HAL
open science

Effect of Enterococcus faecium M-74 strain on selected blood and production parameters of laying hens

Marcela Capcarova

To cite this version:

Marcela Capcarova. Effect of Enterococcus faecium M-74 strain on selected blood and production parameters of laying hens. British Poultry Science, 2010, 51 (05), pp.614-620. 10.1080/00071668.2010.513961 . hal-00638521

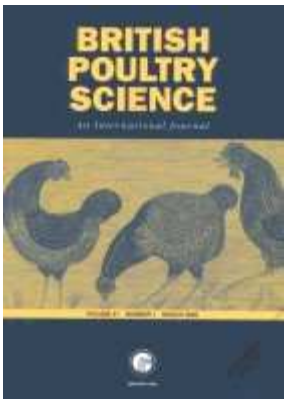
HAL Id: hal-00638521

https://hal.science/hal-00638521v1

Submitted on 5 Nov 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Effect of Enterococcus faecium M-74 strain on selected blood and production parameters of laying hens

Journal:	*British Poultry Science*
Manuscript ID:	CBPS-2009-128.R2
Manuscript Type:	Original Manuscript
Date Submitted by the Author:	11-Feb-2010
Complete List of Authors:	Capcarova, Marcela; Slovak Agricultural University, Animal Physiology
Keywords:	Biochemistry, Fats and fatty acids, Haematology, Laying hens, Probiotics

For Peer Review Only

SCHOLARONE™
Manuscripts

British Poultry Science
CBPS-2009-128
Edited Lewis July 2010, MacLeod August 2010

Effects of *Enterococcus faecium* M 74 strain on selected blood and production parameters of laying hens

M. CAPCAROVA, L. CHMELNICNA[1], A. KOLESAROVA, P. MASSANYI AND J. KOVACIK

Department of Animal Physiology, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, SK – 949 76, Slovak Republic [1]Department of Poultry Science and Small Animal Husbandry, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, SK – 949 76, Slovak Republic

Running title: Probiotics in poultry nutrition

Correspondence to: M. Capcarova, Department of Animal Physiology, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, SK – 949 76, Slovak Republic.
E-mail: marcela.capcarova@uniag.sk

Accepted for publication: 17th March 2010

For Peer Review Only

Abstract: 1. The aim of the present study was to evaluate the functional efficiency of a probiotic strain, *Enterococcus faecium* M 74, in the feed on selected biochemical, haematological and production parameters in ISA Brown hens.

2. Feed in the experimental group was enriched with a probiotic preparation containing of $5x10^9$ viable *Enterococcus* faecium M 74 per g. Blood samples were collected during the egg laying period at 5 (w5), 25 (w25) and 45 (w45) weeks of production. Body weight, rate of lay and egg weight were recorded every 4 weeks during the 48-week laying period.

3. Significantly lower concentrations of total cholesterol and total lipids in blood plasma were observed in the experimental group at all sampling times compared with their respective controls. Concentrations of triglycerides did not differ. Significantly lower concentrations of plasma calcium were found in the experimental group at w5 and w45. Concentrations of inorganic phosphorus in the experimental group were significantly higher at w25 but significantly lower at w45. Erythrocyte count was significantly higher in the experimental group at w25 and w45 when compared with controls. Leucocyte counts were significantly lower in the experimental group at all sampling times. Significantly lower values of haematocrit at w5 and w45 were observed in the experimental group than controls. Body weight, number of eggs and average egg weight were not significantly affected by probiotic addition.

4. In conclusion, the addition of probiotic strain *Enterococcus faecium* M 74 to the feed of ISA Brown hens reduced cholesterol, lipids, calcium, leucocyte counts and haematocrit values in blood plasma in at least two sampling times, while erythrocyte counts were increased. No significant effects of probiotic on triglyceride concentration and egg production parameters were observed.

INTRODUCTION

The European Union has banned the use of antibiotic feed additives due to the negative effect

For Peer Review Only

on human health (Immerseel *et al.*, 2004); antibiotic resistance is among the greatest threats to public health (Finch and Sharland, 2009). Probiotics show promising effects as alternatives to antibiotics as pressure to eliminate growth-promoting antibiotic use increases (Awad *et al.*, 2009).

