The Effect of Phytobiotics, Organic Acid and Humic Acids on the Utility and Egg Quality of Laying Hens

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Abstract

The aim of this study was the assessment of an influence of supplement of dietary herbal additive in combination with organic acids into feed mixture or drinking water of laying hens on performance parameters and egg quality. The Lohmann Brown Lite laying hens (n=30) were divided into 3 groups (n=10), and fed for 20 weeks *ad libitum* with complete feed mixtures (CFM). Hens in the control group received the complete feed mixture (CFM) and drank drinking water without any supplements. In the first experimental group hens received CFM without supplements but phytobiotics (bergamot oil (*Citrus bergamia*), thyme (*Thymus vulgaris*), clove (*Syzygium aromaticum*), pepper (*Piper nigrum*) in combination with the fumaric acid and citric acid at 60 mg per 1 liter of water were added to their drinking water. In the second experimental group was CFM enriched with humic acids in the concentration of 0.5%, and phytobiotcs with organic acids at the same dose as in the first experimental group were added to their drinking water. Monitored parameters: body weight (g), egg production (%), the weight of all produced eggs (g), egg albumen weight (g), egg albumen index, Haugh unit (HU), egg yolk weight (g), egg yolk index, egg yolk colour (°HLR), egg shell weight (g) and egg shell strength ($N.cm^{-2}$). The results showed no significant differences between the both experimental groups and the control group in the parameter body weight of hens ($P>0.05$). The highest average body weight was found in the hens from the second experimental group (values in the order of groups: 1792.22±80.85; 1768.42±55.55; 1820.1±78.56 g±S.D.). We observed positive trend of increasing of egg production by adding of used supplements, especially in the second experimental group with the addition of humic acids, although with no statistically significant difference compared to the control group ($P>0.05$). The mean laying intensity in the order of groups: 90.42; 91.16; 91.56%. We observed statistically significant differences in the quality of egg albumen indicators ($P\leq0.05$), specifically in the egg albumen index (the value in the order of groups: 84.02±16.04, 85.93±16.69, 87.44±18.41) and Haugh units (79.89±7.24, 80.58±7.62, 82.53±12.88 HU) in favour of the second experimental groups. Yolk index was not significantly higher in the second experimental group ($P>0.05$). In other parameters of yolk quality and eggshell quality, monitored values were comparable to those in the control group.

Key words: egg quality, humic acids, laying hens, organic acids, performance parameters, phytobiotics

1. Introduction

Phytobiotics is a term used to describe the natural bioactive substances of plant origin, which affect the growth and health of the animal. Phytobiotics are often applied in the form of essential oils and plant extracts [1]. Herbal plants are a new class of growth promoters and in recent years this feed additives have gained extensive attention in the feed industry. They are a wide variety of herbs, spices, and products derived thereof, and are mainly essential oils. Although numerous reports have demonstrated antioxidative and antimicrobial and immune stimulation efficacy in vitro, respective experimental in vivo evidence is still quite limited. A limited number of experimental comparisons of herbal plants feed additives with antibiotics or organic acid have suggested similar

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effects on the animal gut microflora. Gut microflora has significant effects on host nutrition, health, and growth performance by interacting with nutrient utilization and the development of gut system of the host. In addition, some phytogenic compounds seem to promote intestinal mucus production. However, the future of using herbs in animal feeding will in great measure depend on the knowledge of chemical structure, their value and characteristics of practical herbs or their extract physiological needs and well-being of animal, and, above all on consumer's preferences and expectations [2].

Humic acids are macromolecules that contain humic substances. Humic acids are polymeric carboxylic acids with a complicated structure with a cyclic configuration. They also contain hydroxyl, quinone and semiquinone groups. Various beneficial features can be attributed mainly carboxylic acids. They have antiviral and antiphlogistic effects. The presence of phenols, quinones, carboxylic acids in their structure is linked to their antioxidant, antimutagenic, fungicidal and bactericidal activity [3]. Recent years have seen an increase in the application of humic substances in animal nutrition. In the natural state have humic acids the effect of growth promoter and natural antibiotics. Humic additives generally have a positive effect on production, the health of the laying hens and the quality of chicken eggs from intensive as well as organic and extensive farming [4]. To improve the health and productivity of livestock use different feed supplements rich in humic acids, which have a high absorption capacity, bind a variety of toxic substances and also viruses in the digestive system of animals (mycotoxins, phytotoxins). Humic acids of the available materials have very good buffering capacity, to stabilize the pH throughout the digestive system of animals and promote the proliferation and activity of the symbiotic microflora [5].

