

# THE INFLUENCE OF THE PLANT ESSENTIAL OILS ON INTERNAL QUALITATIVE PARAMETERS AND MICROBIOLOGICAL INDICATORS OF HENS EGGS CONTENT

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## Abstract

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Essential oils are aromatic oily liquids obtained from plant material (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). Besides antibacterial properties, essential oils or their components have been shown to exhibit antiviral, antimycotic, antitoxigenic, antiparasitic, and insecticidal properties. In this experiment the effects of supplementation of the diet for laying hens with thyme and hyssop essential oils on physical and microbiological egg parameters were studied. Hens of laying hybrid ISA Brown ( $n = 72$ ) were randomly divided at the day of hatching into 3 groups ( $n = 26$ ) and fed for 41 weeks on diets which differed in kind of essential oil supplemented. In the first experimental group the feed mixture was supplemented with thyme essential oil addition  $0.25\text{ml.kg}^{-1}$ , in the second one got hyssop essential oil the same dose of  $0.25\text{ml.kg}^{-1}$ . The results suggest that the supplementation of thyme essential oil into laying hens diet statistically significantly increased egg albumen weight, egg albumen percentage portion ( $P < 0.05$ ) and egg yolk colour ( $9.07^a$ ,  $9.47^b$ ,  $9.06^a$ ;  $P < 0.01$ ), compared to the control group and decreased egg yolk percentage ratio. However, the most of qualitative parameters of internal content egg were not with thyme and hyssop essential oils addition significantly influenced. Significant differences in microbiological indicators were found among coliforms bacteria, enterococci, total number count ( $P < 0.001$ ), lactobacilli and mesophilic sporulating aerobes bacteria ( $P < 0.05$ ).

hens, thyme essential oil, hyssop essential oil, egg albumen, egg yolk, microbiological quality

Animal feed additives are used worldwide for many different reasons. Some of them help to cover the needs of essential nutrients and others to increase growth performance (Giannenas et al., 2005). The use of feed additives is more and more questioned by the consumers. Therefore, the feed industry is highly interested in valuable alternatives which could be accepted by the consumers. Probiotics, prebiotics, enzymes and highly available minerals as well as herbs can be seen as alternatives (Capcarova et al., 2008).

Herbs, spices and their extracts (botanicals) have a wide range of activities. They can stimulate feed in-

take and endogenous secretions or have antimicrobial, coccidiostatic or anthelmintic activity. The major field of application of herbs is the protection of animals and their products against oxidation (Wenk, 2003).

Essential oils (EOs) (also called volatile or ethereal oils) are aromatic oily liquids obtained from plant material (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). They can be obtained by expression, fermentation, enfleurage or extraction but the method of steam distillation is the most commonly used for commercial production of EOs (Burt, 2004). The term 'essential oil' is

thought to derive from the name coined in the 16th century by the Swiss reformer of medicine, Paracelsus von Hohenheim; he named the effective component of a drug *Quinta essentia*. An estimated 3000 EOs are known, of which about 300 are commercially important—destined chiefly for the flavours and fragrances market. It has long been recognised that some EOs have antimicrobial properties (Kačániová et al., 2005) and these have been reviewed in the past as have the antimicrobial properties of spices but the relatively recent enhancement of interest in 'green' consumerism has led to a renewal of scientific interest in these substances. Besides antibacterial properties, EOs or their components have been shown to exhibit antiviral, antimycotic, antitoxigenic, antiparasitic, and insecticidal properties. These characteristics are possibly related to the function of these compounds in plants (Burt, 2004). Some essential oils, such as thyme, may be more suitable as an acaricidal product than others, such as pennyroyal, for the use within a commercial poultry system for laying hens (Smith et al., 2009).

Numerous studies have been conducted to evaluate the bacterial contamination of shell eggs during production and processing by sampling eggs, equipment, feed and the hens' reproduction tracts (Jones et al., 2003). For obvious reasons, the majority of these studies were focused on incidence or levels of *Salmonella*, although a few reports have addressed other pathogenic microorganisms and spoilage bacteria (Knappe et al., 2002). Jones et al. (1995) found *Salmonella* on 72% of the environmental samples collected from hen houses, 7.8% of the egg shells before washing and 1.1% of the egg shells after washing. These researchers did not find *Salmonella* in the contents of any of the eggs that they evaluated (Jones et al., 1995). 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. When the cuticle or bloom is deposited by the hen on the shell this acts as a barrier to keep bacteria from entering the egg (Dawson et al., 2001). Knappe et al. (2002) reported that the aerobic plate counts from egg rinses decreased by 2.9 and 1.5 log cfu.ml<sup>-1</sup> for in-line and off-line eggs, 10 respectively when counts on eggs at the transfer belt were compared to counts on eggs after washing. While these studies and others like them have provided critical information regarding direct product contamination, little attention has been given to the areas of indirect product contamination.

