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Variations on the Efficacy of Probiotics in Poultry

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1. Introduction

In face of the current debate about the use of antibiotics as growth promoters, due to the probable relationship with resistance to antibiotics used in human medicine, the presence of antibiotic residues in products of animal origin intended for human consumption and the emergent demand from consumer market for products free from additive residues, it was necessary to search for alternative products that could replace antibiotics used as promoters, without causing losses to productivity or product quality.

An alternative is the use of probiotics, which are products made from living microorganisms or their L-forms (without cell wall). The micro-organisms included as probiotics are usually assumed to be non-pathogenic components of the normal microflora, such as the lactic acid bacteria. However, there is good evidence that non-pathogenic variants of pathogenic species can operate in much the same way as traditional probiotics. For example, avirulent mutants of Escherichia coli, Clostridium difficile, and Salmonella Typhimurium can also protect against infection by the respective virulent parent strain (Fuller, 1995).

In poultry, the early use of probiotics was instituted by Nurmi & Rantala (1973). In their experiments, the authors observed that the intestinal contents of normal adult birds, orally administered to chicks with one day of age, altered their sensitivity to infection by Salmonella spp.

From there, several studies have been made and continue being developed with the use of probiotics. Inconsistent results from the use of probiotics in animal production have been a constraint for the promotion of their use. Variations in the efficacy of probiotics can be due to the difference in microbial species or micro-organism strains used, or with the additive preparation methods (Jin et al., 1998a). However, other factors can justify the variations in the results of probiotic use in poultry, such as origin species, probiotic preparation method, survival of colonizing micro-organisms to the gastrointestinal tract conditions, environment where the birds are raised, management (including the application time and application
route of the probiotic), the immunologic status of the animals, the lineage of the poultry evaluated, as well as age and concomitant use or not of antibiotics.

Thus, the aim of this review is to discuss the use of probiotics in poultry, with emphasis on the type of probiotic and micro-organisms used, action mechanism and its relation with the variations on the results of poultry survey.

2. Type of probiotic and micro-organisms used

There are several types of probiotics available in the market to be used in poultry, with a range of micro-organisms present and, therefore, with different metabolic activities and action modes. Also, they present variations as to the capacity of colonizing the intestine or not, which justifies variations on the results of their use.

Bacillus, Bifidobacterium, Enterococcus, E. coli, Lactobacillus, Lactococcus, Streptococcus, Pediococcus species, and a range of yeast species and non-defined mixed cultures have been used (Fuller, 1992; Patterson & Burkholder, 2003; Kabir et al., 2004; Mountzouris et al., 2007). However, even those belonging to the same species can have different strains and even these different strains from the same species can have different metabolic activities. These bacteria are used alone or in combination (Miles, 1993; Montes & Pugh, 1993).

Non-defined mixed cultures, known as competitive exclusion cultures, are normally related to the treatment of one-day chicks with an indefinite microbiota derived from adult animals resulting in resistance to colonization against pathogenic micro-organisms.

Among the colonizing species, Lactobacillus sp., Enterococcus sp. and Streptococcus sp. are worth mentioning, and among the non-colonizing species, Bacillus spp. (spores) and Saccharomyces cerevisiae (Žikić et al., 2006 apud Perić et al., 2009).

Another characteristic of probiotics is that some micro-organisms are constituted by micro-organisms normal to the intestinal microbiota of poultry, and others by bacteria different from the ones from the digestive tract. According to Kabir (2009) the most commonly used species are: Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus helveticus, Lactobacillus lactis, Lactobacillus salivarius, Lactobacillus plantarum, Streptococcus thermophilus, Enterococcus faecium, Enterococcus faecalis, Bifidobacterium spp. and Escherichia coli, and except for Lactobacillus bulgaricus and Streptococcus thermophilus, all the remaining ones are intestinal strains.

Recently, emphasis has been given to the selection, preparation and application of probiotic strains, especially lactic acid bacteria (Wang & Gu, 2010).

Natural adaptation of lactic acid bacteria to intestinal environment and the lactic acid produced by them have provided advantages for these organisms over other micro-organisms used as probiotic (Guerra et al., 2007).
3. Action mechanisms

The action mechanisms of probiotics (Fig. 1) on the immune system of broiler mucosa are not completely clear. However, it is admitted that probiotics have immune-modulating effects (Cotter, 1994; Erickson & Hubbard, 2000; Edens, 2003; Loddi, 2003; Ng et al., 2009).

According to (Erickson & Hubbard, 2000 and Menten & Loddi, 2003), the bacterium genera present in probiotics that are directly related to the increase in immunity of poultry are *Lactobacillus* and *Bifidobacterium*, mainly when related to diseases affecting the gastrointestinal tract. However, other genera have been related (Hakkinen & Schneitz, 1999; Yurong et al., 2005; Hong et al., 2005).

![Figure 1. Inhibition of enteric bacteria and enhancement of barrier function by probiotic bacteria. Schematic representation of the crosstalk between probiotic bacteria and the intestinal mucosa. Antimicrobial activities of probiotics include the (1) production of bacteriocins/defensins, (2) competitive inhibition with pathogenic bacteria, (3) inhibition of bacterial adherence or translocation, and (4) reduction of luminal pH. Probiotic bacteria can also enhance intestinal barrier function by (5) increasing mucus production (Adapted Ng et al., 2009).](image)

The immune-modulating effect in poultry happens in two ways: (a) from the microbiota, in which the probiotic migrates along the wall of the intestine and is multiplied to a limited extension, or (b) the antigen released by the dead organisms are absorbed and thus stimulate the immune system (Havenaar & Spanhaak, 1994).