In recent years, there has been an increase in the use of organic acids as substitutes for antibiotic growth promoters because of the fear of antibiotic resistance and the implications for human health. Organic acids and their salts have been used in poultry diets and drinking water for decades and seem to elicit a positive response in growth performance. An important objective of dietary acidification is the inhibition of intestinal bacteria competing with the host for available nutrients, and a reduction of possible toxic bacterial metabolites resulting in the improvement of nutrient digestibility, thereby ameliorating the performance of birds and enhancing the specific and non-specific immunity in poultry. Literature shows that short-chain fatty acids, medium-chain fatty acids and other organic acids have more or less pronounced antimicrobial activity, depending on both the concentration of the acid and the bacterial species that is exposed to the acid [6].

The aim of this research was to monitor the effect of phytobiotics, organic acids and humic acids on the performance of laying hens and selected parameters of egg albumen quality, egg yolk quality and egg shell quality of market eggs.

2. Materials and methods

Hens (n=40) of the laying hybrid Lohmann Brown, 17 weeks old, were randomly divided into 3 groups (n=10). Hens received in experimental groups for 20 weeks drank water with contain of mixture of phytobiotics with organic acids or were fed with complete feed mixture with addition of humic acids. 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 useful area provided for one laying hen presented 943.2 cm^2. Each cage was equipped with four nipple drinkers; accession to feed mixture or drinking water was *ad libitum.* The layer hens were kept by the standard bioclimatic conditions. The composition of the basal diet (BD) fed to the laying hens is presented in our previous work [19].

In the control group hens fed complete feed mixture without additions and drank water without any additives. Hens in the first experimental group (E1) were drank a water with 60 $mg.l^{-1}$ of phytobiotics (bergamot oil (*Citrus bergamia*), thyme (*Thymus vulgaris*), clove (*Syzygium aromaticum*), pepper (*Piper nigrum*) with organic acids (combination of the fumaric acid and citric acid) and fed a diet with complete feed mixtures without any additives. Hens in the second experimental group (E2) were drank a water with 60 $mg.l^{-1}$ of phytobiotics (bergamot oil (*Citrus bergamia*), thyme (*Thymus vulgaris*), clove (*Syzygium aromaticum*), pepper (*Piper nigrum*)

with organic acids combination of the fumaric acid and citric acid) and fed a diet with complete feed mixtures with 0.5% of humic acids (humic acids 65%; fluvic acids 5%; Ca 42 278 mg/kg, Mg 5111 mg/kg, Fe 19 046 mg/kg, Cu 15 mg/kg, Zn 37 mg/kg, Mn 142 mg/kg, Co 1.24 mg/kg, Se 1.67 mg/kg, V 42.1 mg/kg, Mo 2.7 mg/kg; humidity 15% from Humic Acid Manufacturer Co., Košice, Slovakia).

Sample Analysis

Eggs of laying hens of Lohmann Brown strain were collected regularly one a month (n=30 per group) and were assessed immediately after collection. Egg weight (g), specific egg weight, albumen weight (g), albumen index, Haugh Units (HU), yolk weight (g), yolk index, yolk colour (HLR), egg shell weight (g), egg shell strength (N/cm^2) were studied. 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 length: 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 colour was evaluated using Hoffman La Roche colour scale (Hoffman–La Roche, Switzerland).

Statistical analysis

Data were analyzed by analysis of variance using the general linear model procedure of the software program Statistical Analysis System. Differences between the indicators were tested using one-way analysis of variance by Duncan's test. Significance was considered at $P \leq 0.05$.

