Iranian Journal of Applied Animal Science

The Use of *Enterococci* as Probiotics in Poultry

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

Received on: 4 Mar 2018
Revised on: 2 Apr 2018
Accepted on: 15 Apr 2018
Online Published on: Dec 2018

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

Online version is available on: www.ijas.ir

ABSTRACT

Enterococci are members of the lactic acid bacteria family and are responsible for many food spoilage and fermentations. Some strains of this microorganism are used as probiotics in humans and animals to improve host immunity. However, some *Enterococci* are important pathogens which cause severe infections. Some strains of *Enterococci* are resistant to common antibiotics. The *Enterococcus faecium* and *Enterococcus faecalis* strains are more common probiotics. Such probiotics are used as an alternative to growth promoting antibiotics, which their use has been restricted. In domestic animals, enterococcal probiotics are mostly used to cure or prevent pathogen infections and immune response and growth performance improvement. This review covers the reports on the application of *Enterococcus* genus as a functional probiotic in poultry. The results suggest that *Enterococcus faecium* is a safe probiotic and improve the immune system and performance of broiler chickens.

KEY WORDS broilers, *Enterococci*, immune system, performance, probiotic.

INTRODUCTION

The intensive systems of broiler chicken production could be accompanied with some stressor factors (Panda *et al.* 2006). In the recent decades, the uncontrolled use of growth promoting antibiotics has been increasing the risk of developing of antibiotic resistant pathogens (Sorum and Sunde, 2001). In 1996, the scientific findings and public concerns, was resulted in ban of growth promoter antibiotic application in the European Union. The new situation, was triggered more intensive research to find new safe animal growth promoter alternatives, such as changing the gut microflora using live non-pathogenic microorganisms with promising effects on birds health and performance which are known as probiotics. Probiotics have shown promising effects as alternatives to growth-promoting (Awad *et al.* 2009).

The positive effects of probiotics on immune responses (Capcarova *et al.* 2008; Lee *et al.* 2008), decreasing pathogenic flora in the intestine has been widely accepted (Crawford, 1979). The lactic acid producing bacteria are the main probiotics and in particular *Lactobacillus acidophilus*, *L. casei*, *L. reuteri* are the base of most lactic acid bacteria based products (Caglar *et al.* 2005). Poultry initiates to eat solid feed immediately after hatching, then probiotics consumption must start in the early ages when gut microflora are not still well developed (Vahjen *et al.* 2002). The probiotic bacteria must also have additional criteria, including resistance to gastrointestinal pH and bile salts which are prerequisites for survival, colonization and action of ingested bacteria in the intestinal tract of the host (Erkkila and Petaja, 2000; Liong and Shah, 2005). The other essential feature to screening bacteria as potential probiotic is sensitivity to antibiotics, because bacteria can contain viru lence factors and antibiotic resistance factors. The most abundant lactic acid bacteria in the intestine of chickens are Lactobacillus and *Enterococcus* (Mitsuoka, 2002). The present review tries to summarize the findings and reports on the *Enterococcus* genus a functional probiotic in poultry.

Enterococcus genus

Until 1980 s, whole the Gram-positive cocci were known as streptococci, however the microbiological progress was reported them to the new genera *Enterococcus*, *Lactococcus* and *Streptococcus* (Schleifer and Kilpper-Bälz, 1984; Devriese *et al*. 1993; Devriese and Pot, 1995). The genus *Enterococcus* is a member of the lactic acid bacteria family and significant bacteria in cheese production, and spoilage. The *Enterococcus* tolerates to high salt and pH then usually is dominant in fermented foods. More than 37 species have been recognized for the genus *Enterococcus* and this genus are found in the environment and many animal and human based materials (Devriese *et al*. 1991; Devriese *et al*. 2003; Franz and Holzapfel, 2006). The probiotic characteristics have been recognized for some enterococcal strains and they have effectively used in human and animals. However, there are also some photogenic enterococcal strains which are responsible for bacteraemia, endocarditis or urinary tract infections in human. The pathogen *Enterococcus* strains are usually antibiotic resistance and there are concerns for the secure application as probiotics (Franz *et al*. 2011). On the other hand, *E. faecium*, *E. faecalis*, *E. hirae*, *E. durans*, and *E. cecorum* are natural residents of the farm animal's intestinal tract, which is an essential factor to probiotic survival (Devriese *et al*. 1991; Devriese *et al*. 1994; Leclercq *et al*. 1996). From a probiotic point of view, the facultative anaerobic bacteria, *E. faecium* and *E. faecalis* are the main enterococcal species and *E. faecium* is permitted by the Association of American Feed Control Officials, fed to broiler chickens as a probiotic supplement (Franz *et al*. 1999; Foulquié-Moreno *et al*. 2006; Zhao *et al*. 2013). The ability of chicken originated *Enterococcus* spp. (*E. faecium* EF55) to produce bacteriocins as potential antimicrobial factors could confirm their probiotic effects for poultry (Laukova *et al*. 2004).

