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REGULAR ARTICLE

EFFECT OF THYME ESSENTIAL OIL ADDITION ON PHYSICAL AND MICROBIOLOGICAL QUALITY OF TABLE EGGS

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ABSTRACT

Essential oils are intensive fragrant, oily liquid substances contained in different parts of the plant. Their function is based on organoleptic effect and stimulation of organism to the production of digestive juices. Result is a higher digestibility and absorption of nutirents. 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 essential oils 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 23 weeks on diets with thyme essential oil supplemented. In the first experimental group the feed mixture was supplemented with thyme essential oil addition in a dose 0.5 g/kg, in the second one some essential oil in a dose 1g/kg. The results suggest that all of qualitative parameters of egg

internal content (yolk weight (g), yolk index, percentage portion egg yolk (%), yolk index, yolk colour (°HLR), albumen weight (g), percentage portion of albumen (%), Haugh Units (HU), albumen index) were with thyme essential oil addition insignificantly influenced (P>0.05). The number of coliforms, enterococci, fungi and yeasts decreased with increasing dose of oil. The number of lactobacilli was zero in all groups.

Key words: laying hens**,** thyme essential oil, egg yolk, egg albumen, microbiological quality

INTRODUCTION

Phytobiotics is a term used to describe the natural bioactive substances of plant origin, which affect the growth and health of the animal. They are often applied in the form of essential oils and plant extracts **(Friedman, 2007).**

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 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 on physiological needs and well-being of animals, and, above all on consumer's preferences and expectations **(Hashemi and Davoodi, 2011).** According to the EU directive relating to feed, they are a group of very aromatic and flavor compounds **(Recoquillay, 2006).** Phytobiotics have the ability to influence important physiological processes in the animal organism. They may affect the intensification of pulses sent through taste and smell to the central nervous system **(Gunther, 1990).**

Based on the results of the work of several authors, essential oils in poultry affect feed intake, weight gain, nutrient utilization **(Amrik and Bilkei, 2004),** egg weight **(Abd El-**

Motaal *et al.* (2008), egg production, body weight of laying hens **(Yannakopoulos *et al.*, 2005; Suchý *et al.*, 2010; Arpášová, 2011),** and feed intake **(Angelovičová *et al.*, 2010).**

Microbiological quality may be determined through reactions between indicators included within the package and metabolites which are produced during microbial growth (**Pavelková, 2012**). Shelf life of foods is dependent on many factors, internal as pH, water activity, nutrient content, occurrence of antimicrobial agents, redox potential, properties of biological structures, such as temperature and external storage, relative humidity, atmospheric composition. These factors have a direct effect on the chemical, biochemical, physical and microbiological spoilage mechanisms of individual foods and their durability (**Pavelková and Flimelová, 2012**).

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. The aerobic plate counts from egg rinses decreased by 2.9 and 1.5 log cfu.ml^{-1} 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 have provided critical information regarding direct product contamination, little attention has been given to the areas of indirect product contamination **Knape *et al.* (2002).**

The aim of this work was to observe the influence of thyme essential oil addition on qualitative parameters of yolk and albumen of laying hens eggs of hybrid Hy-Line Brown in pilot system. The microbiological indicators monitored count of coliforms bacteria, count of *Enterococcus, Lactobacillus,* total count of microorganisms, count of microscopic fungi and yeasts.

MATERIAL 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 23 weeks with diet containing of different amounts of thyme essential oils. 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*. Equipment of cage consisted of 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.

The composition of the basal diet (BD) fed to the laying hens is shown in Tab. 1 and Tab. 2. Analysis of feed mixture was realized at the Department of Animal Nutrition of the SUA in Nitra.

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, N = natrium

In the control group hens received feed mixture with any additions. The diets in the first, and the second experimental groups were supplemented with 0.5 and 1 g/kg oregano essential oil (Calendula a.s. Nová Ľubovňa, SR). Laying hens accepted fodder *ad libitum*. Essential oils used in the experiment were in the feed mixture homogeneously incorporated in the feed mill.

