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REGULAR ARTICLE

THE INFLUENCE OF OREGANO ESSENTIAL OIL AND BEE PRODUCTS ON QUALITATIVE PARAMETERS AND MICROBIOLOGICAL INDICATORS OF TABLE EGGS CONTENT

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ABSTRACT

Phytobiotics are a new group of natural products. They are defined as products derived from plants, which may have a beneficial effect on the gastrointestinal microflora of animals, performance and quality of animal products. In this experiment the effects of supplementation of the diet for laying hens with oregano essential oil, propolis and pollen extract addition on physical and microbiological egg parameters were studied. Hens of laying hybrid Hy-Line Brown (n=40) were randomly divided into 4 groups (n=10) and fed for 23 weeks with diets with oregano essential oil and propolis or pollen supplemented. In the control group hens received feed mixture with no additions. The diets in the first experimental groups was supplemented with 0.5 g/kg oregano essential oil. The feed for second and third experimental

groups of birds consisted of basal diet supplemented with propolis extract and pollen extract of the same dose at 0.5 g/kg. The results suggest that the most of qualitative parameters of egg internal content were not significantly influenced with oregano oil or bee products 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 was significantly higher difference in favor of the experimental group with addition of oregano essential oil at a dose of 0.5 g/kg and in the group with pollen supplement (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.

Key words: oregano essential oil, propolis, pollen, table egg, physical quality, micobiological quality

INTRODUCTION

Removal and restriction of subtherapeutic antibiotics from poultry diets in many parts of the world has amplified interest in improving intestinal health and nutrient utilization **(Applegate *et al.,* 2010).** 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 **(Dahiya *et al.,* 2006).** Among the candidate replacements for antibiotics are competitive exclusion products, probiotics, prebiotics, organic acids, enzymes and plant extracts **(Capcarová *et al.,* 2010).** Feed additives can not replace the negative impact of diet, feeding regime or unbalanced nutrients in the ration. They are not a source of nutrients for poultry. When absent in the ration, the animals have not signs of nutritional deficiency **(Hashemi, Davoodi, 2011).** Based on the work of several authors may have a beneficial effect on the gastrointestinal microflora of poultry **(Kačániová *et al.,* 2007; Nováková et al., 2008; Kačániová et al., 2011),** production parameters, the quality of poultry meat **(Haščík *et al.,* 2008, 2009; Čuboň *et al.,* 2009)** and eggs **(Arpášová *et al.,* 2009; 2010; Gálik, Horniaková, 2010; Arpášová, 2011; Halaj, Golian, 2011).**

Herbs, spices and their extracts (botanicals) have a wide range of activities. They can stimulate feed intake 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**). They also affect the motility of the bowel and improve the integrity of the intestinal lining. Some extracts stimulate the olfactory receptors and taste buds, resulting in an increase in feed intake and increased production of endogenous enzymes **(Panda *et al.,* 2009).** Essential oils are complex mixtures of different organic molecules - terpenes, alcohols, esters, aldehydes, ketones and phenols **(Fletcher *et al.,* 2001).** They are obtained by extraction, fermentation, pressing, but steam distillation method is the most commonly used for commercial production of essential oils. As plant material can be used flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots **(Burt, 2004).** Their mode of action is more indirect, rather based on a comprehensive approach that can support internal defense mechanisms of animals and can therefore be considered sustainable, long-term solution **(Gwendolyn *et al.,* 2002).**

The basis of propolis is a sticky resin **(Valle, 2000)**. Propolis is a natural substance and harmoniously balanced with a strong antibiotic, immunostimulating, anti-inflammatory and antibacterial effects (**Velíková *et al.,* 2000)**. Its biological characteristics improve immune response, depending on the concentration and the dose intensity. Antimicrobial activity of propolis strictly related to its physical structure (**Dias *et al.,* 2012).** The composition of propolis depends on the tree species from which oil is harvested. Propolis can be used to increase the specific immune response after vaccination **(Freitas *et al.,* 2011).**

Bee pollen is a product composed of bees nutritionally valuable components, comprising a considerable amount of polyphenol components which act as effective anti-oxidants (**Aliyazicioglu *et al.,* 2005).** Polyphenolic compounds contained in pollen have the ability to block the free radicals (**Tang *et al.,* 2005**). Antioxidant activity of pollen varies considerably for the different kinds of pollen **(Leja *et al.,* 2007**). Fresh pollen appears to be an ideal food for regenerating intestinal microflora. Pollen extracts can be used as functional food (**Marghita *et al.,* 2009).**

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, Flimelová, 2012**).