Effect of enterococcal probiotics on intestinal microbial population

Vahjen *et al*. (2002) reported that dietary supplementation of *E. faecium* SF68 increased the lactic acid bacteria population in turkey small intestine. This confirms that the enterococci can tolerate turkey gastrointestinal tract condition and control pathogenic bacteria. Samli *et al*. (2007) found that dietary supplement of *E. faecium* could increase lactic acid bacteria colonization in the ileum, and increased their excretion (Samli *et al*. 2007). A multi species probiotic containing *Enterococcus*, *Bifidobacterium* and *Pediococcus* strains applied in the feed and water manipulated the cecal microbial population, such that increased the *Lactobacilli*, *Bifidobacteria* and gram-positive cocci and also reduced the *Salmonella* population in turkey and broilers (Mountzouris *et al*. 2007; Grimes *et al*. 2008; Capcarova *et al*. 2010). In the study of Samli *et al*. (2010), the *E. faecium* probiotic improved the ileal and cecal microbial population and significantly reduced the *Escherichia coli* population. Kralik *et al*. (2004) also demonstrated the effect of dietary supplementation of *E. faecium* on *E. coli* reduction in broilers. The positive effect of *E. faecium* on fecal microflora of broiler has also reported by Kacaniova *et al*. (2006). In another study, *E. faecium* CCM8558 effectively colonized in the intestinal tract of chickens and reduced the *Campylobacter* spp load (Laukova *et al*. 2017). Cao *et al*. (2013) fed *E. faecium* to broilers and showed lower *E. coli* and *C. perfringens* population and higher *Lactobacillus* and *Bifidobacterium* population in the cecum contents. Levkut *et al*. (2009) observed that the *E. faecium* EF55 decreased the cecal population of Salmonella in the infected broilers. Similarly, other researchers have reported that *Lactobacillus acidophilus* and *E. faecium* based probiotics, decrease the *Campylobacter jejuni* count in chicks (Willis and Reid, 2008; Ghareeb *et al*. 2012). Table 1 shows the effects of *E. faecium* based probiotics on intestinal microbial population.

Effect of enterococcal probiotics on poultry performance

Samli *et al*. (2007) reported that dietary supplementation of *E. faecium* NCIMB 10415 improved broiler chickens weight gain and feed efficiency. The same findings have been reported by Mountzouris *et al*. (2007) and Awad *et al*. (2009). In another study using a multi species containing *Lactobacillus*, *Pediococcus*, *Bifidobacterium* and *Enterococcus* strains in feed and water, Mountzouris *et al*. (2007) found a growth rate comparable to feeding 2.5 mg/kg avilamycin antibiotic. Surprisingly, probiotic included in drinking water was more effective than dietary route. Demeterová *et al*. (2009) studied the supplementation of *E. faecium* DSM 7134 and natural humic substances in broiler chickens. The improved feed conversion ratio in birds fed both the supplements together was attributed to the increased phagocytes (Demeterová *et al*. 2009). Capcarova *et al*. (2010) used *E. faecium* probiotic in the diet of broiler chickens and found a decrease in feed intake without any change in feed conversion ratio. Luo *et al*. (2013) reported a normal growth rate in broiler chickens fed *E. faecium* supplement, and Zheng *et al*. (2015) found that dietary *E. faecium* inclusion did not influence the weight gain and feed intake of broilers, however, the feed conversion ratio was improved.