Sample Analysis

Eggs of laying hens of Hy-Line Brown strain were collected regularly once 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 obtained data. Indexes were calculated as the lenght : width ratio. Haught units 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).

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 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 were grown in PDA 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.

RESULTS AND DISCUSSION

The average egg weight and yolk quality parameters in each group for the monitored laying period reflects table 3. Changes in parameters of egg albumen quality due to addition of additives during the laying period provides table 4. Microbiological quality of eggs is given in Table 5.

With increasing dose of thyme oil in experimental groups was slightly decreased egg weight ($P>0.05$). The values in order of the groups 59.85, 59.48 and 59.23 g. Our results do not agree with the results of **Bolükbasi *et al.* (2008**), who found a positive effect of the addition of thyme oil on egg weight. **Bolükbasi and Erhan (2007),** or **Suchý *et al.* (2010)** after the addition of the premix herbs recorded in accordance with our results insignificant effect on egg weight.

Regarding the impact of thyme oil addition in the feed mixture of laying hens, observed yolk weight was higher in the control group, the difference was statistically insignificant ($P>0.05$). The values in the experimental groups with thyme oil were in the order of groups 16.60, 16.35 and 16.20 g. Difference was higher between the second experimental group with the addition of thyme oil at 1 g/kg ($P <0.05$) compared to the control group ($P<0.05$).

Table 3 Influence of thyme essential oil addition into laying hens feed mixture on the alterations of Hy-Line Brown laying hen's egg weight and egg yolk quality

Group	BD control group	BD + thyme essential oil 0.5 g/kg	BD + thyme essential oil 1 g/kg
Egg weight (g)			
mean	59.85	59.48	59.23
SD	3.91	3.77	3.97
CV (%)	6.55	6.34	6.70
P value		0.4740	0.4082
Egg yolk weight (g)			
mean	16.60	16.35	16.20
SD	1.54	1.44	1.52
CV (%)	13.10	8.80	9.40
P value		0.2067	0.0736
Percentage portion of egg yolk (%)			
mean	27.73	27.48	27.31
SD	2.03	2.54	1.99
CV (%)	7.31	9.21	7.29
P value		0.1761	0.1289
Egg yolk index			
mean	47.60	47.23	48.28
SD	3.39	3.66	3.56
CV (%)	7.12	7.74	7.38
P value		0.3530	0.0509
Egg yolk color (°HLR)			
mean	6.51	6.48	6.46
SD	0.52	0.50	0.57
CV (%)	7.98	7.73	8.82
P value		0.1944	0.1277

n=180; Significant difference (P<0.05); °HLR – colored Hoffman La Roche scale; SD = standard deviation; CV = coefficient of variation

Similarly in percentage yolk portion was found a higher value in the control group compared to the experimental groups with different doses of essential oil (P>0.05). **Bölükbaşi and Erhan (2007)** reported a statistically significant lower proportion of yolk in the group with the addition of thyme in 1% concentration compared with eggs from hens of the control group and experimental groups containing 0.1 and 0.5% concentration of thyme. Addition of 1.0% thyme, rosemary, oregano or 0.5% curcuma longa increased egg production, egg mass

and improved feed conversion. Addition of 1.0% curcuma longa increased percentage of yolk weight compared to control group in the experiment of **Radwan *et al.* (2008).**

In our experiment, yolk index was the highest in the experimental group with thyme oil supplement at a dose of 1 g/kg feed (P>0.05). Insignificant differences in the albumen index and yolk index of eggs from hens fed a meal containing a mixture of green tea recorded also **Uuganbayar *et al.* (2006).** Similarly in experiments of **Aydin *et al.* (2006)** with the addition of *Nigella sati*va, **Yalcin *et al.* (2006**), and **Canogullari *et al.* (2009)** with the addition of garlic meal yolk index was not significantly affected.

In assessing the yolk color among the groups indistinctive differences were observed, shades of yellow color on the color scale Hoffman La Roche for the addition of thyme oil were in the normal range. Similarly, in the experiment of **Liu *et al.* (2009),** yolk color was not affected by supplementation of thyme. **Radwan *et al.* (2008) i**ndicated a significant increase in yolk color but with the addition of turmeric.