3. Results and discussion

The aim of the presented study to monitor the effect of phytobiotics, organic acids and humic acids on the performance of laying hens and selected parameters of egg albumen quality, egg yolk quality and egg shell quality of market eggs of Lohmann Brown laying hybrid. Hens in both experimental groups showed the largest producer of eggs quantity, but with no significantly different (P>0.05) between experimental groups and control group. The values in the order of groups: 1357.2; 1368.2; 1373.28 pieces. These results agreed with results of Florou-Paneri et al. (2005) [7] and Cabukt et al. (2006) [8], who observed improvement of egg production of laying hens by adding of phytoaditives. Dobrzański et al. (2009) [9] and et al., (2009) [10] reported that addition of humic substances to the feed for laying hens had not positive effect on egg production. Our results agree with those of Trziszka et al. (2011) [11], and Kazempour et al. (2016) [12] which proved the positive effect of organic acids to egg production.

Egg weight (g) and specific egg weight are presented in Table 1. Egg weight was the highest in control group with the complete feed mixtures without additives. The addition of phytobiotics with organic acids in drinking water and humic acids in complete feed mixtures had not a significant effect (P>0.05) to the egg weight. These results agreed with those of Mahmoud et al. (2010) [13], and Mohebbifar et al. (2010) [14], who did not record statistically differences in egg weight. The specific egg weight were in experimental groups same or very similar to the control group (the values in the order of groups: 1.1; 1.098; 1.1) without statistically significant different (P>0.05).

The influence of phytobiotics, organic acids and humic acids addition on egg yolk quality is presented in Table 2. The results indicate that the addition of phytobiotics, organic acids and humic acids into drinking water and complete feed mixtures for laying hens had no impact on egg yolk weight. Our results are in contrast with those of Youssef et al. (2013) [15], who reported positive effect of organic acids on egg yolk weight. In egg yolk index was recorded among all experimental groups and control group a statistically nonsignificant difference (P>0.05). This agrees with results of Nasirolelslami and Torki (2010) [16]. In egg yolk colour was recorded the best effect in experimental group with 60 $mg.l^{-1}$ of phytobiotics and organics acids in drinking water and 0.5% of humic acids in complete feed mixtures, but with no significantly different (P>0.05) between groups. The influence of phytobiotics, organic acids and humic acids addition on egg albumen quality is presented in Table 3. In experimental groups with clove essential oil supplement was albumen weight comparable to the control group with no significant different (P>0.05).

Table 1. Influence of phytobiotics, organic acids and humic acids on the alterations of Lohmann Brown laying hens' egg weight and specific egg weight

Parameter	C	E1	E2
Egg weight (g)			
mean	59.87	59.70	59.27
S.D.	3.96	3.28	5.37
CV%	6.61	5.49	9.06
min.	51.23	49.33	48.82
max.	72.02	71.32	73.62
P value		0.40	0.10
Specific egg weight (g/cm^3)			
mean	1.1	1.09	1.1
S.D.	0.02	0.02	0.02
CV%	2.45	2.27	2.45
min.	1.08	1.08	1.06
max.	1.11	1.11	1.12
P value		0.17	0.63

C-control group; E1-60 $mg.l^{-1}$ phyhobiotics and organic acids in drinking water; E2-60 $mg.l^{-1}$ of phyhobiotics and organic acids in drinking water and 0.5% of humic acids in complete feed mixtures

Table 2. Influence of phytobiotics, organic acids and humic acids on the alterations of Lohmann Brown laying hens' egg yolk quality

Parameter	C	E1	E2
Egg yolk weight (g)			
mean	16.62	16.44	16.18
S.D.	1.56	1.22	1.37
CV%	9.38	7.42	8.46
min.	13.12	13.62	12.02
max.	20.02	19.42	18.32
P value		0.33	0.06
Egg yolk index			
mean	47.62	47.30	48.77
S.D.	3.41	3.74	3.50
CV%	7.16	7.90	7.17
min.	39.49	37.93	39.12
max.	56.48	56.31	64.53
P value		0.37	0.07
Egg yolk colour (°HLR)			
mean	6.53	6.56	6.57
S.D.	0.54	0.53	0.52
CV%	8.26	7.81	8.07
min.	6.02	6.02	6.02
max.	8.02	8.02	8.02
P value		0.28	0.26