The aim of this work was to observe the influence of thyme and hyssop essential oil addition on qualitative parameters of yolk and albumen of laying hens eggs of hybrid ISA Brown in pilot system. The microbiological indicators monitoring Count of coliforms bacteria, count of *Escherichia coli*, count of *Enterococcus*, *Lactobacillus*, total count of microorganisms, count of mesophilic anaerobic sporulating microorganisms and count of microscopic fungi were observed.

## MATERIALS AND METHODS

### Animals, diets and treatments

Hens (n = 78) of the laying hybrid ISA Brown were randomly divided at the day of hatching into 3 groups (n = 26) and fed for 41 weeks with diet containing different kinds of essential oils, only. The composition of the basal diet (BD) fed to the laying hens is shown in Tab. I.

I: The composition of the basal diet

Component	g.kg <sup>-1</sup>
Wheat ground (10.5% CP)	392
Soybean oil	7
Maize ground (8.3% CP)	230
Soybean extracted ground meal (45% CP, 1.5% fat)	192
Limestone	74
Vitamin-mineral premix	40
Pea ground	35
Rape extrusion	30

1kg of basal diet contained: IU: vit. A, 14071; vit. D3, 3400; m.j.: vit. K, 4.47; thiamine, 6.37; riboflavin, 7.47; pyridoxine, 43.97; cyanocobalamin, 0.35µg; niacin, 67; panthothenic acid, 18.21; biotin, 0.15; folic acid, 0.94; lysine, 8.7; methionine, 3.600; Se, 0.1mg; Zn, 85.21; I, 1.00mg; Co, 0.38mg; Mn, 116.13; Cu, 14.96; Fe, 210.55mg

At the beginning of the experiment, the chickens were placed in a single-level cage battery in groups. After rearing till the age of 4 months, the hens were kept in the three – deck cage technology system, model AGK 200/616. 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<sup>2</sup>. 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. Hens were fed from 1<sup>st</sup> day by the standard feed mixture HYD-10. Laying hens accepted fodder *ad libitum*. In the control group hens received feed mixture without additions, in the first experimental group the feed mixture was supplemented with thyme essential oil addition 0.25ml.kg<sup>-1</sup>; in the second one got hyssop essential oil the same dose of 0.25ml.kg<sup>-1</sup> (*Calendula a.s.* Nová Lubovňa, SR).

### Sample analysis

Eggs of laying hens of hybrid ISA Brown were collected regularly once a month (n = 30 per group) and were assessed immediately after collection. The egg albumen weight (g), egg albumen content (%), egg albumen index, Haugh units (HU), egg yolk weight (g), egg yolk content (%), egg yolk index and egg yolk color (°HLR) were evaluated.

Weight parameters were detected using analytical weighing machine owa labor 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 - 1.7 x egg weight<sup>0.37+7.6</sup>)]. Yolk color was evaluated using Hoffman la Roche color scale (Hoffman-La Roche, Switzerland).

### 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: 5g of egg content was added to the test tube containing 45ml of distilled water. The cells were separated from substrate by shaking on a horizontal shaker (TEIII. Chirana, Piešťany) (30 minutes). In this way prepared basic substance was diluted on to reduce the content of microorganisms on the level below 300 cfu.

#### Media and culture conditions

The number of coliforms bacteria was grown in Endo agar (aerobiosis), at the temperature 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 (microaerophilic), at 37°C during 72 hours. The total number of bacteria was grown in GTK agar (aerobiosis), at 37°C during 48 hours. The mesophilic sporulating aerobes microorganisms were grown in MPA agar (aerobiosis), at 37°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 into 45ml sterile tap-water containing 0.02% Tween 80 and then 30 min shaken. 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<sup>-1</sup> counts in samples were determined after 5 days of incubation.

#### Statistical analysis

The results of observation were evaluated by the statistical programme ANOVA, one-way analysis

of variance with the post hoc Tukey multiple comparison tests with use of Statgraphics.

### RESULTS

Tab. II presents the changes in the egg albumen quality parameters with plant essential oil addition to feed.

In the egg albumen weight ( $P < 0.05$ ) were found the statistically significant differences between the experimental group with thyme essential oil and control group as well as between the experimental group with thyme essential oil and experimental group with hyssop essential oil.

Similar percentage portion of egg albumen was statistically significantly affected by thyme essential oils supplementation ( $P < 0.01$ ). Statistically significant differences ( $P < 0.01$ ) between the experimental groups were found.

Albumen index was not statistically significantly affected by essential oils supplementation ( $P > 0.05$ ).

The Haugh units (HU) score revealed a higher quality of egg albumen in the experimental group of layer hens fed the hyssop essential oil addition. No significant differences ( $P > 0.05$ ) were found.

Tab. III presents the changes of ISA Brown laying hen's egg yolk quality. The egg yolk index value was not affected statistically significantly after essential oils application ( $P > 0.05$ ).