Table 4 Influence of thyme essential oil addition on laying hens feed mixture on the alterations of Hy-Line Brown laying hen's egg albumen quality

Group	BD - control group	BD + thyme essential oil 0.5 g/kg	BD + thyme essential oil 1 g/kg
Egg albumen weight (g)			
mean	37.96	37.48	38.25
SD	4.09	4.01	4.97
CV (%)	10.77	10.69	10.65
P value		0.5585	0.4728
Percentage portion of egg albumen (%)			
mean	63.81	63.01	63.37
SD	6.77	4.80	6.18
CV (%)	10.60	7.57	9.75
P value		0.2931	0.5151
Egg albumen index			
mean	84.00	82.21	82.64
SD	16.01	16.09	16.96
CV (%)	19.08	19.58	20.52
P value		0.2573	0.2512
Haugh Units (HU)			
mean	79.87	80.46	79.85
SD	7.22	7.62	7.76
CV (%)	9.04	10.48	9.76
P value		0.9845	0.9756

n=180; Significant difference (P<0.05); SD = standard deviation; CV = coefficient of variation

Weight differences of albumen detected in our experiment between experimental groups with thyme supplementation compared to the control group were insignificant. The values of egg albumen weight were in the order of groups 37.96, 37.48 and 38.25 g.

The values of the percentage of albumen found among the groups were balanced, slightly lower values were in the experimental group compared to the control group (P>0.05) (values in the order of groups 63.8, 63.0 and 63.4%). Similarly, **Basmacioglu *et al.* (2003)** recorded a significant impact of adding of flax seed to feed on the percentage of albumen. Relative albumen weight significantly decreased in response to essential oil mixture or mannan-oligosaccharide supplementation in the experiment of **Bozkurt *et al.* (2012).**

Table 5 Influence of thyme essential oil addition into laying hens on the alterations of ISA Brown laying hen's egg microbiological quality

Experimental group	Group of microorganisms					
	CB	TNC	E	L	MF	Y
The number of different groups of microorganisms at the beginning of the laying (cfu/g)						
BD control group	$2.86x10^1$	$2.95x10^2$	$1.00x10^1$	<10	<10	$1.82x10^1$
	<10	$2.32x10^2$	$1.50x10^1$	<10	$1.82x10^1$	$2.73x10^1$
	$7.00x10^1$	$1.30x10^3$	$1.50x10^1$	<10	<10	$2.73x10^1$
BD + thyme essential oil 0.5 g/kg	<10	$3.81x10^1$	<10	<10	<10	<10
	<10	$1.82x10^1$	<10	<10	$1.00x10^1$	$1.82x10^1$
	<10	$5.91x10^1$	<10	<10	$1.00x10^1$	<10
BD + thyme essential oil 1 g/kg	<10	$3.81x10^1$	<10	<10	<10	<10
	<10	$3.18x10^2$	$1.82x10^1$	<10	<10	$1.27x10^2$
	<10	$7.91x10^2$	<10	<10	<10	$3.18x10^1$
The number of different groups of microorganisms at the end of the laying period (log cfu/g)						
BD control group	<10	$5.71x10^2$	$2.86x10^1$	<10	<10	<10
	$2.00x10^1$	$2.18x10^2$	$1.18x10^2$	<10	<10	<10
	<10	$3.45.10^2$	$1.23x10^2$	<10	$1.00x10^1$	<10
BD + thyme essential oil 0.5 g/kg	<10	$1.00x10^1$	<10	<10	<10	<10
	<10	$1.00x10^1$	<10	<10	<10	<10
	<10	$1.23x10^2$	$1.50x10^1$	<10	<10	<10
BD + thyme essential oil 1 g/kg	<10	$2.73x10^1$	$8.32x10^1$	<10	<10	<10
	<10	$4.09x10^1$	$3.00x10^1$	<10	<10	<10
	<10	$5.91x10^1$	$2.73x10^1$	<10	<10	$1.00x10^1$

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)