The use of probiotic cultures in the poultry industry has recently become more common (Torres-Rodriguez *et al.*, 2007). Probiotics are available as single microbial strains (*e.g.*, *Bacillus clausii, Lactobacillus*) or as a mix of multiple strains of *Lactobacillus* (*acidophilus, sporogenes, lactis, reuteri, plantarum), Bifidobacterium (bifidum*, *longum, infantis*), *Streptococcus (thermophillus, lactis, fecalis), Saccharomyces boulardii* etc. (Singhi and Baranwal, 2008). They have potential health benefits for conditions such as gastrointestinal infections, genitourinary infections, allergies and certain bowel disorders, all of which afflict a considerable proportion of the global population (Senok *et al.*, 2005). Research over the last century has shown that lactic acid bacteria and certain other micro-organisms can increase resistance to disease and that lactic acid bacteria can be enriched in the intestinal tract by feeding specific carbohydrates (Patterson and Burkholder, 2003).

A single dose of *Lactobacillus* administered intragastrically improved weight gain and food conversion efficiency (Khan *et al*., 2007; Liu *et al*., 2007; Zulkifli *et al*., 2000), had a growth-promoting effect in broiler chickens (Cavazzoni *et al.*, 1998) and modulating effects on immune system of layer- and meat-type chickens (Koenen *et al.*, 2004). The application of *Rhodobacter capsulatus* into diet may be a feasible way to reduce cholesterol concentration and improve the ratio of unsaturated fatty acids to saturated fatty acids in broiler meat (Salma *et al.*, 2007*a*). After *Bacillus subtilis* treatment, egg production, feed consumption and feed conversion efficiency in layers (Li *et al.*, 2006) and growth performance of goslings (Wu *et al.*, 2008) was improved.

Probiotic beneficial action in human and animals was also confirmed by Balevi *et al.*

For Peer Review Only

(2001), Juntunen *et al.* (2001), Wielen *et al.* (2002), Glück and Gebbers (2003), Weese and Arroyo (2003), Gil de los Santos *et al.* (2005), Capcarova *et al.* (2008), Siggers *et al.* (2008), Al-Salami *et al.* (2008) and others.

Based on the data from literature and the trends of research in the field of probiotics, the aim of the present study was to evaluate the functional efficiency of a probiotic strain *Enterococcus faecium* M 74 in the feed on selected biochemical, haematological and production parameters of laying hens.

MATERIAL AND METHODS

Birds, management and diets

Forty ISA Brown laying hens were housed at 20 weeks of age in individual cages with a space allowance of 573 cm^2 and given diet KKZ HYD 10 (Table 1). Feed and water were provided *ad libitum*. Twenty birds acted as controls and 20 were given the experimental diet. Blood collections were made at 5, 25 and 45 weeks of a 48-week laying period. The experimental group of hens received a probiotic preparation with concentration of $5x10^9$ living organisms of *Enterococcus faecium* M 74 in 1 g. Probiotic preparation (Probiotics International Ltd., UK) was in powder form for ease of incorporation into the diet, and added to the feed at the rate of 0.5 g/1 kg to produce colony forming units (CFU) of $2.5x10^9$ in 1 kg of finished feed.

Table 1 near here

Measurements

Blood was obtained from *vena basilica* and samples collected into EDTA treated tubes. A 0.2-ml blood sample was removed by micropipette to micro-capillaries for haematological analyses and placed in a MPW-310 centrifuge for haematocrit measurement. The blood plasma was separated from whole blood by centrifugation at 3000 *g* for 30 mins. Biochemical parameters of mineral profile (calcium and inorganic phosphorus iP), energy profile (total cholesterol, total lipids, triglycerides) and haematological parameters (erythrocytes and

For Peer Review Only

leucocytes counts) were analysed using a semi–automated clinical chemistry analyser Microlab 300 (Vilat Scientific, Dieren, The Netherlands) and Bürker chamber after dilution with Natt-Herich solution. Haematocrit was determined by the micro-haematocrit centrifuge method.

Body weight (BW), rate of lay and egg weight were determined. The birds were weighed during whole laying period at 4-weekly intervals. The average egg weight was the mean of all eggs produced during the 48-week laying period. Mortality was recorded daily.

Statistical analysis

SAS software was used to conduct statistical analyses. Analysis of variance and *t*-test were used to calculate basic statistic characteristics and to determine significant differences between experimental and control groups. The data presented concerning the biochemical and haematological effects of probiotic strain are means of values obtained at three separate sampling ages.

