

A Review on the Lactic Acid Bacteria Probiotic in the Control of Coccidiosis, Campylobacteriosis, and Salmonellosis in Broiler Chickens

Review Article

M. Royan^{1*}

¹ North Region Branch, Agricultural Biotechnology Research Institute of Iran (ABRII), Agricultural Research, Education and Extension Organization (AREEO), Rasht, Iran

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*Correspondence E-mail: m.royan@abrii.ac.ir

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ABSTRACT

Roles of probiotic microorganisms in digestive physiology are contributed to enhancing animals health and reducing the incidence of gastrointestinal diseases. Banning the use of antibiotics as growth promoters in poultry in many countries has been contributed to the increasing use of probiotics because long-term use of antibiotics causes antibiotic resistance of pathogenic bacteria in poultry and subsequently in the man, the vaccines are also costly. Salmonellosis, campylobacteriosis and coccidiosis infections are the most common intestinal diseases in the poultry industry. The pathogens causing these diseases are *Salmonella enteritidis*, *Campylobacter jejuni*, and *Eimeria tenella*, respectively. Probiotics are able to control these diseases. Certainly, controlling the causes of these disease outbreaks will promote the public health. On the other hand, the probiotics increase bird resistance against the invasion of *Eimeria* that may lead to protect against coccidiosis. This review deals to the potential of lactic acid probiotic bacteria to control coccidiosis, campylobacteriosis and salmonellosis.

KEY WORDS broilers, campylobacteriosis, coccidiosis, salmonellosis, probiotic.

INTRODUCTION

Control and prevention of diseases such as coccidiosis, salmonellosis, and campylobacteriosis in the poultry industry are particularly important. Coccidiosis is the most common diseases in broilers, and salmonellosis and campylobacteriosis are two food-borne important diseases in human caused from food animals. Iran's climate is appropriate for the spread of the coccidiosis and has caused great economic losses in the poultry industry (Baba Ahmadi *et al.* 2009). This disease is caused by a parasite known as *Eimeria*. The parasite multiplies in the digestive system, and leads to the mortality, malabsorption and reduced growth in the broilers and decreased egg production in laying hens (Lillehoj *et al.* 2004).

Drugs and live vaccine are two main methods to control this disease. However, due to problems caused by long-term consumption of medicines and high cost of vaccines, the use of more effective and safer methods are needed (Dalloul and Lillehoj, 2006; Williams, 2006). On the other hand, *Salmonella* and *Campylobacter* are the agents of the most important food-borne infectious diseases in humans and the poultry products are the most important sources of human infection with these bacteria. Gram-negative bacterium *Campylobacter jejuni* is the pathogen of this intestinal food borne disease. Since the ban on the use of antibiotic growth stimulants in Europe, problems caused by *Salmonella* have increased. It was reported that 24% of the examined broiler flocks were positive for *Salmonella* colonization (European Food Safety Authority, 2007).

The studies conducted by Iranian researchers representing between 35 and 54 percent of *Salmonella* contamination of poultry flocks in different provinces of Iran (Khannazer and Firouzi, 1998; Soltan Dallal, 2007; Shapouri *et al.* 2009). It was suggested several antibiotic resistances in *Salmonella* isolates from poultry in Iran (Asadpour *et al.* 2014).

Following the Banning antibiotics as growth promoters (AGPs) in the broiler industry, probiotic bacteria could be suitable alternatives to replace AGPs. Probiotics are live microbial supplements that have beneficial effects on the host animal by improving the intestinal microbial balance (Fuller, 1989). Variety of microbial species, including *Lactobacillus*, *Streptococcus*, *Aspergillus*, *Candida*, *Saccharomyces* and *Bacillus* have beneficial effects on performance, microflora, inhibiting pathogens and modifying the immune system in poultry (Zulkifli *et al.* 2000; Kalavathy *et al.* 2003; Kabir *et al.* 2005; Karimi Kivi *et al.* 2015; Pourakbari *et al.* 2016; Pournazari *et al.* 2017; Seidavi *et al.* 2017). By using probiotics, stimulation the acquired immune, changes in the microbiome of the cecum and the production of inhibiting metabolites such as organic acids are the possible mechanisms to reduce pathogens in the digestive tract (Neal-McKinney *et al.* 2012).

Coccidiosis control by lactic acid bacteria probiotics

Coccidiosis is a disease of global emissions and despite using antibiotics and the sanitation and vaccination operations in the control and prevention of the disease, coccidiosis is still considered one of the greatest challenges in the poultry industry (Dalloul and Lillehoj, 2006). The protozoan parasites of the genus *Eimeria* are amplified in the gastrointestinal tract of broiler chickens, leading to tissue damage, reduced growth and increased susceptibility to pathogens (Cook, 1998).

So far, nine species of *Eimeria* have known as the agents of coccidiosis disease. Among these species, three species, including *E. tenella*, *E. brunetti* and *E. necatrix* have a high pathogenicity and have been identified as causes of the clinical form of disease, whereas *E. maxima* and *E. acervulina* are moderate pathogenic forms which cause subclinical disease (Adib Nishaboori *et al.* 2006). The clinical form of disease has symptoms such as watery to bloody diarrhea, anemia, and loss of appetite, weakness and wasting. However, the subclinical form of the disease adversely affects the growth and feed conversion ratio (Adib Nishaboori *et al.* 2006). Adib Nishaboori *et al.* (2006) studied the coccidiosis outbreaks in flocks of laying hens in the Mashhad city (North East of the country) and reported the spread of 47% *E. acervulina*, 53% *E. maxima*, 41% *E. necatri*, and 35% *E. tenella*.

