Effect of Probiotics and Humic Acid on Egg Production and Quality Parameters of Laying Hens Eggs

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Abstract

The aim of our experiment was to evaluate the influence of probiotic preparation based on lactobacillus; probiotic preparation based on enterococci or humic acid on egg production and selected internal egg quality parameters of laying hens' hybrid Lohman Brown Lite. For housing hens (n=60) three storey enriched battery cage was used in which hens were divided in groups (n=15). In the control group of hens complete feed mixtures without any additions were fed. In the first experimental group complete feed mixture was enriched with probiotic enterococci based preparation in a dose of 0.5 g x kg^{-1}. In the second experimental group probiotic lactobacilli based preparation was added to the feed mixture at a dose of 0.5 g x kg^{-1}. The third experimental group was enriched with 0.5% concentration of humic acid. All groups were fed *ad libitum*. Egg production and egg weight were recorded daily. Complete analysis of the table egg quality was used to evaluate quality parameters: yolk weight (g), yolk index, yolk colour (HLR), albumen weight (g), Haugh Units (HU), and albumen index). The results show that supplementation of feed mixture with both kind probiotics as well as humic acid increased egg production ($p>0.05$) (values of average intensity of laying in the order of the groups: 90.3; 92.0; 91.6 and 92.4%). The addition of probiotics or humic acid only slightly influenced egg weight ($p>0.05$). The addition of both kind of probiotics influenced non-significant positive albumen index ($p>0.05$). The supplement of probiotics on lactobacilli based statistically significantly increased Haugh Units ($p\leq0.05$). The supplement of humic acid statistically significantly increased albumen index and Haugh Units ($p\leq0.05$). The others qualitative parameters of egg internal content were with probiotics or humic acids addition insignificantly influenced ($p>0.05$). Doses of supplements used in this study did not significant negatively influenced monitored egg quality parameters. Based on these findings and the beneficial effects of substances on the poultry health confirmed by other authors we recommend use of these substances as supplements to the feed mixtures for laying hens.

Keywords: egg quality, egg production, humic acid, laying hens, probiotics.

1. Introduction

Removing the feed antibiotics from feed for poultry was increased the interest for improving the gastrointestinal health of animals and use of nutrients was increased [1]. The interests of the feed industry are alternatives that would like antibiotics stimulate growth, but do not cause bacterial resistance and can be positive received by consumers. Substances used for poultry have antimicrobial and antioxidant effects. Among the candidate replacements for antibiotics are competitive exclusion products, probiotics, prebiotics, organic acids, enzymes and plant extracts [2]. For the longest time nutritional supplements used are probiotics. Probiotics are defined as microbial food supplements which beneficially affect the host animal by improving its intestinal microbial balance. The probiotics improved feed conversion for the target species, reduced morbidity or mortality and benefits for the consumer through improved product quality. Potentiated probiotics are more effective than their

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components separately. Probiotics enhanced the growth of many domestic animals, improved the efficacy of forage digestion and quantity and quality of milk, meat and egg [3]. The poultry is bred in high concentrations, which causes the animals stress. The use of probiotics is more effective by animals with the evolving microflora or the disruption of the stability. The probiotics are particularly intended for chicken at early age. The disruption of the ecosystem digestive system may be by livestock older age categories (eg. change of feed, weaning, antibiotic therapy), or is need to boost the immune system (illness, stress) [4]. Implementation of probiotics has great potential in delivering promising results by reducing the intestinal pathogenic load and thereby reducing the subsequent contamination in poultry production. Several mechanisms of action have been proposed including resistance to colonization, competitive exclusion, production of toxic and inhibitory compounds, competition for nutrients and stimulation of the immune system [5]. Applegate et al., 2010 [6] found that the probiotic supplement significantly reduced the pathologic findings of reproductive tract, mortality and increased production of hens. Reproductive problems, which often cause a drop in egg production and sudden deaths, can be reduced if the hens treated with probiotics before lor during the laying.

