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ASSESSMENT OF A SYNBIOTIC EFFECT ON BROILER PRODUCTIVE PERFORMANCE AND ANTIOXIDATIVE ENZYMES ACTIVITY

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ABSTRACT

The biological experiment was conducted in order to investigate the influence of synbiotic addition in broiler's nutrition on productive performance and antioxidative status. In the control treatment (T1) the broilers were fed with commercial mixtures of standard composition and quality. Experimental broilers were fed with the same mixtures supplemented with synbiotic (T2) or antibiotic salinomycin (T3). During the preparation period chickens received a starter mixture without additions, thereafter, chickens were fed with finisher mixtures according to the experimental design. At the end of the experiment (day 42) chickens in experimental treatments T2 and T3 achieved significantly ($p<0.05$) higher final body masses compared to the chickens in T1 treatment. Also, after completing the trial, significantly ($p<0.05$) lower feed conversion ratios were noticed in experimental treatments compared to treatment T1. Chicken mortality rate was the lowest in treatment T2 and the highest in treatment T1. The significantly lowest ($p<0.05$) serum malondialdehyde content was recorded in in treatment T2, followed by treatments T3 and T1, respectively, while the highest GSH content was in treatment T2. On the basis of obtained findings, it can be concluded that the addition of synbiotic in chicken's nutrition had positive impact on productive performance and antioxidative protection in broilers blood.

Key words: Antioxidative enzymes; Broiler chicken; Productive performance; Synbiotic.

INTRODUCTION

The wide use of antibiotics as feed additives led to concerns about development of antimicrobial resistance and about transference of antibiotic resistance genes from animal to human microbiota (Castanon, 2007). For this reason, approval for antibiotics use as growth promoters was withdrawn in the European Union since January 1, 2006. As a result of this prohibition, the search for alternative strategies regarding safe food is intensified. Alternatives like probiotics, yeast cultures, organic acids, prebiotics, enzymes, botanicals including extracts and essential oils of some herbs and spices were investigated in numerous studies (Langhout, 2000; Hertrapmf, 2001; Alavi *et al*., 2012; Puvača *et al.*, 2015).

Synbiotic present combination of probiotics and prebiotics, where specific substrate provide the survival of the probiotic organism since its is available for the fermentation (Mokhtari *et al*., 2010). Prebiotics, which are included in category of oligosaccharides, are one of the most important natural products which improve body immunity level, increase resistance against infectious diseases, increase calcium and absorption of magnesium, provide prevention from connecting and colonizing some of intestinal bacteria and increase nutrients absorption (Alavi *et al.,* 2012). On the other hand, microorganisms added as probiotic to broiler's diet, are usually microbes of digestive system, which create balance in population of intestinal bacteria and prevent organism from digestive infections, improve animals performance and increase growth of livestock and birds. Furthermore, probiotics, unlike antibiotics, remains no residues in livestock and birds and they do not create microbial resistance (Alavi *et al*., 2012). Use of these substances in broiler's diet provides health meat without drug residues.

Oxidation is a result of natural metabolic processes, but production of free radicals can adversely affect broiler performance and health since free radical react with proteins, lipids, or fat-soluble and form toxic products, but also decrease the nutrient content of the feed (Delles *et al*., 2014). Synbiotic can play an important role in antioxidative protection of cell and, therefore, prevents cell damage and improve the growth performance of broilers (Li *et al.*, 2012; Rezaei *et al*., 2015). MDA is the main final product of lipid peroxidation and has been often used as a biomarker for radical-induced damage and endogenous lipid peroxidation in urine, blood, and tissues. The most important antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). The SOD catalyzes the dismutation of the superoxide radical anion into hydrogen peroxide, afterwards CAT and GPx acts in conjuction with other enzymes to reduce H2O2 and to terminate lipid peroxidation (Halliwell and Chirico, 1993).

On the basis of previous considerations, the aim of this experiment was to investigate the influence of synbiotic as natural-antioxidant dietary supplements in

broiler chicken's nutrition on production performance and antioxidative enzymes activities.

