The Influence of Oregano Essential Oil and *Rhus Coriaria* L. on Qualitative Parameters and Microbiological Indicators of Hens Eggs Content

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Abstract

In this experiment the effects of supplementation of the diet for laying hens with oregano essential oil and *Rhus coriaria* L. seeds addition on physical and microbiological egg parameters were studied. Hens of laying hybrid Hy-Line Brown (n=30) were randomly divided into 3 groups (n=10) and fed for 20 weeks with diets with oregano essential oil and *Rhus coriaria* L.seeds supplemented. In the control group hens received feed mixture with no additions. The diets in the first experimental groups were supplemented with 1 ml/kg oregano essential oil. The feed for second experimental group of birds consisted of basal diet supplemented with *Rhus coriaria* L. seeds (1% in total feed mixture). The results suggest that the most of qualitative parameters of egg internal content and egg shell were not significantly influenced with oregano oil or *Rhus coriaria* L. seeds addition ($p>0.05$). A statistically significant difference in favour of the experimental groups compared with the control group was observed in two indicators of albumen quality, in the index of albumen and in the Haugh Units ($p<0.05$). The highest total number of bacteria and count of coli forms bacteria was found in the control group. The number of lactobacilli was zero in all groups.

Keywords: micobiological quality, oregano essential oil, physical quality, *Rhus coriaria* L. seeds, table eggs

1. Introduction

Phenolic components of oregano (*Origanum vulgare*), carvacrol (2-methyl-5-phenol) and thymol (2-isopropyl-5-methylphenol), are known primarily for their broad spectrum antimicrobial activity, which has been analyzed *in vitro* in work [1]. Essential oils of oregano exhibit antimicrobial activity against a variety of gram-positive, gram-negative bacteria, yeasts and fungi *in vitro* [2]. Were also tested its antimicrobial effect in food [3]. Also observed fungicidal activity of oregano [4], may therefore be potentially used as a biologically active agent to its toxic effect on bacteria and fungi.

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For the evaluation of essential oil obtained by hydrodistillation of the aerial parts *Origanum onites* L. was tested its effect on alantochorion membrane of fertilized chicken egg. Means of gas chromatography were identified eighty-three components, representing 99.1% of the total number of oil. As main components were identified carvacrol, thymol, p-cymene, and γ-terpinene. By Baydar et al. (2004) [5], the essential oil of oregano contains mainly carvacrol (86.9%) and to a lesser extent, contain γ-terpinene, p-cymene and myrcene. Based on the work of several authors may have essential oils a beneficial effect on the gastrointestinal microflora of poultry [6], production parameters, the quality of poultry meat [7] and eggs [8].

Rhus genus includes more than 250 kinds of shrubs and trees, belongs to the family *Anacardiaceae*. One species of this genus *Rhus coriaria* L. is commonly known as sumac. The main compounds in *Rhus coriaria* L. are tannins

and substantial amounts of flavonoids [9]. Contains tannin was 48% of dry matter in cultivated plants. Fruits summation contains phenolic acids, anthocyanins, and organic acids such as malic, citric and tartaric acid.

Microbiological quality may be determined through reactions between indicators included within the package and metabolites which are produced during microbial growth [10]. The microbiological contamination of egg inside is greatly affected by the ability of the egg shell to stop the invasion of microorganisms and bacteria from entering the egg through the shell's pores [11].

The aim of this work was to observe the influence of oregano essential oil and sumac *(Rhus coriaria*, L.) addition on qualitative parameters of yolk, albumen and shell of laying hens eggs of hybrid Hy-Line Brown in pilot system. The microbiological indicators monitoring count of coliforms bacteria, count of lactobacilli, total count of microorganisms, count of microscopic fungi and yeasts were observed.

2. Materials and methods

Animals, diets and treatments

Hens (n=30) of the laying hybrid Hy-Line Brown, 17 weeks old, were randomly divided into 3 groups (n=10) and fed for 20 weeks with diet containing oregano essential oils or *Rhus coriaria* L. seeds.