In another study in the Mashhad city, Razmi and Klideri (2000), studied the prevalence of subclinical coccidiosis in broiler and reported a 38% incidence of coccidiosis, such that the percent of infected farms with *E. acervulina*, *E. maxima* and *E. tenella* were 97%, 41%, and 12%, respectively. In a study in the west of Iran (Hamadan province), Ghaderkhani *et al.* (2014) reported 31.8% coccidiosis incidence, and 54.3%, 75.7%, and 20% for prevalence of *E. tenella*, *E. necatrix* and *E. maxima*, respectively. A mixture of *Eimeria* species was observed in all the infected farms. Hamidinejat *et al.* (2010) reported a 31.5% incidence of coccidiosis in Khuzestan province (Southwest) in Iran. The use of anti-coccidiosis drugs and vaccination methods are the common methods to control coccidiosis.

Studies have shown that *Lactobacillus* species isolated from different parts of the gastrointestinal tract of poultry are able to inhibit invasion of *Eimeria tenella*, *in vitro* (Tierney *et al.* 2004) as well as *in vivo* studies confirm that probiotics reduce the intestinal lesions caused by *Eimeria* (Lan *et al.* 2004; Lee *et al.* 2007b). Ghasemi *et al.* (2010) reported that using Biomin IMBO symbiotic, which is a combination of *Enterococcus faecium* bacteria and inulin prebiotic has a protective role against coccidiosis in broilers. Primalac, commercial lactic acid bacteria probiotic in the diet of broiler chickens from birth to three weeks of age stimulated the intestinal epithelial lymphocytes, and significantly (about 75%) reduced the *E. acervulina* oocytes (Dalloul *et al.* 2003). In studies on the effects of commercial probiotics in broiler chickens under the challenge of *E. tenella* and *E. acervulina*, the Mitomax commercial probiotic (*Pediococcus acidilactici* and *Saccharomyces boulardii*) 10-38% decreased the prevalence of oocytes and increased the level of antibodies against *Eimeria* in broiler chickens (Lee *et al.* 2007a). The MitoGrow probiotics (*Pediococcus acidilactici*) increased the levels of antibodies against *Eimeria*, however, had no effect on the prevalence of the oocytes (Lee *et al.* 2007b).

Concurrent use of poly-star probiotic and coccidiosis vaccine leads to a protective effect against *Eimeria acervulina* and *E. maxima*, resulting in improved bird performance and condition of the intestine (Ritzi *et al.* 2016). In a recent study, a probiotic compound containing 4 strains of lactic acid (*L. acidophilus*, *L. fermentum*, *L. Planetarium* and *Enterococcus faecium*) significantly reduced the intestinal ulcers caused by *Eimeria*, decreased the expression level pro-inflammatory cytokines such as interleukin and level of gene expression (IL) -1 β , IL-6, and interferon (IFN) - γ significantly increased the anti-inflammatory cytokine IL-10 gene expression (Chih-Yuan *et al.* 2016). Some studies on the effect of probiotics on coccidiosis control are presented in Table 1.

Table 1 The summarized results of some studies on the effect of probiotics on coccidiosis control

Reference	Probiotic bacteria	The probiotic name	The observed effects
Giannenas <i>et al.</i> (2012)	<i>Enterococcus faecium</i> 589, <i>Bifidobacterium animalis</i> 503, <i>Lactobacillus reuteri</i> 514 and multi-species probiotic BIOMIN GmbH	Multi-species probiotic BIOMIN GmbH	In the absence of in-feed anticoccidial drugs, treatment with probiotics could alleviate the impact of <i>E. tenella</i> infection in chicken, help to maintain enteric health and minimize the risk and spread of coccidiosis. Effect of probiotics in oocyst shedding was significantly lower than that exhibited by lasalocid.
Giannenas <i>et al.</i> (2014)	<i>Enterococcus faecium</i> 589, <i>Bifidobacterium animalis</i> 503 and <i>Lactobacillus salivarius</i> 505	PoultryStar®	Probiotic groups presented lesion score values and oocyst numbers that were lower than in control infected birds, but higher than in the lasalocid group. In the duodenum, jejunum and ileum, the highest villus height values were presented by probiotic groups.
Chih-Yuan <i>et al.</i> (2016)	<i>Lactobacillus acidophilus</i> (LASW), <i>L. fermentum</i> (LF33), <i>L. plantarum</i> (LPL05) and <i>Enterococcus faecium</i> (TM39)	Infant feces	Four LAB strains were equally combined into a multistrain formula termed as MF. Seven days post challenge it was found that feeding MF could reduce the cecal lesion scores (LS) of the <i>Eimeria</i> infected chickens. In addition, for chickens in the MF group, the levels of cecal gene expression of proinflammatory cytokines, such as interleukin (IL)-1 β , IL-6, and interferon (IFN)- γ , were found significantly lower, while anti-inflammation cytokine, ie, IL-10, was higher than those of the chickens in the infected group
Lee <i>et al.</i> (2007b)	Pediococcus-based probiotic	MitoGrow	In <i>E. acervulina</i> -infected birds, the MG 0.1 group improved weight gain and reduced oocyst shedding in birds infected with 5000 <i>E. acervulina</i> oocysts. In <i>E. tenella</i> -infected birds, <i>Eimeria</i> -specific antibody levels were higher in the MitoGrow-fed groups, especially in the MG 0.1 birds, compared with the regular diet group. <i>Pediococcus acidilacti</i> based probiotic effectively enhances the resistance of birds and partially protects against the negative growth effects associated with coccidiosis.
Dalloul <i>et al.</i> (2003)	<i>Lactobacillus acidophilus</i> , <i>Lactobacillus casei</i> , <i>Bifidobacterium thermophilum</i> , and <i>Enterococcus faecium</i>	Primalac	Fecal oocyst shedding was lowest in (control+p) birds. Feeding the probiotic reduced shedding oocysts. The data demonstrate for the first time a probiotic-enhanced immunity in vitamin A deficient bird.

