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# EFFICACY OF PROBIOTICS INTAKE ON INTERNAL MILIEU OF HENS

*Martin Mellen* [1], *Anna Kalafová* [2], *Henrieta Arpašová* [3], *Miroslava Kačániová* [4], *Marcela Capcarova**[2]

***Address(es):***
[1] Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Hygiene and Food Safety, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic.
[2] Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Animal Physiology, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic.
[3] Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Department of Poultry Science and Farm Animal Husbandry, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic.
[4] Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Microbiology, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic.

*Corresponding author: marcela.capcarova@uniag.sk

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**ABSTRACT**

The aim of present study was to evaluate the functional efficiency of probiotic preparation on selected blood biochemical parameters of ISA Brown hens. Feed in the experimental group of hens was enriched with a probiotic preparation in the dose of 500g.t$^{-1}$ consisted of freeze-dried cultures: *Lactobacillus bulgaricus LAT 187, L. acidophilus LAT 180, L. helveticus LAT 179, L. delbrueckii ssp. Lactis LAT 182, Streptococcus thermophiles LAT 205, Enterococcus faecium E-253* with concentration of 5.10$^{9}$ (CFU LAB) living organisms in 1 gram. Blood samples were collected in 25 and 48 week of hens' age. Biochemical parameters of mineral profile (calcium, phosphorus, magnesium, sodium, potassium, chlorides), energetic profile (plasma total cholesterol, triglycerides, total proteins, bilirubin, glucose), and activities of serum liver enzymes (aspartate aminotransferase AST, alanine aminotransferase ALT, alkaline phosphatase ALP) were analysed using Ecoline kits and a semi-automated clinical chemistry analyser Microlab 300 (Vilat Scientific, Dieren, The Nederland). Probiotic preparation reduced (P<0.05) serum cholesterol and triglycerides content. No significant effects of probiotic on remaining parameters were confirmed.

**Keywords:** Probiotics, laying hens, blood biochemistry, cholesterol

## INTRODUCTION

Microbial flora of the gastro intestinal tract plays an important role in the health and performance of the poultry **(Yu *et al.*, 1999).** Pathogenic microbial flora competes with the host for nutrients **(*Alp et al.*, 1999).** Probiotics are defined as organisms and substances which contribute to intestinal microbial balance **(Parker, 1974). Fuller (1989)** redefined probiotics as a live microbial feed supplement which benefits the host animal by improving its intestinal microbial balance. The definition given by **FAO (2009)** describes probiotics as live microorganisms, which when administered in adequate amounts, confer a health benefit on the host. Many strains can be used as probiotics; however some features must be followed: a) it should be a strain, which is capable of exerting a beneficial effect on the host animal (e.g. increase growth or resistance to disease), b) it should be non-pathogenic and non-toxic, c) it should be present as viable cells, preferably in large numbers, d) it should be capable of surviving and metabolizing in the gut environment, resistant to low pH and organic acids, and e) it should be stable and capable of remaining viable for periods under storage and field conditions **(Fuller, 1989; Enzema, 2013).** Probiotics can stimulate appetite; improve host intestinal microbial balance and intestinal environment for the processes of digestion and absorption of nutrients. They also inhibit the growth of certain pathogens that produce toxic compounds **(Patterson and Burkholder, 2003).** The beneficial effects of probiotics will depend on a number of factors, including the strain, level of duration and consumption, frequency of exposure, physiological condition of the individual **(Koop-Hoolihan, 2001).** The species currently being used in probiotic preparations varies widely, including *Bacillus, Bifidobacterium, Enterococcus, Lactobacillus, Lactococcus, Streptococcus*, a variety of yeast species and undefined mixed culture **(Kabir, 2009).** Supplementation of probiotics in a basal diet has been shown to be useful for ameliorating the adverse influence of stress **(Deng *et al.*, 2012)**, for inhibition of the adhesion of pathogenic bacteria to the intestinal wall and improvement of immune potency **(Balevi *et al.*, 2001).** Probiotics used in poultry breeding are designed for two reasons: to replace beneficial organisms that is not present in the alimentary tract and to provide the chicken with the effects of beneficial organisms. A number of different cultures and products have been tested in laying hens **(Enzema, 2013)**. Feeding a so called *Lactobacillus* complex to young Leghorn hens resulted in improvement in egg production, feed efficiency **(Krueger *et al.*, 1977; Aghaii *et al.*, 2010)**, decrease in mortality **(Yoruk *et al.*, 2004)**, improvement in egg size and quality **(Haddadin *et al.*, 1996)**. Supplementation of layers diets with *Saccharomyces cerevisiae* increased hen-day egg performance **(Enzema, 2013).** The improvement in productive performance of all poultry species fed with probiotics was mostly due to the fact that the probiotics promoted the metabolic processes of digestion and nutrient utilization. Experimental studies have shown that probiotic dietary supplementation might influence these mechanisms by exerting enzymatic activities, increasing the passage rate of digestion and deconjugating bile salts and acids **(Enzema, 2013)**. Probiotic beneficial action was confirmed in humans and animals by **Balevi *et al.* (2001), Siggerss *et al.* (2008); Capcarova *et al.* (2009; 2010 a,b,c; 2011), Ma *et al.* (2012)** and others.
The present study was conducted on laying hens to evaluate and determinate the effect of probiotic preparation on serum biochemical parameters.

