal microbiota in the caecum has been considered a major factor in the susceptibility of chicks to bacterial infection. A typical microflora of adult birds in the small intestine is established within 2 weeks; however, it was found that the adult caecal microbiota took up to 30 days to develop. Because of the susceptibility of 1-day old chicks to infections, inoculating them with competitive exclusion cultures helps to establish the microbial population and disease resistance. Apart from the commensal bacterial interference in pathogenic infections through a modulating effect on the intestinal mucosa structure, the prevention of colonization by pathogens is achieved in part by synthesis of the anti-microbial compounds including bacteriocins, short-chain fatty acids, hydrogen peroxide, and others (Yang et al. 2009).

Bacteriocins are antimicrobial peptides produced to inhibit or kill other related or unrelated bacteria, and their activity spectrum varies among species. Bacteriocins interact with their targets (i.e. *E. coli*, *Salmonella*, Typhimurium, *Staphylococcus aureus*, *Clostridium perfringens*, *Bacillus* sp., *Liseria* sp., *Klebsiella* sp., *Proteus* sp.) by bonding to the specific receptors and causing cell damage (Śliżewska et al. 2006).

These substances are produced by the lactic acid bacteria, such as *Lactobacillus* spp., *Pediococcus acidilactici*, *Lactococcus lactis* and *Enterococcus faecium* (Balciunas et al. 2013). Moreover, strains genetically engineered to produce bacteriocins, such as avian *E. coli* AvGOB18, can become a useful tool, lowering *Salmonella* Typhimurium and *E. coli* O157:H7 counts in chicken (Wooley et al. 1999), thus possibly playing a role in the protection of human health. This discovery may become even more important since some bacteriocins, such as nisin, has received GRAS (generally recognized as safe) status under 21 CFR 184.1538 and can be used commercially (Joerger 2003).

Members of the normal gut microbiota, mainly spore-forming and non-spore-forming anaerobes, produce short-chain fatty acids (SCFAs), such as lactic, acetic, propionic and butyric acid. The formation of SCFAs is of major relevance for the host, because it enables the host to salvage some of the energy contained in dietary fiber that would otherwise be lost. Additionally, these SCFAs inhibit the growth of many pathogens, such as putrefactive bacteria, including Gram-negative bacteria and some molds (van der Wielen et al. 2000). The main mechanisms of this anti-bacterial action rely on 1) lowering of the pH of the intestinal contents (and also bacterial cytoplasm), and 2) a specific chemical antagonism of SCFAs towards certain bacteria. Un-dissociated lactic acid can be easily transferred into bacterial cells. It then dissociates, acidifying the cytoplasm and releasing potentially toxic hydroxide anions. In contrast, acetic acid denatures bacterial proteins and neutralizes electrochemical cell potential by interacting with cell membranes. Moreover, both acids act synergistically against yeasts, molds and bacteria, such as *Clostridium* and *Salmonella* (Śliżewska et al. 2006). Other SCFAs, such as propionic and butyric acid, are also known to be *Salmonella*-inhibiting factors (Mead 2000). Another anti-bacterial factor, hydrogen peroxide, is produced by some strains of *Lactobacillus*. It unspecifically inhibits or kills a number of bacteria, those which lack antioxidant enzymes, such as peroxidase, superoxide dismutase and catalase (Śliżewska et al. 2006).

The gut microbiota participates in many metabolic pathways. Chickens, like most animals, lack certain enzymes involved in the carbohydrate metabolism, such as glycoside hydrolase, polysaccharide lyase and carbohydrate esterase (Yeoman et al. 2012). These enzymes are important for chicken nutrition, since the birds cannot utilize fiber, starch, cellulose, pectin, etc. A solution to this problem can be provided only by gut microbiota. As mentioned above, the metabolic endpoint of bacterial carbohydrate fermentation is a generation of SCFAs, whose presence limits the population of pathogens, and is a great source of nutrients and energy for the host organism. Many studies show, that SCFAs are willingly assimilated by the host organism. For instance, butyric acid, the most important source of energy for colonocytes, stimulates growth, proliferation and differentiation of these cells, and is essential for the proper development of intestinal villuses (Panda et al. 2009, Donohoe et al. 2011). Many SCFAs – especially acetate, propionate and butyrate – are responsible for absorption of ions: calcium, magnesium and iron by the host (Brassart and Schiffrin 2000).