Role of lactic acid bacteria to prevent campylobacteriosis

The *Campylobacter* infection has called campylobacteriosis and has global emission, so that *Campylobacter jejuni* is one of the main causes of enteritis in children in developed and developing countries and birds, especially poultry are the major source of human infection (Baserisalehi *et al.* 2007). *Campylobacter jejuni* is a common commensal microorganism in broiler chickens which do the colonization in the cecum (Waldenstrom *et al.* 2001). Chicken meat contamination with the bacterium occurs during processing. As a result, a high rate of infection is due to contact with infected poultry carcasses and consumption of contaminated products. *Campylobacter jejuni* is a major cause of diarrhea in Iranian children and the pathogen has been observed in almost 8% of children with acute diarrhea symptoms (Feizabadi *et al.* 2007). Mokhtarian *et al.* (2009) examined the contamination of poultry carcasses in Gonabad city (east of Iran) industrial slaughterhouse and reported that 31% of the samples were positive for *Campylobacter jejuni* and *Campylobacter coli*. Havaei *et al.* (2007) tested the prevalence of *Campylobacter jejuni* and *Campylobacter coli* in the feces of broilers in Isfahan province (middle of Iran) and observed 31% outbreaks in the feces of poultry.

Rahimi and Tajbakhsh (2008) examined the *Campylobacter* contamination of different meats and reported the following infection rates of 68.4%, 56.1%, 27.4% and

11.7% quail, chicken, turkey and ostrich, respectively.

Zendehbada *et al.* (2013) reported that chicken, quail, turkey are the main sources of *Campylobacter* infection in humans and 96.6% of the isolates of *Campylobacter* were resistant to one or more antibiotics.

Rahimi *et al.* (2010) reported *Campylobacter* bacterial infection and antibiotic resistance in poultry carcasses during processing in Ahvaz city in south of Iran and found that 55.4% of the carcasses were contaminated. The sensitivity tests on these samples to 10 antibiotics were showed that 92.9% of the samples were resistant to one or more antibiotics and the resistance to tetracycline at a rate of 78.3% was more common than others. Probiotics such as *Lactobacillus* bacteria are capable of preventing colonization of *C. jejuni* in the intestinal tract through the competitive eliminating (Gaggia *et al.* 2011). The competitive inhibition of *C. jejuni* by *Lactobacillus* is performed through several key mechanisms such as competition for binding sites (Wine *et al.* 2009), co-aggregation with pathogens (Schachtsiek *et al.* 2004), the production of antimicrobial substances, production of hydrogen peroxide (Zhao *et al.* 2006) and lactic acid (Neal-McKinney *et al.* 2012).

Saint-Cyr *et al.* (2017) reported that administration of *Lactobacillus salivarius* SMXD51 isolated from the intestinal tract of poultry have anti *Campylobacter* effect and significantly reduced the pollution in the intestinal contents of broilers.

Baffoni *et al.* (2012) reported that feeding a synbiotic compound contains galacto-oligosaccharide prebiotic and *Bifidobacteria longum* subsp PCB133 to broilers for 14 days resulted in a significant reduction of *Campylobacter jejuni* in the feces of birds.

The *in vitro* and *in vivo* studies of Ghareeb *et al.* (2012) have shown that the combination of specific probiotic of poultry (including *Enterococcus faecium*, *Pediococcus acidilactici*, *Lactobacillus reuteri* and *Lactobacillus salivarius*) isolated from the digestive tract of broiler chickens had antimicrobial activity resulting in reduced colonization of *Campylobacter jejuni* in ceca of broilers. Jayaraman *et al.* (2013) reported that *Bacillus subtilis* PB6 isolated from the gut of healthy chickens that produce antimicrobial substances can be used to control different strains of *Campylobacter*.

Neal-McKinney *et al.* (2012) reported that *Lactobacillus crispatus* JCM5810 isolated from chicken feces had probiotic properties on *in vitro* and *in vivo* tests and was able to reduce the colonization of *Campylobacter jejuni* in the gastrointestinal tract of broiler chickens. The bacteria used in the experiment were selected among 4 strains of *Lactobacillus* bacteria and the lactic acid produced by the bacteria was known as the inhibitory agent against *Campylobacter jejuni*. It has been proven that the probiotic even if added to the diet 7 days prior to slaughter can be effective, which this makes the probiotics affordable for manufacturers. Results of some studies on the effect of probiotics on campylobacteriosis control were presented in Table 2.

The role of lactic acid bacteria to prevent salmonellosis

Salmonella is one of the most common causes of food-borne infectious diseases in the world (Addis and Sisay, 2015). Poultry meat products are the most important sources of *Salmonella* infections in human (Higgins *et al.* 2007; Carter *et al.* 2009). Salmonellosis is mostly caused by moving serotypes, especially *Salmonella enteritidis* and *Salmonella typhimurium*, (Aboudi, 2001; Soltan Dallal 2007; Shapouri *et al.* 2009). Clinical syndromes caused by *Salmonella* are divided into two main groups typhoid fever and non-typhoid salmonellosis. The non-typhoid *Salmonella* have isolated from almost all the animal groups and the poultry and their products are the most important source of human infection and endemic disease in Iran (Soltan Dallal, 2007; Shapouri *et al.* 2009). *Salmonella* not only is a pathogen, but also it has the ability to cause on disease in animals and poultry and only be a member of the intestinal microbial population (Knap *et al.* 2011). Sometimes the *Salmonella* colonization does not affect the body weight or performance of the chicken, however, this asymptomatic can increase the risk of infection transmission to humans through the food chain (Carter *et al.* 2009).

It is known that antibiotics used as growth promoters (AGP) lead to a reduction of *Salmonella* colonies (Dibner and Richards, 2005). In the United States between 1998 and 2006, only 10.9 to 16.3 percent of broiler chickens were positive for *Salmonella* contamination (USDA, 2007). In recent years, concerns about antibiotic resistance have increased (Barza, 2002; Cui *et al.* 2005). Identification of *Salmonella* resistant to several antibiotics, leading to major concerns about the safety of the foods (Callaway *et al.* 2008). The indiscriminate use of antibiotics in poultry flocks is very common in Iran, resulted in the emergence of *Salmonella* resistant to antibiotics (Akbarian *et al.* 2012).