MATERIALS AND METHODS

Chicken Housing and Nutrition: The biological experiment with chickens was performed on total of 750 day-old Cobb 500 hybrid broilers eqally distributed into three dietary treatments with five replicates each. Chickens were reared on floor holding system with chopped straw as litter material in amount of 3 kg/m^2. Chickens were provided with a light regime of 23 h per day during the entire experimental period of 42 days with incandescent light source (5 w/m^2). Heating of chickens was provided locally with infrared light heaters and the whole house was supplied with 2 thermometers linked to the heater ventilation controls. Chickens were watered through a nipple water system with 1 nipple/10–15 broilers. Microclimate conditions were regularly monitored. Feeding program included a two-phase diet as starter and finisher, respectively according to the experimantal design shown in Table 1. Dieatry treatments in the experiment were as follow: T1 (control diet), T2 (synbiotic treatment) and T3 (salinomycin treatment). Chemical composition and energy content of used starter and finisher mixtures is given in Table 2. During the whole trial, feed and drinking water were provided *ad libitum*. Body weight (BW) was monitored at an individual level during the entire experimental period every seven days, as well as the feed intake (FI) and feed conversion ratio (FCR) which were monitored at pen level. Mortality (M) was recorded daily.

Table 1. Experimental design.

Experimental treatments	Additive	Concentration of additives in chicken diets	
		Starter, g/kg 1 – 14 days	Finisher, g/kg 15 – 42 days
T1	Control	0.0	0.0
T2	Synbiotic	0.0	1
T3	Salinomycin	0.0	0.06

Table 2. Chemical composition and energy content of the used mixtures excluding the added additives.

Parameters	Starter 1-14 days	Finisher 15-42 days
Dry matter (%)	86.5	86.8
Crude fat (%)	3.0	4.0
Crude fiber (%)	4.0	5.0
Ash (%)	7.0	8.0
Crude protein (%)	22.0	20.5
Methionine+Cysteine (%)	0.671	0.714
Calcium (%)	0.774	0.783
Phosphorus (available) (%)	0.334	0.383
Lysine (%)	1.15	1.16
Threonine (%)	0.804	0.807
Arginine (%)	1.45	1.46
ME (MJ/kg)	12.5	12.8

**Additives were added on top of the basal diet*

Preparation of blood hemolysate: On day 42, two broilers from each pen (10 per treatment) were randomly selected and blood samples were collected from the wing vein into heparinized test tubes. Subsequently, plasma was separated by centrifugation (2000 g, 10 min) and removed, while remaining red blood cells (RBCs) were washed three times in isotonic NaCl (0.15 M), hereupon RBC hemolysates were prepared by diluting RBCs in ratio 1:10 with ice–cold double distilled H2O, shaken vigorously to force hemolysis, and stored at –70°C (Kostadinović, 1998).

Enzyme assay: The SOD (EC 1.15.1.1) activity has been assayed according to Kostadinovic *et al.* (2011). The GPx (EC 1.11.1.9) activity was determined by spectrophotometric measurement of absorbance at 412 nm with cumenhydroperoxide as the substrate (Chiu and Stults, 1976). Glutathione reductase (EC 1.6.4.2) activity was determined by following the rate of NADPH oxidation as measured by the decrease in the absorbance at 340 nm (Lukaszewicz-Hussain and Moniuszko-Jakoniuk, 2004). The CAT (EC 1.11.1.6.) activity was determined by hydrogen peroxide (H2O2) decomposition

rate, according to Ahn *et al.* (1998). One international unit (IU) was equivalent to one mmol H2O2 consumed/min/mg protein. The determination of POD (EC 1.11.1.7) activity was based on the catalytic oxidation of guaiacole according to Kostadinovic *et al*. (2011). Malondialdehyde concentration (MDA) in serum was measured by 2-thiobarbituric acid (TBA) method (Simmon *et al*., 1974). The glutathione (GSH) content in the blood hemolysate was determined using the method reported by Kostadinović *et al*. (2011). Spectrophotometric method in combination with commercial test ('Dialab', Vienna, Austria) was used for determination of hemoglobin level, necessary for the expression of the enzymatic activities in hemolysed blood.

Statistical analyses: The one way ANOVA analysis and Tukey's post hoc test were performed to assess data differences between groups using Statistica software version 12 (Statistica, 2013). The data means were considered different at $p<0.05$.