The egg yolk percentage portion was significantly decreased ( $P < 0.05$ ) in the experimental group at thyme essential oil addition. Statistically significant differences ( $P < 0.05$ ) between the experimental groups were found as well.

The egg yolk colour (°HLR) of analyzed eggs in experimental group receiving thyme essential oil enriched diet was significantly higher (9.07<sup>a</sup>, 9.47<sup>b</sup>, 9.06<sup>a</sup>;  $P < 0.01$ ), respectively.

The results of egg microbiological quality in control and experimental groups are shown in Tab. IV. The highest count of coliforms bacteria was determined in control group and the lowest count of coliforms bacteria was found in group with hyssop essential oil. The number of coliforms bacteria was confirmed by significant differences ( $P < 0.001$ ) among experimental groups and control group. No significant differences ( $P > 0.05$ ) were found between experimental groups.

The highest number of count *Escherichia coli* were monitored in control group and the lowest number in group with thyme essential oil.

The number of enterococci ranged from 0.154 log cfu.g<sup>-1</sup> in group with thyme essential oil to 2.074 log cfu.g<sup>-1</sup> in control group. The number of enterococci showed significant differences ( $P < 0.001$ ) between experimental group with thyme essential oil and control group and significant differences ( $P < 0.001$ ) were found between experimental groups. Similar differences ( $P < 0.05$ ) between experimental and control groups at the number of lactobacilli were found.

II: Influence of thyme and hyssop essential oil addition into laying hens feed mixture on the alterations of ISA Brown laying hen's egg weight and egg albumen quality

Group	BD - Control group	BD + thyme essential oil 0.25ml.kg <sup>-1</sup>	BD + hyssop essential oil 0.25ml.kg <sup>-1</sup>	SEM
<b>Egg weight (g)</b>				
mean	58.32	58.74	58.19	0.42
S.D.	4.96	4.96	5.35	
CV (%)	8.50	8.45	9.19	
minimum	46.1	43.9	44.0	
maximum	71.3	71.8	70.1	
<b>Egg albumen weight (g)</b>				
mean	37.98 <sup>a</sup>	38.67 <sup>b</sup>	37.87 <sup>a</sup>	0.26
S.D.	3.18	3.24	3.40	
CV (%)	8.37	8.39	8.98	
minimum	30.10	29.4	28.40	
maximum	48.80	47.8	47.80	
<b>Percentage portion of albumen (%)</b>				
mean	65.18 <sup>a</sup>	65.87 <sup>b</sup>	65.13 <sup>a</sup>	0.18
S.D.	2.23	2.25	2.11	
CV (%)	3.43	3.42	3.24	
minimum	59.4	61.1	57.34	
maximum	71.86	70.9	72.21	
<b>Egg albumen height (mm)</b>				
mean	7.95	7.91	7.71	0.10
S.D.	1.12	1.21	1.30	
CV (%)	14.05	15.32	16.94	
minimum	4	4	4	
maximum	11	11	11	
<b>Egg albumen index</b>				
mean	11.57	11.52	11.33	0.18
S.D.	1.86	2.28	2.37	
CV (%)	16.04	19.79	20.95	
minimum	4.94	5.16	5.84	
maximum	15.39	18.03	17.70	
<b>Haugh units of egg albumen (HU)</b>				
mean	84.97	88.33	86.67	0.57
S.D.	8.66	6.87	5.41	
CV (%)	10.19	7.79	6.25	
minimum	59.38	76.21	76.52	
maximum	99.42	103.36	98.28	

Distinct letters in superscripts within a row mean significant differences ( $P < 0.05$ );  $n = 150$ ; Values are means.

The number of lactobacilli ranged from 1.156 log cfu.g<sup>-1</sup> in group with thyme essential oil to 1.570 log cfu.g<sup>-1</sup> in control group and hyssop essential oil as well. The highest total number of bacteria was found in control group and the lowest count was in group with hyssop essential oil. Significant differences ( $P < 0.05$ ;  $P < 0.001$ ) at the total number of bacteria were found between experimental groups and control group.

The lowest number of mesophilic sporulating aerobes bacteria was found in group with thyme es-

sential oil and the highest count was in control group and in group with hyssop essential oil. Significant differences ( $P < 0.05$ ) of mesophilic sporulating aerobes bacteria were found between experimental group with hyssop essential oil and control group.

Number of microscopic fungi ranged from 0.079 log cfu.g<sup>-1</sup> in group with thyme essential oil to 0.585 log cfu.g<sup>-1</sup> in group with hyssop essential oil.

No significant differences ( $P > 0.05$ ) were found at the number of count *Escherichia coli* and number of microscopic fungi.

