

ISSN 1682-8356  
ansinet.org/ijps



INTERNATIONAL JOURNAL OF  
**POULTRY SCIENCE**

**ANSI***net*

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan  
Mob: +92 300 3008585, Fax: +92 41 8815544  
E-mail: editorijps@gmail.com

## Selected Blood Biochemical and Haematological Parameters in Turkeys after an Experimental Probiotic *Enterococcus faecium* M-74 Strain Administration

Marcela Capcarova, A. Kolesarova, P. Massanyi and J. Kovacic  
Department of Animal Physiology, Slovak University of Agriculture in Nitra,  
Tr. A. Hlinku 2, SK-949 76, Slovak Republic

**Abstract:** In this study selected biochemical (cholesterol, total lipids, triglycerides, calcium, inorganic phosphorus) and haematological parameters (erythrocytes and leucocytes count, haematocrit) in blood of turkeys, BIG 6 breed, after probiotic strain *Enterococcus faecium* M-74 administration added to the feed mixture and drinking water during 12 weeks of feeding were analyzed. Animals were divided into three groups: control and two experimental groups P1 and P2. Preparation with *Enterococcus faecium* M-74  $5 \cdot 10^9$  CFU (colonies forming units) in amount of 300g/1000kg (0.03%) to the feed mixture of P1 group was supplemented. Animals in group P2 received probiotic preparation from the drinking water (structured doses from  $2 \cdot 10^8$  to  $30 \cdot 10^8$  daily for animal according to age and live weight). The lowest concentration of total lipids in control group  $4.79 \pm 0.30$  mmol/L was found as compared with values  $4.91 \pm 0.34$  mmol/L in P1 group and  $5.34 \pm 0.48$  mmol/L in the P2 group. The highest average concentration of triglycerides in the control group  $3.38 \pm 0.09$  mmol/L was observed. In the experimental groups the concentration of this parameter was significantly lower ( $2.74 \pm 0.10$  mmol/L in P1 group and  $2.75 \pm 0.21$  mmol/L in P2 group). The lowest average concentration of cholesterol  $3.73 \pm 0.11$  mmol/L was detected in control group, followed by  $3.77 \pm 0.19$  mmol/L in P1 and  $3.78 \pm 0.29$  mmol/L in P2 group. The highest values of calcium and inorganic phosphorus were found in control group ( $2.44 \pm 0.03$  mmol/L and  $1.81 \pm 0.09$  mmol/L). Slightly higher values without significant differences in both experimental groups were found. In the case of haematological parameters any significant differences were found in erythrocytes and leucocytes count and haematocrit.

**Key words:** *Enterococcus faecium*, blood biochemistry, haematology, turkeys

### INTRODUCTION

The intestinal microbiota, epithelium and immune system provide resistance to enteric pathogens. Recent data suggest that resistance is not solely due to the sum of the components, but that cross-talk between these components is also involved in modulating this resistance. Inhibition of pathogens by the intestinal microbiota has been called bacterial antagonism, bacterial interference, barrier effect, colonization resistance, and competitive exclusion (Patterson and Burkholder, 2003).

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Reid *et al.*, 2003). According to Shane (2008) probiotics are bacteria and yeast that have a beneficial effect on the maintenance of health. Probiotic microorganisms are inherently present in fermented food products and according to Kamiya *et al.* (2008) they are live microbial feed supplement which beneficially affects the host animals by improving its microbial balance. Therapy with probiotics is an effort to reduce or eliminate potential pathogens and toxins, to release nutrients, antioxidants, growth factors and coagulation factors, to stimulate gut motility and to modulate innate and adaptive immune defense mechanisms via the normalization of altered gut flora

(Singhi and Baranwal, 2008). Mechanisms by which probiotic bacteria affect the microecology of the gastrointestinal tract are not well understood, but at least three mechanisms of action have been proposed: production/presence of antibacterial substances (e.g., bacteriocins or colicins), modulation of immune responses and specific competition for adhesion receptors to intestinal epithelium. The rapid establishment of bacterial communities has been thought to be essential for the prevention of colonization by pathogenic bacteria (Nava *et al.*, 2005). Probiotics exhibit strain-specific differences in their resistance to acid and bile, ability to colonize the gastrointestinal tract, clinical efficacy and benefits to the health of the host (Pham *et al.*, 2008). It is becoming increasingly accepted by consumers that live lactic acid bacteria do exert health benefits when eaten. In addition, it is also becoming recognized that not all probiotic bacteria are equal (Dekker *et al.*, 2007). The application of living bacteria as probiotics in food or food supplements requires a careful safety assessment (Wassenaar and Klein, 2008). Results of Yurong *et al.* (2005) suggested that probiotics enhance intestinal mucosal immunity of chicken at the early age. Carina *et al.* (2000) concluded that *Enterococcus faecium* J96 can protect newly hatched chicks from *Salmonella Pullorum* infection.

