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REGULAR ARTICLE

EFFECT OF PROBIOTIC SUPPLEMENTATION ON SELECTED INDICES OF ENERGY PROFILE AND ANTIOXIDANT STATUS OF CHICKENS

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ABSTRACT

The objective of the present study was to evaluate the effect of two probiotic strains *Lactobacillus fermentum* CCM 7158 and *Enterococcus faecium* M 74 given to the drinking water on the some parameters of energy profile (cholesterol, triglycerides, total protein, glucose) and antioxidant status (total antioxidant status – TAS, albumins, bilirubin) of female broiler chickens. The experiment was conducted on hybrid Hybro (n=180). The feeding period lasted 42 days. Experimental chickens of E1 group received a probiotic preparation in drinking water with concentration of 1.10^9 colony forming units (CFU) of *L. fermentum* CCM 7158 in 1 g of nutrient medium and experimental chickens of E2 group concentration of 2.10^9 CFU of *E. faecium* M-74 in 1 g of nutrient medium. The control group of animals received water without any additives. TAS in both probiotic groups was significantly increased. No significant differences were found in other parameters.

Keywords: probiotic, antioxidant status, albumin, bilirubin, broiler chickens

INTRODUCTION

Nowadays, after the antibiotics prohibition as growth stimulators in animal nutrition we tested and used probiotics as biologically active compounds or bee products (**Haščík et al., 2011 a,b; Pochop et al., 2011**).

Probiotics are defined as live microbial food supplements, which beneficially influence human **(Songisepp et al., 2005)** and animals health **(Carroll et al., 2007; Capcarova et al., 2011; Lee et al., 2008; Novakova et al., 2008; Gratz et al., 2010)**. They are nonpathogenic bacteria that can promote bird health by reducing pathogen colonization **(Stringfellow et al., 2011)**. Their efficiency was demonstrated for the treatment of gastrointestinal disorders, respiratory infections, and allergic symptoms. In most cases, evidence for a beneficial effect was obtained by studies using animal models **(Travers et al., 2011)**.

Nutrition plays a key role in maintaining the prooxidant-antioxidant balance **(Cowey 1986)**. Under physiological conditions the reactive species figure a crucial role in primary immune defense **(Diplock et al., 1998)**. But prolonged excess of reactive species is highly damaging for the host biomolecules and cells, resulting in dysbalance of the functional antioxidative network of the organism and leading to substantial escalation of pathological inflammation **(Petrof et al., 2004)**. Several studies reported the antioxidant activity of probiotic bacteria using assays in vitro **(Shen et al., 2011)**.

Lactic acid bacteria are evaluated as beneficial bacteria by their product of acids (lactic acid), bacteriocin-like substances or bacteriocins **(Strus et al., 2001)**. Widely accepted probiotics contain different lactic acid producing bacteria: bifidobacteria, lactobacilli or enterococci **(Mikalsaar and Zilmer 2009)**. For the chicken, the intestinal lactic acid bacteria are mainly *Lactobacillus* and *Enterococcus* **(Mitsuoka 2002)**.

Based on the data from literature the aim of the present study was to evaluate the effect of two probiotic strains *Lactobacillus fermentum* CCM 7158 and *Enterococcus faecium* M 74 given to the drinking water on some parameters of energy profile and antioxidant status of female broiler chickens.

MATERIAL AND METHODS

Experimental design and animal management

The experiment was conducted on female broiler chickens, hybrid Hybro (n=180). Animals were stabled in a 3-etage cage technology (MBD, Slovak Republic) consisted of 18 cages with proportions 75x50 cm – 0.375 m^2. Chickens were fed with complete feed mixture KKZ (Boskop, a.s., Trencin, Slovak Republic) as follows: KKZ HYD – 01 (powdery form) from Day1 till Day 21 of feeding and KKZ HYD – 02 (granular form) from Day 22 till Day 42 of feeding. Ingredients and nutrient composition of diets are shown in Table 1. Feed and water were provided on an *ad libitum* basis from containers on the front of the cages.

Animals were kept in thermoneutral hall (from Day1 33^{o}C until final 19^{o}C). In closed hall thermo aggregate was installed and experimental conditions with defined temperature and humidity were simulated by sensor. Simulated conditions were continually monitored using electronic recorder (Hivus s.r.o., Zilina, Slovak Republic).

Chickens were divided into three groups (control - C and experimental groups – E1 and E2). The feeding period lasted 42 days. Experimental chickens of E1 group received a probiotic preparation (IPC s.r.o., Kosice, Slovak Republic) in drinking water with concentration of 1.10^9 colony forming units (CFU) of *Lactobacillus fermentum* CCM 7158 in 1 g of nutrient medium with supporting components maltodextrin and oligofructose (1 % in preparation).