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 was *ad libitum.* To equipment of cage belonged roosts, place for rooting in ashes–synthetic grass, nest and equipment for shortening of clutches. The layer hens were kept by the standard bioclimatic conditions.

In the control group hens received feed mixture without additions. The diets in the first experimental group were supplemented with 1 ml/kg oregano essential oil (Calendula a.s. Nová Ľubovňa, SR). The feed for second experimental group of birds consisted of BD supplemented with *Rhus coriaria* L. seeds in the dose 1% in total feed mixture. Laying hens accepted fodder *ad libitum.*

All kinds of feed supplements used in the experiment were homogenously incorporated into the feed mixture in the feed mill.

Sample Analysis

Eggs of laying hens of Hy-Line Brown strain were collected regularly one a month (n=30 per group) and were assessed immediately after collection. The egg weight (g), egg yolk weight (g), egg yolk index, egg yolk color (HLR), albumen weight (g), egg albumen index and Haugh units (HU) 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 heigh –1.7x egg $weight^{0.37}$+7.6)]. Yolk color was evaluated using Hoffman la Roche color scale (Hoffman–La Roche, Switzerland) and egg shell compactness was detected using Egg Crusher 1.1 (VEIT Electronics, Czech Republic). 6 analyzes were performed. The experiment lasted 20 weeks.

Microbiological indexes

Determination of CFU counts in egg

Plate diluting method was applied for quantitative CFU counts determination of respective groups of microorganisms in 1g of substrate. Nutrient medium in Petri dishes was inoculated with 1ml of egg samples on surface in three replications. Homogenized samples of eggs were prepared in advance by sequential diluting based on decimal dilution system application. Stock suspension (10^{-1}) was prepared as follows: 5 g of egg content was added to the test tube containing 45 ml of distilled water.

Media and culture conditions

The number of coliforms bacteria was grown in Endo agar (aerobiosis), at 37°C during 24 hours. *Escherichia coli* were grown in Violet red bile agar (aerobiosis), at 37°C during 24 hours. Enterococci were grown in Slanetz-Bartley agar (aerobiosis), at 37°C during 48 hours. Lactobacilli were grown in Rogosa agar (microaerophillia), at 37°C during 72 hours. The total number of bacteria was grown in GTK agar (aerobiosis), at

30°C during 48 hours. The composition of these nutritive substrates was according to the directions for use declared by the producer (Biomark laboratories). Bacteria were determined according to Holt et al. (1994). For determinations of fungal colony-forming units (CFU) 5g samples of egg were soaked in 45 ml sterile tap-water containing 0.02% Tween 80 and then 30 min shaked. Dilutions (from 10^{-1} to 10^{-5}) in sterile tap-water with 0.02% Tween 80 were prepared and 1-ml aliquots were inoculated on each of three plates of Czapek-Dox agar with streptomycin (to inhibit the bacterial growth). Petri dishes were inoculated using the spread-plate technique and incubated at 25°C. Total fungal CFU/g counts in samples were determined after 5 days of incubation.

Statistical analysis

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

3. Results and discussion

Egg weight, yolk quality, albumen quality and shell quality indicators in each group for the observed laying period expresses Table 1. The microbiological quality during the period provided in Tables 2 and 3.

After add oregano oil and *Rhus coriaria* seeds, egg weight values were in the order of groups 59.85±3.91; 59.10±5.22; 60.1±04.27 g (±SD). In the group with option of oregano has been reported not significantly lower difference compared to the control group ($p>0.05$). In the group with option summation has been reported not significantly higher difference compared with the control group ($p>0.05$). Florou-Paneri et al. (2005) [12] after addition of oregano in accordance with our results recorded in relation to the weight of the eggs a insignificant impact.

Senköylü et al. (2004) [13] indicated a reduction in egg weight using phytobiotics, though not to a statistically significant difference. Yang et al. (2003) [14] observed after the addition of green tea significantly negative impact phytobiotics.