Table 1 The effects of *Enterococcus* probiotic on poultry intestinal microbial population

Probiotic	Bird	Effect on intestinal microbial population	Source
E. faecium SF68	Turkey	Increase in lactic acid bacteria	Vahjen *et al.* (2002)
E. faecium	Broilers	Increase in lactic acid bacteria	Samli *et al.* (2007)
Multi species probiotic containing *Enterococcus*, *Bifidobacterium* and *Pediococcus* strains	Broilers	Increases in the *Lactobacilli*, *Bifidobacteria* and gram-positive cocci and also	Mountzouris *et al.* (2007)
Direct-fed microbial (Primalac) containing *E. faecium*	Turkey	Reduced the *Salmonella* population	Grimes *et al.* (2008)
E. faecium	Broilers	Reduced the *Escherichia coli* population	Samli *et al.* (2010)
E. faecium	Broilers	Reduced the *Escherichia coli* population	Kralik *et al.* (2004)
E. faecium CCM8558	Broilers	Reduced the *Campylobacter* spp. population	Laukova *et al.* (2017)
E. faecium	Broilers	Lower *E. coli* and *C. perfringens* and higher *Lactobacillus* and *Bifido bacterium* populations	Cao *et al.* (2013)
E. faecium EF55	Broilers	Decreased the population of *Salmonella*	Levkut *et al.* (2009)
Lactobacillus acidophilus and *E. faecium* based	Broilers	Decrease the *Campylobacter jejuni* population	Ghareeb *et al.* (2012)

Cao *et al.* (2013) found that *E. faecium* probiotic improved the growth rate of chickens experimentally infected with pathogenic *E. coli* K88. In the study of Majidi-Mosleh *et al.* (2017), *in ovo* injection of *E. faecium* had no effect on hatchability our growth performance in broiler chickens. Zheng *et al.* (2016) suggested that dietary *E. faecium* feeding may change the partitioning of nutrients, consequently, improve nutrient utilization. Table 2 shows the effects of *E. faecium* based probiotics on performance traits of broilers.

Effect of enterococcal probiotics on poultry meat quality

Zheng *et al.* (2015) used 2D-DIGE-based proteomics to study the proteome changes in the meat of broilers fed *E. faecium* probiotic.

The *E. faecium* supplement increased pH, water holding capacity and meat colour of pectoral muscle, however reduced abdominal fat content. They suggested that meat quality alterations following *E. faecium* feeding were due to changes in expression of 22 proteins in the pectoral muscle, such that dietary *E. faecium* probiotic improved meat quality of broilers.

This was due to the changes in expression of proteins responsible for energy and carbohydrate metabolism, cytoskeleton, and also molecular chaperones. These proteins are the main controllers of pH and water holding capacity of meat. The pectoral muscle of broiler chickens fed *E. faecium* supplement had also reduced the cooking loss and drip loss (Zheng *et al.* 2015).

Effect of enterococcal probiotics on intestinal morphology in poultry

The gastrointestinal tract is responsible for the uptake of nutrients, remove pathogens and immune response. Luo *et al.* (2013) reported that dietary supplementation of *E. faecium* increased gut microvilli and influenced immune organ development and mucosal structure chickens. They also showed that dietary supplement of *E. faecium* had a pronounced effect on genes expression related to the intestinal tissue development and epithelium maturation, and genes-responsible for digestion and absorption of nutrients. The mucin is a very glycosylated protein that is synthesized by the goblet cell in epithelial tissues and acts as a protective barrier (Marin *et al.* 2008).

In the study of Luo *et al.* (2013), *E. faecium* supplementation led to down-regulation of mucin-2 which is a member of mucin protein family, which can combine to pathogens as part of the immune response (Johansson *et al.* 2011). Samli *et al.* (2007) also reported that dietary supplementation of *E. faecium* increased the villus height in jejunum and ileum of broilers, which could enhance the digestive and absorptive capacity of the intestinal tract because of a higher absorptive surface area, up-regulation of brush border enzymes and enhancing nutrient transport mechanisms (Amat *et al.* 1996).