Several studies indicate that *Salmonella* infection rates in poultry flocks of Iran were 19.5-40% (Khannazar and Firoouzi, 1998). But the contamination rate in meat and meat products was different in different regions and different years. Akbarian *et al.* (2012) collected samples from 150 region and broiler breeder, layers and broiler flocks of different ages and also hatchery factories and slaughterhouse in different areas of Iran and found that the highest rates of *Salmonella* infection at a rate of 32% was related to broiler chicken flocks. Use of commercial probiotic supplements on the competitive elimination of *Salmonella*, such as Aviguard and BROILACT, significantly reduced the colonization of *Salmonella* in broilers (Nakamura *et al.* 2002). Different types of probiotic products such as single-strain or multi-strain probiotics are able to reduce the *Salmonella* infection in poultry, however, the multi-strain probiotics have been more successful (Timmerman *et al.* 2004; Chapman *et al.* 2011).

The FloraMax-B11 a commercial lactic acid bacteria based probiotic consisting of 11 bacteria belonging to the genus *Lactobacillus* has been one of the most successful strategies against *Salmonella* in the poultry industry, which is used in drinking water (Higgins *et al.* 2008; Vicente *et al.* 2008; Prado-Rebolledo *et al.* 2016).

In general, lactic acid producing bacteria, accelerate the development of a normal microflora in the intestines of chickens and turkey, this leads to increased resistance to *Salmonella* infection (Tellez *et al.* 2012). A combination of *Lactobacillus salivarius* and *Enterococcus faecium* PXN33 excluders was an effective competitive inhibitor for *Salmonella* in poultry and over a course of 43 days usage resulted in a reduction of *Salmonella* colonization in the ileum, colon and cecum of broiler chicken (Carter *et al.* 2017). The *in vitro* study of these bacteria showed that reducing the acidity caused by the lactic acid produced by the bacteria caused the *Salmonella* inhibition, *in vitro* (Carter *et al.* 2017). Marietto-Gonçalves (2014) reported that the use of *Lactobacillus* probiotics in broilers infected with the *Salmonella typhimurium* resulted in removal of the pathogen by increasing blood heterophiles.

Table 2 The summarized results of some studies on the effect of probiotics on campylobacteriosis control

Reference	Probiotic bacteria	Bacteria origin	The observed effects
Mañes-Lázaro <i>et al.</i> (2017)	<i>Lactobacillus johnsonii</i> FI9785	Poultry	Two levels of <i>L. johnsonii</i> FI9785 administrated a week apart reduced <i>C. jejuni</i> colonization in the caecal contents, however this biocontrol seemed dependent upon a high level of early colonization by the probiotic. The microbial population in the chicken gut was altered by the probiotic treatment. The results showed the potential of <i>L. johnsonii</i> FI9785 as a competitive exclusion factor in poultry against <i>C. jejuni</i> .
Baffoni <i>et al.</i> (2012)	<i>B. longum</i> subsp. <i>Longum</i> (PCB133)	Adult	The galactooligosaccharide was combined with <i>B. longum</i> subsp. <i>longum</i> PCB133, possessing <i>in vitro</i> antimicrobial activity against <i>C. jejuni</i> . <i>C. jejuni</i> concentration in poultry feces was significantly reduced in chickens administered with the synbiotic mixture.
Saint-Cyr <i>et al.</i> (2017)	<i>Lactobacillus salivarius</i> SMXD51	Chicken ceca	A significant reduction of 2.81 log in <i>Campylobacter</i> loads was observed and 73% of chickens treated with the culture exhibited <i>Campylobacter</i> loads below 7 log ₁₀ cfu/g.
Cean <i>et al.</i> (2015)	<i>Lactobacillus paracasei</i> J. R, <i>L. rhamnosus</i> 15b, <i>L. lactis</i> Y, and <i>L. lactis</i> FOa	Human	The four probiotic strains had a significant effect on <i>C. jejuni</i> invasion of chicken primary cells, with the strongest inhibitory effect detected when a combination of four was administered. These four new probiotic strains are able to cause modifications in the chicken intestinal mucosa and can reduce the ability of <i>C. jejuni</i> to invade, <i>in vitro</i> , and to colonize, <i>in vivo</i> .
Ghareeb <i>et al.</i> (2012)	<i>Enterococcus faecium</i> , <i>Pediococcus acidilactici</i> , <i>Lactobacillus salivarius</i> , and <i>Lactobacillus reuteri</i>	Chicken gut	1-d-old broiler chicks received 2 mg/bird per day of a multispecies probiotic product via the drinking water. Probiotic treatment reduced cecal colonization by <i>C. jejuni</i> at both 8 and 15 d postchallenge.
Nishiyama <i>et al.</i> (2014)	<i>Lactobacillus gasseri</i> SBT2055 (LG2055)	Human feces	<i>In vitro</i> study: Reduction in the number of <i>C. jejuni</i> cells adhering to and internalized by human epithelial cells demonstrated that LG2055 is an organism that effectively and competitively excludes <i>C. jejuni</i> . <i>In vivo</i> : chicks treated with LG2055 had significantly reduced cecum colonization by <i>C. jejuni</i> . The results of the colonization assay suggest that treatment with LG2055 could be useful in suppressing <i>C. jejuni</i> colonization of the chicks at early growth stages.
Neal-McKinney <i>et al.</i> (2012)	<i>L. crispatus</i>	Chicken isolates	The lactic acid produced by <i>L. crispatus</i> JCM5810 inhibits <i>C. jejuni</i> growth and colonization <i>in vitro</i> and <i>in vivo</i> . <i>L. crispatus</i> most effectively inhibited the growth of all <i>C. jejuni</i> strains tested when compared with <i>L. acidophilus</i> , <i>L. gallinarum</i> and <i>L. helveticus</i> .