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 this acts as a barrier to keep bacteria from entering the egg. When eggs are washed, however, 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 (**Dawson *et al.*, 2001**).

The aim of this work was to observe the influence of oregano essential oil, propolis and pollen extract additions 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 lactobacilli, total count of microorganisms, count of microscopic fungi and yeasts.

MATERIAL AND METHODS

Animals, diets and treatments

Hens (n=40) of the laying hybrid Hy-Line Brown, 17 weeks old, were randomly divided into 4 groups (n=10) and fed for 23 weeks with diet containing of different amounts of oregano essential oils, propolis or pollen extracts.

At the beginning of the experiment, the hens were kept in 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.

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 at 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
Methionine (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 the Feed Mixture
MEN ($MJ.kg^{-1}$)	11.5
CP ($g.kg^{-1}$)	177
LYS ($g.kg^{-1}$)	8.81
MET ($g.kg^{-1}$)	4.17
M + C ($g.kg^{-1}$)	7.41
THR ($g.kg^{-1}$)	6.27
LA ($g.kg^{-1}$)	19.0
Ca ($g.kg^{-1}$)	39.1
Pavail. ($g.kg^{-1}$)	3.8
Na ($g.kg^{-1}$)	1.5

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

In the control group hens received feed mixture with no additions. The diet in the first experimental group was supplemented with 0.5 g/kg oregano essential oil (Calendula a.s. Nová Ľubovňa, SR).

The feed for the second and third experimental groups of birds consisted of basal diet (BD) supplemented with propolis extract and pollen extract of the same dose 0.5 g/kg. Laying hens accepted fodder *ad libitum*.

Propolis samples were collected from the Slovak republic. Hand collected propolis samples were kept dried in the dark until processing. Propolis samples were extracted for a week with 100 ml of 70% ethanol, at room temperature to obtain the extract (**Blonska *et al.*, 2004)**.

The bee pollen was collected in Slovakia, Nitra region. The freshly collected bee pollen was dried at 40 °C with the protection from light and ground into powder. The material (1 kg) was extracted with 70% ethanol three times under reflux for 2 h. After filtration and centrifugation (1700×g, 30 min), the combined solution was concentrated under reduced pressure in rotavatour at the temperature 45 °C to evaporate the solvent and finally dried in high vacuum.

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 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

Plate diluting method was applied for quantitative cfu counts determination of respective groups of microorganisms in 1 g of substrate. Nutrient medium in Petri dishes was inoculated with 1ml of egg mass 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.

The number of coliforms bacteria were 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 were 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 using the SAS software.

RESULTS AND DISCUSSION

Egg weight and yolk quality indicators in each group for the observed laying period expresses Table 3. Quality indicators of albumens during the period as provided in Table 4, the microbiological quality is shown in Tables 5 and 6.

The average weight of analyzed eggs in all groups receiving oregano oil and bee products enriched diets was of 59.85 ± 3.91; 59.25 ± 5.35; 59.70 ± 3.26 and 60.35 ± 4.64 g ($\bar{x}$ ± SD), (P>0.05), respectively.