Effect of adding of oregano oil into feed mixture at higher doses caused a significant decrease in yolk weight. The values in the order of groups: 16.60±1:54, 16.15±1.33; 16.53±1.55 g ($\overline{x}$ ±SD). Statistically significant effect of plant essential oils on this indicator found Canogullari et al. (2009) [15], respectively Bozkurt et al. (2012) [16].

Yolk index. In our experiment, the recorded among all test groups and a control group a statistically significant difference ($p>0.05$), similar to the below cited authors with different kinds of phytobiotics. Not significantly higher values compared to the control group were observed in our experiment in groups of oregano oil supplement. Marginally significant difference was experimental group with complementary Rhus L. coriaria compared to the control group. Minor differences in yolk index of eggs from hens fed a meal containing a mixture of green tea in its attempt had also Yalcin et al. (2006) [17], respectively Canogullari et al. (2009) [15] with the addition of garlic meal.

Yolk color. In this indicator were observed significant differences between the groups. Shades of yellow colors on the color scale Hoffman La Roche were in the normal range for the add oregano oil and sumac. Radwan et al. (2008) [18] in accordance with the conclusions of our experiment detected after the addition of oregano significant differences in color shade yolk, but after adding turmeric effect was significantly pronounced. Significant increase in the intensity yolk addition of 2% concentration of green tea also noted Yang et al. (2003) [19], respectively. Yodseranne et al. (2003) [20] as well as Botsoglou et al. (2005) [21] in the experimental group with the addition of saffron in a dose of 20 mg.kg. On the contrary Ayerza et al. (2002) [22] reported paler color of the yolk by the addition of sage.

In groups with oregano supplement was albumen weight relatively balanced (37.96±09.04, 37.45±4.95, 37.98±4.7 g (±SD). Higher values of the albumen indicate in their experiment with the addition of *Nigella sativa* [23]. Sahinler et al. (2005) [24] in accordance with our results did not record the impact of the add phytobiotics. Results of Bozkurt et al. (2012) [16] found that supplementation diet with essential oil mixture provided increments in eggshell weight, however relative albumen weight was significantly decreased in response to essential oil mixture.

In parameter index of the albumen were in both experimental groups recorded significantly higher mean values compared to the control group ($p<0.05$). The values in the order of groups were

84.00±16.02; 88.81±16.45; 89.39±16.08 HU ($\overline{x}$ ±SD). A significant difference in the index of albumen in the group with the addition of garlic meal found out [15].

Table 1 Influence of oregano essential oil and *Rhus coriaria* L. addition into laying hens feed mixture on the alterations of Hy-Line Brown laying hen's egg weight, egg yolk quality, egg albumen quality and egg shell quality

Group	BD-control group	BD+oregano essential oil 1 ml/kg	BD+sumac(*Rhus coraria* L.) 1%
Egg weight (g)			
mean	59.85	59.10	60.15
SD	3.91	5.22	4.27
CV (%)	6.55	8.83	7.44
p value		0.0556	0.1273
Egg yolk weight (g)			
mean	16.60	16.15	16.53
SD	1.54	1.33	1.55
CV (%)	9.29	8.23	9.37
p value		0,0419	0.3825
Egg yolk index			
mean	47.60	48.33	48.80
SD	3.39	3.65	6.66
CV (%)	7.12	7.25	13.10
p value		0.1322	0.0518
Egg yolk color (°HLR)			
mean	6.51	6.47	6.56
SD	0.52	0.52	0.50
CV (%)	7.98	8.07	7.60
p value		0.1322	0.2084
Egg albumen weight (g)			
mean	37.96	37.45	37.98
SD	4.09	4.95	4.07
CV (%)	10.77	13.21	10.71
P value		0.6959	0.6728
Egg albumen index			
mean	84.00	88.81	89.39
SD	16.02	16.45	16.08
CV (%)	19.08	18.51	17.99
p value		0.0042	0.0019
Haugh Units (HU)			
mean	79.87	82.53	84.80
SD	7.22	7.96	11.97
CV (%)	9.04	9.76	14.10
p value		0.0409	0.0001
Egg shell weight (g)			
mean	5.70	5.49	5.63
SD	0.52	0.48	0.41
CV (%)	9.09	8.74	7.28
p value		0.0617	0.0631
Egg shell strength (N/cm^2)			
mean	27.81	28.41	27.23
SD	6.00	6.36	5.91
CV (%)	21.56	21.62	23.46
p value		0.0688	0.6437
Egg shell thickness (μm)			
mean	376.50	387.22	380.22
SD	24.46	25.77	28.98
CV (%)	6.50	6.65	7.58
p value		0.0321	0.0619