Unlike carbohydrate fermentation, metabolism of proteins, amino acids and nitrogen is a multi-dimensional issue. SCFAs, the major end products from carbohydrate fermentation, are also produced from proteins by reductive deamination. However, fermentation of amino acids also results in a population of toxins and carcinogens, dangerous to the host's organism. Among these substances p-cresol, phenol and hydrogen sulfide were proven to have a direct toxic effect on human and animal colonic epithelial cells (Pedersen et al. 2002, Attene-Ramos et al. 2006, Windey et al. 2012). Ammonia, another protein utilization product, is of no nutritive value and was proven to decrease broiler performance and to increase mortality (Reece et al. 1980). Therefore, although microbial fermentation of proteins which escaped from the host's digestive tract seems positive and beneficial, it contributes new problems. Latshaw and Zhao (2011) suggested that, in order to defeat these problems, protein content in a feed diet should be reduced. Other data implied usage of pro- and prebiotics, which acidify the intestinal content, decrease the protein fermentation inside the GIT and lower the activity of enzymes responsible for formation of toxins (De Preter et al. 2006).

Another metabolic pathway, the microbial metabolism of lipids, is less controversial. Gut microbionts, especially lactobacilli, enterococci, bifidobacteria, *Clostridium* spp. and *Bacteroides* spp., deconjugate bile acids, which leads to a poor fat emulsification, and therefore reduce fat absorption and fat storage (Begley et al. 2006). Moreover, the gut microbionts produce fatty acids, such as conjugated linoleic acid (CLA). CLA is important for human and animal nutrition. Several studies report that CLA demonstrates a significant increase in hepatic catalase activity in chickens, and may be associated with reduction of fat in animals (Rahman et al. 2001, Dunshea et al. 2002). Moreover, CLA has been shown to have an anti-carcinogenic activity.

Studies on mice models and clinical reports of patients after antimicrobial treatment indicate a possible

effect of the gut microbiota on vitamin production for the host (Farthig 2004). In cases of patients treated with antibiotics, it has been observed that a decreased number of gut microbionts manifested with reduced vitamin K2 (menaquinone) concentrations, and thus as a vitamin K deficiency and coagulopathy (Alkhalil and Tate 2009). The same model of action is predicted for animals, chickens included. Moreover, lactic acid bacteria and other gut microbionts are also responsible for synthesis of folate and vitamin B2 (riboflavin) (Burgess et al. 2009).

Probiotic bacteria in poultry production

Composition of chicken gut microbiota depends on the animal's age, especially at the early stages of life (starting with embryos), genotype, farming conditions/environment and – most importantly – on diet and feed additives (Binek et al. 2000, Hume et al. 2003, Lu et al. 2003, Kizerwetter-Świda and Binek 2008, Zulkifli et al. 2009, Danzeisen et al. 2011). It has been proven that by altering chicken gut microbiota, we can stimulate growth and possibly decrease the chance of contamination of their carcasses, which happens very often during and after slaughter (Olsen et al. 2003, Posch et al. 2006). Therefore, our efforts to make poultry farming more efficient and safe rely on the use of probiotics, prebiotics and a combination of both – synbiotics.

The bird GIT is shorter and the gastric juice has a lower pH in comparison to the mammalian GIT. Therefore, populations of microorganisms colonizing the chicken GIT should demonstrate higher acid tolerance in shorter periods of time (Ehrmann et al. 2002). Bacteria which fulfill such requirements effectively colonize a host's GIT and confer a health benefit, are considered as probiotics.