According to Loddi (2003) and Nunes (2008), antigens (lipopolysaccharides and peptidoglycans) are constantly released in intestinal lumen. On the other hand, this release is increased during infectious processes, once these components are fundamental in the development and maintenance of local immune response (Hamann et al., 1998; Loddi, 2003).
since they have chemotactic effect on epithelial cells and cells related to mucosa immunity, and induce changes in the intestinal epithelium of the host.

The chemotactic effect is accomplished by mediators such as cytokines, metaloproteins (elastase and cathepsin), prostaglandins, oxygen and nitrogen reactive metabolites, elevating the production of IgA, IgM and IgG immunoglobulins, activating differentiation and proliferation of NK (Natural Killer), CD3, CD4 and CD8 lymphocytes, increasing the migration of lymphocyte T and the production of interferon (Fuller 1989; Jin et al., 1997; Erickson & Hubbard, 2000; Edens, 2003; Loddi, 2003; Zhang et al., 2007; Neurath, 2007; Ng et al., 2009).

The changes induced by probiotics in the intestinal epithelium are accentuated by the decrease in luminal pH, antimicrobial activity and secretion of antimicrobial peptides inhibiting bacterial invasion and blocking the adhesion to epithelial cells. In this sense, they improve the intestinal barrier elevating the production of cytokines (TNF-α, IFN-γ, IL-10 and IL-12) (Arvola et al., 1999), which in turn, induce the secretion of IgA in the intestinal mucosa, causing the release of mucins (Gupta & Garg, 2009).

Mucins, the layer of glycoproteins that when in contact with water, form a film that lubricates and protects the intestinal epithelium against pathogens, forming a physical barrier between the epithelium and the content from the intestinal lumen (Oliveira-Sequeira et al., 2008), keeping the bacteria in a safe place in the intestinal lumen (Mattar et al., 2002).

Studies suggest that the inhibiting effect of bacterial translocation by *Lactobacillus casei* GG *in vivo* and *in vitro* could be related with the regulation of the MUC-2 gene, which promotes the expression of mucin by goblet cells (Mattar et al., 2002).

In the intestine, probiotics interact with enterocytes, goblet cells, M cells from Peyer’s patches, isolated follicles that are extended through the mucosa and submucosa in the small intestine, forming GALT (Gut Associated Lymphoid Tissue) and immune cells among them, intraepithelial lymphocytes. These interactions result in an increase in the number of IgA-producing cells accompanied by the production of secretory IgM and IgA that are particularly important to the immunity of the mucosa, contributing to the barrier against pathogenic micro-organisms (Szajewska et al., 2001).

Thus, in the modulation of the immune response, the suppression of potential pathogens has been observed (Majarmaa, 1997), through the increase of intestinal motility (Gupta & Garg, 2009), increase in the population of intraepithelial lymphocytes in the intestinal epithelium (Dalloul et al., 2003), removal of pathogens (Patterson & Burkholder, 2003), modification of intestinal microbiota (Shane, 2001; Salzman et al., 2003), and increase in the height of intestinal villi (Iji et al., 2001). Added to these effects, the capacity of bacterial groups to develop a fimbria network that blocks the linking location of some enteric pathogens.

Another relevant aspect is related to different bacterial genera, which colonize and are developed, producing an almost permanent exclusion environment, known as competitive
exclusion mechanism, which represents the competition for adhesion locations to the membrane of goblet cells, enteroendocrine cells and enterocytes in the intestinal mucosa, which promote a status of physical barrier to the mucosa by creating a special integrity system, preventing intestinal pathogens from becoming established (Rantala & Nurmi, 1974; Soerjadi et al., 1982; Salminen & Isolauri, 1996). Therefore, a mechanism proposal was described by Revolledo et al. (2006) for poultry receiving supplementation of competitive exclusion products, probiotics or immunostimulants (Fig. 2).

As well as this mechanism, there is an antagonist effect through the secretion of substances that inhibit the growth and development of pathogenic bacteria (Fig. 1), such as bacteriocines, organic acids and hydrogen peroxide (Patterson & Burkholder, 2003; Oumer et al., 2001; Mazmanian et al., 2008). As well as these, other benefits from the use of probiotics are: increase of enzymatic activity inducing absorption and nutrition (Hooper et al., 2002; Timmerman et al., 2005) and inhibition of procarcinogenic enzymes (Gill, 2003).