Table 3 The summarized results of some studies on the effect of probiotics on salmonellosis control

Reference	Probiotic bacteria	The observed effects
Olnood <i>et al.</i> (2015)	<i>Lactobacillus johnsonii</i>	<i>L. johnsonii</i> (109 cfu/chick) was administered to chicks individually by oral gavage on days 1, 3, 7 and 12. The chicks were individually challenged with <i>S. sofia</i> (107 cfu/chick) by oral gavage on d 2, 8 and 13. <i>L. johnsonii</i> reduced the number of <i>S. sofia</i> in the gut environment. The probiotic strain <i>L. johnsonii</i> may increase the VFA concentration after inoculation.
Carter <i>et al.</i> (2017)	<i>Lactobacillus salivarius</i> 59 and <i>Enterococcus faecium</i> PXN-33	1 × 10 ⁹ cfu of probiotic and 5 × 10 ⁴ cfu of <i>S. enteritidis</i> (at day 2). Colonization of poultry by <i>Salmonella enteritidis</i> S1400 is reduced by combining administration of <i>Lactobacillus salivarius</i> 59 and <i>Enterococcus faecium</i> PXN-33.
Youssef <i>et al.</i> (2011)	<i>Lactobacillus acidophilus</i>	Incorporation of probiotic and/or prebiotic with vaccination with live attenuated <i>S. typhimurium</i> vaccine gave better results in decreasing the percentage of <i>S. typhimurium</i> reisolation from the internal organs of the chickens than in case of vaccination alone
Mountzouris <i>et al.</i> (2009)	Crop (<i>Lactobacillus reuteri</i>), jejunum (<i>Enterococcus faecium</i>), ileum (<i>Bifidobacterium animalis</i>) caecum (<i>Pediococcus acidilactici</i>) and <i>Lactobacillus salivarius</i>	Microbiological analysis of cecal digesta in 42-d-old broilers revealed that while broilers in treatment C+ were 100% <i>Salmonella</i> positive, the broilers in treatments probiotic were 50% positive and in addition they had lower <i>Salmonella</i> levels (cfu/g digesta) by 2.7 logs compared to C+.

Vicente *et al.* (2008) reported the use of commercial probiotic FM-B11 for 3 days in the drinking water of broilers an hour after challenge with *Salmonella typhimurium*, decreased the recovered pathogens in the cecal contents.

In the inhibition of *Salmonella*, use of *L. reuteri* needs a special attention because the bacteria produce a particular metabolite, reuterin, which has antimicrobial activity against *Salmonella*, *E. coli* and *Campylobacter* (Mulder *et al.* 1997).

Unlike the other *Lactobacillus* cultures, *in ovo* injection of *L. reuteri* had no negative effects on the hatchability and reduced the *Salmonella* and *E. coli* colonies after hatching. In addition, deaths due to exposure to *Salmonella* and *E. coli* were reduced in the hatchery (Edens *et al.* 1997). *Lactobacillus plantarum* has receptors sensitive to mannose which is something unusual in Gram-positive bacteria. Because of this receptor *Lactobacillus plantarum* can compete with gram-negative pathogenic bacteria for the intestinal

junction sites, which leads to the reduction of *Salmonella* infection in poultry (Bengmark, 2001). Table 3 summarizes the results of some studies on the effect of probiotics on salmonellosis.

CONCLUSION

Creating a balanced flora in the gastrointestinal tract and stimulating the immune system have attributed to the use of probiotics. Several studies have provided clear evidence that certain strains of probiotics can stimulate several aspects of innate immunity as well as humoral immunity. According to experiments conducted on probiotics as an alternative to antibiotics, it appears that probiotics have the ability to inhibit important pathogens of poultry industry including *Salmonella*, *Complyobacter* and coccidiosis. It can be said that there is a positive effect of multi-strain probiotics and the combination of probiotics and prebiotics in controlling pathogens.