RESULTS AND DISCUSSION

Production performances: Significant influence of synbiotic on production performance of broiler chickens is shown in Table 3. During the finisher phase, diet supplementation with synbiotic exerted the stimulating effect and led to significant differences ($p<0.05$) in body weight in relation to treatments T1 and T3. However, significant ($p>0.05$) difference between treatments T1 and T3 was absent. It is assumed that the intestinal microbiota plays a major role in the normal nutritional, physiological, immunological, and protective functions of the host animals (Vispo and Karasov, 1997). The interaction between the probiotic strain and the intestinal microbiota may be based on aggregation with pathogenic bacteria, competitive adhesion to epithelial receptors, production of specific substances (organic acids, bacteriocins, dipicolinic acid), or competition for nutrients (Netherwood *et al*., 1999). On the other hand, second possible mechanism of probiotic action could be modifications of the structure and function of the intestinal epithelium. Thus, Caspary (1992) reported that increase in the villus height suggests an increased surface area capable of greater absorption of available nutrients. Recently, it was shown that addition of probiotic containing *Enterococcus faecium* microorganism to broiler diets increased the jejunal villus height (Chichowski *et al*., 2007) and ileal villus height (Samli *et al*., 2007). Subsequently, the investigation of Awad *et al.* (2008) showed changes in the mucosal architecture in terms of increased ileal villus height to crypts depth in birds fed with synbiotic supplemented diet. Samli *et al*. (2007) reported longer villi in the ileum of broilers with slight improvement in feed efficiency after dietary addition of *Enteroccocus faecium*. This finding is similar to the result reported by Aluwong *et al*. (2013), who claimed the supplementation with yeast probiotic improved growth rate of male broiler chickens. Furthermore, prebiotics which are non-digestible oligosaccharides may affect influence on microbial composition and/or activity, assisting in such a way a beneficial microflora that suppresses the growth of pathogens through different mechanisms.

After the completion of the finisher period, broilers in treatment T2 exhibited a higher feed intake (2876 g) compared to broilers in treatments T1 (2842 g) and T3 (2827 g). However, observing the whole experimental period, there was no significant differences ($p<0.05$) in feed intake between treatments. This observation could also be explained by improved digestion and absorption of nutrient in the digestive tract due to the presence of live bacteria cells of *Enterococcus faecium*.

Table 3. Productive performances of broiler chickens.

Exp. time	Parameter	T1	T2	T3	SEM
	Body weight (g)	1286^{b}	1351^{a}	1298^{b}	19
15-42 d	Feed intake (g)	2842^{c}	2876^{b}	2827^{a}	101
	Feed conversion ratio (kg/kg)	2.21^{a}	2.12^{b}	2.18^{a}	0.05
	Mortality (%)	1.26^{a}	0.53^{b}	1.05^{a}	0.04
	Body weight (g)	2107^{b}	2200^{a}	2210^{a}	14
0-42 d	Feed intake (g)	4001^{a}	3988^{a}	4011^{a}	36
	Feed conversion ratio	1.90^{a}	1.81^{b}	1.81^{b}	0.02
	Mortality (%)	1.75^{a}	0.70^{b}	0.75^{b}	0.11

SEM - standard error (n = 5); $^{a-c}$Treatments with different letter indexes in the same column are statistically significantly different ($P<0.05$).

In the study reported by Samli *et al*. (2007) it was shown that the addition of *E. faecium* to broiler diet increased the ileal villus height and enhanced broiler performance with respect to weight gain and FCR. Therefore, it was assumed that in present study addition of synbiotic (*Enterococcus faecim* +

fructooligosaccharides) in finisher phase led to a significantly ($p<0.05$) lower feed conversion ratio in treatment T2 (2.12 kg/kg) compared to treatments T1 (2.21 kg/kg) and T3 (2.18 kg/kg). Furthermore, improvement in FCR in broilers fed with synbiotic was recorded throughout the experimental period, but without significant differences compared to treatment T3. Dhama *et al.* (2015) recorded that probiotic is responsible for the feed conversion ratio decrease, causing the body weight gain increase, which is achieved by improving the digestion by balancing the resident gut micro flora. The results achieved in present study are in accordance with the observation by Mahdavi *et al*. (2013) who stated the broilers fed with diet supplemented with probiotic have lower FCR values. From obtained results it could be concluded that addition of synbiotic and salinomycin in broilers nutrition led to significantly better feed conversion to body mass compared to basal diet.