RESULTS

Blood biochemical parameters

Clinical examination showed no changes in the health status of the control and experimental groups. The effects of probiotic strain *Enteroccocus faecium* on selected biochemical parameters are shown in Table 2.

Table 2 near here

The concentration of total cholesterol in blood plasma was significantly lower in the experimental group with probiotic supplementation compared with controls at all sampling times (week 5, $P < 0.05$; weeks 25 and week 45, $P < 0.01$).

Significantly lower mean concentrations of total lipids were found at all sampling ages in comparison with controls. The lowest lipid concentration was found at week 45 in the experimental group and the highest concentration at week 5 in the control group. Mean concentrations of triglycerides were not significantly different throughout the study and

For Peer Review Only

ranged from 12.51 mM to 13.06 mM in both groups.

Mean concentrations of plasma calcium were lower in the experimental group than controls at all three sampling times, but the difference was only significant at weeks 5 and 45. Mean concentrations of inorganic phosphorus were in a narrow range from 1.81mM to 1.92 mM in both groups, but that of the experimental group was significantly higher than controls after 25 weeks of production but significantly lower at week 45.

Haematological parameters

Erythrocyte counts at week 5 were similar for the two groups, significantly higher at week 25 in the experimental group compared with the control group, but significantly lower than controls at week 45 (Table 3). Mean leucocytes counts were significantly lower at all sampling ages in the experimental group compared with controls (P <0.001). The haematocrit values fell within narrow limits in both the experimental and control groups, but the mean haematocrit values in the experimental group were significantly lower than controls at weeks 5 and 45.

Table 3 near here

Production parameters

Results of production parameters are shown in Table 4. Although the BW of birds given the probiotic strain treatment was higher than controls at all times, the differences between groups were not statistically significant. *Enterococcus faecium* strain had slight beneficial effect on egg production, with the experimental group averaging 308 eggs in the 48 weeks compared with 285 eggs for the control group (data not shown). The average egg weight was also higher in the experimental group than the controls, but again the difference was not statistically significant. Only one bird died during whole experimental period, but a post-necropsy investigation confirmed that the treatment had no negative effect on the health and internal organs of the experimental hens. No other treatment-related sickness was found.

Table 4 near here

DISCUSSION

For Peer Review Only

In the present experiment, the probiotic strain *Enterococcus faecium* M 74 significantly decreased concentrations of total cholesterol as well as total lipid in blood plasma. Since a large proportion of the cholesterol in the body is synthesised *de novo*, mostly in the liver, the search for feed additives to inhibit cholesterol biosynthesis has long been pursued as a means of lowering the concentrations of plasma cholesterol (Regnstrom *et al.*, 1992). Regulation of the serum cholesterol level is important to prevent atherosclerosis, as it has been shown that atherosclerosis could be suppressed by controlling the concentration of cholesterol in the serum (Kapila *et al.*, 2009).

Lower concentrations of cholesterol in yolk and blood in connection with probiotic strains supplementation have been described by Panda *et al.* (2003), Kurtoglu *et al.* (2004) Li *et al.* (2006) and Salma *et al.* (2007*b*). Reduced blood plasma cholesterol concentrations effected by *Lactobacillus acidophilus* were shown in some studies, and cholesterol decrease was detected to be transient (de Roos and Katan, 2000). The mechanism of that action is still not clear, though Buck and Gilliland (1994) and Pereira and Gibson (2002) suggested that *Lactobacillus* was able to assimilate the cholesterol molecules. In a similar study, de Smet *et al.* (1994) suggested that probiotic bacteria increase the synthesis of unconjugated bile acids, thus contributing to the regulation of serum cholesterol, which is the precursor of bile acids. The result of increased synthesis of these acids reduces the concentration of cholesterol. After adding probiotics to the diet, the cholesterol concentrations in the liver and blood serum were significantly lower in cockerels (Endo *et al.*, 1999) and in rats (Hosono, 2000, 2001).