Humic acids are natural substances formed by decomposition of biomass plants and microorganisms. Humic substances are used for their detoxifying, antiseptic and antifungal properties and as natural growth stimulator. Play an important role in protecting the gastrointestinal tract against infections and positively affect its function. They affect the microbes' proteins and carbohydrates metabolism destroying the pathogenic bacteria and viruses. They have anti-inflammatory effects, bind free radicals and stimulate the receptors of immune system. They have good buffering capacity and can stabilize the pH in the digestive system. They increase the absorption of iron and [7]. Humic acids are among the most active substances with antioxidant effect [8]. Ozturk et al., 2014 [9] and Nagaraju et al., 2014 [10] agree that the use of humic acids in the diet of broiler chickens have a positive impact on meat quality, increasing weight gains and improves the immune system. The impact on breeding environment by applying humic acids on litter was established. The ammonia in the environment increases, improving health of the poultry and the cost of the removal of the manure are smaller.

Manure may be used to enrich the soil humic acids and nitrogen [11]. Zraly et al., 2008 [12] studied the effect of humic acid on the accumulation of mercury in the organs and muscles of chickens. The results indicated lower levels of mercury in the liver, kidney, brain, and muscle tissue. Bubel et al., 2015 [13] investigated the effect of humic substances on biochemical parameters of blood hens. Gladkowski et al., 2011 [14] found that the fatty acid profile by the yolk was significantly influenced and increase the proportion of polyunsaturated fatty acids.

The aim of this research paper was to monitor the effect of probiotic enterococci based preparation or probiotic lactobacilli based preparation or humic acid on **e**gg production, egg weight and selected qualitative parameters of table eggs.

2. Materials and methods

Animals, diets and treatments

Hens (n=60) of the laying hybrid Lohman Brown Lite, 17 weeks old, were randomly divided into 4 groups (n=15) and fed for 21 weeks with diet containing of probiotic lactobacilli based preparation or probiotic enterococci based preparation or humic acid. At the beginning of the experiment, the hens were kept in the three–deck cage technology system, model AGK 2000/616. The technology system was in accordance with requirements specified by the Directive 1999/74 EC. The layer hens were kept by the standard bioclimatic conditions. The composition of the basal diet (BD) fed to the laying hens is shown in Tab. 1 and Tab. 2.

In the control group hens received feed mixture without additions. In the first experimental group complete feed mixture was enriched with probiotic enterococci based preparation (International probiotic company, Košice) in a dose of 0.5 g/kg. In the second experimental group probiotic lactobacilli based preparation was added to the feed mixture at a dose of 0.5 g/kg (International probiotic company, Košice). The third experimental group was enriched with 0.5% concentration of humic acid (Humac, s.r.o Košice). Laying hens accepted fodder *ad libitum*.

Table 1. Composition of the trial diets

Component	Participation in the Diet (%)
Wheat	26.30
Rye	15.00
Barley	20.00
Soybean meal (47% crude protein)	22.00
Soybean oil	2.50
Fat	2.00
Monocalcium phosphate	1.70
Calcium carbonate	9.14
Natrium chloride (38% Na)	0.30
Sodium bicarbonate (28% Na)	0.10
Methionin (99% DL-Methionin)	0.16
Vitamin Premix	0.40
Mineral Premix	0.10
Choline chloride	0.20
Caroten premix	0.10

Table 2 Nutrient content in the trial diets

Nutrient	Nutrient Content in Mixture
MEN (MJ.kg^{-1} of DM)	11.5
CP (g.kg^{-1} of DM)	177
LYS (g.kg^{-1} of DM)	8.81
MET (g.kg^{-1} of DM)	4.17
M + C (g.kg^{-1} of DM)	7.41
THR (g.kg^{-1} of DM)	6.27
LA (g.kg^{-1} of DM)	19.0
Ca (g.kg^{-1} of DM)	39.1
Pavail. (g.kg^{-1} of DM)	3.8
Na (g.kg^{-1} of DM)	1.5

*MEN: metabolisable energy for poultry, CP: crude protein, LYS: lysine, MET: methionine, M+C: methionine plus cysteine, THR: threonine, LA: linoleic acid, Ca: calcium, Pavail: available phosphorus, Na: natrium.