REFERENCES

- Abboudi B. (2001). Evaluation of *Salmonella* contamination of local and industrial chickens and eggs in Shiraz. *J. Med. Sci. Health Shiraz Univ.* **6**, 40-43.
- Addis M. and Sisay D. (2015). A review on major food borne bacterial illnesses. *J. Trop. Dis.* **3**, 176-181.
- Adib Nishaboori M., Razmi G.H. and Kalidari G.H. (2006). A study of coccidiosis in the pullets of laying hens in Mashhad area. *Anim. Sci. J. (Pajouhesh and Sazandegi)*. **19**, 35-31.
- Akbarian R., Peighambari S.M., Morshed R. and Yazdani A. (2012). Survey of *Salmonella* infection in Iranian poultry flocks. *Iranian Vet. J.* **8**, 5-10.
- Asadpour Y., Mohammadi M., Pourbakhsh S.A. and Rasa M. (2014). Isolation, serotyping and antibiotic resistance of *Salmonella* isolated from chicken carcasses in Guilan province. *Iranian Vet. J.* **9**, 5-13.
- Baba Ahmadi E., Khosravi A., Shamsi M. and Hooshmandfar R. (2009). The anti-coccidial evaluation of quim coccid, pharm coccid, amprolium and monensia against *Eimeria tenella* *in vitro*. *Sci. J. Ilam Univ. Med. Sci.* **18**, 37-43.
- Baffoni L., Gaggia F., Di Gioia D., Santini C., Luca Mogna L. and Biavati B. (2012). A *Bifidobacterium* based synbiotic product to reduce the transmission of *C. jejuni* along the poultry food chain. *Int. J. Food. Microbiol.* **157**, 156-161.
- Barza M. (2002). Potential mechanisms of increased disease in humans from antimicrobial resistance in food animals. *Clin. Infect. Dis.* **34**(3), 123-125.
- Baserisalehi M., Bahador N. and Kapadous B.P. (2007). Isolation and characterization of *Campylobacter* spp. from domestic animals and poultry in South of Iran. *Pakistan J. Biol. Sci.* **10**, 1519-1524.
- Bengmark S. (2001). Pre-, pro- and synbiotics. *Curr. Opin. Clin. Nutr. Metab. Care.* **4**, 571-579.
- Callaway T.R., Edrington T.S., Anderson R.C., Byrd J.A. and Nisbet D.J. (2008). Gastrointestinal microbial ecology and the safety of our food supply as related to *Salmonella*. *J. Anim. Sci.* **86**(14), 163-172.
- Carter A.J., Adams M.R., Woodward M.J. and La Ragione R.M. (2009). Control strategies for *Salmonella* colonization of poultry: The probiotic perspective. *Food Sci. Technol.* **5**, 103-115.
- Carter A.J., Adams M.R., Roberto M., LaRagione J. and Woodward M. (2017). Colonization of poultry by *Salmonella enteritidis* S1400 is reduced by combined administration of *Lactobacillus salivarius* 59 and *Enterococcus faecium* PXN-33. *Vet. Microbiol.* **199**, 100-107.
- Cean A., Stef L., Simiz S., Julean C., Dumitrescu G., Vasile A., Pet E., Drinceanu D. and Corcionivoschi N. (2015). Effect of human isolated probiotic bacteria on preventing *Campylobacter jejuni* colonization of poultry. *Foodborne Pathog. Dis.* **12**, 122-130.
- Chapman C.M., Gibson G.R. and Rowland I. (2011). Health benefits of probiotics: Are mixtures more effective than single strains? *European J. Nutr.* **50**, 1-17.
- Chih-Yuan C., Li-Tsen C., Yue-Cheng C., Chun-Li L., Yi-Yang L. and Hau-Yang T. (2016). Use of a probiotic to ameliorate the growth rate and the inflammation of broiler chickens caused by *Eimeria tenella* infection. *J. Anim. Res. Nutr.* **1**(10), 1-7.
- Cook G.C. (1998). Small intestinal coccidiosis and emergent clinical problems. *J. Infect.* **16**, 213-219.
- Cui S., Zheng B., Ge J. and Meng J. (2005). Prevalence and antimicrobial resistance of *Campylobacter* spp. and *Salmonella* serovars in organic chickens from Maryland retail stores. *Appl. Environ. Microbiol.* **71**, 4108-4111.
- Dalloul R.A. and Lillehoj H.S. (2006). Poultry coccidiosis: Recent advancements in control measures and vaccine development. *Expert Rev. Vaccines.* **5**, 143-163.
- Dalloul R.A., Lillehoj H.S., Shellem T.A. and Doerr J.A. (2003). Enhanced mucosal immunity against *Eimeria acervulina* in broilers fed a *Lactobacillus*-based probiotic. *Poult. Sci.* **82**, 62-66.
- Dibner J.J. and Richards J.D. (2005). Antibiotic growth promoters in agriculture: History and mode action. *Poult. Sci.* **84**, 634-643.
- Edens F.W., Parkhurst C.R., Casas I.A. and Dobrogorz W.J. (1997). Principles of ex ovo competitive exclusion and *in ovo* administration of *Lactobacillus reuteri*. *Poult. Sci.* **76**, 179-196.
- European Food Safety Authority (2007). Preliminary report on analysis of the baseline study on the prevalence of *Salmonella* in laying hen flocks of *Gallus gallus*. Available at: <http://www.efsa.europa.eu/en/efsajournal/pub/81r.htm> Accessed Jul. 2012.
- Feizabadi M.M., Dolatabadi S. and Zali M.R. (2007). Isolation and drug-resistant patterns of *Campylobacter* strains cultured from diarrheic children in Tehran. *Japanese J. Infect. Dis.* **60**, 217-219.
- Fuller R. (1989). Probiotics in man and animals. *J. Appl. Bacteriol.* **66**, 365-378.
- Gaggia F., Di Gioia D., Baffoni L. and Biavati B. (2011). The role of protective and probiotic cultures in food and feed and their