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

Haugh Units. We had a statistically significantly higher values in both experimental groups compared to the control group ($p<0.05$). The values in the order of groups: 79.87±7.22; 82.53±7.96; 84.80±11.97 HU (±SD). In accordance with our findings [12] observed after the addition of oregano a positive impact on this indicator, although not statistically significant difference.

Table 2 Influence of oregano essential oil and *Rhus coriaria* L. addition into laying hens feed mixture on the alterations of Hy-Line brown laying hen's egg microbiological quality at the beginning of the laying (CFU/g)

Experimental group	Group of microorganisms					
	CB	TNC	E	L	MF	Y
BD control group	$2.85x10^1$	$1.95x10^2$	$1.00x10^1$	<10	<10	$1.52x10^1$
	<10	$1.32x10^2$	$1.50x10^1$	<10	$1.85x10^1$	$2.33x10^1$
	$3.00x10^1$	$1.30x10^3$	$1.50x10^1$	<10	<10	$2.33x10^1$
BD+oregano essential oil 1 g/kg	<10	<10	<10	<10	<10	<10
	<10	$1.00x10^1$	<10	<10	<10	<10
	<10	$1.00x10^1$	<10	<10	<10	<10
BD+*Rhus coriaria* L. seeds 1%	<10	$1.00.10^1$	<10	<10	<10	$1.00.10^1$
	<10	$1.10.10^2$	<10	<10	$1.00.10^1$	<10
	<10	$1.00.10^1$	<10	<10	<10	<10

CB–Count of coliforms bacteria (cfu/g), TNC–total number count (CFU/g), E–Count of enterococci (CFU/g) L–Number of lactobacilli (CFU/g), MF–Count of microscopic fungi (CFU/g), Y–yeasts (CFU/g)

In the experimental group with oregano coliform bacteria counts were zero. Similarly, calculations were zero coliform bacteria in the experimental group with the addition of sumac. In the group where the oregano oil was used at 1 ml/kg, the total plate count ranged between 0 and $1.00x10^1$ CFU/g. Similarly, in samples of eggs from experimental groups with complementary sumac, the number of enterococci decreased with increasing dose. The number of lactobacilli were <10 in all experimental group. The highest total number of bacteria was found in control group. Number of microscopic fungi ranged from <10 CFU/g in experimental groups to $1.85x10^1$ CFU/g in control group. Number of yeasts ranged from <10 CFU/g in experimental group to $2.33x10^1$ CFU/g in control group.

Table 3. Influence of oregano essential oil *and Rhus coriaria* L. seeds addition into laying hens feed mixture on the alterations of Hy-Line brown laying hen's egg microbiological quality at the end of the laying (CFU/g)

Experimental group	Group of microorganisms					
	CB	TNC	E	L	MF	K
BD control	$3.86x10^1$	$2.95x10^2$	$1.00x10^1$	<10	<10	$1.82x10^1$
	<10	$2.32x10^2$	$1.50x10^1$	<10	$2.82x10^1$	$2.73x10^1$
	$4.00x10^1$	$1.30x10^3$	$1.50x10^1$	<10	<10	$2.73x10^1$
BD+oregano essential oil 1 g/kg	<10	<10	<10	<10	<10	<10
	<10	$1.14x10^1$	<10	<10	<10	<10
	<10	$2.73x10^1$	<10	<10	<10	<10
BD+*Rhus coriaria* L. 1%	<10	$2.73.10^1$	<10	<10	<10	<10
	<10	$1.73.10^1$	<10	<10	<10	<10
	<10	$1.25.10^1$	<10	<10	<10	<10