Sample Analysis

Total number of eggs produced during of all experimental period and their weights were recorded daily. Eggs of laying hens of Lohmann Brown Lite strain were collected regularly one a month (n=30 per group) and were assessed immediately after collection. The egg weight (g), egg albumen weight (g), egg albumen index, Haugh units (HU), egg yolk weight (g), egg yolk index and egg yolk color (HLR) were evaluated. All these parameters were detected using routine methods. Weight parameters were detected using analytical weighting machine and the growth intensity and percentage contents were calculated from weight data. Indexes were calculated as the lenght: width ratio. Haught units (HU) detected egg quality as relation of albumen weight and egg weight [100 log.(dense albumen height–1.7x egg weight$^{0.37}$+7.6)]. Yolk color was evaluated using Hoffman la Roche color scale (Hoffman–La Roche, Switzerland).

Statistical analysis

Statistical analysis was done using one–way analysis of variance (ANOVA) with the post hoc Duncan's multiple comparison test in the program SAS.

3. Results and discussion

Effect of probiotics and humic acid on the egg production and egg mass in average during laying period provided Table 3. Egg albumen quality indicators in each group for the observed laying period express Table 4. Quality indicators yolk during the period provided in Table 5.

We found a positive effect of the two probiotic strains, as well as humic acids on egg production, but with no statistically significant difference.

Table 3. Influence of probiotics preparation and humic acid addition into laying hens feed mixture on the production parameters of Lohmann Brown Lite laying hens

Parameter	Groups			
	Control BD	E1	E2	E3
		BD+*Lactobacillus fermentum* 0.5 g/kg	BD+*Enterococci faecium* 0.5 g/kg	BD+humic acid 0.5% concentration
Egg production per hen (ks)	135.5	138.1	137.4	138.6
Average intensity of laying (%)	90.3	92.0	91.6	92.4
Average egg weight (g)	57.36	58.05	57.89	58.19
Egg mass production on the KD (g)	51.81	53.44	53.02	53.86
per 1 henne (kg)	7.77	8.01	7.95	8.06

Zhang et al., 2012 [15] introduced that addition *Lactobacillus salivarius* and *Bacillus subtilis* to the diets of layer hens, caused highly significant ($p<0.05$) increases in egg production and daily egg yield. Similarly, Zhang and Kim, 2013 [16] in the experiment reported, that the laying hens fed with feed mixture with probiotics (*Enterococcus faecium* DSM 7134) were higher production, weight of the eggs and the shell. On the contrary in the experiment of Capcarova et al., 2010 [17] were not significantly affected by probiotic strain *Enterococcus faecium* M 74 the number of eggs and average egg weight. No significant effects of probiotic on triglyceride concentration and egg production parameters were observed. Xu, et al., 2006 [18] investigated the effect of dried cultures of *Bacillus subtilis* on the quality of the eggs of laying hens. After addition of 500 mg of *Bacillus subtilis* culture results showed an increase in egg production, feed consumption and improved feed utilization laying hens. According to Gallazzi et al., 2008 [19], groups of hens fed with probiotics in feed had significantly higher egg production, specific weight of and Haugh units of egg white. Mo et al., 2004 [20] added to the feed of laying hens three types of probiotics. Compared to the control group was egg production, egg quality and the number of intestinal lactic acid bacteria improved, or a significantly increased. Similarly Balevi et al., 2001 [21] recorded in a group with complement of 500 ppm probiotic products lower feed conversion and significantly lower number of damaged eggs.

Results of our experiment are consistent with results of Rosa et al., 2012 [22]. Humic substances supplementation resulted in no significant differences on hen weight, laying percentage, egg weight, or feed conversion ratio ($p>0.05$).

In our experiment was not recorded significant difference among the groups in egg weight ($p>0.05$). There were no significant differences between groups in the average egg weight, daily feed intake and feed conversion in similarly experiments conducted by Bong, 2011 [23], and Sobczak et al., 2015 [24]. The results in the experiment of Lei et al., 2013 [25] showed that dietary supplementation with 0.01 and 0.03% *Bacillus licheniformis* significantly increased egg production and egg mass. However, no significant differences were observed in egg weight. According to Kang et al. 2015, [26] aimed at investigate the effect of dietary supplementation of non-fermented (SB) and Lactobacillus-fermented sea buckthorn (LFSB) on the laying performance of Hy-line Brown laying hens. There were no differences in egg weight and feed intake between the SB and LFSB groups and the basal diet groups. On the contrary Ribeiro et al., 2014 [27] investigated the effect of *Bacillus subtilis* on production and quality of the egg shell. They noticed significant ($p<0.05$) increase in egg production, egg weight, egg mass, weight and thickness of the shell.