Regarding the finisher phase, the highest mortality rate (1.26%) was recorded in the control treatment, while the lowest mortality rate was noticed in treatment T2 (0.53%). Mortality rate of 1.05% was recorded in treatment T3 and there was statistically significantly ($p<0.05$) higher rate in comparison to treatment T2. After the completion of the experimental period, it was noticed significant ($p<0.05$) differences in mortality rate between treatments T1 and T2, while differences between treatment T2 and T3 was absent. It could be noticed, that both of feed additives (synbiotic and salinomycin) had beneficial effect on the livability of broilers compared to control broilers.

Serum antioxidative enzyme activities in broiler chickens: The results of antioxidant indices in hemolysed blood are shown in Table 4. Broilers of treatment T2 had the highest ($p<0.05$) GPx activity compared to other two treatments, wherein almost the same tendency was observed in POD activity. The activity of SOD did not have differences among treatments.

The MDA activity in blood was significantly lower ($p<0.05$) in the group supplemented with synbiotic compared to the treatment T1, whit no statistically significant difference ($p>0.05$) compared to treatment T3. The lowest GSH content was noticed in treatment T1 (22.5 U/ml) with no statistically significant difference ($p>0.05$) compared to treatment T3 (27.0 U/ml), but both of this treatments statistically significantly differ ($p<0.05$) from treatment T2 (29.0 U/ml). CAT activity was the highest in treatments T3, followed by treatment T2 with statistically significant difference ($p<0.05$) compared to treatment T1. Also, the GSR activity was the highest in treatment T2 with statistically significant differences ($p<0.05$) compared to treatments T1 and T3.

Free radicals represent products of normal metabolism, but could induce body damage if they are present in excessive levels. It has been generally recognized that SOD, GPx, and CAT are the most important antioxidant enzymes in scavenging the oxygen free radical (Zhang *et al*., 2009). Hence, it could be noticed that increasing activities of SOD and GPx would subsequently enhance the capacity of broilers to clear out the oxygen free radicals (Zhang *et al*., 2009). Along with increased activities of SOD and CAT activities, MDA concentration in the serum has been reduced. Since MDA is one of the most the most known secondary products of lipid peroxidation, determination of MDA level is far the most popular indicator of oxidative damage to cells and tissues. Hence, the reduced serum MDA level in treatment T2 as compared with control broilers indicated that lipid peroxidation was reduced by synbiotic via enhancing antioxidative action.

Table 4. Antioxidative enzyme activities in broiler chickens.

Parameter	T1	T2	T3	SEM
GPx (Umol/L)	5.10^{c}	7.36^{a}	6.21^{b}	0.04
POD (Umol/L)	0.35^{c}	1.09^{a}	0.49^{b}	0.05
CAT (U/ml)	22.0^{b}	31.5^{a}	39.2^{a}	4.9
GSR (U/ml)	32.8^{b}	36.0^{a}	33.6^{b}	1.4
GSH (U/ml)	22.5^{b}	29.0^{a}	27.0^{b}	4.0
SOD (Umol/L)	60.1^{a}	58.0^{a}	65.2^{a}	5.1
MDA (Umol/L)	8.81^{a}	6.63^{b}	7.08^{b}	0.5

SEM - standard error (n = 5);
$^{a-c}$Treatments with different letter indexes in the same column are statistically significantly different ($P<0.05$).

Capcarova *et al.* (2010) and Rajput *et al.* (2013) also reported that certain microorganisms could help in the oxidation resistance, scavenge hydroxyl radical and increase antioxidant capacity. Investigation of Kullisaar *et al*. (2002) showed that administration of the probiotic in broiler nutrition significantly reduce MDA content. Results obtained in this study are also in agreement with statement by Hutt *et al.* (2009) who noticed increase in GSH content after the consumption of synbiotic.

Conclusions: From reported findings, it can be concluded that the dietary addition of synbiotic resulted in enchanced production performance of broiler chickens.

Furthermore, this biological experiment showed significant effect of added synbiotic in chicken nutrition on blood antioxidative status. Thus, added synbiotic acted as powerful supplement and could be used as alternative to antibiotics, growth promoters and as antioxidant.

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