## III: Influence of thyme and hyssop essential oil addition on the alterations of ISA Brown laying hen's egg yolk quality

Group	BD - Control group	BD + thyme essential oil 0.25ml.kg <sup>-1</sup>	BD + hyssop essential oil 0.25ml.kg <sup>-1</sup>	SEM
<b>Egg yolk weight (g)</b>				
mean	14.62	14.50	14.64	0.17
S.D.	2.04	1.96	2.07	
CV (%)	13.98	13.50	14.10	
minimum	9.40	10.20	9.10	
maximum	18.50	17.90	19.60	
<b>Percentage portion of egg yolk (%)</b>				
mean	25.00 <sup>a</sup>	24.64 <sup>b</sup>	25.09 <sup>a</sup>	0.18
S.D.	2.24	2.17	2.05	
CV (%)	8.95	8.80	8.15	
minimum	17.64	20.04	18.46	
maximum	30.27	29.30	30.89	
<b>Egg yolk index</b>				
mean	52.29	52.13	51.90	0.52
S.D.	5.68	7.43	5.94	
CV (%)	10.88	14.26	11.44	
minimum	40.47	41.3	40.0	
maximum	65.63	68.75	65.60	
<b>Egg yolk color (°HLR)</b>				
mean	9.07 <sup>a</sup>	9.47 <sup>b</sup>	9.06 <sup>a</sup>	0.16
S.D.	2.11	1.92	2.00	
CV (%)	23.26	20.33	22.15	
minimum	5	6	6	
maximum	13	13	12.5	

Distinct letters in superscripts within a row mean significant differences ( $P < 0.05$ );  $n = 150$ ; Values are means. °HLR – colored Hoffman La Roche scale.

## IV: Influence of thyme and hyssop essential oil addition into laying hens on the alterations of ISA Brown laying hen's egg microbiological quality

Group	BD - Control group	BD + thyme essential oil 0.25ml.kg <sup>-1</sup>	BD + hyssop essential oil 0.25ml.kg <sup>-1</sup>	SEM
<b>Count of coliforms bacteria (log cfu.g<sup>-1</sup>)</b>				
mean	2.46 <sup>a</sup>	0.95 <sup>b</sup>	0.32 <sup>b</sup>	0.12
S.D.	0.31	0.75	0.37	
CV (%)	12.42	212.03	109.66	
minimum	1.95	0.00	0.00	
maximum	2.03	1.94	0.95	
<b>Count of <i>Escherichia coli</i> (log cfu.g<sup>-1</sup>)</b>				
mean	0.35	0.07	0.30	0.17
S.D.	0.12	8.22	0.58	
CV (%)	333.94	316.23	195.70	
minimum	0.00	0.00	0.00	
maximum	2.51	0.70	1.80	
<b>Count of enterococci (log cfu.g<sup>-1</sup>)</b>				
mean	2.07 <sup>a</sup>	0.15 <sup>b</sup>	1.70 <sup>a</sup>	0.19
S.D.	0.51	0.52	0.75	
CV (%)	24.40	61.28	44.04	
minimum	1.30	0.00	0.60	
maximum	2.88	1.87	2.80	

Group	BD - Control group	BD + thyme essential oil 0.25ml.kg <sup>-1</sup>	BD + hyssop essential oil 0.25ml.kg <sup>-1</sup>	SEM
<b>Number of lactobacilli (log cfu/g)</b>				
mean	1.57 <sup>a</sup>	1.16 <sup>b</sup>	1.57 <sup>a</sup>	0.15
S.D.	0.57	0.25	0.57	
CV (%)	36.11	21.63	36.11	
minimum	0.69	0.78	0.69	
maximum	2.32	1.53	2.32	
<b>Total number count (log cfu.g<sup>-1</sup>)</b>				
mean	3.225 <sup>a</sup>	2.233 <sup>b</sup>	2.075 <sup>b</sup>	0.19
S.D.	0.38	1.04	0.41	
CV (%)	11.69	46.92	19.67	
minimum	2.49	0.00	1.43	
maximum	3.70	3.42	2.54	
<b>Count of mesophilic sporulating aerobes bacteria (log cfu.g<sup>-1</sup>)</b>				
mean	2.436 <sup>a</sup>	2.242 <sup>b</sup>	2.437 <sup>a</sup>	0.19
S.D.	0.593	0.989	0.186	
CV (%)	26.179	44.112	7.633	
minimum	2.29	0.17	2.09	
maximum	3.67	3.30	2.67	
<b>Count of microscopic fungi (log cfu.g<sup>-1</sup>)</b>				
mean	0.578	0.079	0.585	0.20
S.D.	0.975	0.128	0.804	
CV (%)	168.781	163.03	136.75	
minimum	0.00	0.00	0.00	
maximum	2.42	0.39	2.13	

Distinct letters in superscripts within a row mean significant differences ( $P < 0.05$ );  $n = 10$ ; Values are means.

## DISCUSSION

The results of this experiment indicated that the majority of parameters of egg internal content quality were not significantly influenced by supplementation of laying hens with thyme and hyssop essential oils.