Ramasamy et al., 2009 [28] recorded significantly increase of hens egg weight in average during laying period in the group with lactobacillus addition in compare to the control group.

End of the trial there were no significant effects of dietary boric acid and humate inclusion on feed intake, egg weights and yields, egg quality parameters (shell thickness, breaking strength, and shape index) compared with control in the experiment of Hakan et al., 2012 [29]. In the experiment of Yörük et al., 2004 [30], the production of eggs in the experimental groups with the addition of humate and probiotic strains was higher, but the difference was not statistically significant.

In all treatment groups in our experiment was egg mass higher in comparison with control group. Effects of feeding different postbiotic metabolite combinations produced by *Lactobacillus plantarum* strains on egg quality and production performance of laying hens evaluated [31]. No significant difference (p>0.05) was found among the treatment groups on overall feed intake, egg weight, egg mass and feed conversion efficiency. In the experiment of Ribeiro et al., 2014 [27] results showed that egg component were not influenced (p>0.05) by the treatments. Dietary supplementation with *Bacillus subtilis* at 8x10 (5) CFU/g feed compared to control increased (p<0.05) egg production (g/kg) in 2.63% and egg mass in 3.96%. Probiotic-1 (1 g/kg *Bacillus subtilis*) group exhibited the maximum (p<0.05) increase in egg production, egg weight, egg mass, eggshell weight, and eggshell thickness, compared with Probiotic–0.5 (0.5 g/kg *Bacillus subtilis*) and the control groups in the trial of Abdelqader et al., 2013 [32].

Table 4. Influence of probiotics preparation and humic acid addition into laying hens feed mixture on the alterations of Lohmann Brown Lite hen's egg weight and egg albumen quality

Statistical charakteristic	Groups			
	Control	E1	E2	E3
	BD	BD+*Enterococci faecium* 0.5 g/kg	BD+*Lactobacillus fermentum* 0.5 g/kg	BD+humic acid 0.5% concentration
Egg weight (g)				
mean	59.10	59.68	59.28	59.85
S.D.	3.22	3.26	3.36	3.91
CV%	5.44	5.43	5.67	6.55
min.	48.10	49.30	48.80	48.10
max.	73.20	71.30	73.60	73.20
P value		0.3685	0.0689	0.0556
Egg albumen weight (g)				
mean	37.45	38.06	37.53	37.96
S.D.	4.95	4.01	4.50	4.09
CV%	13.21	10.50	11.99	10.77
min.	28.80	30.20	28.00	31.90
max.	63.50	56.90	50.10	59.50
P value		0.7622	0.6408	0.6957
Egg albumen index (%)				
mean	84.00	85.91	87.42	88.81
S.D.	16.02	16.67	16.39	16.45
CV%	19.08	19.40	18.74	18.51
min.	47.05	38.71	39.63	42.10
max.	138.46	142.86	139.51	138.46
P value		0.2634	0.0583	0.0042
Haugh Units (HU)				
mean	79.87	80.56	82.51	81.53
S.D.	7.22	7.60	8.06	7.96
CV%	9.04	9.43	9.77	9.76
min.	62.68	59.05	61.32	55.72
max.	95.96	99.80	99.63	98.55
P value		0.3528	0.0180	0.0409

n=180; Significant difference (P<0.05); Values are means

Egg albumen weight in our experiment was not with probiotics or humic acid addition to the feed mixture of laying hens significantly affected. Egg quality (Haugh unit, relative weights of the albumen and yolk, specific gravity, shell thickness, and yolk color) was not affected by probiotic PrimaLac supplementation in the trial of Tang et al., 2015 [33]. In albumen index was recorded in our experiment statistically significant difference only in favour of the group with humic acid addition (p≤0.05). In the experimental groups with probiotics supplement were values non-significnatly higher in compare to the control group (p>0.05). Feeding supplemental carnitine, humic substances or carnitine+humic substances resulted in increases in body weight gain ($p<0.05$) in the experiment of Yalcin et al., 2006 [34]. Dietary treatments did not significantly affect daily feed intake, daily metabolizable energy intake, egg production, egg weight, feed efficiency, mortality, egg shape index, egg breaking strength, egg shell thickness, egg albumen index, egg yolk index, egg Haugh unit and the percentages of egg shell, albumen and yolk. In all experimental groups of our experiment were Haug Units higher in compare to the control group. In experimental groups with probiotics addition was recorded statistically significant difference in group with lactobacilli based probiotics supplement. On the contrary in experiment of Panda et al., 2003 [35] or Haugh unit were unaffected by feed supplemented with the probiotic *Lactobacillus sporogenes.*