In poultry breeding species of the genus *Lactobacillus*, i.e. *L. salivarius*, *L. animalis*, *L. crispatus*, *L. johnsonii* have the greatest chance to become probiotics. However, it should be noted that only certain strains of these species demonstrate desirable probiotic activities (Ehrmann et al. 2002, La Ragione et al. 2004, Kizerwetter-Świda and Binek 2005, 2006, 2009, Taheri et al. 2009).

Mechanisms of probiotic action of *Lactobacillus* strains include synthesis of SCFA, lactic acid and bacteriocins, and competition for nutrients with other inhabitants of the intestine, all of which were reviewed in the previous chapter. Probiotic activity also depends on the stabilization of the epithelial barrier, mucin secretion induction, adherence to the epithelium, aggregation skills, and many others. As has been demonstrated on mouse and human epithelial cells, lactobacilli are responsible for protection against cytokine-induced disruption of epithelial integrity, promotion of cell growth in human and mouse colon epithelial cells *in vitro* (Yan et al. 2007), modulation of the mucosal and systemic immune responses (Harzallah and Belhadj 2013), and stimulation of the goblet cells for mucus secretion. For instance, *Lactobacillus acidlactici* A4 has been proven to induce the upregulation of MUC2 mucin, which prevented *E. coli* O157:H7 from attachment to HT-29 human colonic epithelial cells (Kim et al. 2008). Moreover, Gopal et al. (2001) showed the *in vitro* inhibitory effect of *L. acidophilus* HN017, *L. rhamnosus* DR20 and *L. rhamnosus* GG against adhesion of *E. coli* O157:H7 to various human intestinal cell lines. Interestingly, with mucus-secreting cell-line HT29-MTX, the adhesion indices of *Lactobacillus* strains were 2-3 times higher compared to non-mucus-secreting cell lines.

Many species of *Lactobacillus* (i.e. *L. casei* subsp. *casei*, *L .rhamnosus*, *L. reuteri* and *L. salivarius*) demonstrate good adherence abilities to epithelial cells. Buck et al. (2005) and Pretzer et al. (2005) found several cell surface proteins acting as adhesion factors in *L. acidophilus* NCFM and *L. plantarum* WCFS1, which are two proteins similar to the adhesion protein R28 from *Streptococcus pyogenes* (LBA1633 and LBA1634), a fibronectin-binding protein (LBA1148), mucin-binding protein (LBA1392) and a mannose-specific adhesin Msa. Moreover, the mannose-specific adhesin found in *L. plantarum* is similar to those which enteric pathogens, such as enteropathogenic *E. coli* (EPEC), *Salmonella* Enteritidis and *Salmonella* Typhimurium, use to bind to human epithelial cells. Therefore, probiotic strains were predicted to compete for attachment sites, inhibit pathogen adherence and protect the host against infections. This theory of excluded attachment has been proven right in case of *L. acidophilus* and *L. fermentum* strains protecting against *Salmonella* Pullorum and Typhimurium, however not against *Salmonella* Enteritidis and *E. coli* in chicken (Jin et al. 1998). Another study shows that *L. salivarius* has better adhesive abilities to chicken mucus than *L. salivarius* and *L. brevis* strains. Moreover, *L. salivarius* demonstrate different adhesion ability to mucus from different intestinal regions (Kizerwetter-Świda and Binek 2006). Corresponding observations were made by Ma et al. (2006). Moreover, authors suggest that the use of *L. acidophilus* and *L. fermentum* mixture may improve the exclusion of *Salmonella* Typhimurium and *E. coli* from the chicken GIT to a higher extent than a single species of *Lactobacillus*.