Bölükbaşı and Erhan (2007) found that feed conversion and egg productions of laying hens were improved by thyme supplementation at level 0.1 and 0.5%.

In our experiment was egg albumen weight positively influenced by thyme essential oil addition. Contrary Sahinler et al. (2005) showed that egg albumen weight, egg weight, and egg production were not affected by *Ferula eleaocytris* root powder treatment. Yucca supplementation to the diet did not affect feed intake, egg production, feed conversion efficiency, body weight gain, egg shell, white weight, shell thickness and shape index but significantly reduced number of cracked eggs in experiment of Kutlu et al. (2001).

The addition of flax seed to diets in experiment of Basmacioglu et al. (2003) did not cause any negative effect on albumen ratio, egg yolk and egg shell qualitative parameters. The egg production of hens fed a diet containing 4.32% flax seed was significantly higher than the controls. The percentage portion of

albumen was in our experiment significantly higher in experimental group with thyme essential oil supplementation.

Alike Yalcin et al. (2006), or Yalcin et al. (2007) reported no significant effect on egg production, egg shell quality and egg albumen index by garlic powder addition.

There were observed no significant differences in albumen index and yolk index of eggs for layers fed by diets containing green tea powders ( $P > 0.05$ ) in experiment of Uganbayar et al. (2006). These results are in agreement with findings of our experiment.

The addition of 3 ml.kg<sup>-1</sup> *Nigella sativa* oil to laying hens feed led to a significant decrease in the Haugh unit of the egg in experiment of Bölükbaşı et al. (2009).

In our experiment was not recorded any significant differences among the groups in Haugh Units ( $P > 0.05$ ). Our results are in agreement with Yalcin et al. (2006), or Uganbayar et al. (2006) in the experiment of which garlic powder addition showed no significant difference. Hosseini et al. (2008) present the lowest Haugh unit and shell thickness with 10% safflower seed supplementation but the different treatments were not statistically significant.

In the experiment of Sahinler et al. (2005) egg yolk weight was not affected by *Ferula eleaocytris* supplementation, this is in agreement with results of our experiment. Yalcin et al. (2006) recorded in this parameter no significant influence of phytobiotics complement too. There was a significant reduction in egg yolk cholesterol concentration when the dietary level of garlic powder was increased from 0 to 10 g.kg<sup>-1</sup>. Yucca supplementation to the diet did not affect yolk weight (Kutlu et al., 2001).

The addition of flax seed to diets did not cause any negative effect on egg yolk ratio in experiment of Basmacioglu et al. (2003). There were significant effects of dietary treatments on yolk rate of egg in experiment Bölükbaşı and Erhan (2007). Hens receiving the diets containing 1% thyme had significantly lower yolk rate compared to those fed the control and the diet containing 0.1 and 0.5% thyme. These results are in accordance to our experiment in which thyme essential oil addition significantly decreased yolk rate.

Supplementation of poultry diets with antioxidant substances seems to be an efficient tool for improving the oxidative stability of eggs. The effect of dietary tocopheryl acetate supplementation on enhancing lipid stability in egg yolk has been repeatedly reported (Lopez-Bote et al., 1998). The use of aromatic plant extracts including thyme (Botsoglou et al., 1997) and rosemary (Galobart et al., 2001) have also been demonstrated to delay lipid oxidation in eggs when used in hen feeding.

Results of Botsoglou et al. (2005) showed no significant differences in egg production, feed intake, feed conversion ratio, egg weight and shape, yolk shape, Haugh units and shell thickness among treatments. Similarly in the experiment of Yalcin et al. (2006) garlic powder addition did not significantly affect egg yolk index. In the experiments of Botsoglou et al. (2005) or Uganbayar et al. (2006) results showed no significant differences in yolk index. In our experiment was not recorded significant different among the groups too.

In the experiment Botsoglou et al. (2005) yolk color was significantly improved in the saffron at 20 mg.kg<sup>-1</sup> diet SAF group compared to all other groups. These results agree with our experiment, in

which thyme essential oil addition significantly increased egg yolk color.

Possible transfer of the antioxidant constituents of the essential oil of oregano or tocopherol into hen organism through feeding might inhibit the chain reaction involved in oxidation of the consumed lipids, thus decreasing the oxidation products transferred into the yolk (Florou-Paneri et al., 2005). The higher malondialdehyde found increased oxidative stability of egg yolks when a thyme extract was fed to laying hens (Botsoglou et al., 1997). In pertinent studies with extracts of other aromatic plants, Galobart et al. (2001) found that the dietary supplementation of a rosemary extract to laying hens had no effect on lipid oxidation of eggs. In contrast, Krause and Ternes (2000) and Botsoglou et al. (1997) observed an improvement of the oxidative stability of egg yolk when carnolic acid, the main antioxidant constituent of rosemary, and a thyme extract, respectively, were used as dietary supplement in laying hens.