CB–Count of coliforms bacteria (CFU/g), TNC–total number count (CFU/g), E–Count of enterococci (CFU/g), L–Number of lactobacilli (CFU/g), MF–Count of microscopic fungi (CFU/g), K–kvasinky (CFU/g)

The highest count of coliforms bacteria at the end of the laying was determined in control group. The number of enterococci ranged from <10 CFU/g^{-1} in group with oregano essential oil 1g/kg to $1.50x10^1$ CFU/g in control group. The number of lactobacilli were <10 in all experimental group. The highest total number of bacteria was found in control group. Number of yeasts ranged from <10

CFU/g in experimental group to $2.73x10^1$ CFU/g in control group.

4. Conclusions

The results suggest that the most of qualitative parameters of egg internal content and egg shell were not significantly influenced with oregano oil or *Rhus coriaria* L. seeds addition ($p>0.05$). A statistically significant difference in favor of the experimental groups compared with the control group was observed in two indicators of albumen quality, in the index of albumen and in the Haugh Units ($p<0.05$). The highest total number of bacteria and count of coliforms bacteria was found in the control group. The number of lactobacilli was zero in all groups.

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References

1. Nostro, A., Roccaro, A. S., Bisignano, G., Marino, A., Cannatelli, M. A., Pizzimenti, F. C., Cioni, P. L., Procopio, F. and Blanco, A. R., Effects of oregano, carvacrol and thymol on *Staphylococcus aureaus* and *Staphylococcus epidermidis* biofilms, Journal of Medical Microbiology, 2007, 56, 519-523.
2. Viuda-Martos, M., Ruiz-Navajas, Y., Fernández-López, J., Pérez-Álvarez, J. A., Antifungal activities of thyme, clove and oregano essential oils, Journal of Food Safety, 2007, 27, 91–101.
3. Gutierrez, J., Bourke, P., Lonchamp, J. And Barry-Ryan, C., Impact of plant essential oils on microbiological, organoleptic and quality markers of minimally processed vegetables, Innovative Food Science & Emerging Technologies, 2009, 10, 195–202.
4. Salgueiro, L. R., Cavaleiro, C., Pinto, E. et al., Chemical composition and antifungal activity of the essential oil of *Origanum virens* on Candida species, Planta Medica, 2003, 69, 9, 871-874
5. Baydar et, A. L., Baydar, H., Sağdiç, O., Özkan, O. and Karadoğan, T., Antibacterial activity and composition of essential oils from Origanum, Thymbra and Satureja species with commercial importance in Turkey, Food Control, 2004, 15, 169–72.
6. Kačániová, M., Nováková, I. and Haščík, P., Význam probiotík a ich význam na mikroflóru gastrointestinálneho traktu hydiny. Nitra, SPU, 2011, 181 s. ISBN 978-80-552-0655-4
7. Čuboň, J., Haščík, P., Kačániová, M., Arpášová, H. and Prívara, Š., Vplyv podávania biologicky účinných látok na technologické a nutričné vlastnosti vybraných produktov živočíšneho pôvodu. Nitra, SPU, 2009, 116 s. ISBN 978-80-552-0291-4.
8. Arpášová, H., Fytobiotiká ako náhrada antibiotických stimulátorov rastu aich vplyv na úžitkovosť akvalitu vajec sliepok znáškového typu. Nitra, SPU, 2011, 101 s. ISBN 978-80-552-0555-7.
9. Zalacain et al., Optimalisation of extraction and identification of gallotannins from sumac leaves, Biosys. Eng. 2003, 84, 2, 211-216
10. Pavelková, A., Intelligent packaging as device for monitoring of risk factors in food, Journal of microbiology, biotechnology and food sciences, 2012, 2, 1, 282-292.
11. Dawson, R. C., Cox, J. M., Almond, A. and Moses, A. Food Safety Risk Management in Different Egg production System. Kingstone: Rural Industries Research and Development Corporation, 2001, pp. 50, ISBN 0 6242 58333 1.
12. Florou-Paneri, P., Nikolakakis, I., Giannenas, I., Koidis, A., Botsoglou, E., Dotas, V., Mitsopoulos, I., Hen performance and egg quality as affected by dietary oregano essential oil and tocopheryl acetate supplementation, International Journal of Poultry Science, 2005, 4, 7, 449-454.
9. Gündüz, G. T., Gönül, S. A. and Karapinar, M. 2010. Efficacy of sumac and oregano in the inactivation of *Salmonella typhimurium* on tomatoes, International Journal of Food Microbiology, 2010, 141, 1-2, 39-44.
13. Senköylü, N., Akyürek, H., Samli, H. E., and Yurdakurban, N., Performance and egg weight of laying hens fed on the diets with various by- product oils from the oilseed extraction refinery, Pakistan Journal of Nutrition, 2004, 3, 1, 38-42.
14. Yang, C. J., Uuganbayar, D., Sun, S. S. and Firman, J. D., Effect of dietary green tea on productivity and egg composition in laying hens, Animal Science Journal, 2003, 81, 204.
15. Canogullari, S., Karaman, M., Erdogan, Z., Baylan, M., Kucukgul, A., Duzguner, V. and Kemali Ozugur, A., Effect of garlic powder on egg yolk and serum cholesterol and performance of laying hens, Bulletin of the Veterinary Institute in Pulawy, 2009, 53, 3, 515-519.
16. Bozkurt, M., Kucukyilmaz, K., Catli, A. U., Cinar, M., Bintas, E. and Coven, F., Performance, egg quality, and immune response of laying hens fed diets supplemented with mannan-oligosaccharide or an essential oil mixture under moderate and hot environmental condi, Poultry Science, 2012, 91, 6, 1379-1386.
17. Yalcin, S., Onbasilar, E. E., Reisli, Z. and Yalcin, S., Effect of garlic powder on the performance, egg traits and blood parameters of laying hens, Journal of