Figure 2. Proposed interactions between competitive exclusion products, probiotics or immunostimulants, and avian intestinal immunity. SIgA =secretory IgA; CE=competitive exclusion; IEC =intraepithelial cell; IEL=intestinal intraepithelial lymphocyte; LPL=lamina propria lymphocytes (activated T lymphocytes); dendritic cell or macrophage =antigen-presenting cells (APC); LB=B lymphocyte; LT=T lymphocyte; M cells =cells for the transport of antigens from the intestinal lumen into the gut-associated lymphoid tissue; SC =secretory component; endocytosis =process in which a substance gains entry into a cell without passing through the cell membrane; transcytosis=process of transport of substances across an epithelium layer by uptake on one side of the epithelial cell into a coated vesicle that might then be sorted through the trans-Golgi network and transported to the opposite side of the cell.
Proposed Mechanisms. Antigen uptake: 1. Antigen can be recognized directly by IEL, signals are sent to LT in the lamina propria. 2. When antigen is taken in by M cells using transcytosis process, there are 2 possible mechanisms to stimulate the immune response: a) antigen is directly taken in by macrophages or dendritic cells, which are able to process and present to LT in the lamina propria, or b) antigen activates B cells, which stimulate LT in the lamina propria. 3. Antigen uptake can be made by IEC using endocytosis process. The IEC are able to act as APC and process the antigen, antigen is presented to LT in the lamina propria. SIgA production: activated LT (LPL) produces cytokines, which stimulate LB activation, and finally plasma cells, produce IgA. The IgA acquires the secretory component on the IEC and is able to internalize into IEC; finally SIgA is available in the intestinal lumen to exert surface protection. (Revolledo et al., 2006).

4. Variations on the efficacy of probiotics in poultry

As described before, there is a large range of micro-organisms used as probiotics, with variations in species and strains of the same species, and therefore, they present variations in its metabolic activity and justify variations in the results of their use. However, other factors can justify the variations in the results of using probiotics in poultry, such as the origin species, probiotic preparation method, survival of colonizing micro-organisms in the gastrointestinal tract conditions, the environment where the birds are raised, management (including probiotic application time and application route), the immunologic state of the animals, the lineage of poultry evaluated, as well as age and concomitant use of antibiotics.

Fuller (1986) emphasizes that the specificity of adhesion of lactobacilli (one of the most used probiotic genre in poultry) to epithelial cells is specific host and if the colonization is reached, it is essential to administer bacteria that have been originated form the host species for which they are being given.

On the other hand, it is worth mentioning that there are probiotics presenting efficacy even though they have not been isolated from the original host species. As an example, one can mention the works developed by Impey et al. (1984) and Schnetz & Nuotio (1992) showing that the natural microbiota of chicken (Broilact®) and turkeys provide reciprocal protection for chicks and poults.

Regarding the probiotic preparation method, Fuller (1975) reports that even the carbohydrate source used in the growth media during the preparation of probiotic can affect the micro-organism’s ability in adhering to the intestinal epithelium of poultry and the adhesion capacity also changed during its growth cycle. Therefore, notes that even if two strains are identical, the form which they have been prepared can cause variations in the result (Fuller, 1995).

Several beneficial effects of the use of Lactobacillus as probiotics are reported in literature in relation to the productive performance of poultry (Kalbane et al., 1992; Nahashon et al., 1996; Jin et al., 1998a; Kalavathy et al., 2003; Schocken-iturrino et al., 2004). Thus, studies on the proteomics of Lactobacillus have been made with the objective of allowing its better
growth and/or survival by means of appropriate preservation methods (De Angelis & Gobbetti, 2004) to obtain a better performance with its use.

In a study developed by Desmond et al. (2001), the authors have shown that in order to increase the viability of probiotic strains of *Lactobacillus paracasei* NFBC 338 during spray-drying, a pre-stressing of the culture by exposure to temperature of 52°C for 15 minutes increased in 700 fold the survival of the strain (in reconstituted skimmed milk) during caloric stress and 18 fold during spray drying when compared to non-adapted cells, demonstrating that the probiotic preparation method can aid for a larger survival time and consequent results obtained.

It is important to mention that as well as the genetic variation among species, other environmental factors during the preparation of probiotics (pH, water activity, salts and preservative content) influence in the resistance of *Lactobacillus* to caloric stress and spray drying (Casadei et al., 2001; Desmond et al., 2001).

Also, for a micro-organism to be selected to be used as probiotic, it is necessary that it can be able to overcome some barriers that would be harmful to its survival in the gastrointestinal tract. Mills et al. (2011) report that before probiotic bacteria can start to perform its physiological role in the intestine, they should support a number of tensions to ensure it reaches the target site in sufficient number to elucidate its effect. According to the authors, first the bacterium must be processed in an appropriate manner to allow oral consumption and be able to resist the inhospitable conditions imposed during its passage through the gastrointestinal tract.

In order to be in a highly viable state during processing, storage and intestinal transit, bacteria go through adverse conditions including temperature, acidity, bile, exposure to osmotic and oxidative stress both in the production matrix and during intestinal transit (Corcoran et al., 2008). Thus, the benefit from the use of probiotics is the result of the growth of organisms and generation of some beneficial functions in the intestinal tract (Jin et al., 1998a), being that the efficacy in the use of *Lactobacillus* as probiotics depends not only in the proliferation of bacteria in the intestinal tract, but also that they survive through the stomach.

This is due to the fact that every food ingested (including the probiotics provided in feed) is submitted to a gastric pH ranging between 2 and 4 that can cause the death of bacteria going through the stomach in 10 to 100 fold (Fuller, 1986).

Regarding the nutritional status of the animals, studies have shown that improvements in the performance of broilers have been seen when feed does not contain all nutrients in appropriate quantities.