In the group with addition of 0.5 g/kg of oregano oil has been detected insignificantly lower difference compared to the control group (P>0.05). **Florou-Paneri *et al.* (2005**) after addition of oregano in accordance with our results recorded in relation to the weight of the eggs insignificant impact. In the experiment of **Galal *et al.* (2008)** laying hens fed by diets containing 100 and 150 mg of propolis produced significantly heaviest egg mass compared to control group. The hens fed by diets with addition of 50 mg of propolis were intermediate. **Senköylü *et al.* (2004)** indicated a reduction in egg weight using phytobiotics, though statistically insignificant difference. **Yang *et al.* (2003**) observed after the addition of green tea significantly negative impact of phytobiotics. Average egg weight in the group with the

supplement of propolis was on the level of the control group, addition of pollen increased egg weight (P>0.05).

Table 3 Influence of oregano essential oil, propolis extract and pollen extract addition into laying hens feed mixture on the alterations of Hy-Line Brown laying hen's egg yolk quality

Group	BD - control group	BD + oregano essential oil 0.5 g/kg	BD + propolis extract 0.5 g./kg	BD + pollen extract 0.5 g/kg
Egg weight (g)				
mean	59.85	59.25	59.70	60.35
SD	3.91	5.35	3.26	4.64
CV (%)	6.55	9.02	5.46	7.70
P value		0.0889	0.0542	0.1123
Egg yolk weight (g)				
mean	16.60	16.16	16.44	16.57
SD	1.54	1.35	1.20	1.37
CV (%)	9.29	8.35	7.30	8.32
P value		0.0524	0.1556	0.2196
Percentage portion of egg yolk (%)				
mean	27.73	27.28	27.53	27.27
SD	2.03	1.95	1.85	2.60
CV (%)	7.31	7.15	6.72	9.53
P value		0.1591	0.3382	0.1691
Egg yolk index				
mean	47.60	48.75	47.29	47.64
SD	3.39	3.48	3.16	3.77
CV (%)	7.12	6.72	6.68	7.91
P value		0.0532	0.1871	0.4508
Egg yolk color (°HLR)				
mean	6.51	6.55	6.42	6.38
SD	0.52	0.50	0.48	0.53
CV (%)	7.98	7.63	8.32	8.12
P value		0.2421	0.3112	0.3551

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

The effect of oregano oil supplementation on the feed mixture of layers caused an insignificant decrease in yolk weight. Dietary propolis extract yolk weight decreased slightly (P>0.05), the value of the experimental group with the pollen addition was abreast of the control group. The values were found in the order of groups: 16.60 ± 1.54; 16.16 ± 1.35; 16.44 ± 1.20 and 16.57 ± 1.37 g ($\bar{x}$ ± SD). Insignificant impact of phytobiotics state also **Chowdhury *et al.* (2002),** in an experiment in which been established tendency of higher yolk weights. On the contrary a significant improvement in yolk weigh was observed as a

result of plant essential oils supplementation in experiment of **Canogullari *et al.* (2009),** or **Bozkurt *et al.* (2012).**

The percentage of yolk was relatively balanced among the groups, resulting in statistically insignificant differences. **Radwan *et al.* (2008)** indicated after the addition of thyme, oregano and curcuma increase the percentage of yolk, but statistically insignificant.

In the yolk index was observed among all our experimental groups and the control group statistically insignificant difference (P>0.05). These findings are consistent with the work of several authors, who added to feed different types phytobiotics. Insignificantly higher values compared to the control group were observed in our experiment in the group with oregano oil supplement at dose 0.5 g/kg of feed mixture. Minor differences in yolk index of eggs from hens fed a meal containing a mixture of green tea reported also **Yalcin *et al.* (2006),** or **Canogullari *et al.* (2009)** with the addition of garlic meal. In groups with addition of propolis and pollen in our experiment were recorded values at the level of the control group. Similarly, **Seven *et al.* (2008)** recorded that vitamin C or propolis supplementation did not affect the yolk index.