In the study of Cao *et al.* (2013), dietary inclusion of *E. faecium* increased villi height and decreased crypt depth in the jejunum and the same effect was observed using an antibiotic. Chichlowski *et al.* (2007) found that a multi-strain probiotic containing *Lactobacilli*, *Thermophilum*, *Bifidobacterium*, and *E. faecium* increased villus height and decreased the crypt depth in jejunum, compared with the control group or birds fed salinomycin. Therefore, it seems that dietary *E. faecium* probiotic could play a positive role in the small intestinal morphology of broilers.

Effect of enterococcal probiotics on immune responses in poultry

There have are several reports in the literature of the positive effects of enterococcal probiotics on poultry immune response. Zheng *et al.* (2016), suggested that the improved production efficiency in the broiler chickens fed with *E. faecium* supplement could be attributed to lower nutrient costs for immune response and more available nutrient for growth of birds.

Table 2 The effects of *Enterococcus* probiotic on performance of broilers

Probiotic	Effect on performance	Source
E. faecium NCIMB 10415	Improved weight gain and feed efficiency	Samli *et al.* (2007)
Multi species containing *Lactobacillus*, *Pediococcus*, *Bifidobacterium* and *Enterococcus* strains	A growth rate comparable to feeding 2.5 mg/kg avilamycin antibiotic	Mountzouris *et al.* (2007)
E. faecium DSM 7134 and natural humic substances	Improved feed conversion ratio in birds fed both the supplements together	Demeterová *et al.* (2009)
E. faecium	Decrease in feed intake without any change in feed conversion ratio	Capcarova *et al.* (2010)
E. faecium	Normal growth rate	Luo *et al.* (2013)
E. faecium	Did not influence the weight gain and feed intake of but, the feed conversion ratio was improved	Zheng *et al.* (2015)
E. faecium	Improved the growth rate of chickens experimentally infected with pathogenic *E. coli* K88	Cao *et al.* (2013)
E. faecium	Improve nutrient utilization	Zheng *et al.* (2016)

In the study of Luo *et al.* (2013), the relative weights of intestine, spleen and Bursa Fabricius were heavier in chickens fed *E. faecium* probiotic, they concluded that dietary supplementation of *E. faecium* could improve immune organ development. They also found that the *E. faecium* probiotic decreased the inflammation and oxygen stress conditions in the intestinal mucosa of broilers. This means less energy costs and probably explains the improved feed conversion ratio. In the study of Majidi-Mosleh *et al.* (2017), the antibody titre against Newcastle disease virus, antibody titre against sheep red blood cells and cell-mediated immune response was not influenced in *E. faecium* fed broilers.

Cao *et al.* (2013), studied the pattern of immune system related gene expression in response to dietary *E. faecium* supplementation. They observed an up-regulation of Interleukin 4 (IL-4) which has a key role in the immune responses, in the jejunal mucosa of chicks fed *E. faecium* probiotic. In birds treated with *E. faecium,* the expression of tumor necrosis factor alpha (TNF-α), a key cytokine responsible for systemic inflammation and is one of the cell signaling proteins involved in the acute phase reaction, was also increased in the jejunal mucosa. Birds fed *E. faecium* probiotic had higher levels of secretory immunoglobulin A (S-IgA) in jejunal mucosa, which is an important factor in protecting organs such as oral cavity, intestine, and lungs from invading pathogens. As a matter of fact, the majority of invading pathogens makes first contacts with the host at mucosal level, particularly in the agasreo-intestinal tract, and S-IgA is known as the first protective ban (Muir *et al.* 1998).

Phagocytic action is an important constituent of the cellular innate immunity system and has a vital role in host protection against pathogens. Laukova *et al.* (2017) reported that Phagocytic activity was considerably increased in chickens fed *E. faecium* probiotic and attributed it to the ability of *E. faecium* CCM8558 strain to promote the toll-like receptors (TLRs).

The TLRs are pattern detection receptors that act as pathogens invading sensors and are vital for the start the innate inflammatory and adaptive immune reactions (Shang *et al.* 2008).

There are also reports on the effects of *E. faecium* probiotic on up-regulation of MIF, IFN-β, MD-2, and CD14 immune system related proteins in chickens (Karaffova *et al.* 2017). Table 3 shows the effects of *E. faecium* based probiotics on immune system of broilers.