Vahjen *et al.* (2002) described that *Enterococcus faecium* stimulates other lactic acid bacteria in the small intestine, especially lactobacilli. After repeated cycles of exposure of *Lactobacillus delbrueckii* in urea-rich medium under anaerobic environment, the organisms were demonstrated to lower plasma urea concentration *in vitro* (Chow *et al.*, 2003). Positive effect of probiotics in turkeys was published by many authors (Johannsen *et al.*, 2004; Cetin *et al.*, 2005; Torres-Rodriguez *et al.*, 2007).

The aim of this study was to analyze selected blood biochemical and haematological parameters (cholesterol, total lipids, triglycerides, calcium, inorganic phosphorus, erythrocytes and leucocytes count and hematocrite) of turkeys after probiotic strain *Enterococcus faecium* M-74 administration.

### MATERIALS AND METHODS

In this study the blood samples of turkeys, BIG 6 breed (n = 30), were analyzed in order to find the effect of probiotic preparations added to the feed mixture and drinking water during 12 weeks of feeding. Animals were divided into three groups with 10 turkeys in each group (control and two experimental groups P1 and P2). All groups were fed with feed mixture HYD 14 (NORM TYP). Experimental animals received probiotic preparation. Preparation with *Enterococcus faecium* M-74  $5.10^9$  colony producing units in amount of 300g/1000kg (0.03%) of the feed mixture was served to group P1. Groups P2 received probiotic preparation to drinking water (structured doses from  $2.10^8$ - $30.10^8$  daily for animal according to age and live weight).

After end of experiment blood was obtained from *vena basilica* by macromethod. The blood serum was separated from whole blood by centrifugation at 3000rpm for 30 minutes. The blood serum was used for evaluation of selected parameters of fat metabolism (cholesterol, total lipids, triglycerides) and mineral profile (calcium, inorganic phosphorus). Blood in the amount of 0.2ml was removed by micropipette to micro capillaries and placed to MPW-310 centrifuge for haematocrite measurement (erythrocytes and leucocytes count and hematocrit). Biochemical parameters (cholesterol, total lipids, triglycerides, calcium, inorganic phosphorus) were measured by semi-automated clinical chemistry analyzer Microlab 300 (Vilat Scientific, Dieren, The Netherlands) and haematological devise (erythrocytes and leucocytes count and hematocrite) PICOSCALE PS-4 (Capcarova *et al.*, 2008; Kolesarova *et al.*, 2008). Analysis of variance and T-test were used to calculate basic statistic characteristics and to determine significant differences between experimental and control groups.

### RESULTS

In this study selected parameters of metabolism after probiotic treatment were analyzed (Fig. 1-8). The average

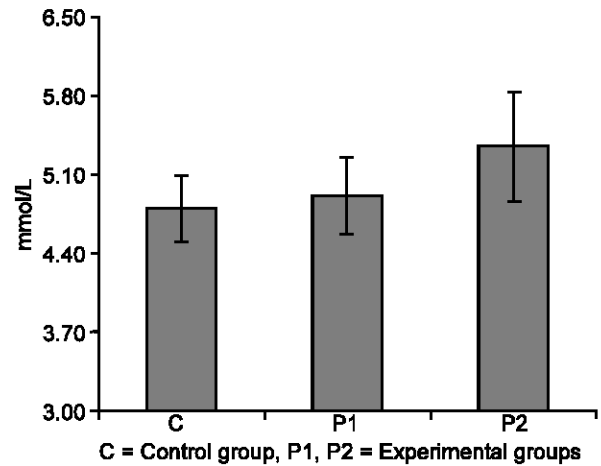


Fig. 1: Effect of *Enterococcus faecium* M-74 strain on the total lipids concentration of turkeys