In our study were found all microorganisms which were monitored and these results are not in agreement with others authors.

Dawson et al. (2001), reported, that microbiological contamination, quality and factors of albumen and yolk are very important. Egg white contains a low concentration of the enzyme lysozyme. This enzyme has been shown to have the capability of breaking down the cell walls of some bacteria. Egg white also has a high pH which acts as a retardant for bacteria growth. Other enzymes are also found in egg yolk (peptidase, catalase, amylase, etc.) which help to keep it free from bacteriological contamination. In addition, the egg yolk has a coating called the vitelline membrane which also protects it. Thus, egg albumen and yolk have many defense mechanisms which help prevent microbiological contamination. Dawson et al. (2001) also state, that when eggs are washed, this removes most if not all of the cuticle from the shell surface. Thus, bacteria have an easier time entering the egg after washing. Even when the cuticle is removed, the two inner shell membranes help prevent bacteria from entering the egg. These barriers provide a good line of defense against invading bacteria.

## SÚHRN

### Vplyv rastlinných silíc na parametre a mikrobiologickú kvalitu vnútorného obsahu vajec nosníc

Cieľom práce bolo sledovanie účinkov krmív pre nosnice s prídavkom tymianovej a yzopovej silice v množstve 0.25 ml.kg<sup>-1</sup> na hmotnosť vajec, vaječných bielkov a kvality žltka. V práci bola ďalej sledovaná mikrobiologická kvalita vajec po aplikácii esenciálnych olejov do krmiva. Nosnice hybridu ISA Brown (n = 72) boli náhodne rozdelené v deň vyliahnutia do troch skupín (n = 26) a kŕmené po dobu 41 týždňov s rozdielnym druhom pridaného esenciálneho oleja. Vajcia nosníc boli zbierané pravidelne, v denných intervaloch a raz mesačne (n = 30 v každej skupine) boli bezprostredne po odbere analyzované. Hodnotená bola hmotnosť vaječného bielka (g), obsah vaječného bielka (%), index vaječného bielka, Haughove jednotky (HU), hmotnosť vaječného žltka (g), obsah vaječného žltka (%), index vaječného žltka a farba žltka (°HLR). Z mikrobiologických parametrov boli sledované počty koliformných baktérií, počty buniek *Escherichia coli*, počty buniek rodu *Enterococcus*, *Lactobacillus*, celkový počet

mikroorganizmov, počet mezofilných anaeróbne sporujúcich mikroorganizmov a počet mikroskopických húb. Štatisticky významné rozdiely boli zistené medzi experimentálnymi skupinami a v skupine s prídavkom tymiánovej silice a kontrolnou skupinou v hmotnosti vaječného bielka ( $P < 0,05$ ). Podobne percento vaječných bielkovín bolo štatisticky významne ovplyvnené prídavkom tymiánovej silice ( $P < 0,01$ ). Vyššie hodnoty Haughových jednotiek (HU) ukázali vyššiu kvalitu vaječných bielkovín v experimentálnej skupine nosníc kŕmených zmesou s tymiánovou aj yzopovou silicou, ale rozdiely neboli v porovnaní s kontrolnou skupinou štatisticky významné ( $P > 0,05$ ). V indexe vaječného bielka a indexe hodnoty žltka neboli dosiahnuté štatisticky významné rozdiely po aplikácii éterických olejov ( $P > 0,05$ ). Percentuálny podiel vaječného žltka sa výrazne znížil ( $P < 0,05$ ) v experimentálnej skupine s prídavkom tymiánovej silice. Farba vaječného žltka ( $^{\circ}$  HLR) analyzovaných vajec v experimentálnej skupine obohatených tymiánovým éterickým olejom bola významne vyššia ( $P < 0,01$ ). Najvyšší počet koliformných baktérií bol zistený v kontrolnej skupine a najnižší počet koliformných baktérií bol zistený v skupine s yzopovým éterickým olejom. V počte koliformných baktérií boli potvrdené štatisticky významné rozdiely ( $P < 0,001$ ) u experimentálnych skupín v porovnaní s kontrolnou skupinou. Najvyšší počet buniek *Escherichia coli* bol zaznamenaný v kontrolnej skupine, a najnižší počet bol v skupine s tymiánovou silicou. Počet enterokokov v skupine s tymiánovým éterickým olejom bol  $0,154 \log \text{ cfu.g}^{-1}$ , najvyšší počet a to  $2,074 \log \text{ cfu.g}^{-1}$  bol zaznamenaný v kontrolnej skupine. Počet enterokokov ukázal štatisticky významné rozdiely ( $P < 0,001$ ) medzi experimentálnymi skupinami, významné rozdiely ( $P < 0,001$ ) boli zistené aj medzi experimentálnou skupinou s prídavkom tymiánovej silice a kontrolnou skupinou. Podobné rozdiely boli zistené v počte laktobacilov medzi experimentálnymi skupinami a kontrolnou skupinou ( $P < 0,05$ ). Počty laktobacilov sa pohybovali v rozmedzí od  $1,156 \log \text{ cfu.g}^{-1}$  v skupine s tymiánovým éterickým olejom do  $1,570 \log \text{ cfu.g}^{-1}$  v kontrolnej skupine a tiež v skupine s yzopovým éterickým olejom. Najvyšší celkový počet baktérií bol zistený v kontrolnej skupine a najnižší počet bol v skupine s yzopovým éterickým olejom. Štatisticky významné rozdiely ( $P < 0,05$ ;  $P < 0,001$ ) boli zistené v celkovom počte baktérií medzi experimentálnymi skupinami a kontrolnou skupinou. Najnižší počet mezofilne aeróbne sporujúcich baktérií sa zaznamenal v skupine s tymiánovým éterickým olejom, vyšší počet bol v kontrolnej skupine a v skupine s yzopovým éterickým olejom. Významné rozdiely ( $P < 0,05$ ) v počte mezofilne anaeróbne sporujúcich baktérií boli zistené medzi experimentálnou skupinou s tymiánovým éterickým olejom a kontrolnou skupinou, ako aj medzi experimentálnymi skupinami navzájom. Počet mikroskopických húb bol v rozmedzí od  $0,079 \log \text{ cfu.g}^{-1}$  v skupine s tymiánovou silicou do  $0,585 \log \text{ cfu.g}^{-1}$  v skupine s yzopovou silicou.