- impact in food safety. *Trends Food Sci. Technol.* **22**, 58-66.
- Ghaderkhani J., Sadeghi-Dehkordi Z. and Bahrami M.A. (2014). Prevalence of coccidiosis in broiler chicken farms in Western Iran. *J. Vet. Med.* **4**, 23-29.
- Ghareeb K., Awad W.A., Mohnl M., Porta R., Biarnés M., Böhm J. and Schatzmayr G. (2012). Evaluating the efficacy of an avian-specific probiotic to reduce the colonization of *Campylobacter jejuni* in broiler chickens. *Poult. Sci.* **91**, 1825-1832.
- Ghasemi H.A., Shivazad M., Esmaeilnia K., Kohram H. and Karimi M.A. (2010). The effects of a synbiotic containing *Enterococcus faecium* and inulin on growth performance and resistance to coccidiosis in broiler chickens. *J. Poult. Sci.* **47**, 149-155.
- Giannenas I., Tsalie E., Triantafyllou E., Hessenberger S., Teichmann K., Mohnl M. and Tontis D. (2014). Assessment of probiotics supplementation via feed or water on the growth performance, intestinal morphology and microflora of chickens after experimental infection with *Eimeria acervulina*, *Eimeria maxima* and *Eimeria tenella*. *Avian Pathol.* **43**(3), 209-216.
- Hamidinejat H., Seifiabad Shapouri M.R., Mayahi M. and Pourmehdi Borujeni M. (2010). Characterization of *Eimeria* species in commercial broilers by PCR based on ITS1 regions of rDNA. *Iranian J. Parasitol.* **5**, 48-54.
- Havaei S.A., Pishva E., Tabibian A., Rabbani M.F., Haghshenas F. and Narimani T. (2007). Incidence of *Campylobacter jejuni* and *C. coli* producing cytolethal distending toxin isolated from broiler using cell culture method in the Isfahan region. *Iranian J. Med. Microbiol.* **1**, 23-17.
- Higgins J.P., Higgins S.E., Vicente J.L., Wolfenden A.D., Tellez G. and Hargis B.M. (2007). Temporal effects of lactic acid bacteria probiotic culture on *Salmonella* in neonatal broilers. *Poult. Sci.* **86**, 1662-1666.
- Higgins S.E., Higgins J.P., Wolfenden A.D., Henderson S.N., Torres-Rodriguez A., Tellez G. and Hargis B.M. (2008). Evaluation of a *Lactobacillus*-based probiotic culture for the reduction of *Salmonella enteritidis* in neonatal broiler chicks. *Poult. Sci.* **87**, 27-31.
- Jayaraman S., Thangavel G., Kurian H., Mani R., Mukkalil R. and Chirakkal H. (2013). *Bacillus subtilis* PB6 improves intestinal health of broiler chickens challenged with *Clostridium perfringens*-induced necrotic enteritis. *Poult. Sci.* **92**, 370-374.
- Kabir S.M.L., Rahman M.M., Rahman M.B., Hosain M.Z., Akand M.S.I. and Das S.K. (2005). Viability of probiotics in balancing intestinal flora and effecting histological changes of crop and caecal tissues of broilers. *Biotechnology.* **4**, 325-330.
- Kalavathy R., Abdullah N., Jalaludin S. and HO Y.W. (2003). Effects of *Lactobacillus* cultures on growth performance, abdominal fat deposition, serum lipids and weight of organs of broiler chickens. *Br. Poult. Sci.* **44**, 139-144.
- Karimi Kivi R., Dadashbeiki M. and Seidavi A.R. (2015). Growth, body characteristics and blood parameters of ostrich chickens receiving commercial probiotics. *Span J. Agric. Res.* **13**, 1-11.
- Khannazar H. and Firouzi R. (1998). Evaluation of *Salmonella* contamination of broiler chickens slaughtered in slaughterhouses in Shiraz and determining their serotype. *Anim. Sci. J. (Pajouhesh and Sazandegi)*. **11**, 98-100.
- Knap I., Kehlet A.B., Bennedsen M., Mathis G.F., Hofacre C.L., Lumpkins B.S., Jensen M.M., Raun M. and Lay A. (2011). *Bacillus subtilis* (DSM17299) significantly reduces *Salmonella* in broilers. *Poult. Sci.* **90**, 1690-1694.
- Lan Y., Xun S., Tamminga S., Williams B.A., Verstegen M.W. and Erdi G. (2004). Real-time PCR detection of lactic acid bacteria in cecal contents of *Eimeria tenella*-infected broilers fed soybean oligosaccharides and soluble soybean polysaccharides. *Poult. Sci.* **83**, 1696-1702.
- Lee S.H., Lillehoj H.S., Park D.W., Hong Y.H. and Lin J.J. (2007a). Effects of *Pediococcus*- and *Saccharomyces*-based probiotic (MitoMax) on coccidiosis in broiler chickens. *Comp. Immunol. Microbiol. Infect. Dis.* **30**, 261-268.
- Lee S.H., Lillehoj H.S., Dalloul R.A., Park D.W., Hong Y.H. and Lin J.J. (2007b). Influence of *Pediococcus*-based probiotic on coccidiosis in broiler chickens. *Poult. Sci.* **86**, 63-66.
- Lillehoj H.S., Min W. and Dalloul R.A. (2004). Recent progress on the cytokine regulation of intestinal immune responses to *Eimeria*. *Poult. Sci.* **83**, 611-623.
- Mañes-Lázaro R., Van Diemen P.M., Pin C., Mayer M.J., Stevens M.P. and Narbad A. (2017). Administration of *Lactobacillus johnsonii* FI9785 to chickens affects colonization by *Campylobacter jejuni* and the intestinal microbiota. *Br. Poult. Sci.* **58**(4), 373-381.
- Marietto-Gonçalves G.A., Curotto S.M.R., Baptista A.A.S., Donato T.C., Takahira R.K., Sequeira J.L. and Andreatti Filho R.L. (2014). Effects of *Lactobacillus* probiotic, P22 bacteriophage and *Salmonella typhimurium* on the heterophilic burst activity of broiler chickens. *Brazilian J. Poult. Sci.* **16**, 257-264.
- Mokhtarian D.H., Mohsenzadeh M., Ghahramani M., Moshki M. and Fani M.J. (2009). Detection and identification of *Campylobacter jejuni* and *Campylobacter coli* from poultry carcasses slaughtered in Gonabad poultry slaughterhouse. *Ofogh-e-Danesh.* **15**(3), 78-81.
- Mountzouris K.C., Balaskas C., Xanthakos I., Tzivinikou A. and Fegeros K. (2009). Effects of a multi-species probiotic on biomarkers of competitive exclusion efficacy in broilers challenged with *Salmonella enteritidis*. *Br. Poult. Sci.* **50**, 467-478.
- Mulder R.W., Havenaar A.W. and Huisintveldt J.H.J. (1997). Intervention strategies the use of probiotics and competitive exclusion microfloras against contamination with pathogens in pigs and poultry. Pp. 187-207 in Probiotics. R. Fuller, ed., Chapman and Hall, London, United Kingdom.
- Nakamura A., Ota Y., Mizukami A., Ito T., Ngwai Y.B. and Adachi Y. (2002). Evaluation of aviguard, a commercial competitive exclusion product for efficacy and alter-effect on the antibody response of chicks to *Salmonella*. *Poult. Sci.* **81**, 1653-1660.
- Neal-McKinney J.M., Lu X., Duong T., Larson C.L., Call D.R. and Shah D.H. (2012). Production of organic acids by probiotic lactobacilli can be used to reduce pathogen load in poultry. *PLoS One.* **7**, e43928.
- Nishiyama K., Seto Y., Yoshioka K., Kakuda T., Takai S. and Yamamoto Y. (2014). *Lactobacillus gasseri* SBT2055 Reduces infection by and colonization of *Campylobacter jejuni*. *PLoS One.* **9**, e108827.