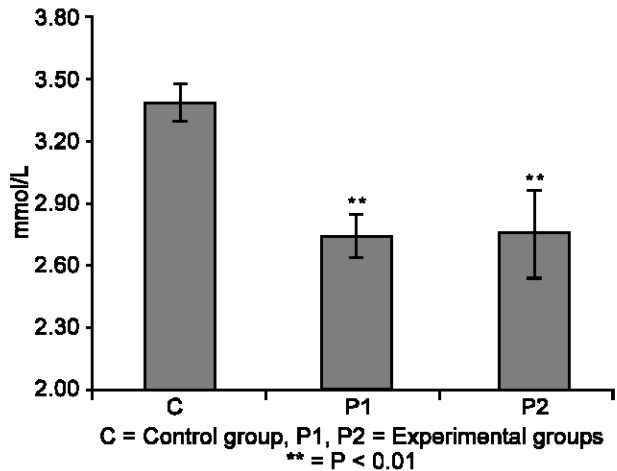


Fig. 2: Effect of *Enterococcus faecium* M-74 strain on the triglycerides concentration of turkeys

concentration of total lipids was the lowest in control group  $4.79 \pm 0.30$ mmol/L. In experimental groups it was  $4.91 \pm 0.34$ mmol/L in P1 group and the highest value in the P2 group  $5.34 \pm 0.48$ mmol/L. Increased tendency of this parameter in experimental groups with probiotic supplement in comparison with control group was detected, however, results were not significant ( $P > 0.05$ ). In the case of triglycerides the highest average concentration was found in the control group  $3.38 \pm 0.09$ mmol/L. In the experimental groups the concentration of this parameter was significantly lower ( $P < 0.01$ ) in comparison with control group ( $2.74 \pm 0.10$ mmol/L in P1 group and  $2.75 \pm 0.21$ mmol/L in P2 group). In the control group the lowest average concentration of cholesterol  $3.73 \pm 0.11$ mmol/L was found. Insignificantly higher concentrations were in P1 and P2 groups ( $3.77 \pm 0.19$ mmol/L and  $3.78 \pm 0.29$ mmol/L). Results did not confirm the effect of

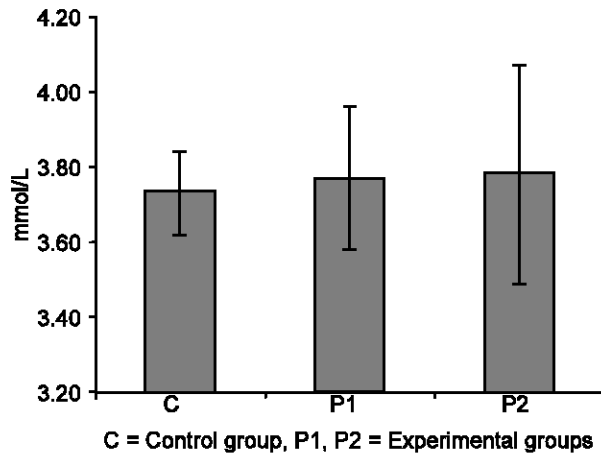


Fig. 3: Effect of *Enterococcus faecium* M-74 strain on the cholesterol concentration of turkeys

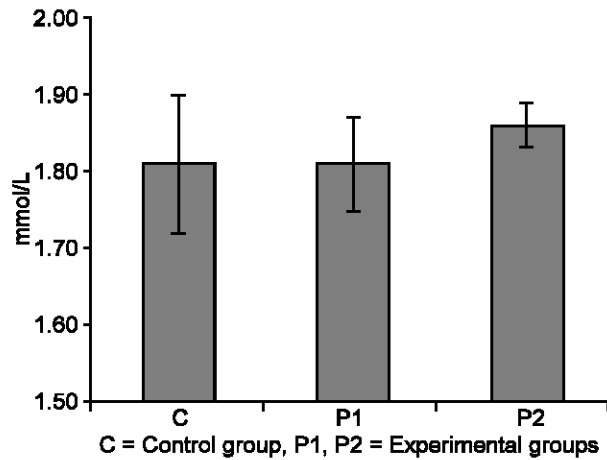


Fig. 5: Effect of *Enterococcus faecium* M-74 strain on the phosphorus concentration of turkeys