Experimental chickens of E2 group received a probiotic preparation (Probiotics International Ltd., UK) in drinking water with concentration of 2.10^9 CFU of *Enterococcus faecium* M 74 in 1 g of nutrient medium with dextrose (1 % in preparation). Quantization of drinking water and probiotic preparations are presented in Table 2. The control group of animals received water without any additives.

Table 1 Diet composition of feed mixture KKZ HYD-01 and KKZ HYD-02

Ingredients	Units	KKZ HYD - 01	KKZ HYD - 02
Crude protein	$g.kg^{-1}$	min. 210	min. 190
ME	$MJ.kg^{-1}$	min. 12	min. 12
Ash matter	$g.kg^{-1}$	max. 70	max. 70
Fiber	$g.kg^{-1}$	max. 35	max. 40
Lysine	$g.kg^{-1}$	min. 11	min. 9.5
Methionine and cistine	$g.kg^{-1}$	min. 7.5	min. 7.5
- from that methionine	$g.kg^{-1}$	min.4.5	min.4
Linoleic acid	$g.kg^{-1}$	min. 10	min. 10
Calcium	$g.kg^{-1}$	min. 8	min. 7
Phosphorus	$g.kg^{-1}$	min. 6	min. 5
Sodium	$g.kg^{-1}$	1.2 – 3	1.2 – 2.5
Manganese	$mg.kg^{-1}$	min. 50	min. 50
Iron	$mg.kg^{-1}$	min. 60	min. 60
Copper	$mg.kg^{-1}$	min. 6	min. 6
Zinc	$mg.kg^{-1}$	min.50	min.50
Vitamin A	$i.u.kg^{-1}$	min. 10000	min. 8000
Vitamin B_2	$mg.kg^{-1}$	min. 4	min. 3
Vitamin B_{12}	$\mu g.kg^{-1}$	min. 20	min. 20
Vitamin D_3	$i.u.kg^{-1}$	min. 1200	min. 1200
Vitamin E (α tokoferol)	$mg.kg^{-1}$	min. 15	min. 15

Chickens 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. SK PC 30008).

Table 2 Design of experimental intervention

Group of animals	Week of age	Total amount of drinking water per day (l)	Quantization of probiotic strain (g)	CFU in 1 ml of drinking water
E1 (n=60) *Lactobacillus fermentum* CCM 7158	1	2.50	6.60	$2.64x10^6$
	2	3.50	6.60	$1.90x10^6$
	3	4.60	3.70	$8.04x10^5$
	4	6.70	3.70	$5.52x10^5$
	5	8.60	3.70	$4.30x10^5$
	6	10.60	3.70	$3.49x10^5$
E2 (n=60) *Enterococcus faecium* M 74	1	2.50	5.04	$4.03x10^6$
	2	3.50	2.10	$1.2x10^6$
	3	4.60	2.10	$9.13x10^5$
	4	6.70	2.10	$6.27x10^5$
	5	8.60	2.10	$4.88x10^5$
	6	10.60	2.10	$3.96x10^5$

Legend: CFU - colony forming units

Blood sampling and analyses

After 42 days of feeding chickens were slaughtered and blood samples were obtained. The blood serum was separated from whole blood by centrifugation at 3000 g for 30 minutes. The following parameters in serum (total proteins, glucose, cholesterol, triglycerides) were determined using Ecoline kits and automatic analyzer Microlab 300 (Merck®, Germany) according to manufacturer conditions.

Total antioxidant status, albumin and bilirubin content of chicken blood was assayed by spectrophotometer Genesys 10 using antioxidant RANDOX kits (Randox Labs., Crumlin, UK) and spectrophotometer Genesys 10 (Thermo Fisher Scientific Inc, USA) according to the manufacturer's instructions.

Statistical analysis

SAS software and Sigma Plot 11.0 (Jandel, Corte Madera, USA) was used to conduct statistical analyses. One-way ANOVA test was used to calculate basic statistic characteristics

and to determine significant differences between experimental and the control groups. 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

There are a few sound data in the literature about the impact of probiotics on metabolic functions of the host **(Mikelsaar and Zilmer, 2009)**. As was previously published by **Agawane and Lonkar (2004)**, addition of probiotic to broiler feed resulted in significant improvement concerning hematobiochemical parameters. A large spectrum of indices measured in healthy adult volunteers or in animals studies showed that the use of *L. fermentum* and *E. faecium* strains were safe regarding the physiological values of principal markers of carbohydrates and lipids or lipid-like compounds (glucose, triglycerides, cholesterol), several metabolites (bilirubin) and several other biochemical indices such as blood calcium and iron **(Mitsuoka 2002; Mikelsaar and Zilmer 2009)**.