In research developed by Dilworth & Day (1978), the authors verified that the effect of supplementation with *Lactobacillus spp.* on the growth of body mass and feed conversion in broilers is significantly greater when the methionine, cystine and lysine levels in the feed are reduced.
Likewise, Kos & Wittner (1982) have not found improvement in the growth and feed conversion of broilers by the addition of probiotics in feed containing all nutrients in appropriate quantities.

Equally, Mikulec et al. (1999) demonstrated the favorable influence that probiotics have on the growth of body mass and improvement in feed conversion of broilers when the level of crude protein in the diet was not efficient.

Regarding the environment where the animals are raised, studies have demonstrated influence of environmental stress on the results of probiotic research. According to Weinack et al. (1985), the physiological stress induced by high or low environmental temperatures or withdrawal of food and water interfere either with the colonization of protective micro-organisms or reduces the protection provided by the probiotic.

However, Fuller (1986) reports that the stressor agent must be present before any effect of the probiotic supplement can be observed and that there will only be stimulus to growth if the depressor agent is present, that is, the author emphasizes that for the evidence of improvement on the performance of animals, the breeding environment must not be free from challenges. In experimental conditions, the absence of beneficial results can be justified by this statement.

Montes & Pugh (1993) reported similar results and showed that in birds, the best results with the use of probiotics happened when the birds were submitted to stress conditions, being by the increase or decrease of temperature, transportation, vaccination and overcrowding. In these conditions, an imbalance in the intestinal microbiota is created and the body defense mechanisms are decreased (Jin et al., 1997), which by the supplementation of probiotics, such problems would be minimized, evidencing differences in the performance results.

In literature, several treatment methods using probiotics are described, such as through feed, addition to drinking water, spraying on the birds, inoculation via cloaca or in embryonated eggs (in ovo), through the litter used, in gelatin capsules and intra-esophagus (Schneitz, 1992; Ziprin et al., 1993).

This way, the administration route of probiotics can determine an improvement or worsening in the intestinal colonization capacity by the bacteria present in the product used. Direct inoculation in esophagus/crop (intra-esophageal) is the most efficient (Stavric, 1992), although in practical terms it has little viability.

One justification for the absence of results with the use of probiotics in drinking water can be the presence of residual chlorine and the fact of the product becoming inefficient before all chicks have received the micro-organisms in the appropriate dose (Seuna et al., 1978), and sometimes, chicks do not drink water before feeding, which makes the protection uneven within the herd (Schneitz et al., 1991).
Also, according to Siriken et al. (2003), the duration of treatment can be an important factor in the effect of a probiotic on the intestinal microbiota, once probiotics can be given only once or periodically, in weekly or daily intervals. Despite the little knowledge regarding the minimum required dose to evidence the effects of probiotics, experiments in mice, humans and pigs have indicated that the effect decreases when the probiotic is discontinued (Cole & Fuller, 1984; Goldin & Gorbach, 1984).

Lan et al. (2005) reported that for the microbiota to be established in the small intestine and in the caecum, it is necessary approximately two and from six to seven weeks, respectively. Particularly for controlling the population of \textit{Escherichia coli}, Fuller (1977) reports that such control is dependent on the presence of sufficient number of \textit{Lactobacillus} and that from the results of \textit{in vitro} tests, it seems to be necessary at least $10^7$ colony forming units per gram (CFU/g).

Currently, the modern broiler and turkey lineages present high weight gain capacity. However, when compared with lineages of slower growth, they are more susceptible to infectious diseases (Korver, 2012).

According to the same author, modern broilers and turkeys present a depressed systemic innate immune response to allow fast growth, once the deviation of nutrients to the development of systemic inflammatory response is minimum, and despite presenting better immunity mediated by cells, there is evidence of increase in the mortality among fast-growth poultry when compared with slow-growth ones, which might justify differences in the effects between the different bird lineages.

Regarding age, the paper by Mohan et al. (1996) found that beneficial effects of probiotics were seen during the initial growth phase, happening before 28 days and not after 49 days of age.

Certainly, during the initial stages of life, the intestinal microbiota is in an unstable condition, and the micro-organisms given orally probably find a niche where they can occupy (Fuller, 1995). Therefore, Siriken et al. (2003) reported that the existence of an intestinal microbiota at the time of administration and the health of the host must be considered when a probiotic is supplemented for the suppression of pathogenic bacteria.

It should also be noticed that some micro-organisms that can act as probiotics do not resist the action of some antibiotics or anticoccidial used in the feed of birds (Jin et al., 1997, 1998a; Tournut, 1998).

Other factors that might justify the variations in the effects of probiotics in poultry are: variations in the persistence of administered strains (relative intestinal concentration) (Siriken et al., 2003; Huyghebaert et al., 2011), stability during the manufacturing of feed (Huyghebaert et al., 2011), absence of statistical analysis of data in previous studies, experimental protocols not clearly defined, micro-organisms not identified (Simon et al., 2001), viability of organisms not verified (Fuller, 1995; Simon et al., 2001), as well as the fact
that in many studies, the origin of micro-organisms in probiotics was not reported (Siriken et al., 2003).