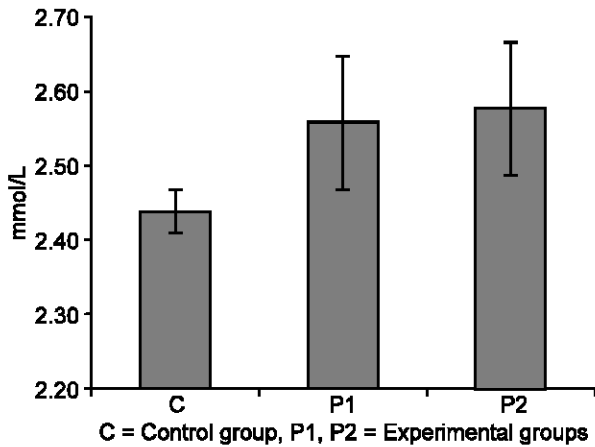


Fig. 4: Effect of *Enterococcus faecium* M-74 strain on the calcium concentration of turkeys

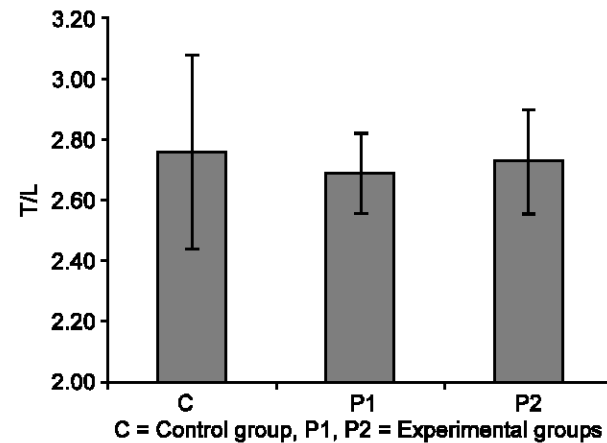


Fig. 6: Effect of *Enterococcus faecium* M-74 strain on the erythrocyte count of turkeys

probiotic preparation on the level of cholesterol in blood serum of turkeys ( $P > 0.05$ ).

Besides fat metabolism also some mineral parameters in turkey blood were observed. The lowest average concentration of calcium in blood serum was found in control group  $2.44 \pm 0.03$  mmol/L, followed by  $2.56 \pm 0.09$  mmol/L in P1 group and the highest value ( $2.58 \pm 0.09$  mmol/L) was found in P2 group. Any significant differences ( $P > 0.05$ ) were found among the groups. The highest average concentration of inorganic phosphorus was measured in P2 group with probiotic supplement to the drinking water ( $1.86 \pm 0.03$  mmol/L). Other values were  $1.81 \pm 0.06$  mmol/L in P1 group and  $1.81 \pm 0.09$  mmol/L in control group.

The effect of probiotic preparation was observed also in relation to haematological parameters as erythrocytes and leucocytes count and haematocrit. Decrease of average erythrocytes count in groups with probiotic

addition ( $2.69 \pm 0.13$  T/L and  $2.73 \pm 0.17$  T/L) versus control group ( $2.76 \pm 0.32$  T/L) was found. Results were not significant ( $P > 0.05$ ). A similar result was obtained for leucocytes count. The highest average count of white blood cells was in control group  $28.46 \pm 0.59$  G/L. In experimental group number of white blood cells decreased ( $27.36 \pm 0.30$  G/L in P1 group and  $27.28 \pm 0.24$  G/L in P2 group). The differences were not significant ( $P > 0.05$ ). The average values of haematocrit were very similarly in all groups. In control group- $38.6 \pm 2.30\%$ , in P1 group- $38.60 \pm 1.67\%$  and in P2 group- $37.60 \pm 2.30\%$ . Any significant differences ( $P > 0.05$ ) among the groups of turkey were detected suggesting any health alterations.

Generally, the results of this study describe significantly decreased concentration of triglycerides in turkeys after probiotic administration and for other analyzed blood parameters only a tendencies were detected.

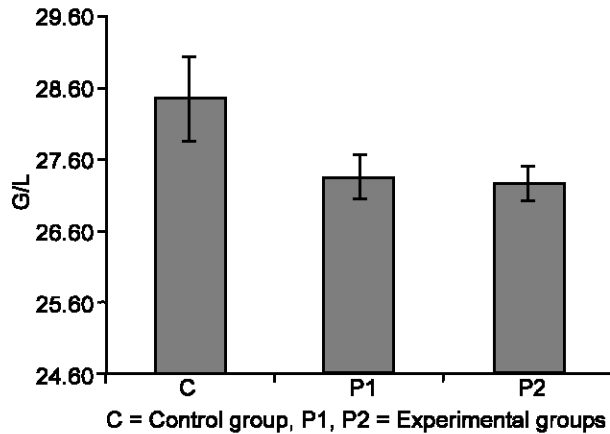


Fig. 7: Effect of *Enterococcus faecium* M-74 strain on the leucocyte count of turkeys