Table 5. Influence of probiotics preparation and humic acid addition into laying hens feed mixture on the alterations of Lohmann Brown Lite hen's egg yolk quality

Statistical charakteristic	Groups			
	Control BD	E1 BD+*Enterococci faecium* 0.5 g/kg	E2 BD+*Lactobacillus fermentum* 0.5 g/kg	E3 BD+humic acid 0.5% concentration
Yolk weight (g)				
mean	16.59	16.40	16.28	16.25
S.D.	1.54	1.20	1.35	1.33
CV%	9.28	7.32	8.29	8.18
min.	13.10	13.60	13.00	13.10
max.	20.00	19.40	19.30	20.30
P value		0.3113	0.0524	0.0519
Yolk index (%)				
mean	47.60	47.28	48.75	48.33
S.D.	3.39	3.72	3.48	3.65
CV%	7.12	7.86	6.72	7.25
min.	39.47	37.91	39.10	36.59
max.	56.76	56.29	64.51	64.35
P value		0.3530	0.0532	0.1322
Yolk colour (HLR)				
mean	6.51	6.54	6.55	6.47
S.D.	0.52	0.51	0.50	0.52
CV%	7.98	7.81	7.63	8.07
min.	6.00	6.00	6.00	6.00
max.	8.00	8.00	8.00	8.00
P value		0.2617	0.2421	0.2288

n=180; Significant difference ($P<0.05$); Values are means. HLR–colored Hoffman La Roche scale

Yolk weight was not with addition of probiotics or humic acid in our experiment statistically significantly influenced. Similarly, results of Loh et al., 2014 [31] show no significant differences in yolk weight between the treatment groups. A similar conclusion in experiment with *Saccharomices cerevisiae* focus on the quality of hen eggs coming authors [36]. The results of the experiment show that the mass of white and the yolk or Haugh units were not significantly affected. On the contrary, Yousefi et. Al., 2007 [37] has been observed of significantly higher weight and the thickness of the shell and the yolk weight. The results of Ergin et al., 2009 [38] indicated that the supplementation of 30 ppm humic acid into the diet may increase the egg shell strength without affecting egg production and feed efficiency compared to control counterparts. Egg production (% hen-day) in the group with addition of 90 ppm dietary humic acid in liquid form was higher ($p<0.05$) than control group. Egg weight, feed conversation ratio and yolk weight were not affected by dietary humic substances.

In the yolk index were found out no statistically significant differences among the groups in our experiment. Hong et al., 2002 [39] indicate the index of the yolk increase with increasing proportion of probiotics in each group. On the contrary, Yalcin et al., 2002 [40] reported significant differences in hens live weight and feed consumption, and also in the yolk index. Yolk color values in experimental groups were in each month and the average for the experiment very balanced and therefore the differences between the groups were statistically no significant (p>0.05). Our results are consistent with the findings of Kalavathy et al., 2005 [41]. Yolk color in our experiment, were not significantly affected probiotic supplement, which is inconsistent with the findings [18]. In contrast to our results also Yodseranne et al., 2003 [42] reported a beneficial effect of probiotics and Hong et al., 2002 [39] reported tend to improve the color of the yolk in the group supplemented with probiotics. Sobczak et al., 2015 [24] recorded no significant differences between the groups in average egg weight, laying performance (%), daily feed intake or feed conversion. Eggs laid by probiotic preparation of *Bacillus subtilis* group hens received significantly higher scores for yolk color (Roche yolk color fan) and albumen quality (Haugh units) in comparison with eggs laid by control group hens.

4. Conclusions

In all experimental groups was recorded higher egg production compared to the control group. The addition of humic acid in 0.5% concentration significantly increased egg albumen index, supplement of probiotic products based on lactobacilli significantly increased egg albumen index and Haugh Units. The results suggest that the egg weight, egg albumen weight, yolk index and yolk colour were not significantly influenced with probiotics or humic acid addition (p>0.05).

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