Effect of enterococcal probiotics on blood parameters in poultry

There are reports on the effects of probiotics in altering the chicken blood lipid fractions as an index of body metabolism (Panda *et al.* 2006). Capcarova *et al.* (2010) showed that the *E. faecium* M 74 probiotic reduced levels of total cholesterol and also total lipids in blood plasma. The blood cholesterol concentration is an important factor to prevent atherosclerosis, and it's known that atherosclerosis could be controlled by adjusting the blood cholesterol level (Kapila *et al.* 2009).

De Smet *et al.* (1994) found that probiotics increase the production of unconjugated bile acids, therefore involve in the regulation of blood cholesterol, which is the precursor substance of bile acids. Capcarova *et al.* (2008) reported that blood bilirubin concentration increased in broilers fed *E. faecium* M 74 and addition of a probiotc containing *L. fermentum* and *E. faecium* caused in an increase in serum calcium and iron concentration and a lower blood triglyceride level. The higher serum calcium concentration could be a positive effect for the animals to reach a more strength bone and growth rate.

Capcarova *et al.* (2010) studied the effect of *E. faecium* M74 strain on blood parameters of laying hens, and found a reduced levels of calcium, lipids, cholesterol, haematocrit values and leucocyte counts in plasma, although the triglyceride level was not altered and the erythrocyte counts were greater than before.

Table 3 The effects of *Enterococcus* probiotic on immune system responses of broilers

Probiotic	Effects on broilers immune system	Source
E. faecium	Heavier weights of intestine, spleen and fabricius bursa Decreased the inflammation and oxygen stress conditions in the intestinal mucosa	Luo *et al*. (2013)
E. faecium	No effects on the antibody titre against Newcastle disease virus and sheep red blood cells and cell-mediated immune response	Majidi-Mosleh *et al*. (2017)
E. faecium	Higher levels of secretory immunoglobulin A (S-IgA) in jejunal mucosa Up-regulation of Interleukin 4 (IL-4) Increase in Tumor necrosis factor alpha (TNF-α) in the jejunal mucosa	Cao *et al*. (2013)
E. faecium CCM8558	Phagocytic activity was considerably increased	Laukova *et al*. (2017)
E. faecium	Up-regulation of MIF, IFN-β, MD-2, and CD14 immune system related proteins	Karaffova *et al*. (2017)

Probiotic application was not changed the egg production parameters. The effect of *E. faecium* CCM8558 strain on blood lipid fractions could be attributed to the fact that the probiotic is a lactic acid producing bacteria, release bile degrading enzymes, deconjugates bile, and reduces pH. These alterations could decrease blood triglycerides or cholesterol levels (Bovdisova and Capcarova, 2015).

In the experiment of Capcarova *et al*. (2008), supplementation of *E. faecium* M 74 strain in drinking water had no effect on serum hepatic enzymes, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (GGT) and glutamate dehydrogenase (GLDH). However, the antioxidant potential of *E. faecium* M 74 strain added was proved, such that multivalent anti-oxidativity (TAS) was increased, which is an index to inhibit the exogenous and endogenous oxidative stress.

CONCLUSION

To date, the *Enterococcus faecium* are known as safe probiotic microorganisms which enhance the immune system and performance of broiler chickens. However, various factors may affect the potential probiotic effects and more investigations may be needed to conclusively reveal the involved mechanisms.

REFERENCES

Amat C., Planas J.M. and Moreto M. (1996). Kinetics of hexose uptake by the small and large intestine of the chicken. *American J. Physiol.* **271,** 1085-1089.

Awad W., Ghareeb K., Abdel-Raheem S. and Böhm J. (2009). Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. *Poult. Sci*. **88,** 49-56.

Bovdisova I. and Capcarova M. (2015). Effect of a probiotic product on the content of cholesterol and triglycerides in the blood serum of laying hens. Pp. 42-45 in Proc. Conf. Young Sci., Nitra, Slovak Republic.

Caglar E., Kargul B. and Tanboga I. (2005). Bacteriotherapy and probiotics' role on oral health. *Oral Dis*. **11,** 131-137.