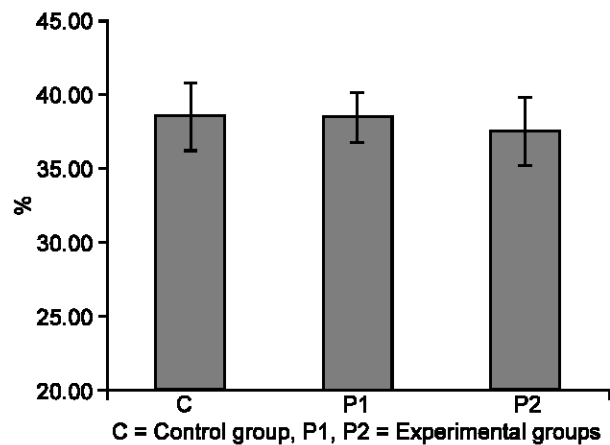


Fig. 8: Effect of *Enterococcus faecium* M-74 strain on the haematocrit value of turkeys

## DISCUSSION

Bacterial species that have traditionally been regarded as safe are used in probiotics. The main strains used include lactic acid bacteria and bifidobacteria that inhibit the intestinal tracts of human and animals (Holzapfel *et al.*, 2001; Ishibashi and Yamazaki, 2001). There are indications that probiotic intervention may lead to changes in serum global lipid profiles (Kekkonen *et al.*, 2008). In our study an increase of concentration of total lipids in experimental groups with probiotic supplement in comparison with control group was found. According to Umeki *et al.* (2004) the concentrations of serum total lipids, triacylglycerol, total cholesterol and phospholipids were significantly reduced in rats fed with probiotic strain *Lactobacillus rhamnosus*. We found significantly ( $P < 0.01$ ) lower average concentration of triglycerides in the experimental groups versus control group. In the control group the lowest average concentration of cholesterol in

comparison with experimental group is reported. The differences were not significant. Findings of Liang and Shah (2005) suggest that strains of lactobacilli could remove cholesterol via various mechanisms and may be promising candidates for use as a dietary adjunct to lower serum cholesterol *in vivo*. Pereira and Gibson (2002) published that mechanistically, probiotic bacteria ferment food-derived indigestible carbohydrates to produce short-chain fatty acids in the gut, which can then cause a decrease in the systemic levels of blood lipids by inhibiting hepatic cholesterol synthesis and/or redistributing cholesterol from plasma to the liver. Furthermore, some bacteria may interfere with cholesterol absorption from the gut by deconjugating bile salts and therefore affecting the metabolism of cholesterol, or by directly assimilating cholesterol. The results of Salma *et al.* (2007) revealed that the supplementation of *Rhodobacter capsulatus* in diet reduced cholesterol and triglyceride concentrations in broiler meat. In addition, the concentrations of serum cholesterol and triglyceride and hepatic cholesterol and triglyceride were also reduced by dietary *Rhodobacter capsulatus*. Compared with the control diet, *Rhodobacter capsulatus* supplemented diet reduced the ratio of low-density lipoprotein-cholesterol to high-density lipoprotein-cholesterol. Human serum samples showed increase of triacylglycerols in the probiotic group during the intervention (Kekkonen *et al.*, 2008). In the study of Hlivak *et al.* (2005) the administration of *Enterococcus faecium* M-74 probiotic strain was associated with reduction of serum cholesterol concentration by 12% after 56 weeks.

In the case of some mineral parameters the lowest average concentration of calcium in blood serum was found in control group in comparison with P1 and P2 group with probiotic strain supplement, however, results were not significant. The highest average concentration of inorganic phosphorus was detected in P2 group with probiotic supplement to the drinking water. Other values were insignificantly lower. According to Panda *et al.* (2003) the addition of probiotic significantly increased serum calcium and reduced the concentrations of cholesterol in the serum and yolk of layers. Serum phosphorus was not influenced by supplementation with probiotic used in this study. As found by Stropfova *et al.* (2006) the concentration of calcium was significantly higher after application of strain *Enterococcus faecium*. On the other hand, cholesterol was significantly lower in the *Enterococcus faecium* group of animals. The findings of Gilman and Gashman (2006) suggested that bacteria can enhance intestinal calcium uptake, if not calcium transport.