Interestingly, the findings of Vesterlund et al. (2005) couldn't be more different. These authors suggest that *L. rhamnosus* GG does not inhibit

Salmonella Typhimurium's attachment the human epithelium. On the contrary, it is *Salmonella* that has higher adhesion indices than *L. rhamnosus* GG as measured by confocal laser scanning microscopy (CLSM) on unprocessed intestinal biopsy tissue in displacement assay. Differences between these findings may be explained by the use of different intestinal cells – enterocytes from intestinal culture cell-lines vary from those obtained from intestinal biopsy tissues in regard to a normal microbiota's presence and diverse mucin content. It is possible that the number and type of binding sites for bacteria on mucus may be very different in respect of the enterocyte nature. Curiously, both the studies of Kizerwetter-Świda and Binek (2006) and Ma et al. (2006) used natural intestinal tissues of chickens, and the findings were as described previously – they matched the well-established theory of positive inhibitory effect of lactobacilli against pathogens. As to natural microbiota versus probiotics, Garriga et al. (1998) found that one strain of *L. salivarius* was able to colonize and overcome the resident microbiota in the crop and the caecum of the chickens. This is an important factor, since indigenous microbiota may influence the adhesion abilities of probiotic strains to the epithelium of the GIT (Ouwehand et al. 2002).

Another important feature of lactobacilli is the ability to auto- and co-aggregate. It has been reported that bacteria demonstrating a high auto-aggregation capacity, also show good adhesion properties to the mucus. Auto-aggregation allows some probiotic strains to reach the cell mass necessary for the achievement of their functionality, and to form a barrier which mechanically prevents pathogen-mucosa contact (Tuo et al. 2013). On the other hand, co-aggregation with potential pathogens allows to interact closely with undesirable bacteria. All these attributes become useful when competing against enteric pathogens. *L. salivarius*, *L. plantarum*, *L. rhamnosus* and *L. casei* strains demonstrate auto- and co-aggregation (Garriga et al. 1998, Tuo et al. 2013). However, it should be noted that a strong auto-aggregation capacity is not always combined with a strong co-aggregation property. For instance highly auto-aggregative *L. rhamnosus* GG, one strain of *L. agilis* and two strains of L. *reuteri* demonstrated average co-aggregative abilities with pathogenic bacteria. Interestingly, the co-aggregative bacteria are almost always auto-aggregative (Ehrmann et al. 2002, Tuo et al. 2013).

Lactobacilli demonstrate antagonism against numerous pathogens, such as *Salmonella* sp., *Campylobacter* spp. and certain strains of *Escherichia coli*. These bacteria are highly pathogenic not only for humans but, except for *Campylobacter* spp., also for birds. However, it should be noted that campylobacteria cause negative effect on the growth performance of chickens, and should be eliminated from poultry products. Many studies reveal the positive effect of lactobacilli on lowering the potentially pathogenic bacterial counts. For instance, Watkins et al. (1982) indicated that prophylactic inoculation of germ-free chicks with *L. salivarius* reduced shedding of *E. coli* from 100 to 47% compared to the control. The importance of these findings is that certain probiotic strains can prevent *E. coli* O157:H7 infections, which thus far have been a great danger for patients at the extremes of age. Other strains of *E. coli* can become dangerous as well. Research shows that *E. coli* may transform from commensal to pathogen by acquiring virulence-encoding genetic material (i.e. genes encoding adhesins, enterotoxins, invasins and cytotoxins) from other pathogens through horizontal gene transfer (Farthing 2004), which is highly possible since Qu et al. (2008) showed that mobile DNA fragments are the major functional component of a caecal microbiome.

Another causative agent of food-borne illnesses is *Salmonella* sp. Poultry products, especially eggs, are the main sources of this bacterium. As for prevention against salmonellosis, probiotics seem even more efficient than they have been in the case of *E. coli*. A study of Jin et al. (1996) using the agar spot test and the well diffusion assay, showed that twelve *Lactobacillus* isolates (including *L. brevis*, *L. fermentum*, *L. acidophilus* and *L. crispatus*) were able to inhibit the growth of *Salmonella* Enteritidis, *Salmonella* Pullorum, *Salmonella* Typhimurium and *Salmonella* Blockley to higher degrees compared to the inhibition against *E. coli*. Moreover, *Salmonella* Pullorum proved to be more sensitive to the antibacterial activity of lactobacilli than *Salmonella* Typhimurium and *Salmonella* Enteritidis. Inhibition occurred due to synthesis of organic acids by lactobacilli. Moreover, single oral gavage of *L. salivarius* was shown to prevent *Salmonella* Enteritidis colonization in the chicken GIT for 21 days (Pascual et al. 1999).