- Olnooda C.G., Beskia S.M., Chocta M. and Ijia P.A. (2015). Use of *Lactobacillus johnsonii* in broilers challenged with *Salmonella sofia*. *Anim. Nutr.* **1**, 203-212.
- Pourakbari M., Seidavi A.R., Asadpour L. and Martínez Marin A.L. (2016). Probiotic level effects on growth performance, carcass traits, blood parameters, cecal microbiota, and immune response of broilers. *Ann. Brazilian Acad. Sci.* **88**, 1011-1021.
- Pournazari M., Qotbi A.A., Seidavi A.Z. and Corazzin M. (2017). Prebiotics, probiotics and thyme (*Thymus vulgaris*) for broilers: Performance, carcass traits and blood variables. *Rev. Colomb Cienc. Pecu.* **30**, 3-10.
- Prado-Rebolledo O.F., Delgado-Machuca J.J., Macedo-Barragan R.J., GarciaMarquez L.J., Morales-Barrera J.E., Latorre J.D., Hernandez-Velasco X. and Tellez G. (2016). Evaluation of a selected lactic acid bacteria-based probiotic on *Salmonella enterica* serovar enteritidis colonization and intestinal permeability in broiler chickens. *Avian Pathol.* **22**, 1-17.
- Rahimi E. and Tajbakhsh E. (2008). Prevalence of *Campylobacter* species in poultry meat in the Isfahan city, Iran. *Bulgaria J. Vet. Med.* **11**, 257-262.
- Rahimi E., Momtaz H. and Bonyadian M. (2010). PCR detection of *Campylobacter* sp. from turkey carcasses during processing plant in Iran. *Food Control.* **21**, 692- 694.
- Razmi G.R. and Kalideri G.A. (2000). Prevalence of coccidiosis in broiler-chicken farms in the municipality of Mashhad, Khorasan, Iran. *Preven. Vet Med.* **44**, 247-253.
- Ritzi M.M., Abdelrahman W., van-Heerden K., Mohnl M., Barrett N.W. and Dalloul R.A. (2016). Combination of probiotics and coccidiosis vaccine enhances protection against an *Eimeria* challenge. *Vet Res.* **47**, 111-117.
- Saint-Cyr M.J., Haddada N., Taminiou B., Poezevara T., Quesne S., Amelot M., Daube G., Marianne Chemaly M., Dousset X. and Guyard-Nicodème M. (2017). Use of the potential probiotic strain *Lactobacillus salivarius* SMXD51 to control. *Int. J. Food Microbiol.* **247**, 9-17.
- Schachtsiek M., Hammes W.P. and Hertel C. (2004). Characterization of *Lactobacillus coryniformis* DSM 20001T surface protein Cpf mediating coaggregation with and aggregation among pathogens. *Appl. Environ. Microbiol.* **70**, 7078-7085.
- Seidavi A.R., Dadashbeiki M., Alimohammadi-Saraei M.H., Van Den Hoven R., Laudadio V. and Tufarelli V. (2017). Effects of dietary inclusion level of a mixture of probiotic cultures and enzymes on broiler chickens immunity response. *Environ. Sci. Pollu. Res.* **24**, 4637-4644.
- Shapouri R., Rahnama M. and Egbalzadh S.H. (2009). A survey on the prevalence of *Salmonella* serotypes in chicken meat and eggs and determine their antibiotic susceptibility in the Zanjan city. *J. Biol. Sci.* **2**, 71-63.
- Soltan Dallal M.M. (2007). Comparing the prevalence of bacterial contamination of red meat and packaged and non-packaged chicken in shops and department stores of south of Tehran. *J. Shahid Sadoughi Univ. Med. Sci.* **1**, 43-35.
- Tellez G., Pixley C., Wolfenden R.W., Layton S.L. and Hargis B.M. (2012). Probiotics/direct fed microbials for *Salmonella* control in poultry. *Food Res. Int.* **45**, 628-633.
- Tierney J., Gowing H. and Van Sinderen D.V. (2004). *In vitro* inhibition of *Eimeria tenella* invasion by indigenous chicken *Lactobacillus* species. *Vet. Parasitol.* **122**, 171-182.
- Timmerman H.M., Koning C.J., Mulder L., Rombouts F.M. and Beynen A.C. (2004). Monostrain, multistrain and multispecies probiotics-a comparison of functionality and efficacy. *Int. J. Food Microbiol.* **96**, 219-233.
- USDA. (2007). Progress Report on *Salmonella* testing of raw meat and poultry products, 1998-2006. Washington, DC., Food Safety and Inspection Service, USDA.
- Vicente J.L., Torres-Rodriguez A., Higgins S.E., Pixley C., Tellez G., Donoghue A.M. and Hargis B.M. (2008). Effect of a selected *Lactobacillus* spp. based probiotic on *Salmonella enterica* serovar enteritidis-infected broiler chicks. *Avian Dis.* **52**, 143-146.
- Waldenstrom J., Broman T., Carlsson I., Hasselquist D. and Olsen B. (2001). Prevalence of *Campylobacter* spp. in migrating wild birds in Sweden-ecological considerations of carriership. *Int. J. Med. Microbiol.* **291**, 37-38.
- Williams R.B. (2006). Relative virulences of a drug-resistant and a drug-sensitive strain of *Eimeria acervulina*, a coccidium of chickens. *Vet. Parasitol.* **135**, 15-23.
- Wine E., Gareau M.G., Johnson-Henry K. and Sherman P.M. (2009). Strain-specific probiotic (*Lactobacillus helveticus*) inhibition of *Campylobacter jejuni* invasion of human intestinal epithelial cells. *FEMS Microbiol. Lett.* **300**, 146-152.
- Youssef G.A., Ezzeldeen N.A., Mostafa M.A. and Sherif N.A. (2011). Effects of isolated *Lactobacillus acidophilus* as a probiotic on chicken vaccinated and infected with *Salmonella typhimurium*. *Global Vet.* **7(5)**, 449-455.
- Zendehbada B., Arian A.A. and Alipour A. (2013). Identification and antimicrobial resistance of *Campylobacter* species isolated from poultry meat in Khorasan province, Iran. *Food Control.* **32**, 724-727.
- Zhao T. and Doyle M.P. (2006). Reduction of *Campylobacter jejuni* on chicken wings by chemical treatments. *J. Food Prot.* **69**, 762-767.
- Zulkifli I., Abdulkah N., Azrin N.M. and HO Y.W. (2000). Growth performance and immune response of two commercial broiler strains fed diets containing *Lactobacillus* cultures and oxytetracycline under heat stress conditions. *Br. Poult. Sci.* **41**, 593-597.