Our findings showed insignificant decrease of average erythrocytes count in groups with probiotic addition versus control group. A similar result was in the case of leucocytes count. The highest average count of white

blood cells was in control group. In experimental group both counts were insignificantly lower. The average value of haematocrit was in similar range, without significant differences in all groups. Cetin *et al.* (2005) observed in turkey that the probiotic supplementation caused statistically significant increases the erythrocyte count, haemoglobin concentration and haematocrit values. Total leukocyte and differential leukocyte counts were not affected by probiotic supplementation. The concentrations of haemoglobin, haematocrit, red blood cell count and index of phagocytic activity of leukocytes were significantly higher after application of strain *Enterococcus faecium* (Strompfova *et al.*, 2006). The intestinal bacterium *Enterococcus faecium* NCIMB 10415 (*E. faecium* SF68) has been used for more than a decade as a probiotic strain in animal nutrition as well as in the prevention and treatment of diarrhoea in humans (Macha *et al.*, 2004).

**Conclusion:** In conclusion, selected biochemical (cholesterol, total lipids, triglycerides, (calcium, inorganic phosphorus) and haematological (erythrocytes and leucocytes count and hematocrite) parameters were analyzed. In our study significant decreases of triglycerides was determined after probiotic administration in turkeys. Other findings were not significant. Some positive effects of probiotic supplement on health state in turkeys are described.

#### ACKNOWLEDGMENT

We would like to express our gratitude to Ing. Peter Cupka for technical assistance. This study was supported by Grant Agency of Slovak University of Agriculture in Nitra 727/05400.

#### REFERENCES

Capcarova, M., A. Kolesarova, H. Arpasova, P. Massanyi, N. Lukac, J. Kovacik, A. Kalafova and M. Schneidgenova, 2008. Blood biochemical dynamics and correlations in laying hens after experimental nickel administration. *Int. J. Poult. Sci.*, 7: 538-547.

Carina Audisio, M., G. Oliver and M.C. Apella, 2000. Protective effect of *Enterococcus faecium* J. 96, a potential probiotic strain, on chicks infected with *Salmonella Pullorum*. *J. Food Prot.*, 63: 1333-7.

Cetin, N., B.K. Güçlü and E. Cetin, 2005. The effects of probiotic and mannanoligosaccharide on some haematological and immunological parameters in turkeys. *J. Vet. Med. A Physiol. Pathol. Clin. Med.*, 52: 263-7.

Chow, K.M., Z.C. Liu, S. Prakash and T.M. Chang, 2003. Free and microencapsulated *Lactobacillus* and effects of metabolic induction on urea removal. *Artif. Cells Blood Substit. Immobil. Biotech.*, 31: 425-34.

Dekker, J., M. Collett, J. Prasad and P. Gopal, 2007. Functionality of probiotics-potential for product development. *Forum Nutr.*, 60:196-208.

Gilman, J. and K.D. Cashman, 2006. The effect of probiotic bacteria on transepithelial calcium transport and calcium uptake in human intestinal-like Caco-2 cells. *Curr. Issues Intest. Microbiol.*, 7: 1-5.

Hlivak, P., J. Odraska, M. Ferencik, L. Ebringer, E. Jahnova and Z. Mikes, 2005. One-year application of probiotic strain *Enterococcus faecium* M-74 decreases serum cholesterol levels. *Bratisl. Lek. Listy*, 106: 67-72.

Holzappel, W.H., P. Haberer, R. Geisen, J. Björkroth and U. Schillinger, 2001. Taxonomy and important features of probiotic microorganisms in food and nutrition. *Am. J. Clin. Nutr.*, 73: 365-373.

Ishibashi, N. and S. Yamazaki, 2001. Probiotics and safety. *Am. J. Clin. Nutr.*, 73: 465-470.

Johannsen, S.A., R.W. Griffith, I.V. Wesley and C.G. Scanes, 2004. *Salmonella enterica serovar typhimurium* colonization of the crop in the domestic turkey: influence of probiotic and prebiotic treatment (*Lactobacillus acidophilus* and lactose). *Avian. Dis.*, 48: 279-86.

Kamiya, T., M. Shikano, T. Wada, M. Sasaki and T. Joh, 2008. The efficacy of probiotics in gastrointestinal disease. *Nippon. Rinsho.*, 66: 1385-90.