## MATERIAL AND METHODS

### Birds, management and diet

The experiment was conducted on laying hens in age of 17 weeks, ISA Brown (n=12). Hens were fed with feed mixture HYD 10 (Table 1). Animals were housed in furnished cages; model AGK 200/616 according to European directive 1999/74 ES. Hens were healthy and their condition was judged as good at the commencement of the experiment. Conditions of animal care, manipulations and use corresponded with the instruction of ethical commission. Care and use of animals and experimental devices met the requirement of the certificate of Authorization to Experiment on Living Animals (State Veterinary and Food Institute of Slovak Republic, no. Ro 397/09-221/3a).
Space allowance for one animal was 943.2 cm$^2$. Feed and water were provided on an *ad libitum* basis. Hens were kept by standard bioclimatic conditions. The

temperature and humidity were controlled by automatic device HDL TRH-D/LP (Hivus s.r.o., Zilina, Slovak Republic).

Laying hens were divided into two groups, one control group (n=6) and one experimental group (n=6). The experimental period lasted 7.5 months. Experimental hens received a probiotic preparation in the feed mixture in the dose of 500 $g.t^{-1}$. Probiotic preparation (Prochema, Geschäftbereich Agro, Wien, Austria) consisted of freeze-dried cultures: *Lactobacillus bulgaricus LAT 187, L. acidophilus LAT 180, L. helveticus LAT 179, L. delbrueckii ssp. Lactis LAT 182, Streptococcus thermophiles LAT 205, Enterococcus faecium E-253* with concentration of $5.10^9$ (CFU LAB) living organisms in 1 gram.

**Table 1** Diet composition of feed mixture HYD-10

| Ingredients | KKZ HYD-10 |
|---|---|
| Crude protein | min. 153 g/kg |
| ME | min. 11.5 MJ/kg |
| Ash matter | max. 160 g/kg |
| Fiber | max. 60 g/kg |
| Lysine | min. 7 g/kg |
| Methionine and cistine | min. 6 g/kg |
| - from that 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 | min. 10 mg/kg |

**Measurements**

Blood was obtained from *vena basilica* and samples were collected into tubes for biochemical analysis. The blood collections were realized two times, in 25 and 48 week of hens' age. The blood serum was separated from whole blood by centrifugation at 3000 g for 30 minutes. Biochemical parameters of mineral profile (calcium, phosphorus, magnesium, sodium, potassium, chlorides), energetic profile (plasma total cholesterol, triglycerides, total proteins, bilirubin, glucose), and activities of serum liver enzymes (aspartate aminotransferase AST, alanine aminotransferase ALT, alkaline phosphatase ALP) were analysed using Ecoline kits and a semi-automated clinical chemistry analyser Microlab 300 (Vilat Scientific, Dieren, The Nederland) according to manufacturer instructions.