A study performed by Weese (2002) with eight veterinary and five human probiotics showed that only three from the eight veterinarian products provided data regarding its content; the majority of the products had less quantity than the one declared and five products lacked one or more strains declared; and three products had different strains from the ones declared in the package.

Similar work was developed by Lata et al. (2006), where it was verified that among the five probiotics evaluated, four presented information on validity date, species and amount of bacterium per gram of product. The three products containing Enterococcus faecium in its composition presented the amount of bacteria as declared in its label. However, the presence of Lactobacillus sp. was also found, which was not specified in the labels. In the product containing Bacillus subtilis and Lactobacillus paracasei in its composition, only Bacillus subtilis was found in amounts lower than the one declared.

With all these possible variations, it is not surprising that probiotics not always grant the desired result, but the fact that significant results are obtained show that the correct use of probiotics, under appropriate conditions and using the correct administration method, justify the fact that probiotics are an efficient food supplement in animal breeding.

5. Research results from the use of probiotics in poultry

5.1. Performance of poultry

Using two commercial probiotics, the first composed with Bacillus subtilis (150 g/ton feed) and the second with Lactobacillus acidophilus and casei, Streptococcus lactis and faecium, Bifidobacterium bifidum and Aspergillus oryzae (1 kg/ton feed) for broilers in the period of one to 14 days of age, Pelicano et al. (2004) observed an improvement in feed conversion up to 21 days of age in animals receiving probiotics, regardless of the composition, in relation to the group without any addition. However, there were no significant differences for the total breeding period (1-42 days), demonstrating that the period of treatment with probiotic might influence the performance results.

Improvement in the performance of broilers has been reported by several researchers (Dilworth & Day, 1978; Jin et al., 1996; Mohan et al., 1996; Yeo & Kim, 1997; Santoso et al. 1995; Jin et al., 1998a; Cuevas et al., 2000; Fritts et al., 2000; Kabir et al., 2004; Huang et al., 2004; Schocken-Iturrino et al., 2004; Gil de los Santos et al., 2005; Mountzouris et al., 2007; Rigobelo et al., 2011).

On the other hand, works performed by (Loddi et al. 2000; Lima et al. 2003; Willis & Reid, 2008) have not shown any benefit for the use of probiotics in any breeding phase of broilers.

In Japanese quails (Coturnix coturnix japonica), Sahin et al. (2008) evaluated the effect of different concentrations (0.5, 1 and 1.5 g/Kg feed) of a symbiotic (probiotic + prebiotic) on
the diet of animals and have not found differences among the treatments in relation to body weight gain, feed conversion rate and carcass yield.

In a similar way, Otutumi et al. (2010) evaluated the effect of including a probiotic based on *Lactobacillus* spp added through drinking water and feed to meat quails in the period of one to seven days of age on the performance in the period of one to 35 days of age and have not found differences in weight gain, feed conversion and carcass yield. However, the animals receiving the probiotic presented lower feed consumption (*P*<0.05), without affecting weight gain.

Yang (2009) compiled several studies with diverging results regarding the performance of broilers with the use of probiotics (Table 1). Faria Filho et al. (2006) performed a meta-analysis study resulting from 35 tests involving probiotics in Brazil between 1995 and 2005. Based on the results, the authors concluded that the usage of probiotics is a viable technique for improvement on the development of broilers.

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Probiotics</th>
<th>Improvement (%)</th>
<th>Reference</th>
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<tbody>
<tr>
<td>BWG (g/bird)</td>
<td>1892</td>
<td>1920</td>
<td>+1</td>
<td>Liu et al (2007)</td>
</tr>
<tr>
<td>FCR (g/g)</td>
<td>1.75</td>
<td>1.74</td>
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<tr>
<td>BWG (g/bird)</td>
<td>2216</td>
<td>2237</td>
<td>+1</td>
<td>Mountzouris et al (2007)</td>
</tr>
<tr>
<td>FCR (g/g)</td>
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<td>1.78</td>
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<td></td>
</tr>
<tr>
<td>BWG (g/bird)</td>
<td>2784</td>
<td>2720</td>
<td>-2</td>
<td>Murry et al (2006)</td>
</tr>
<tr>
<td>FCR (g/g)</td>
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</tr>
<tr>
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<tr>
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<tr>
<td>Mortality (%)</td>
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<td>Mortality (%)</td>
<td>1290</td>
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<td>Jin et al (1998b)</td>
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<tr>
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<tr>
<td>Mortality (%)</td>
<td>6.7</td>
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Table 1. Growth performance and/or mortality rate of birds to probiotic supplementation.

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*,

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1. BWG = Body Weight Gain.
2. FCR = Feed Conversion Ratio.
3. ADG = Average daily gain.
Bifidobacterium thermophilus and Enterococcus faecium, improved egg size and lowered feed cost in laying hens. Moreover, probiotics increase egg production (Kurtoglu et al., 2004; Yörük et al., 2004; Panda et al., 2008) and quality (Kurtoglu et al., 2004; Panda et al., 2008) of chickens.

In laying Japanese quails, Ayasan et al. (2005) observed improvement in the feed conversion efficiency, while reducing egg shell thickness but not affected on feed intake, egg production, egg shell weight, egg shape index and numbers of eggs after six weeks of application of 120 ppm probiotic based on Yucca schidigera in feed.