According to Kurtoglu *et al.* (2004), supplementation of 250, 500 and 750 mg/kg probiotic increased egg production, reduced the number of cracked eggs, and decreased the egg yolk and serum cholesterol concentrations. Serum triglyceride concentrations were reduced by diet supplementation with 500 and 750 mg/kg probiotic. There are indications that probiotic supplementation of the diet may lead to changes in serum global lipid profiles, as

For Peer Review Only

reflected in increased triglyceride levels (Kekkonen *et al.*, 2008). The results of Xiao *et al.* (2003) showed the potential of the probiotic *Bacterium longum* strain to improve the plasma lipid panel in rats when added to diet. The hypocholesterolaemic effect of *Lactobacillus* strains is attributed to its ability to reduce serum and liver total cholesterol concentrations, as was demonstrated in the hamster (Chiu *et al.*, 2006). However, results from Greany *et al.* (2008) do not support a beneficial effect of *Lactobacillus acidophilus* strain DDS-1 or *Bifidobacterium longum* strain UABL-14 on plasma lipids in young women and men who have a normal cholesterol concentration. Simon *et al.* (2006) suggested that *Lactobacillus fermentum* did not appear to produce a major change in serum lipid fractions in humans, but a small effect could not be excluded.

A lower mean concentration of plasma calcium was found at all weeks in the experimental group in comparison with the control group. These findings are in contrast with work of Panda *et al.* (2003), who reported significantly higher serum calcium levels following probiotic supplementation. Mean concentrations of iP in our experiment were within narrow limits in both groups of birds. No clear effect of probiotic supplementation on iP in blood plasma was observed and results seem to be inconclusive. Similarly, Panda *et al.* (2003), could not confirm an effect of probiotic supplementation on the concentration of plasma inorganic P.

In another report, Panda *et al.* (2006) confirmed that addition of a probiotic strain (*Lactobacilu sporogenes)* had a positive effect on bone breaking strength and bone ash content and could be attributed to the favourable environment in intestinal tract created by the feeding of a probiotic strain. This probiotic supplementation may have helped to digest and absorb more calcium and subsequently support bone metabolism resulting in a higher bone density and strength.

Tang *et al.* (2007) showed that fermenting calcium-fortified soya milk with selected

For Peer Review Only

probiotics can potentially enhance the calcium bioavailability of calcium-fortified soya milk due to an increase in calcium solubility and bioactive isoflavone aglycone enrichment.

Cetin *at al.* (2005) concluded that probiotic supplementation resulted in significant increases in the erythrocyte count, haemoglobin concentration and haematocrit values, but had no effect on total leucocytes and differential leucocytes counts. In contrast, the current study only showed significantly higher erythrocytes counts in the experimental group at week 25. We found significantly lower leucocyte counts in all observed weeks, which is in contrast to the findings of Cetin *at al.* (2005) and Zhou *at al.* (2000), who reported no significant effect of probiotics on leucocyte count.

The current data show significantly lower mean haematocrit values in hens with probiotic administration, which concurs with the report of Agawane and Lonkar (2004), who found a significant decrease in the haemoglobin and packed cell volume in birds with probiotic treatment.

Production parameters were not influenced by probiotic supplementation during our experiment, which is in agreement with the findings of Balevi *et al.* (2001) that egg weight was not influenced by probiotic addition. Kurtoglu *et al.* (2004) and Yörük *et al.* (2004) reported increased egg production, reduced mortality, and improved feed conversion efficiency with probiotic supplementation but with no improvement in egg quality. Irshad (2006) found that poultry growth was promoted with gradually increasing doses of probiotics, up to 1 g per 10 kg feed. However, doses of probiotics higher than that may have had reversed effect on poultry growth.

In conclusion, the addition of probiotic strain *Enterococcus faecium* M 74 to the diet of ISA Brown hens lowered cholesterol, lipids, calcium, leucocyte counts and haematocrit, and increased erythrocyte counts, in blood plasma in at least two sampling times. The effect of probiotic on iP was inconclusive and no effect was observed on triglyceride concentrations or

For Peer Review Only

egg production parameters.

ACKNOWLEDGMENTS

The authors are grateful to Jenifer Poston B.S. and Ida Holaskova PhD for linguistic revision of this paper.

REFERENCES

AGAWANE, S.B. & LONKAR, P.S. (2004) Effect of probiotic containing *Saccharomyces boulardii* on experimental ochratoxicosis in broilers: hematobiochemical studies. *Journal of Veterinary Science*, **4**: 359-67.

AL-SALAMI, H, BUTT, G. TUCKER, I. & MIKOV, M. (2008) Influence of the semisynthetic bile acid MKC on the ileal permeation of gliclazide *in vitro* in healthy and diabetic rats treated with probiotics. *Methods and Finding*, **2**: 107-13.