Values of albumen index in test groups with thyme were not significantly lower than in the control group (P>0.05). The average values of egg albumen index: 84.00, 82.21, 82.64. **Yalcin *et al.* (2007)** found an insignificant effect on egg production, shell quality, and egg albumen index after the addition of garlic meal. A statistically significant difference was found in the index of the albumen, when using addition of flour garlic in doses of 0.5, 1 and 2%, indicated **Canogullari *et al.* (2009).**

In the first and second experimental group with the addition of thyme oil were Haugh units of albumen very similar to the value in the control group (79.87, 80.46 and 79.85 HJ). **Botsoglou *et al.* (2005),** or **Liu *et al.* (2009)** reported in accordance with our findings statistically insignificant differences in this indicator. **Yalcin *et al.* (2006), Uuganbayar *et al.* (2006),** or **Canogullari *et al.* (2009)** found significant differences by adding of garlic powder. **Hosseini *et al.* (2008)** presented the lowest Haugh units and shell thickness at 10% addition of saffron seeds, but the differences were not statistically significant. The addition of 3 ml/kg oil of *Nigella sativa* in the feed of laying hens significantly reduced Haugh Units of albumen in the experiment of **Bölükbasi *et al.* (2009).**

The number of microorganisms in eggs at the beginning and end of the laying is shown in the table 5. The highest count of coliforms bacteria at the beginning of the laying was determined in control group and the lowest count of coliforms bacteria was found in both experimental groups with thyme essential oil. The number of enterococci ranged from <10 cfu/g in group with thyme essential oil 0.5 g/kg to $1.82x10^1$ cfu/g in group with thyme essential oil 1g/kg. The number of lactobacilli were <10 in all experimental group. The highest total number of microorganisms was found in control group ($1.30x10^3$) and the lowest count was in group with thyme essential oil ($1.82x10^1$). Number of microscopic fungi ranged from <10 cfu/g in group with thyme essential oil to $1.82x10^1$ cfu/g in control group. Number of yeasts ranged from <10 cfu/g in group with thyme essential oil 0.5 g/kg to $3.18x10^1$ cfu/g in thyme essential oil 1 g/kg.

The highest count of coliforms bacteria at the end of the laying was determined in control group and the lowest count of coliforms bacteria was found in both experimental group with thyme essential oil. The number of enterococci ranged from <10 cfu/g^{-1} in group with thyme essential oil 0.5 g/kg to $1.23x10^2$ cfu/g in control group. The number of lactobacilli were <10 in all experimental group. The highest total number of microorganisms was found in control group ($5.71x10^2$) and the lowest count was in group with thyme essential oil 0.5 g/kg ($1.00x10^1$). Number of microscopic fungi ranged from <10 cfu/g in group with thyme essential oil to $1.00x10^1$ cfu/g in control group. Number of yeasts ranged from <10

cfu/g in group with thyme essential oil 0.5 g/kg to $1.00x10^{1}$ cfu/g in thyme essential oil 1 g/kg.

Kačániová *et al.* (2007) in their study found, that microbiological quality of eggs from ecological breeding had zero number of coliforms bacteria, *Escherichia coli*, enterococci, lactobacilli and microscopic fungi. The total number of microorganisms ranged from 1.30 to 2.55 log cfu.g^{-1}. The average of total bacterial number of eggs in samples from organic farming was 2.00 log cfu.g^{-1}. The number of mesophilic aerobic sporulated microorganisms in samples of eggs ranged from 1.11 to 2.67 log cfu.g^{-1}. The average number of sporulated aerobic mesophilic microorganisms in samples of eggs was 1.92 log cfu.g^{-1}.

CONCLUSION

In this experiment the effects of supplementation of the diet for laying hens with thyme essential oils on egg weight, egg albumen and egg yolk quality were studied. This study was intended to determine microbiological quality of eggs after essential oil application into the feed as well. The egg weight, yolk weight, yolk percentage portion, yolk index, yolk colour, albumen weight, albumen percentage portion, albumen index and Haugh Units were affected statistically insignificantly after thyme essential oils application ($P>0.05$). The yolk index was affected most favourably. The number of coliforms, enterococci, fungi and yeasts decreased with increasing dose of oil. The number of lactobacilli was zero in all groups.

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