Energy profile of chicken's blood

The results of selected parameters of energy profile are shown in Table 3. In this study female chickens without probiotic supplement (C group) and those with probiotic administration (E1 and E2 groups) showed no significant differences ($P>0.05$) in the content of cholesterol, triglycerides, total protein and glucose in blood serum.

Table 3 Effect of probiotic strains addition on parameters of energy profile of chickens

Parameter	Units	C	E1	E2
Cholesterol	$mmol.^{-1}$	4.42±0.03	4.60±0.15	5.08±0.38
TG	$mmol.l^{-1}$	0.50±0.08	0.39±0.03	0.49±0.02
Total protein	$g.l^{-1}$	35.08±1.65	33.51±3.32	30.58±1.61
Glucose	$mmol.l^{-1}$	9.83±0.55	10.60±1.55	10.33±0.42

Legend: C – control group (without probiotic strains addition), E1 – experimental group (*Lactobacillus fermentum* CCM 7158), E2 – experimental group (*Enterococcus faecium* M74), TG - triglycerides, values shown are mean±SD (standard deviation), differences were not significant (P>0.05)

In our previous study dietary probiotics caused significant decrease of triglycerides content in blood of chickens **(Capcarova et al., 2010a; Capcarova et al., 2011)** and cholesterol content in hens **(Capcarova et al., 2010b)**. In this paper the triglycerides content decreased in both experimental groups when compared to the control group, but differences remained insignificant (P>0.05). The discrepancies are probably due to use of different strains, different kinds of subjects and length of treatment periods **(Jahreis et al., 2002)** and also probably due to various doses and concentrations of probiotic strains added to the host organism

Antioxidant status of chicken's blood

The results are presented in Table 4. According to our results TAS showed a significant (P<0.05) increase in both experimental groups (E1 and E2) with probiotic strains additions against the control group. No significant differences (P>0.05) in the bilirubin and albumin contents were detected between the control group and both probiotic groups (E1 and E2).

Table 4 Effect of probiotic strains addition on antioxidants parameters of chickens

Antioxidant parameter	Units	C	E1	E2
TAS	$mmol.^{-1}$	0.66 ± 0.03^{a}	0.82 ± 0.07^{b}	0.78 ± 0.01^{b}
Albumins	$g.l^{-1}$	13.55±0.08	12.32±1.11	12.49±0.97
Bilirubin	$mmol.l^{-1}$	3.46±0.57	4.10±0.77	4.82±1.02

Legend: C – control group (without probiotic strains addition), E1 – experimental group (*Lactobacillus fermentum* CCM 7158), E2 – experimental group (*Enterococcus faecium* M74), TAS – total antioxidant status, ^{a,b} Means with different superscripts within the same row differ significantly (P<0.05), Values shown are mean±SD (standard deviation)

There are several evidences in the literature reported that lactobacilli have antioxidant properties **(Kapila and Sinha, 2006; Mikelsaar and Zilmer, 2009)**. An increase of total antioxidant status was registered together with an improved bioquality of LDL particles of sera of volunteers after consumption of the symbiotic strains (*L. fermentum, L. paracasei, Bifidobacterium longum*) for 3 weeks **(Milkesaar and Zilmer, 2009)**. Results of this study confirmed our previous results **(Capcarova et al., 2010a, Capcarova et al., 2011)**.

Lactobacillus ssp. in a food product to humans comprising increases in TAS **(Songisepp et al., 2005; Mikelsaar et al., 2007)**. Strains and their lysates had physiologically relevant multivalent antioxidativity (TAS) to overcome the exogenous and endogenous oxidative stress of the host **(Mikelsaar and Zilmer, 2009)**.

CONCLUSION

In this paper the addition of two probiotic strains *L. fermentum* CCM 7158 and *E. faecium* M 74 to the drinking water for female broiler chickens resulted in some changes in internal milieu of animals. Antioxidant status of chickens after consumption of probiotic strains was significantly increased in both probiotic groups. The content of bilirubin, albumin, cholesterol, triglycerides, total protein and glucose did not differ among the groups. To our knowledge, there are few such similar studies concerning the effect of probiotic strains given in the drinking water and their effect on internal milieu, antioxidant status and production parameters of broiler chickens. Research on the field of probiotics will be worthy of further investigation.

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