AWAD, W.A., GHAREEB, K., ABDEL-RAHEEM, S. & BÖHM, J. (2009) Effects of dietary inclusion of probiotic and symbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. *Poultry Science*, **88**: 49-56.

BALEVI, T., UCAN, U.S., COSKUN, B., KURTOGLU, V. & CETINGUL, I.S. (2001) Effect of dietary probiotic on performance and humoral immune response in layer hens. *British Poultry Science*, **4**: 456-61.

BUCK, L.M. & GILLILAND, S.E. (1994) Comparison of freshly isolated strains of *Lactobacillus acidophilus* of human intestinal origin for ability to assimilate cholesterol during growth. *Journal of Dairy Science*, **77:** 2925-33.

CAPCAROVA, M., KOLESAROVA, A., MASSANYI, P. & KOVACIK, J. (2008) Selected blood biochemical and haematological parameters in turkeys after experimental probiotic *Enterococcus faecium* M 74 strain administration. *International Journal of Poultry Science,* **7:** 1194-1199.

CAVAZZONI, V., ADAMI, A. & CASTROVILLI, C. (1998) Performance of broiler

For Peer Review Only

chickens supplemented with *Bacillus coagulans* as probiotic. *British Poultry Science*, **39**: 526-9.

CETIN, N., GÜÇLÜ, B.K & CETIN, E. (2005) The effects of probiotic and mannanoligosaccharide on some haematological and immunological parameters in turkeys. *Journal of Veterinary Medicine, A,* **6**: 263-7.

CHIU, C.H., LU, T.Y., TSENG, Y.Y. & PAN, T.M. (2006) The effects of Lactobacillus-fermented milk on lipid metabolism in hamsters fed on high-cholesterol diet. *Applied Microbiology and Biotechnology*, **2**: 238-45.

DE ROOS, N.M. & KATAN, M.B. (2000) Effects of probiotic bacteria on diarrhoea, lipid metabolism, and carcinogenesis: a review of papers published between 1988 and 1998. *American Journal of Clinical Nutrition*, **2**: 405-11.

DE SMET, I., VAN HOORDE, L., DE SAEYER, M., VAN de WOESLYNE, M. & 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. *Microbial ecology in health and disease*, **7:** 315-29**.**

ENDO, T., NAKANO, M., SHIMIZU, S., FUKUSHIMA, M. & MIYOSHI, S. (1999) Effects of a probiotic on the lipid metabolism of cocks fed on a cholesterol-enriched diet. *Bioscience, Biotechnology and Biochemistry*, **9**: 1569-75.

FINCH, R. & SHARLAND, M. (2009) November and beyond: observations on the EU Antibiotic Awareness Day. *Journal of Antimicrobial Chemotherapy,* **63**: 633-5.

FOOKS, L.J. & GIBSON, G.R. (2002) Probiotics as modulators of the gut flora. *British Journal of Nutrition*, **1**: 39-49.

GIL DE LOS SANTOS, J.R., STORCH, O.B. & GIL-TURNES, C. (2005) *Bacillus cereus var. toyoii* and *Saccharomyces boulardii* increased feed efficiency in broilers infected with *Salmonella enteritidis*. *British Poultry Science*, **46:** 494-7.

For Peer Review Only

GLÜCK, U. & GEBBERS, O. (2003) Ingested probiotics reduce nasal colonization with pathogenic bacteria *(Staphylococcus aureus, Streptococcus pneumoniae,* and β-hemolytic streptococci). *American Journal of Clinical Nutrition*, **77**: 517-20.

GREANY, K.A., BONORDEN, M.J., HAMILTON-REEVES, J.M., MCMULLEN, M.H., WANGEN, K.E, PHIPPS, W.R., FEIRTAG, J., THOMAS, W. & KURZER, M.S. (2008) Probiotic capsules do not lower plasma lipids in young women and men. *European Journal of Clinical Nutrition*, **2**: 232-7.

HOSONO, U. A. (2000) Effect of administration of *Lactobacillus gasseri* on serum lipids and faecal steroids in hypercholesterolemic rats. *Journal of Dairy Science*, **8**: 1705-11.

HOSONO, U. A. (2001) Hypocholesterolemic effect of *Lactobacillus gasseri* SBT0270 in rats fed a cholesterol-enriched diet. *Journal of Dairy Research*, **4**: 617-24.