**Statistical Analyses**

Sigma Plot 9.0 (Jandel, Corte Madera, USA) was used to conduct statistical analyses. T-test was used to calculate basic statistic characteristics and to determine significant differences between the experimental and the control group. Data presented were given as mean and standard deviation (SD). Differences were compared for statistical significance at the level $P<0.05$.

**RESULTS AND DISCUSSION**

Microflora inhabiting the gastrointestinal tract of animals interacts with host animals and their populations vary with animal species, site along the gastrointestinal tract, age, diet, and environment **(Yeo and Kim, 1997).** Probiotics have a significant role in normalization of colonic physiologic function and barrier integrity of conjunctions of the cells with a reduction in mucosal pro-inflammatory cytokine levels **(Madsen *et al*., 2001).**

**Serum mineral parameters**

The effect of probiotic strains on selected serum mineral parameters are listed in Table 2 and 3. Analysis of the first and second blood collections revealed no significant differences ($P>0.05$) in measured parameters between the control and the experimental group. Similar results were obtained in previous studies with poultry **(Capcarova *et al*., 2010a, 2010c, 2011).**

**Table 2** Effect of probiotic strains on serum mineral parameters of hens of first blood collection

| Parameter ($mmol.l^{-1}$) | C | E |
|---|---|---|
| **Ca** | 5.957±0.863 | 5.917±0.432 |
| **P** | 1.803±0.145 | 1.713±0.074 |
| **Mg** | 1.157±0.140 | 1.175±0.124 |
| **Na** | 151.6±2.4 | 152.3±3.2 |
| **K** | 4.120±0.458 | 4.027±0.485 |
| **Cl** | 114.9±2.1 | 115.2±2.8 |

Ca – calcium, P – phosphorus, Mg – magnesium, Na – sodium, K – potassium, Cl - chlorides C - control group (without probiotic strains supplement); E - experimental group with probiotic strains addition, values shown as means ± SD

**Table 3** Effect of probiotic strains on serum mineral parameters of hens of second blood collection

| Parameter ($mmol.l^{-1}$) | C | E |
|---|---|---|
| **Ca** | 6.740±0.299 | 6.627±0.162 |
| **P** | 1.780±0.620 | 2.273±0.365 |
| **Mg** | 0.572±0.167 | 0.858±0.118 |
| **Na** | 156.7±1.2 | 157.7±2.3 |
| **K** | 3.573±0.140 | 3.413±0.399 |
| **Cl** | 122.9±2.7 | 120.3±1.8 |

Ca – calcium, P – phosphorus, Mg – magnesium, Na – sodium, K – potassium, Cl - chlorides C - control group (without probiotic strains supplement); E - experimental group with probiotic strains addition, values shown as means ± SD,

**Serum parameters of energy profile**

It is reported that probiotic supplementation can depress cholesterol concentration in poultry breeding **(Haddadin *et al*., 1996).** In this study probiotic preparation caused significant decrease ($P<0.05$) in cholesterol and triglycerides content in hens' blood in the first blood collection (Table 4) and also decrease in triglycerides content in the second blood collection (Table 5). Other parameters of energy profile of hens were not influenced by probiotic preparation ($P>0.05$). These results are in correspondence with previous studies with probiotics in hens **(Capcarova *et al*., 2010a),** turkeys **(Capcarova *et al*., 2008),** and chickens **(Capcarova *et al*., 2010c, 2011)**. Similarly, results of **Aghaii *et al*. (2010)** showed significant effect of probiotics on decrease of serum cholesterol and triglycerides in laying hens. The use of probiotic may active the lactic acid producing bacteria, production of enzymes disintegrating bile salts and de-conjugating them as well as reduction of the pH in the intestinal tract. These changes can be effective in reducing the cholesterol and triglycerides. Probiotics can also assimilate cholesterol and de-conjugated bile acids what can lead to reduction in serum cholesterol level **(Aghaii *et al*., 2010).** Lower concentration of cholesterol in blood in connection with probiotics has been described in various studies using various animals (**Panda *et al*., 2003; Hosono, 2001; Endo *et al*., 1999).**