the Science of Food and Agriculture, 2006, 86, 9, 1336–1339
18. Radwan Nadia, L., Hassan, R. A., Qota, E. M., Fayek, H. M., Effect of natural antioxidant on oxidative stability of eggs and productive and reproductive performance of laying hens, International Journal of Poultry Science, 2008, 7, 2, 134-150.
19. Yang, C. J., Uuganbayar, D., Sun, S. S., Firman and J. D., Effect of dietary green tea on productivity and egg composition in laying hens, Animal Science Journal, 2003, 81, 204.
20. Yodseranne, R., Chantsavang, S. and Bunchasuk, C. Effect of dietary supplementation of mixed culture compost, plantand animal bioextract compost on layer´s productive performance and egg quality, Animal and Veterinary Medicine, 2003, 119–126.
21. Botsoglou, N., Florou-Paneri, P., Botsoglou, E., Dotas, V., Giannenas, I., Koidis, A. and Mitrakos, P., The effect of feeding rosemary, oregano, saffron and alpha-tocopheryl acetate on hen performance and oxidative stability of eggs, South African Journal of Animal Science, 2005, 35, 3, 143-151.
22. Ayerza, R. and Coates, W., Dietary levels of chia: influence on hen weight, egg production and sensory quality, for two strains of hens, British of Poultry Science., 2002, 2, 283-290.
23. Aydin, R., Bal, A. M., Ozugur, K. A., Toprak, C. H. H., Kamalak, A., Karaman, M., Effects of black ssed (*Nigella sativa* L.) supplementation on feed efficiency, egg yield parameters and Shell Quality in Chickens, Pakistan Journal of Biological Sciences, 2006, 9, 2, 243-247.
24. Sahinler, S., Sahin, A., Gorgulu, O., Ferula eleaochytris powder effect in layer diet on feed intake and some egg parameters using a multivariate analysis method for repeated measures, Journal of Applied Animal Research, 2005, 27, 1, 29-33.