IMMERSEEL, F.V., DE BUCK, J., PASMANS, F., HUYGHEBAERT, G., HAESEBROUCK, F. & DUCATELLE, R. (2004) *Clostridium perfringens* in poultry: an emerging threat for animal and public health. *Avian Pathology*, **33**: 537-49.

IRSHAD, A. (2006) Effect of probiotics on broilers performance. *International Journal of Poultry Science,* **5**: 593-7.

JUNTUNEN, M., KIRJAVAINEN, P.V., OUWEHAND, A.C., SALMINEN, S.J. & ISOLAURI, E. (2001) Adherence of probiotic bacteria to human intestinal mucus in healthy infants and during rotavirus infection. *Clinical and Diagnostic Immunology*, **2**: 293-6.

KAPILA, S., VIBHA, P. & SINHA, R. (2009) Antioxidative and hypocholesterolemic effect of *Lactobacillus casei* ssp. *Casei* (biodefensive properties of lactobacilli). *Indian Journal of Medical Science,* **60**: 361-370.

KEKKONEN, R.A, SYSI-AHO, M., SEPPANEN-LAAKSO, T., JULKUNEN, I., VAPAATALO, H., ORESIC, M. & KORPELA, R. (2008) Effect of probiotic

For Peer Review Only

Lactobacillus rhamnosus GG intervention on global serum lipidomic profiles in healthy adults. *World Journal of Gastroenterology*, **20**: 3188-94.

KHAN, M., RAOULT, D., RICHET, H., LEPIDI, H. & LA SCOLA, B. (2007) Growth-promoting effects of single-dose intragastrically administered probiotics in chickens. *British Poultry Science*, **6**: 732-5.

KOENEN, M.E., KRAMER, J., VAN DER HULST, R., HERES, L., JEURISSEN, S.H. & BOERSMA, W.J. (2004) Immunomodulation by probiotic lactobacilli in layer- and meat-type chickens. *British Poultry Science*, **45**: 355-66.

KURTOGLU, V, KURTOGLU, F., SEKER, E., COSKUN, B., BALEVI, T. & POLAT, E.S. (2004) Effect of probiotic supplementation on laying hen diets on yield performance and serum and egg yolk cholesterol. *Food Additives & Contaminants*, **9**: 817-23.

LI, L., XU, C.L., JI, C., MA, Q., HAO, K., JIN, Z.Y. & LI, K. (2006) Effects of a dried *Bacillus subtilis* culture on egg quality. *Poultry Science*, **2**: 364-8.

LIU, J.R., LAI, S.F., YU, B. (2007) Evaluation of an intestinal *Lactobacillus reuteri* strain expressing rumen fungal xylanase as a probiotic for broiler chicken fed on a wheat-based diet. *British Poultry Science*, **48**: 507-14.

PANDA, A.K., REDDY, M.R., RAMA RAO, S.V & PRAHARAJ, N.K. (2003) Production performance, serum/yolk cholesterol and immune competence of white leghorn layers as influenced by dietary supplementation with probiotic. *Tropical Animal Health and Production*, **1**: 85-94.

PANDA, A.K., RAMARAO, S.V., RAJU, M.V.L.N. & SHARMA, S.R. (2006) Dietary supplementation of *Lactobacillus sporogenes* on performance and serum biochemico-lipid profile of broiler chickens. *Journal of Poultry Science*, **43**: 235-240.

PATTERSON, J.A. & BURKHOLDER, K.M. (2003) Application of prebiotics and probiotics in poultry production. *Poultry Science*, **4**: 627-31.

For Peer Review Only

PEREIRA, D.I.A. & GIBSON, G.R. (2002) Cholesterol assimilation by lactic acid bacteria and Bifidobacteria isolated from the human gut. *Applied and Environmental Microbiology*, **68:** 4689-96.

REGNSTROM, J., NILLSON, J., TORNVALL, P., LANDOU, C. & HAMSTEN, A. (1992) Susceptibility to low-density lipoprotein oxidation and coronary atherosclerosis in man. *Lancet,* **339**: 1183-1186.

SALMA, U., MIAH, A.G., MAKI, T., NISHIMURA, M. & TSUJII, H. (2007a) Effect of dietary *Rhodobacter capsulatus* on cholesterol concentration and fatty acid composition in broiler meat. *Poultry Science*, **9**: 1920-26.