Campylobacter jejuni is one of the most common food-borne pathogens, and chickens are the main source of these bacteria. It is estimated that 50-90% of chicken carcasses are contaminated at the time of sale (Qu et al. 2008). In fact, fowls (and birds in general) are prone to *C. jejuni* colonization more than any other animals. Commercially raised chicken are usually colonized by *C. jejuni* within 5-6 weeks of age (Sahin et al. 2001), and remain as a reservoir throughout their lifespan. In order to reduce human infections with these bacteria, it is necessary to reduce the colonization in the chickens. Several studies have brought up the subject of the effect of probiotic lactobacilli against *C. jejuni*. Murry et al. (2006) reported

that diet supplementation with *Lactobacillus* sp. lowered the amount of *C. jejuni* recovered from carcass rinses, and the effect was similar to that observed after coccidiostatic and antibiotic treatment. Chaveerach et al. (2004). showed the positive effect of *Lactobacillus* and *Enterococcus* strains *in vitro*, yet only native bacteria isolated from adult chickens (not milk isolates) prevented young broilers from *Campylobacter* colonization. Interestingly, colonization of *C. jejuni* in the chicken GIT reduced *Klebsiella peumoniae*, *Citrobacter diversus* and *Escherichia coli* (013:H-) as well (Schoeni and Doyle 1992).

As for protection against Gram-positive bacteria, lactobacilli demonstrate very good antibacterial activity, much better than in the case of Gram-negative ones. Two studies of Kizerwetter-Świda and Binek (2005, 2009) showed that *L. salivarius* inhibits the growth of *Clostridium perfringens*, *Salmonella* Enteritidis and *E. coli*; however, inhibition of *C. perfringens* reached higher values than inhibition of other bacteria. This was determined *in vitro* and *in vivo*. Similar results were reported by La Ragione et al. (2004) and Murry et al. (2004, 2006) for *L. johnsonii*, *L. plantarum* and *L. fementum*, and these authors showed a positive antibacterial activity against *C. perfringens*.

Antimicrobial activity of probiotics against Gram-positive and Gram-negative bacteria varies, and this has to do with production of bacteriocins. Some inhibit taxonomically related Gram-positive bacteria, whilst others are active against a much wider range of Gram-positive and Gram-negative bacteria as well as yeasts and molds (Harzallah and Belhadj 2013). For instance, *Lactococcus lactis* produces lacticin, which inhibits the growth of yet another anaerobe – *Clostridium difficile*. Unfortunately, use of lacticin alone lowers populations of lactobacilli and bifidobacteria (Rea et al. 2007). Moreover, bacteriocin Abp118 produced by *L. salivarius* was shown to inhibit *Listeria monocytogenes*, *Bacillus*, *Staphylococcus*, *Enterococcus* and *Salmonella* species (Corr et al. 2007, Harzallah and Belhadj 2013).

Bifidobacteria are most commonly used for commercial probiotic purposes in human nutrition, whereas only a few *Bifidobacterium* species have been used in animals (Wasilewska et al. 2008). Bifidobacteria produce lactic and acetic acid in large amounts, larger than lactobacilli. Similarly to lactobacilli, bifidobacteria partake in stabilization of the gastrointestinal barrier, modulation of the local and systemic immune responses, inhibition of the pathogenic invasion and promotion of the bioconversion of unavailable dietary compounds into bioactive healthy molecules (Rossi and Amaretti 2010).