C-control group; E1-60 $mg.l^{-1}$ phyhobiotics and organic acids in drinking water; E2-60 $mg.l^{-1}$ of phyhobiotics and organic acids in drinking water and 0.5% of humic acids in complete feed mixture

The values in the order of groups: 37.98 g; 38.08 g and 37.55 g. In parameter index of the albumen were in experimental groups with phytobiotics, organic acids and humic acids in both groups higher values (the values in the order of groups: 84.02; 85.93; 87.44), but only in the group of hens with 60 $mg.l^{-1}$ of phytobiotics and organic acids in drinking water and humic acids in complete feed mixtures was recorded significantly higher values compared to the control group ($P<0.05$). In the

groups with addition 60 mg.l^{-1} of phytobiotics and organic acids in drinking water and humic acids in complete feed mixtures was found the significantly different (P<0.05) in Haugh Units compared to the control group. This agree with results by other authors [17, 18], which found a positive effect of phytobiotics to the egg albumen index and Haugh Units.

The influence of phytobiotics, organic acids and humic acids addition on egg yolk quality is presented in Table 4. In both experimental groups with phytobiotics, organic acids and humic acids were egg shell weight (the values in the order of groups: 5.72; 5.73 and 5.64 g) and egg shell strength (the values in the order of groups: 27.83; 27.60 and 27.78 N.cm^{-2}) comparable to the control group with no significant different (P>0.05). Our results are inconsistent with the result of Akhtar et al., (2003) [18], who found a positive effect of phytobiotics to the shell quality.

Table 3. Influence of phytobiotics, organic acids and humic acids on the alterations of Lohmann Brown laying hens' egg albumen quality

Parameter	C	E1	E2
Egg albumen weight (g)			
mean	37.98	38.08	37.55
S.D.	4.11	4.03	4.52
CV%	10.82	10.58	12.03
min.	31.92	30.22	28.02
max.	59.52	56.92	50.12
P value		0.782	0.66
Egg albumen index			
mean	84.02	85.93	87.44
S.D.	16.04	16.69	18.41
CV%	19.09	19.42	21.05
min.	47.07	38.73	29.65
max.	138.48	142.88	118.53
P value		0.2834	0.0438
Haugh Units (HU)			
mean	79.89	80.58	82.53
S.D.	7.24	7.62	12.88
CV%	9.06	9.45	15.60
min.	62.7	59.07	31.34
max.	95.98	99.82	99.65
P value		0.3728	0.038

C-control group; E1-60 mg.l^{-1} phyhobiotics and organic acids in drinking water; E2-60 mg.l^{-1} of phyhobiotics and organic acids in drinking water and 0.5% of humic acids in complete feed mixtures

Table 4. Influence of phytobiotics, organic acids and humic acids on the alterations of Lohmann Brown laying hens' egg shell quality

Parameter	C	E1	E2
Egg shell weight (g)			
mean	5.72	5.73	5.64
S.D.	0.54	0.52	0.40
CV%	9.44	9.07	7.09
min.	3.92	4.22	4.12
max.	7.12	6.52	6.82
P value		0.74	0.56
Egg shell strength weight (N/cm^2)			
mean	27.83	27.60	27.78
S.D.	6.02	5.26	6.29
CV%	21.63	19.05	22.64
min.	16.42	17.02	14.27
max.	42.02	40.07	44.97
P value		0.45	0.47

C-control group; E1-60 $mg.l^{-1}$ phyhobiotics and organic acids in drinking water; E2-60 $mg.l^{-1}$ of phyhobiotics and organic acids in drinking water and 0.5% of humic acids in complete feed mixtures

4. Conclusion

Based on the obtained results, it can be concluded that addition of phytobiotics and organic acids had no significantly impact to the egg production and egg quality. The results suggest that egg weight, specific egg weight, yolk weight, yolk index, yolk colour, albumen weight, shell weight and shell strength were not significantly influenced with phytobiotics and organic acids addition ($P>0.05$). Only in the E2 group with addition of phytobiotics and organic acids in drinking water and with humic acids in complete feed mixtures were recorded significantly ($P<0.05$) positive effect on albumen index and Haugh Units.

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