Cao G.T., Zeng X.F., Chen A.G., Zhou L., Zhang L., Xiao Y.P. and Yang C.M. (2013). Effects of a probiotic, *Enterococcus faecium*, on growth performance, intestinal morphology, immune response, and cecal microflora in broiler chickens challenged with *Escherichia coli* K88. *Poult. Sci.* **92,** 2949-2955.

Capcarova M., Kolesarova A., Massanyi P. and Kovacik J. (2008). Selected blood biochemical and haematological parameters in turkeys after experimental probiotic *Enterococcus faecium* M 74 strain administration. *Int. J. Poult. Sci*. **7,** 1194-1199.

Capcarova M., Weiss J., Hrncar C., Kolesarova A. and Pal G. (2010). Effect of *Lactobacillus fermentum* and *Enterococcus faecium* strains on internal milieu, antioxidant status and body weight of broiler chickens. *J. Anim. Physiol. Anim. Nutr*. **94,** 215-224.

Chichlowski M., Croom W.J., Edens F.W., MacBride B.W., Qiu R., Chiang C.C., Daniel L.R., Havenstein G.B. and Koci M.D. (2007). Microarchitecture spatial relationship between bacteria and ileal, cecal colonic in chicks fed a direct-fed microbial, PrimaLac, and salinomycin. *Poult. Sci*. **86,** 1121-1132.

Crawford J.S. (1979). Probiotics in animal nutrition. Pp. 45-55 in Proc. Arkansas Nutr. Conf., Arkansas, USA.

Demeterová M., Mariscáková R., Pistl J., Nad P. and Samudovská A. (2009). The effect of the probiotic strain *Enterococcus faecium* DSM 7134 in combination with natural humic substances on performance and health of boiler chickens. *Berl. Munch. Tierarztl. Wochenschr*. **122,** 370-377.

De Smet I., Van Hoorde L., De Saeyer M., Van De Woeslyne M. and Verstraele W. (1994). *In vitro* study of bile salt hydrolase (BSH) activity of BSH isogenic *Lactobacillus plantarum* 80 strains and estimation of cholesterol lowering through enhanced BSH activity. *Microb. Ecol. Health Dis*. **7,** 315-329.

Devriese L.A., Pot B. and Collins M.D. (1993). Phenotypic identi fi cation of the genus *Enterococcus* and differentiation of phylogenetically distinct enterococcal species and species groups. *J. Appl. Bacteriol.* **75,** 399-408.

Devriese L.A., Hommez J., Pot B. and Haesebrouck F. (1994). Identification and composition of the streptococcal and enterococcal flora of tonsils, intestines and faeces of pigs. *J. Appl. Bacteriol*. **77,** 31-36.

Devriese L.A. and Pot B. (1995). The genus *Enterococcus*. Pp. 327-367 in The Lactic Acid Bacteria. : The Genera of Lactic

Acid Bacteria. B.J.B. Wood and W.H. Holzapfel, Eds. Blackie Academic, London.

Devriese L.A., Hommez J., Wijfels R. and Haesebrouck F. (1991). Composition of the enterococcal and streptococcal intestinal flora of poultry. *J. Appl. Bacteriol.* **71,** 46-50.

Devriese L., Baele M. and Butaye P. (2003). The genus *Enterococcus*. Pp. 101-107 in The Prokaryotes. M. Dworkin, S. Falkow, E. Rosenberg, K.H. Schleifer and E. Stackebrandt, Eds. Springer-Verlag Berlin Heidelberg Publisher, Germany.

Erkkila S. and Petaja E. (2000). Screening of commercial meat starter cultures at low pH and in the presence of bile salts for potential probiotic use. *Meat Sci*. **55,** 297-300.

Foulquié-Moreno M.R., Sarantinopoulos P., Tsakalidou E. and De Vuyst L. (2006). The role and application of *Enterococci* in food and health. *Int. J. Food Microbiol.* **106,** 1-24.

Franz C.M., Holzapfel W.H. and Stiles M.E. (1999). *Enterococci* at the crossroads of food science? *Int. J. Food Microbiol*. **47,** 1-24.

Franz C.M. and Holzapfel W.H. (2006). The enterococci. Pp. 557-613 in Emerging Foodborne Pathogens. Y. Motarjemi and M. Adams, Eds. Woodhead Publishing, Sawston, United Kingdom.