Some strains of *Bifidobacterium breve* were found to prevent *E. coli* O157:H7 (STEC, Shiga-toxin producing *E. coli*) colonisation in the mouse GIT. The main mechanism of this action was via acetic acid synthesis by *Bifidobacterium* strains, resulting in reduction of the luminal pH (Asahara et al. 2004). Acetic acid was also shown to prevent translocation of toxin from the gut lumen further into the bloodstream. Moreover, the presence of *B. breve* inhibited production of the Shiga toxin by STEC *in vitro*. More extensive research on *Bifidobacterium longum* subspecies revealed that only certain subspecies of *B. longum* can prevent STEC-induced epithelial apoptosis, and thereby protect against upregulation of inflammation-related genes in mice (Fukuda et al. 2012). This was explained by the positive effect of *B. longum* metabolites (such as acetate) on upregulation of mice genes involved in cellular energy metabolism during anti-inflammatory response, and on prevention of the increasing permeability of epithelial cells induced by STEC-related cell death.

Another useful feature of bifidobacteria is their ability to adhere to the mucus and epithelial cells. Some strains of *B. animalis* demonstrated good adhesive capacity to the rat mucus and rat epithelial cell lines (Wasilewska et al. 2008), and *B. lactis*, *B. breve* and *B. infantis* to human epithelial cell lines. Moreover, certain strains of theses species were shown to inhibit enteropathogenic *E. coli*, *Yersinia pseudotuberculosis* and *Salmonella* Typhimurium attachment to human cell lines (Gopal et al. 2001). Liu et al. (2010) claimed that *B. lactis* was able to inhibit *Salmonella* Typhimurium attachment to INT-407 cells, which was due to competition for attachment sites. Moreover, these authors demonstrated the protective activity of *B. lactis* against acute inflammatory responses induced by *Salmonella* sp.

Unfortunately, neither anti-inflamation activity nor the adhesive ability of *Bifidobacterium* strains are described for the chicken GIT. Little is known about the inhibition of enteropathogens by bifidobacteria of chicken origin. Baffoni et al. (2012) observed a reduction of *C. jejuni* counts after administration of *B. longum*-based synbiotic. This anti-*Campylobacter* effect of *B. longum* is suspected to rely on antibacterial metabolite production and synthesis of unidentified proteinaceous substances. Moreover, *B. longum* was found in feces of chickens six days after probiotic treatment (Santini et al. 2010). This fact may be useful when constructing bifidobacterium-based probiotics. Another study demonstrates reduction of total aerobic bacteria, coliforms and clostridia in chickens which received *B. bifidum*; however, this concerned the reduction of cellulitis in broiler chickens, not changes of microbial community inside the GIT (Estrada et al. 2001).

Certain representatives of *Enterococcus* spp. are also considered as probiotic. It was shown that some strains of *E. faecium* stimulate the growth and metabolic activity of lactobacilli inside the bird GIT (Vahjen et al. 2002, Samli et al. 2007). Moreover, Vahjen et al. (2002) observed an increased concentration of lactic acid, which was probably related to the increased metabolic activity of lactobacilli. Another study suggests that a diet supplemented with probiotic mixture containing *Lactobacillus*, *Bifidobacterium*, *Enterococcus* and *Pediococcus* strains increases populations of these four groups of bacteria in the gut; however, it does not change the amounts of total counts of aerobes, coliforms, and *Bacteroides* spp. populations (Mountzouris et al. 2007). As for protection against enteropathogens, enterococci, mostly *E. faecium*, show good inhibitory activity against enteropathogenic *Escherichia coli*, *Salmonella* Gallinarum, *S.* Enteritidis, *S.* Typhimurium, *S.* Pullorum and *S.* Duselforf. Interestingly, *E. faecium* and *E. faecalis* strains isolated from domestically reared chickens demostrated higher inhibitory activity against enteropathogens than strains of industrial chicken origin. The antibacterial action was due to secretion of lactic acid and bacteriocins, known as enterocins (Lauková et al. 2004, Miteva and Boytcheva 2008).