SALMA, U., MIAH, A.G., TAREQ, K.M., MAKI, T. & TSUJII, H. (2007b) Effect of dietary *Rhodobacter capsulatus* on egg-yolk cholesterol and laying hen performance. *Poultry Science*, **4**: 714-9.

SENOK, A.C., ISMAEEL, A.Y. & BOTTA, G.A. (2005) Probiotics: facts and myths. *Clinical Microbiology and Infection*, **12**: 958-66.

SIGGERS, R.H., SIGGERS, J., BOYE, M., THYMANN, T., MØLBAK, L., LESER, T., JENSEN, B.B. & SANGILD, P.T. (2008) Early administration of probiotics alters bacterial colonization and limits diet-induced gut dysfunction and severity of necrotizing enterocolitis in preterm pigs. *Journal of Nutrition*, **8**: 1437-44.

SIMONS, L.A., AMANSEC, S.G. & CONWAY, P. (2006) Effect of *Lactobacillus fermentum* on serum lipids in subjects with elevated serum cholesterol. *Nutrition, Metabolism & Cardiovascular Diseases*, **8**: 531-5.

SINGHI, S.C. & BARANWAL, A. (2008) Probiotic use in the critically ill. *Indian Journal of Pediatrics*, **6**: 621-7.

TANG, A.L., SHAH, N.P., WILCOX, G., WALKER, K.Z. & STOJANOVSKA, L. (2007) Fermentation of calcium-fortified soymilk with *Lactobacillus*: effects on calcium

For Peer Review Only

solubility, isoflavone conversion, and production of organic acids. *Journal of Food Sciences*, **9**: 431-6.

TORRES-RODRIGUEZ, A., DONOGHUE, A.M., DONOGHUE, D.J., BARTON, J.T., TELLEZ, G. & HARGIS, B.M. (2007) Performance and condemnation rate analysis of commercial turkey flocks treated with a *Lactobacillus* spp.-based probiotic. *Poultry Science*, **3**: 444-6.

WEESE, J. S. & ARROYO, L. (2003) Bacteriological evaluation of dog and cat diets that claim to contain probiotics. *Canadian Veterinary*, **3**: 212-6.

WIELEN, P., LIPMAN, L.J., KNAPEN, F. & BIESTERVELD, S. (2002) Competitive exclusion of *Salmonella enterica* serovar enteritidis by *Lactobacillus crispatus* and *Clostridium lactatifermentans* in a sequencing fed-batch culture. *Applied and Environmental Microbiology*, **2**: 555-9.

WU, L.Y., TAN, R.B. & SHI, K.J. (2008) Effect of a dried *Bacillus subtilis* culture on gosling growth performance. *British Poultry Science*, **49**: 418-22.

XIAO, J.Z., KONDO, S., TAKAHASHI, N., MIYAJI, K., OSHIDA, K., HIRAMATSU, A., IWATSUKI, K., KOKUBO, S. & HOSONO, A. (2003) Effects of milk products fermented by *Bifidobacterium longum* on blood lipids in rats and healthy adult male volunteers. *Journal of Dairy Science*, **7**: 2452-61.

YÖRÜK, M.A., GÜL, M., HAYIRLI, A. & MACIT, M. (2004) The effects of supplementation of humate and probiotic on egg production and quality parameters during the late laying period in hens. *Poultry Science*, **1**: 84-8.

ZHOU, J.S., SHU. Q., RUTHERFURD, K.J. PRASAD, J., BIRTLES, M.J., GOPAL, P.K. & GILL, H.S. (2000) Safety assessment of potential lactic acid bacterial strains *Lactobacillus rhamnosus* HN001, *Lb. acidophilus* HN017, and *Bifidobacterium lactis* HN019 in BALB/c mice. *International Journal of Food Microbiology,* **56**: 87-96.

For Peer Review Only

ZULKIFLI, I., ABDULLAH, N., AZRIN, N.M. & 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. *British Poultry Science*, **41:** 593-7.