**Table 4** Effect of probiotic strains on parameters of hens' energy profile of first blood collection

| Parameter | C | E |
|---|---|---|
| **TP ($g.l^{-1}$)** | 57.34±4.44 | 56.60±7.84 |
| **Glucose ($mmol.l^{-1}$)** | 11.25±1.73 | 9.50±2.13 |
| **Cholesterol ($mmol.l^{-1}$)** | $4.79±1.02^a$ | $3.35±0.86^b$ |
| **TAG ($mmol.l^{-1}$)** | $23.73±2.4^a$ | $15.64±2.89^b$ |
| **Bilirubin ($mmol.l^{-1}$)** | 16.25±4.02 | 14.04±5.89 |

TP – total proteins, TAG- triglycerides, C - control group (without probiotic strains supplement); E - experimental group with probiotic strains addition, values shown as means ± SD, a-b means significant difference between groups ($P<0.05$)

**Table 5** Effect of probiotic strains on parameters of hens' energy profile of second blood collection

| Parameter | C | E |
|---|---|---|
| **TP ($g.l^{-1}$)** | 65.12±10.17 | 62.50±2.03 |
| **Glucose ($mmol.l^{-1}$)** | 11.90±1.47 | 11.93±1.79 |
| **Cholesterol ($mmol.l^{-1}$)** | 4.68±0.69 | 4.40±0.51 |
| **TAG ($mmol.l^{-1}$)** | $15.06±2.9^a$ | $22.43±1.94^b$ |
| **Bilirubin ($mmol.l^{-1}$)** | 30.19±6.21 | 29.13±11.68 |

TP – total proteins, TAG- triglycerides, C - control group (without probiotic strains supplement); E experimental group with probiotic strains addition, values shown as means ± SD, a-b means significant difference between groups ($P<0.05$)

**Serum liver enzymes parameters**

In this study activity of serum liver enzymes were not affected by probiotic inclusion (Table 6 and 7). Statistical analysis showed no significant differences between the groups of hens ($P>0.05$), what was confirmed also in our previous studies with probiotics in poultry. Results of present study are in agreement with

studies on probiotics in poultry (**Capcarova *et al*., 2010c Capcarova *et al*., 2011).**

**Table 6** Effect of probiotic strains on serum liver enzymes of hens of first blood collection

| Parameter (μkat.l$^{-1}$) | C | E |
|---|---|---|
| **AST** | 2.57±0.30 | 2.54±0.44 |
| **ALT** | 0.14±0.04 | 0.10±0.03 |
| **ALP** | 12.30±4.10 | 14.18±3.05 |

AST - aspartate aminotransferase, ALT - alanine aminotransferase, ALP - alkaline phosphatase, C - control group (without probiotic strains supplement); E - experimental group with probiotic strains addition, values shown as means ± SD

**Table 7** Effect of probiotic strains on serum liver enzymes of hens of second blood collection

| Parameter (μkat.l$^{-1}$) | C | E |
|---|---|---|
| **AST** | 2.28±0.10 | 2.67±0.95 |
| **ALT** | 0.11±0.05 | 0.15±0.06 |
| **ALP** | 14.11±5.30 | 15.35±5.76 |

AST - aspartate aminotransferase, ALT - alanine aminotransferase, ALP - alkaline phosphatase, C - control group (without probiotic strains supplement); E - experimental group with probiotic strains addition, values shown as means ± SD

The improvement in metabolic processes after probiotic treatment in hens could be due to improved development of the gut and increased microvilli height which led to the enlargement of the microvilli's absorptive surface and enabled the optimal utilization of nutrients **(Enzema, 2013).**

## CONCLUSION

In the condition of present experiment, probiotic inclusion was effective in decreasing the hens' serum cholesterol and triglycerides content. However, further studies are needed to evaluate these effects.

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