slepký, tymiánový esenciálny olej, yzopový esenciálny olej, bielok, žltok, mikrobiálna kvalita

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## REFERENCES

- ARPASOVA, H., CAPCAROVA, M., KALAFOVA, A., LUKAC, N., KOVACIK, J., FORMICKY, G., MASSANYI, P., 2007: Nickel induced alteration of hen body weight, egg production and egg quality after an experimental peroral administration. *Journal of Environmental Science and Health*, 42, 8: 913–918. ISSN: 0360-1234.
- BASMACIOGLU, H., CABUK, M., UNAL, K., OZKAN, K., AKKAN, S., YALCIN, H., 2003: Effects of dietary fish oil and flax seed on cholesterol and fatty acid composition of egg yolk and blood parameters of laying hens. *South African Journal of Animal Science*, 33, 4: 266–273. ISSN: 0375-1589.
- BÖLÜKBAŞI, S. C., ERHAN, M. K., 2007: Effect of Dietary Thyme (*Thymus vulgaris*) on Laying Hens Performance and *Escherichia coli* (*E. coli*) Concentration in Feces. *International Journal of Natural and Engineering Sciences*, 1, 2: 55–58. ISSN: 1307-1149.
- BÖLÜKBAŞI, S. C., KAYNAR, O., ERHAN, M. K., URUPAN, H., 2009: Effect of feeding *Nigella sativa* oil on laying hen performance, cholesterol and some proteins ratio of egg yolk and *Escherichia coli* count in feces. *Archiv für Geflügelkunde*, 73, 3: 167–172. ISSN: 0003-9098.
- BOTSGLOU, N. A., YANNAKOPOULOS, A. L., FLETOURIS, D. J., TSERVENI-GOUSSI, A. C., FORTOMARIS, P., 1997: Effect of dietary thyme on the oxidative stability of egg yolk. *Journal of Agricultural and Food Chemistry*, 45, 10: 3711–3716. ISSN: 0021-8561.
- BOTSOGLOU, N., FLOROU-PANERI, P., BOTSGLOU, E., DOTAS, V., GIANNENAS, I., KOIDIS, A., MITRAKOS, P., 2005: 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*, 35, 3: 143–151. ISSN: 0375-1589.
- BURT, S., 2004: Essential oils: their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology*, 94, 33: 223–253. ISSN: 0168-1605.
- CAPCAROVÁ, M., KOLESÁROVÁ, A., MASSANYI, P., KOVÁČIK, J., 2008: Selected Blood Biochemical and Haematological Parameters in Turkeys after an Experimental Probiotic *Enterococcus*