For Peer Review Only

Table 1. *Diet composition of feed formulation KKZ HYD-10*

Ingredients	KKZ HYD-10
Crude protein	min. 153 g/kg
ME	min. 11.5 MJ/kg
Ash	max. 160 g/kg
Fibre	max. 60 g/kg
Lysine	min. 7 g/kg
Methionine and cystine	min. 6 g/kg
- of which methionine	min. 3.5 g/kg
Linoleic acid	min. 15 g/kg
Calcium	min. 28 - 45 g/kg
Phosphorus	min. 5 g/kg
Sodium	1.2 – 2.5 g/kg
Manganese	min. 40 mg/kg
Iron	min. 40 mg/kg
Copper	min. 4 mg/kg
Zinc	min. 60 mg/kg
Retinol	min. 5333 mg/kg
Riboflavin	min. 4 mg/kg
Cyanocobalamin	min. 0.01 mg/kg
Cholecalciferol	min. 1066 mg/kg
Vitamin E (α-tocopherol)	min. 10 mg/kg

For Peer Review Only

***Table 2**. Effects of dietary treatment (Enterococcus faecium M 74) on some blood biochemical parameters of ISA Brown laying hens*

		Control group			Experimental group		
		Sampling times (weeks of production)					
		5	25	45	5	25	45
cholesterol	mean	3.96	3.53	3.17	3.80*	2.92**	2.63**
mM	SD	0.27	0.30	0.52	0.31	0.53	0.50
total lipids	mean	11.05	10.29	10.20	10.71*	10.11*	9.80*
g/L	SD	0.32	0.45	0.37	0.24	0.28	0.26
triglycerides	mean	12.64	12.97	12.91	12.51	12.92	13.06
mM	SD	0.25	0.46	0.66	0.45	0.18	0.44
calcium	mean	8.28	7.94	8.12	7.88*	7.88	7.93*
mM	SD	0.11	0.47	0.09	0.27	0.60	0.16
phosphorus	mean	1.86	1.81	1.92	1.85	1.85*	1.88*
mM	SD	0.06	0.05	0.05	0.05	0.08	0.07

Significantly different from the control values of the same week (* $P < 0.05$; ** $P < 0.01$)

For Peer Review Only

Table 3. *Effects of dietary treatment (Enterococcus faecium M 74) on some blood haematological parameters of ISA Brown laying hens*

		Control group			Experimental group		
		Sampling times (weeks of production)					
		5	25	45	5	25	45
erythrocytes T/l	mean	3.39	3.04	3.06	3.40	3.08*	3.03*
	SD	0.08	0.05	0.07	0.06	0.05	0.10
leucocytes G/l	mean	24.49	24.38	24.43	21.25***	20.60***	20.71***
	SD	0.54	0.15	0.13	0.61	0.11	0.06
hematocrit %	mean	33.85	32.44	33.43	33.09*	32.48	32.72*
	SD	0.52	0.39	0.49	0.39	0.34	0.53

Significantly different from the control values of the same week (* P <0.05; ** P <0.01)

For Peer Review Only

Table 4. *Effect of dietary treatment (Enterococcus faecium M 74) on body weight, rate of lay and egg weight of ISA Brown hens*

	Weeks of production											
	4	8	12	16	20	24	28	32	36	40	44	48
Body weight (g)												
C	1656	1702	1773	1749	1727	1735	1771	1781	1830	1825	1889	1907
SD	135	152	151	147	164	161	169	196	178	202	201	198
E	1702	1759	1803	1816	1787	1792	1834	1870	1887	1894	1941	1958
SD	142	124	121	125	155	165	152	173	192	199	210	215
Rate of lay (eggs/100 bird.d)												
C	91	93	95	95	92	96	95	92	92	87	85	80
SD	9.8	11.2	5.8	6.6	9.0	5.6	5.9	8.8	8.1	10.1	11.1	17.7
E	93	95	94	98	93	97	95	93	90	83	85	84
SD	8.1	6.6	7.9	4.0	7.8	4.8	6.7	9.4	10.2	17.2	14.8	15.4
Egg weight (g)												
C	51.2	57.5	62.1	63.3	63.5	62.7	62.9	63.6	63.4	62.9	61.9	63.5
SD	5.4	3.6	2.8	3.1	2.9	2.5	2.5	3.8	2.8	3.0	9.5	3.6
E	52.6	58.9	62.9	65.1	64.5	63.7	63.9	64.2	63.9	62.2	64.0	63.2
SD	4.1	4.4	3.9	3.7	3.7	3.3	2.9	4.1	4.0	6.5	4.0	4.2

No significant differences between groups (P >0.05)

For Peer Review Only