5.2. Exclusion of pathogens and immunity

One of the action mechanisms of the previously mentioned probiotics was the competitive exclusion, which plays an important role in the prevention of enteric colonization by pathogenic micro-organisms, among them, Salmonella spp.

According to Scanlan (1997), three mechanisms present an important role in the prevention of enteric colonization of chicks by Salmonella spp. previously supplemented by competitive exclusion cultures: a) the micro-organisms constituting the competitive exclusion culture establish an enteric flora before exposure to Salmonella spp.; b) the micro-organisms from the inoculated flora compete with Salmonella spp. for essential nutrients, and c) the beneficial micro-organisms produce concentrations of volatile fatty acids that lower the intestinal pH and are bacteriostatic for Salmonella spp.

Several authors (Hinton & Mead, 1991; Stavric, 1992; Blankenship et al., 1993) reported that these exclusion cultures seem to be more effective against the colonization by Salmonella in the cecum. However, some authors have reported their inefficacy (Stavric et al., 1991).

Table 2 shows that in several works there was a high percentage of reduction in the colonization by Salmonella spp with the use of probiotics in broilers.

<table>
<thead>
<tr>
<th>Researchers</th>
<th>Probiotic</th>
<th>Treatment with probiotic</th>
<th>Reduction (%) in the colonization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menconi et al. (2011)</td>
<td>Lactic acid bacteria</td>
<td>1 h post challenge</td>
<td>95% SH(^4)</td>
</tr>
<tr>
<td>Knap et al. (2011)</td>
<td>Bacillus subtilis</td>
<td>Diet (1 to 42 days of age)</td>
<td>58% SH(^6)</td>
</tr>
<tr>
<td>Higgins et al. (2010)</td>
<td>Lactic acid bacteria</td>
<td>1 h post challenge</td>
<td>4 -76% SE(^5)</td>
</tr>
<tr>
<td>Higgins et al (2007)</td>
<td>Lactic acid bacteria</td>
<td>1 h post challenge</td>
<td>60 -72% SE(^5); 92-96% ST(^5)</td>
</tr>
</tbody>
</table>

Table 2. Effectiveness of probiotics in the prevention of Salmonella colonization in broiler chicken.

\(^1\) SE = Salmonella Enteritidis; ST = Salmonella Typhimurium; SH = Salmonella Heidelberg.

\(^2\) 24 h after treatment, cecal tonsil.

\(^3\) 42 days of age – drag swabs.

\(^4\) Data related to experiments 1, 2 & 3.
Mountzouris et al. (2010), studying inclusion levels of a probiotic composed by *Lactobacillus reuteri, Enterococcus faecium, Bifidobacterium animalis, Pedicoccus acidilactici* and *Lactobacillus salivarius*, found that the inclusion of $10^9$ and $10^{10}$ CFU/kg feed provided benefit in modulation of the composition of cecal microflora. Particularly, they reduced the concentration of coliforms in the cecum (log CFU/g of wet digesta) at 14 and 42 days of age in broilers. Also, the authors have found an increase in the concentration of *Bifidobacterium* and *Lactobacillus* at 42 days of age. Thus, the supplementation of probiotic in the indicated concentrations has been efficient as modulation of beneficial microbiota and reducing the studied pathogens.

According to Leandro et al. (2010), the early use of probiotics establishes a balance in microbial flora against pathogenic bacteria, thus, using probiotic constituted by *Enterococcus faecium, Lactobacillus casei, L. plantarum* inoculated *in ovo* at the dose of $10^6$ CFU/g per egg has avoided the colonization of the gastrointestinal tract of broilers challenged with 0.1 mL aqueous solution containing $1.36\times 10^6$ CFU *Salmonella Enteritidis*, inoculated via crop. Therefore, broilers challenged early (post eclosion) and not receiving probiotics presented reduction of *Salmonella* in gastrointestinal tract (crop and cecum) of the birds and a better performance.

La Ragione & Woodward (2003) verified that the administration of viable spores of *Bacillus subtilis* to birds free from specific pathogens challenged with *C. perfringens* reduced the number of pathogens in the spleen, duodenum, colon and cecum, reporting similar results with a probiotic based on *Lactobacillus johnsonii* (La Ragione et al., 2004).

Haghghi et al. (2006) shown that a commercial probiotic containing *Lactobacillus acidophilus, Bifidobacterium bifidum,* and *Streptococcus faecalis* stimulated the production of antitoxin αIgA from *C. perfringens* in the intestine of non-vaccinated chicks.

In meat quails, Otutumi et al. (2010) evaluated the effect of probiotics based on *Lactobacillus spp* administered in the period of one to seven days of age on the counting of *Lactobacillus spp*, enterobacteria and *Escherichia coli* in the small intestine (at 7 and 14 days of age) and have not observed changes in the counting with the use of probiotic. However, it is worth mentioning that when evaluating the microbial population in the intestine, there is a very large standard deviation, which many times makes it difficult to identify differences by the use of inappropriate statistical models. And despite having used appropriate statistical analysis, the results were not significant.