In the yolk color were observed insignificant differences among the groups, shades of yellow color on the color scale Hoffman La Roche were in the normal range at oregano oil addition as well as both species of bee products. In the groups with the addition of oregano in our experiment were balanced values compared with the control group. In supplement of plant extracts and their possible influence on the color tint yolk, plays an important role in the plant species used. **Radwan *et al.* (2008)** in accordance with the conclusions of our experiment revealed insignificant differences in yolk color shade after the addition of oregano oil, but after adding of turmeric significant effect was significant. Significant increase in the intensity of yolk color by adding 2% concentration of green tea also recorded **Yang *et al.* (2003**), or **Yodseranee *et al.* (2003**), as well as **Botsoglou *et al.* (2005**) in experimental group with the addition of saffron at 20 mg/kg. On the contrary, **Ayerza, Coates (2002**) reported paler color of the yolk by the addition of sage. Regarding addition of propolis and pollen in our experiment was found slightly lighter shade in the groups with addition of both bee products.
In the experimental groups were albumen weight relatively balanced (37.96 ± 09.04; 37.53 ± 4.50; 38.09 ± 4.00 and 37.93 ± 3.60 g ($\overline{x}$ ± SD), respectively (P>0.05). Higher values of albumen weight indicate in their experiment with the addition of *Nigella sativa* **Aydin *et al.* (2006**). **Kutlu *et al.* (2001),** or **Sahinler *et al.* (2005)** reported in accordance with our results insignificant effect of phytobiotics supplement on albumen weight. Results of **Bozkurt *et al.* (2012)** found that supplementation of diet with essential oil mixture provided increments in

eggshell weight, however relative albumen weight was significantly decreased in response to essential oil mixture

Table 4 Influence of oregano essential oil, propolis extract and pollen extract addition into laying hens feed mixture on the alterations of Hy-Line Brown laying hen's egg albumen quality

Group	BD - control group	BD + oregano essential oil 0.5 g/kg	BD + propolis extract 0.5 g./kg	BD + pollen extract 0.5 g/kg
Egg albumen weight (g)				
mean	37.96	37.53	38.09	37.93
SD	4.09	4.50	4.00	3.60
CV (%)	10.77	11.99	10.50	9.49
P value		0.6408	0.3810	0.4791
Percentage portion of egg albumen (%)				
mean	63.81	63.35	63.87	63.17
SD	6.77	4.92	6.36	4.51
CV (%)	10.60	7.76	9.96	7.14
P value		0.5151	0.4658	0.1470
Egg albumen index				
mean	84.00	88.42	86.57	90.62
SD	16.02	18.39	17.91	16.88
CV (%)	19.08	21.03	20.69	52.29
P value		0.0483	0.0965	0.0003
Haugh Units (HU)				
mean	79.87	82.51	80.58	82.63
SD	7.22	12.86	7.60	8.03
CV (%)	9.04	15.59	9.43	9.77
P value		0.0181	0.1813	0.0035

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

In our experiment, the values of the percentage of albumen fairly balanced, as is apparent from the average values obtained (Table 5). Similarly **Basmacioglu *et al.* (2003)** recorded insignificant impact of addition of flax seed into feed to the percentage of albumen. **Cherian *et al.* (2009)** found after addition of feedstuff with *Camelina sativa* at a concentration of 10% and 15% significant reduction in yolk weight, percentage of yolk from egg weight and increase the percentage of albumen, compared with the control group.

In the index of the albumen were in all experimental groups recorded higher average values compared to the control group. Significantly higher index of albumen was in the experimental group with the oregano oil (0.5 g/kg) and in the experimental group with pollen supplement ($P<0.05$). The value of albumen index in test group with the addition of propolis, was also higher than in the control group, but statistically insignificant difference. The values were found in the order of the groups 84.00 ± 16.02; 87.42 ± 18.39; 86.57 ± 17.91 and 90.62

± 16.88 ($\bar{x}$ ± SD). A significant difference in the albumen index in the group with the addition of garlic meal found **Canogullari *et al.* (2009).**

Similarly, as in the albumen index also in Haugh Units were recorded average higher values in all experimental groups compared to the control group. Significantly higher Haugh Units were in the experimental group with oregano oil and in the experimental group with pollen supplement ($P<0.05$). Index of albumen in the experimental group with the addition of propolis was also higher than in the control group, but the difference was not statistically significant ($P>0.05$). Values of Haugh Units in order the groups: 79.87 ± 7.22; 82.51 ± 12.86; 80.58 ± 7.60 and 82.63 ± 8.03 HJ ($\bar{x}$ ± SD). Similarly, **Florou–Paneri *et al.* (2005),** observed after the addition of oregano oil positive impact on this indicator although statistically insignificant difference. In the experiment of **Sahin *et al.* (2008)** with propolis supplementation albumen index and Haugh Units were not affected.