As for species of *Pediococcus*, their probiotic activity inside the bird GIT is not as well described. *P. acidlactici* is proven to have a positive effect on lowering the number of coliforms, and may reduce enteropathogen amounts as well. The antagonistic activity of pediococci depends on lactic acid production and secretion of bacteriocins, known as pediocins (Taheri 2010). Pediocin of pediococci strains isolated from cucumber brine demonstrated good antagonistic activity against *Clostridium* spp., *Staphylococcus* spp. and also against lactobacilli, leuconostocs, streptococci, bacilli and other pediococci, but not against *Salmonella* Typhimurium, *E. coli* and yeasts *in vitro* (Fleming et al. 1975, Daeschel and Klaenhammer 1985).

Unlike previously described bacteria, bacilli can be administered orally as cells and spores. This seems to be the greatest advantage of *Bacillus* strains, since spores are resistant to heat and conditions inside the GIT, especially to gastric acid and bile salts. *Bacillus subtilis* KD1 was shown to increase tenfold lactobacilli counts in the intestinal tracts of broilers compared to untreated chickens (Wu et al. 2011). On the other hand, *B. subtilis* PB6 was also responsible for inhibition of *Clostridium perfringens*, *C. difficile*, *Streptococcus pneumoniae*, *Campylobacter jejuni* and *C. coli*. Studies show that inhibition occurs mainly by production of bacteriocins, which in the case of *B. subtilis* was subtilin (Teo and Tan 2005).

Concluding remarks

Intestinal microbiota is composed of a highly complex community of various microorganisms which are vital to many aspects of a normal host physiology. They contribute to many aspects of the intestinal tract development, and serve as a deterrent to pathogen colonization. Bacterial metabolism results in the production of several by-products with an antimicrobial effect, such as peroxides and various acids. Intestinal microbes harbor substantial amounts of genes encoding enzymes involved in the breakdown of non-digestible dietary components with harvest of energy ingested, but not digested by the host. They also demonstrate trophic effects on the intestinal epithelium, by favoring the development of intestinal microvilli, and play a fundamental role in the maturation of the host's innate and adaptive immune response. Host-microbe interactions are also relevant in control of the gut microbiota composition. An imbalance between the commensal microbionts and the host's response to such imbalance are considered to be involved in the pathogenesis of a variety of intestinal disorders. The main mechanisms regulating the equilibrium of the microbial ecology in the gut are still poorly understood and include competition for colonization sites, competition for nutrients, production of toxic compounds, stimulation of the immune system, and triggering the expression of multiple cell-signaling processes (Hume et al. 2003, Amit-Romach et al. 2004, Zulkifli et al. 2009, Yeoman et al. 2012, Binek 2012).

In commercial poultry production, there are no natural microbiota providers since chickens are hatched in the clean environments of a hatchery. This is why the use of competitive exclusion products and probiotic microorganisms enabling an early and rapid colonization of chickens with healthy bacteria is a common approach in poultry production.

A variety of microbial species have been used as probiotics, including species of *Bacillus*, *Bifidobacterium, Enterococcus*, *E.coli*, *Lactobacillus*, *Lactococcus*, *Streptococcus*, a variety of yeast species and undefined mixed cultures. Most probiotic bacteria were originally isolated from healthy hosts. This means that probiotics have virtually no distinguishing characteristics from commensal organisms, except for their beneficial effects when consumed. Their effects are probably dependent on factors involving both the host and the specific probiotic strains. The positive effect of the lactic acid bacteria and certain other microorganisms on host resistance to disease occurs in numerous and diverse ways. These include maintenance of barrier function, maturation and homeostasis of the immune system, and effects on other surrounding or infecting

bacteria. Additionally, they may control inflammation by reducing pro-inflammatory mediators and increasing anti-inflammatory mediators, as well as by restoration of commensal microbe equilibrium after antibiotic treatment. A number of studies also demonstrated a positive and comparable to certain antibiotics effect of probiotics on growth performance. In this respect probiotics are of current interest, because they offer biological alternatives which should find acceptance by both producers and consumers (Paterson and Burkholder 2003, Klose et al. 2006).

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