Siriken et al. (2003) investigated the effect of two probiotics, alone and in combination with an antibiotic on the caecal flora of Japanese quail (*Coturnix coturnix japonica*) and no significant differences were detected among treatments for pH values and total count of aerobic bacteria, lactobacilli, enterobacteriaceae, coliforms, enterococci, salmonellae, except for sulphite-reducing anaerobic bacteria ($P<0.001$).

Unfortunately, more than 80% of gut bacteria cannot be cultured under current laboratory conditions, limiting assessment of the effects of probiotics on the gut microbiota. This drawback, however, has been overcome today to a large extent by employing molecular techniques (Ajithdoss et al., 2012).
The suggested mechanism by which probiotics might exert their protective or therapeutic effect against enteric pathogens include non immune mechanisms, such as the stabilization of the gut mucosal barrier, increasing the secretion of mucus, improving gut motility, and therefore interfering with their ability to colonize and infect the mucosa; competing for nutrients; secreting specific low molecular weight antimicrobial substances (bacteriocins) (Delgado et al., 2007; Liu et al., 2011), and influencing the composition and activity of the gut microbiota (regulation of intestinal microbial homeostasis) (Castilho et al., 2012).

5.3. Carcass quality and blood parameters

The quality of broiler meat as well as the reduction of fat levels in the carcass have been a constant concern of researchers. Thus, research directed to the improvement of meat quality has been made including the use of probiotics.

Santoso et al. (1995) demonstrated that the supplementation of *Bacillus subtilis* at the dose of 20g/Kg feed increased the level of phospholipids in blood serum, but reduced the concentration of phospholipids in carcass and triacylglycerol in liver, carcass and blood serum, as well as decreasing the percentage of abdominal fat. This parameter was also evaluated by Denli et al. (2003), who proved that the supplementation of *Saccharomyces cerevisiae* on the diet has decreased the weight and percentage of abdominal fat in broilers.

Equally, Pietras (2001) demonstrated that *L. acidophilus* and *Streptococcus faecium* decreased the plasmatic protein concentrations and the total cholesterol and high density lipoprotein (HDL) cholesterol levels, and that the meat from supplemented broilers presented a significant increase in protein content.

Other works with supplementation of probiotics based on *Lactobacillus spp.* demonstrated similar results, with reduction in the total cholesterol and low density lipoprotein (LDL) cholesterol levels (Kalavathy et al., 2003; Taherpour et al., 2009) and triglycerides (Kalavathy et al. 2003) in blood serum of broilers.

In Japanese quails with 4 weeks of age, Homma e Shinohara (2004) studying the effect of a commercial probiotic based on *Bacillus cereus toyoi* on the accumulation of abdominal fat verified that at eight weeks (four weeks of probiotic supplementation period), birds fed the control diet with probiotic had significantly less abdominal fat than those fed without the probiotic.

Moreover, probiotic supplementation has been shown to reduce the cholesterol concentration in egg yolk (Abdulrahim et al., 1996; Haddadin et al., 1996) and serum in chicken (Mohan et al., 1996; Jin et al., 1998a).

According to Matur & Eraslan (2012), hypocholesterolemic effect of probiotics depends on the species of the bacteria, and can occur by the assimilation of cholesterol from either endogen or hexogen origin in the intestinal tract, or de-conjugating bile acids by lactic acid bacteria (Gilliland et al., 1990) or the cholesterol and free bile acids bind to the cell surface of micro-organisms or co-precipitate with the free bile acids by probiotics (Guo & Zhang,
However, recent research has revealed that probiotics affect gene expression of carrier proteins responsible for cholesterol absorption (Matur & Eraslan, 2012).

Regarding the microbiological quality of meat, Bailey et al. (2000) proposed that competitive exclusion cultures for broilers can be used to reduce contamination by *Salmonella Enteritidis* in processed carcasses, reducing therefore the exposure of consumers to food-borne infections.

Likewise, Estrada et al. (2001) observed a tendency to reduce total aerobic bacteria, coliforms and clostridia in broilers receiving *Bifidobacterium bifidum*, and proven a reduction in the number of carcass condemnation by cellulites in animals supplemented, and recently, Lilly et al. (2011) observed 86% reduction in contamination by *Salmonella* before slaughtering in broilers receiving probiotic with combination of *Lactobacillus acidophilus*, *Enterococcus faecium*, *Lactobacillus plantarum* and *Pediococcus acidilactici*.

Regarding the organoleptic quality, Kabir (2009), studying the supplementation of a commercial probiotic (Protexin® Boost, Novartis) in the ratio of 2g probiotic for every 10 liters of drinking water until 36 days of age in broilers, observed that the probiotic supplementation improved the organoleptic quality of broiler meat right after slaughtering, as well as after 21 days storage in freezer.

### 5.4. Bone quality in broilers

The surveys aiming the reduction in growth time in poultry, together with the increase of its live weight, have led to the development of broilers known as conformation or yield type. However, the development of this new broiler came together with some undesirable aspects associated to the fast growth which have compromised the performance of the birds (Leeson & Summers, 1988).

Among these aspects, it is notable the increase in bone problems, once the genetic selection for a high growth rate has promoted higher breast muscle weight when compared to the muscles and bones in legs, and therefore, this unbalanced redistribution of weight has increased the leg problems in poultry (Yalcin et al., 2001).