Kekkonen, R.A., M. Sysi-Aho, T. Seppanen-Laakso, I. Julkunen, H. Vapaatalo, M. Oresic and R. Korpela, 2008. Effect of probiotic *Lactobacillus rhamnosus* GG intervention on global serum lipidomic profiles in healthy adults. *World. J. Gastroenterol.*, 14: 3188-94.

Kolesarova, A., J. Slamecka, R. Jurcik, F. Tataruch, N. Lukac, J. Kovacik, M. Capcarova, M. Valent and P. Massanyi, 2008. Environmental levels of cadmium, lead and mercury in brown hares and their relation to blood metabolic parameters. *J. Environ. Sci. Health*, A43: 646-650.

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

Macha, M., D. Taras, W. Vahjen, A. Arini and O. Simon, 2004. Specific enumeration of the probiotic strain. *Arch. Anim. Nutr.*, 58: 443-52.

Nava, G.M., L.R. Bielke, T.R. Callaway and M.P. Castañeda, 2005. Probiotic alternatives to reduce gastrointestinal infections: the poultry experience. *Anim. Health Res. Rev.*, 6: 105-18.

Panda, A.K., M.R. Reddy, S.V. Rama Rao and N.K. Praharaj, 2003. Production performance, serum/yolk cholesterol and immune competence of white leghorn layers as influenced by dietary supplementation with probiotic. *Trop. Anim. Health. Prod.*, 35: 85-94.

Patterson, J.A. and K.M. Burkholder, 2003. Application of prebiotics and probiotics in poultry production. *Poult. Sci.*, 82: 627-31.

- Pereira, D.I. and G.R. Gibson, 2002. Effects of consumption of probiotics and prebiotics on serum lipid levels in humans. *Crit. Rev. Biochem. Mol. Biol.*, 37: 259-81.
- Pham, M., D.A. Lemberg and A.S. Day, 2008. Probiotics: sorting the evidence from the myths. *Med. J. Aust.*, 188: 304-8.
- Reid, G., J. Jass, M.T. Sebulsy and J.K. McCormick, 2003. Potential uses of probiotics in clinical practice. *Clin. Microbiol. Rev.*, 16: 658-72.
- Salma, U., A.G. Miah, T. Maki, M. Nishimura and H. Tsujii, 2007. Effect of dietary *Rhodobacter capsulatus* on cholesterol concentration and fatty acid composition in broiler meat. *Poult. Sci.*, 86: 1920-6.
- Shane, A.L., 2008. Applications of probiotics for neonatal enteric diseases. *J. Perinat. Neonatal. Nurs.*, 22: 238-43.
- Singhi, S.C. and A. Baranwal, 2008. Probiotic use in the critically ill. *Ind. J. Pediatr.*, 75: 621-7.
- Strompfová, V., M. Marcináková, M. Simonová, S. Gancarcíková, Z. Jonecová, L. Sciranková, J. Koscová, V. Buleca, K. Cobanová and A. Lauková, 2006. *Enterococcus faecium* EK13-an enterocin a-producing strain with probiotic character and its effect in piglets. *Anaerobe*, 12: 242-8.
- Torres-Rodriguez, A., A.M. Donoghue, D.J. Donoghue, J.T. Barton, G. Tellez and B.M. Hargis, 2007. Performance and condemnation rate analysis of commercial turkey flocks treated with a *Lactobacillus* spp.-based probiotic. *Poult. Sci.*, 86: 444-6.
- Umeki, M., K. Oue, S. Mochizuki, Y. Shirai and K. Sakai, 2004. Effect of *Lactobacillus rhamnosus* KY-3 and cellobiose as synbiotics on lipid metabolism in rats. *J. Nutr. Sci. Vitaminol.*, 50: 330-4.
- Vah jen, W., A. Jadamus and O. Simon, 2002. Influence of a probiotic *Enterococcus faecium* strain on selected bacterial groups in the small intestine of growing turkey poults. *Arch. Tierernahr.*, 56: 419-29.
- Wassenaar, T.M. and G. Klein, 2008. Safety aspects and implications of regulation of probiotic bacteria in food and food supplements. *J. Food. Prot.*, 71: 1734-41.
- Yurong, Y., S. Ruiping, Z. Shimin and J. Yibao, 2005. Effect of probiotics on intestinal mucosal immunity and ultrastructure of cecal tonsils of chickens. *Arch. Anim. Nutr.*, 59: 237-46.