Franz C.M., Huch M., Abriouel H., Holzapfel W. and Gálvez A. (2011). *Enterococci* as probiotics and their implications in food safety. *Int. J. Food Microbiol.* **151,** 125-140.

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.

Grimes J.L., Rahimi S., Oviedo E., Sheldon B.W. and Santos F.B.O. (2008). Effects of a direct-fed microbial (Primalac) on turkey poult performance and susceptibility to oral *Salmonella* challenge. *Poult. Sci*. **87,** 1464-1470.

Johansson M.E., Larsson J.M. and Hansson G.C. (2011). The two mucus layers of colon are organized by the MUC2 mucin, whereas the outer layer is a legislator of host-microbial interactions. *Proc. Natl. Acad. Sci. USA.* **108(1),** 4659-4665.

Kacaniova M., Kmet V. and Cubon J. (2006). Effect of *Enterococcus faecium* on the digestive tract of poultry as a probiotic. *Turkish J. Vet. Anim. Sci.* **30,** 291-298.

Kapila S., Vibha P. and Sinha R. (2009). Antioxidative and hypocholesterolemic effect of *Lactobacillus casei* ssp. Casei (biodefensive properties of *Lactobacilli*). *Indian J. Med. Sci*. **60,** 361-370.

Karaffova V., Marcinkova E., Bobikova K., Herich R., Revajova V., Stasova D., Kavulova A., Levkutova M., Levkut Jr M., Laukova A., Sevcikova Z. and Levkut S.M. (2017). TLR4 and TLR21 expression, MIF, IFN-β, MID-2, CD14 activation, and sIgA production in chickens administered with EFAL41 strain challenged with *Campylobacter jejuni*. *Folia Microbiol*. **62,** 89-97.

Kralik G., Milakovic Z. and Ivankovic S. (2004). Effect of probiotic supplementation on the performance and the composition of the intestinal microflora in broilers. *Acta Agraria KaposvÃ¡ Riensis*. **8,** 23-31.

Laukova A., Guba P., Nemcova R. and Marekova M. (2004). Inhibition of *Salmonella enterica* serovar Dusseldorf by enterocin A in gnotobiotic Japanese quails. *Vet. Med. Czech*. **49,** 47-51.

Laukova A., Pogany Simonova M., Chrastinova L., Kandricakova A., Scerbova J., Placha I., Cobanova K., Formelova Z., Ondruska L., Strkolcova G. and Strompfova V. (2017). Beneficial effect of bacteriocin strain *Enterococcus* du-rans ED26E/7 in model experiment using broiler rabbits. *Czech J. Anim. Sci*. **62,** 168-177.

Leclercq H., Devriese L.A. and Mossel D.A.A. (1996). Taxonomical changes in intestinal (faecal) *Enterococci* and streptococci: Consequences on their use as indicators of faecal contamination in drinking water. *J. Appl. Bacteriol.* **81,** 459-466.

Lee N.K., Yun C.W., Kim S.W., Chang H.I., Kang C.W. and Paik H.D. (2008). Screening of *Lactobacilli* derived from chicken feces and partial characterization of *Lactobacillus acidophilus* A12 as an animal probiotics. *J. Microbiol. Biotechnol*. **18,** 338-342.

Levkut M., Pustl J., Lauková A., Revajova V., Herich R., Ševcíková Z., Strompfova V., Szaboova R. and Kokincakova T. (2009). Antimicrobial activity of *Enterococcus faecium* 55 against *Salmonella enteritidis* in chicks. *Acta Vet. Hung.* **57,** 13-24.

Liong M.T. and Shah N.P. (2005). Acid and bile tolerance and cholesterol removal ability of lactobacilli strains. *J. Dairy Sci.* **88,** 55-66.

Luo J., Zheng A., Meng K., Chang W., Bai Y., Li K., Cai H., Liu G. and Yao B. (2013). Proteome changes in the intestinal mucosa of broiler (*Gallus gallus*) activated by probiotic *Enterococcus faecium*. *J. Proteomics*. **91,** 226-241.