From an economic point of view, there is a great concern by the companies with the losses regarding bone anomalies in broilers, since they have contributed for the reduction in productivity and increase in mortality, as well as condemnation of whole carcasses or during the processing of meat.

The most prevalent bone problems in broilers are tibial dyschondroplasia, chronic painful lameness in older or reproductive broilers, condrodistrophy or bone angular deformity, valgus-varus angular deformities, spondylolisthesis, rickets, epiphyseal separation, femoral necrosis, curled toes and rupture of gastrocnemius tendon (Julian, 1998; Angel, 2007).

The etiology of bone abnormalities is generally complex and apparently it is not related to a single factor, and sometimes there is an overlapping among etiology, pathology and clinical signs of these conditions. Factors affecting the intestinal epithelium, leading to the reduction of nutrient absorption, as well as anti-nutritional factors of the ingredients can induce leg
disorders caused by nutritional imbalance. Thus, genetics, handling, nutrition, hygiene and diseases will influence the occurrence of leg problems under field or experimental conditions. Therefore, even if the content of diets seems to be adequate, bone abnormalities can appear (Waldenstedt, 2006).

Although studies demonstrate probable influence of probiotics, prebiotics and symbiotics on the bone characteristics of poultry, it is not well established the relation between probiotics and mineral absorption or bone growth (Mutus et al., 2006).

Plavnick & Scott (1980) observed lower incidence of tibial dyschondroplasia and greater bone resistance in broilers receiving yeast extract supplementation. Likewise, Mutus et al. (2006) observed that at 42 days of age, the thickness of medial and lateral wall, tibia-tarsal index, percentages of ashes and phosphorus and the diameter of the medullar channel of the tibia in broilers fed with diets containing probiotics were higher than those receiving the control diet without supplementation.

Although the bone abnormality score has not been influenced, Panda et al. (2006) described positive effects of diets supplemented with *Lactobacillus sporogenes* (100mg/kg) on bone resistance to breakage and ash content from broiler tibiae. According to the authors, the supplementation of diets with probiotics resulted in higher serum concentration of calcium, which might explain the better resistance and ash concentration of bones.

Positive results as to morphometric (weight, length, tibia-tarsi and tibia-tarsal indexes, lateral and medial wall thickness), mechanical (elasticity module and draining tension) and mineral composition parameters (ashes, calcium and phosphorus) in the tibia of broilers receiving probiotics (150mg/kg) in feed were observed by Ziaie et al. (2011). According to the authors, the supplementation of diet with antibiotic substitutes can increase digestibility and availability of nutrients (such as calcium and phosphorus) due to the development of a desirable microflora in the digestive tract, which in turn results in an increase in mineral retention and bone mineralization.

Nahashon et al. (1994) reported a positive correlation between the diets containing probiotics (*Lactobacillus*) and the retention of calcium and phosphorus in laying hens. On the other hand, in a study with broilers, Maiorka et al. (2001) have not observed changes in the plasmatic levels of calcium and phosphorus of the broilers at 40 days of age receiving probiotic supplementation (*Bacillus subtilis*).

Working with broilers, Angel et al. (2005) demonstrated that the addition of probiotics based on *Lactobacillus* (0.9kg/ton) in feed has improved the retention of calcium and phosphorus by birds receiving feed that supply to their nutritional demands. However, birds receiving moderate density (18% less calcium and phosphorus in relation to the recommendation of the National Research Council - NRC) and low density feed (25% less calcium and phosphorus in relation to the recommendation by NRC) supplemented with probiotics presented bone breaking resistance and ash concentration in tibia similar to those receiving the control feed, without addition of additive. Data revealed that probiotics based on lactobacillus can improve the retention of nutrients, allowing its usage in feeds with lower nutritional levels, reducing excretion and costs.
Guçlu et al. (2011) analyzed the effect of different probiotic inclusion levels on the productive performance and quality of breeder quail eggs and reported that the improvement in the thickness of the shell observed with the addition of probiotic would probably be related with the greater absorption of calcium in the birds’ intestines.

According to Scholz-Ahrens et al. (2007), as well as the stimulation of calcium entering enteroctyes, another probable action mechanism of probiotics on bone health is the degradation of the mineral-phytic acid complex.

Lan et al. (2002) evaluated the effect of supplementation of an active culture of *Mitsuokella jalaludinii* (a kind of bacteria present in the rumen of cattle) in broiler feeds with high and low concentrations of non-phytate phosphorus and observed improvement in the performance, in the values of apparent metabolizable energy, in protein and dry matter digestibility, in the usage of calcium, phosphorus and copper, and bone mineralization of broilers receiving feed with lower concentrations of non-phytate phosphorus.

6. Conclusion

As it can be seen, the results of research available in literature with the use of probiotics are very variable, once several factors can interfere, such as the type of probiotic, its action mode, its interaction with the host and breeding environment. However, evidences presented in relation to the benefit of its use justify the continuity of research with the objective of expanding the knowledge on its action mechanism, its immune-modulation effect and methodologies that aid the maintenance of its viability for use in animal feed. Currently, research has evaluated the genomes of various probiotic species and the term “probiogenomics” has been proposed to denote the sequencing and analysis of probiotic genomes, for further development of strains and assessment of the safety of probiotics in order to aid the propagation of using probiotics in human and animal feed.

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7. References