- faecium* M – 74 Strain Administration. *International Journal of Poultry Science*, 7, 12: 1194–1199. ISSN: 1682-8356.
- DAWSON, R. C., COX, J. M., ALMOND, A., MOSES, A., 2001: Food Safety Risk Management in Different Egg production System. Kingstone, *Rural Industries Research and Development Corporation*, 50.
- FLOROU-PANERI, P., NIKOLAKAKIS, I., GIANNENAS, I., KOIDIS, A., BOTSOGLOU, N., DOTAS, V., MITSOPOULOS, I., 2005: Hen performance and egg quality as affected by dietary oregano essential oil and tocopheryl acetate supplementation. *International Journal of Poultry Science*, 4, 7: 449–454. ISSN: 1682-8356.
- GIANNENAS, I. A., FLOROU-PANERI, P., BOTSOGLOU, N. A., CHRISTAKI, E., SPAIS, A. B., 2005: Effect of supplementing feed with oregano and/or alpha-tocopheryl acetate on growth of broiler chickens and oxidative stability of meat. *Journal of Animal and Feed Sciences*, 14, 3: 521–535. ISSN: 1230-1388
- GALOBART, J., BARCHETA, A. C., BAUCCELLS, M. D., CODONY, R., TERNES, D. W., 2001: Effect of dietary supplementation with rosemary extract and  $\alpha$ -tocopheryl acetate on lipid oxidation in eggs enriched with T3-fatty acids. *Poultry Science*, 80, 4: 460–467. ISSN: 0032-5791.
- HOLT, G. J., KRIEG, N. R., SNEATH, P. H. A. et al., 1994: *Bergey's Manual of Determinative Bacteriology*. Baltimore: Williams and Wilkins, 787.
- HOSSEINI VASHAN, S. J., AFZALI, N., MALLEKANEH, M., NASSERI, M. A., ALLAHRESAN, A., 2008: The Effect of Different Concentrations of Safflower Seed on Laying Hen's Performance, Yolk and Blood Cholesterol and Immune System. *International Journal of Poultry Science*, 7, 5: 470–473. ISSN: 1682-8356.
- JONES, F. T., RIVES, D. V., CAREY, K. B., 1995: *Salmonella* contamination in commercial eggs and an egg production facility. *Poultry Science*, 74, 4: 753–757. ISSN: 0032-5791.
- JONES, D. R., NORTHCUTT, J. K., MUSEGROVE, M. T., CURTIS, P. A., ANDERSON, K. E., FLETCHER, D. L., COX, N. A., 2003: Survey of shell egg processing plant sanitation programs: Effects on egg contact surfaces. *Journal of Food Protection* 66, 8: 1486–1489. ISSN: 0362-028X.
- KAČÁNIOVÁ, M., BOBČEK, R., KMEŤ, V., ANGELOVIČOVÁ, M., 2005: *Feed supplements as a substitute for antibiotics and other applications*. Nitra: SPU, 78 p.
- KNAPE, K. D., CHAVES, C., BURGESS, R. P., COUFAL, S. D., CAREY, J. B., 2002: Comparison of egg-shell surface microbial populations for in-line and off-line commercial egg processing facilities. *Poultry Science* 81, 5: 95–698. ISSN: 0032-5791.
- KRAUSE, E., TERNES, W., 2000: Bioavailability of the antioxidative Rosmarinus officinalis compound carbonic acid in eggs. *European Food Research and Technology*, 210, 3: 161–164. ISSN: 1438-2377.
- KUTLU, H. R., GORGULU, M., UNSAL, I., 2001: Effects of dietary *Yucca schidigera* powder on performance and egg cholesterol content of laying hens. *Journal of Applied Animal Research*, 20, 1: 49–56. ISSN: 0971-2119.
- LOPEZ-BOTE, C. J., GRAY J. I., GOMAA, E. A., FLEGAL, C. J., 1998: Effect of dietary administration of oil extracts from rosemary and sage on lipid oxidation in broiler meat. *British Poultry Science*, 39, 2: 235–240. ISSN: 0007-1668.
- SAHINLER, S., SAHIN, A., GORGULU, O., 2005: Ferula eleoachyris 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*, 27, 1: 29–33. ISSN: 0974-1844.
- SMITH, T. J., GEORGE, D. J., SPARAGANO, O. A. E., SEAL, C., SHIEL, R. S., GUY, J. V., 2009: A pilot study into the chemical and sensorial effect of thyme and pennyroyal essential oil on hens eggs. *International Journal of Food Science and Technology*, 44, 9: 1836–1842. ISSN: 0950-5423.
- UGANBAYAR, D., SHIN, I. S., YANG, C. J., 2006: Comparative performance of hens fed diets containing Korean, Japanese and Chinese green tea. *Asian-Australasian Journal of Animal Sciences*, 19, 8: 1190–1196. ISSN: 1011-2367.
- WENK, C., 2003: Herbs and botanicals as feed additives in monogastric animals. *Asian-Australasian Journal of Animal Sciences*, 16, 2: 282–289. ISSN: 1011-2367.
- YALCIN, S., ONBASILAR, E. E., REISLI, Z., YALCIN, S., 2006: Effect of garlic powder on the performance, egg traits and blood parameters of laying hens. *Journal of the Science of Food and Agriculture*, 86, 9: 1336–1339. ISSN: 0022-5142.
- YALCIN, S., ONBASILAR, I., ONBASILAR, I., SEHU, A., YALCIN, S., 2007: The effects of dietary garlic powder on the performance, egg traits and blood serum cholesterol of laying quails. *Asian-Australasian Journal of Animal Sciences*, 20, 6: 949–947. ISSN: 1011-2367.

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