Majidi-Mosleh A., Sadeghi A., Mousavi S.N., Chamani M. and Zarei A. (2017). Ileal *MUC2* gene expression and microbial population, but not growth performance and immune response, are influenced by *in ovo* injection of probiotics in broiler chickens. *British Poult. Sci*. **58,** 40-45

Marin F., Luquet G., Mari B. and Medakovic D. (2008). Molluscan shell proteins: primary structure, origin, and evolution. *Curr. Top. Dev. Biol.* **80,** 209-276.

Mitsuoka T. (2002). Research in intestinal flora and functional foods. *J. Int. Microbiol*. **15,** 57-89.

Mountzouris K.C., Tsistsikos P., Kalamara E., Nitsh S., Schatzmayr G. and Fegeros K. (2007). Evaluation of the efficacy of a probiotic containing *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, and *Pediococcus* strains in promoting broiler performance and modualting cecal microflora composition and metabolic activities. *Poult. Sci.* **86,** 309-317.

Muir W.I., Bryden W.L. and Husbandw A.J. (1998). Evaluation of the efficacy of intraperitoneal immunization in reducing *Salmonella typhimurium* infection in chickens. *Poult. Sci*. **77,** 1874-1883.

Panda A.K., Ramarao S.V., Raju M.V.L.N. and Sharma S.R. (2006). Dietary supplementation of *Lactobacillus* sporogenes on performance and serumbiochemico-lipid profile of broiler chickens. *J. Polt. Sci*. **43,** 235-240.

Samli H.E., Senkoylu N., Koc F., Kanter M. and Agma A. (2007). Effects of *Enterococcus faecium* and dried whey on broiler perforance, gut histomorphology and intestinal microbiota. *Arch. Anim. Nutr*. **61,** 42-49.

Samli H.E., Dezcan S., Koc F., Ozduven M.L., Okur A.A. and Senkoylu N. (2010). Effects of *Enterococcus faecium* supplementation and floor type on performance, morphology of erythrocytes and intestinal microbiota in broiler chickens. *British Poult. Sci*. **51,** 564-568.

Schleifer K.H. and Kilpper-Bälz R. (1984). Transfer of *Streptococcus faecalis* and *Streptococcus faecium* to the genus *Enterococcus nomrev* as *Enterococcus faecalis* comb. nov. and *Enterococcus faecium* comb. nov. *Int. J. Syst. Bacteriol.* **34,** 31-34.

Shang L., Fukata M., Thirunarayanan N., Martin A.P., Arnaboldi P., Maussag D., Berin C., Unkeless J.C., Mayer L., Abreu M.T. and Lira S.A. (2008). TLR signaling in small intestinal epithelium promotes B cell recruitment and IgA production in lamina propria. *Gastroenterology*. **135,** 529-538.

Sorum H. and Sunde M. (2001). Resistance to antibiotics in the normal flora of animals. *Vet. Res*. **32,** 227-241.

Vahjen W., Jadamus A. and Simon O. (2002). Influence of probiotic *Enterococcus faecium* strain on selected bacterial groups in the small intestine of growing turkey poults. *Arch. Anim. Nutr*. **56,** 419-429.

Willis W.L. and Reid L. (2008). Investigating the effects of dietary probiotic feeding regimens on broiler chicken production and *Campylobacter jejuni* presence. *Poult. Sci*. **87,** 606-611.

Zhao X., Guo Y., Guo S. and Tan J. (2013). Effects of *Clostridium butyricum* and *Enterococcus faecium* on growth performance, lipid metabolism, and cecal microbiota of broiler chickens. *Appl. Microbiol. Biotechnol*. **97,** 6477-6488.

Zheng A., Luo J., Meng K., Li J., Zhang S., Li K., Liu G., Cai H., Bryden W.L. and Yao B. (2015). Proteome changes underpin improved meat quality and yield of chickens (*Gallus gallus*) fed the probiotic *Enterococcus faecium*. *BMC Genomics.* **15,** 1167-1171.

Zheng A., Luo J., Meng K., Li J., Bryden W.L., Chang W., Zhang S., Wang L.X.N., Liu G. and Yao B. (2016). Probiotic (*Enterococcus faecium*) induced responses of the hepatic proteome improves metabolic efficiency of broiler chickens (*Gallus gallus*). *BMC Genomics.* **17,** 89-97.