Table 5 Influence of oregano essential oil, propolis and pollen extracts addition into laying hens feed mixture on the alterations of ISA 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 0.5 g/kg	<10	$1.96x10^1$	<10	<10	<10	<10
	<10	$1.16x10^1$	<10	<10	<10	$1.00x10^1$
	<10	$1.78x10^1$	<10	<10	<10	<10
BD +propolis extract 0.5 g/kg	<10	$2.76x10^1$	$1.00x10^1$	<10	<10	<10
	<10	$2.81x10^1$	<10	<10	<10	<10
	<10	$2.33x10^1$	$1.00x10^1$	<10	<10	<10
BD + pollen extract 0.5 g/kg	<10	$1.00x10^1$	<10	<10	<10	<10
	<10	$1.30x10^2$	$1.00x10^1$	<10	<10	<10
	<10	$1.36x10^1$	<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)

The number of microorganisms in eggs at the beginning 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 experimental group with oregano essential oil and bees products. The number of enterococci ranged from <10

cfu/g in group with essential oil and bees products 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 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 6 Influence of oregano essential oil, propolis and pollen extracts addition into laying hens feed mixture on the alterations of ISA Brown laying hen's egg microbiological quality at the end of the laying (cfu/g)

Experimental	**Group of microorganisms**					
group	**CB**	**TNC**	**E**	**L**	**MF**	**Y**
BD control	$3.86x10^1$	$2.95x10^2$	$1.00x10^1$	<10	<10	$1.82x10^1$
group	<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	<10	$2.54x10^1$	$1.00x10^1$	<10	<10	<10
essential oil	<10	$3.40x10^1$	$3.50x10^1$	<10	<10	<10
0.5 g/kg	<10	$2.46x10^1$	$1.00x10^1$	<10	<10	<10
BD +propolis extract	<10	$1.00x10^1$	$2.00x10^1$	<10	<10	<10
0.5 g/kg	<10	$1.00x10^2$	$1.40x10^1$	<10	<10	<10
	<10	$1.24x10^2$	$1.90x10^1$	<10	<10	<10
BD + pollen extract	<10	$2.18x10^1$	$3.60x10^1$	<10	<10	<10
0.5 g/kg	<10	$3.76x10^1$	$8.60x10^1$	<10	<10	<10
	<10	$3.33x10^1$	$6.80x10^1$	<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)

The number of microorganisms in eggs at the end of the laying showed the table 6. 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 experimental group with oregano essential oil and bees products. The number of enterococci ranged from $1.00x10^1$ cfu/g in group with oregano essential oil 0.5 g/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 microscopic fungi ranged from <10 cfu/g in experimental groups to $2.82x10^1$ cfu/g in control group. Number of yeasts ranged from <10 cfu/g in experimental group to $2.73x10^1$ cfu/g in control group.

Dawson *et al.* (2001) state, 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 to prevent microbiological contamination.

CONCLUSION

The most of qualitative indicators of egg yolk and egg albumen (percentage portion og yolk (%), yolk index, yolk colour (°HLR), albumen weight (g), percentage portion of albumen (%)) were not with oregano oil or bee products addition significantly influenced ($P>0.05$). The egg yolk weight was insignificantly decreased in the experimental group with oregano essential oil addition in a dose 0.5 g/kg. 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 were significantly higher differences in favor of the experimental groups with complement oregano essential oil and in the group with pollen supplement. The addition of oregano essential oil, propolis or pollen affected positively the microbiological quality of internal contents of the egg.

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