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Use of Tea Tree Essential Oil (*Melaleuca alternifolia*) in Laying Hen's Nutrition on Performance and Egg Fatty Acid Profile as a Promising Sustainable Organic Agricultural Tool

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Abstract: The level of production in a variety of organic production systems is often lower than in other traditional production systems. In poultry production, there is also a direct negative effect of the small scale regarding sustainable organic poultry production. Regardless of differences between organic and conventional production systems, this experiment aimed to investigate the usage of tea tree *Melaleuca alternifolia* (Maiden and Betche) Cheel essential oils as a natural alternative to antibiotics in hen nutrition on productive parameters, table egg quality and eggs fatty acid profile as a promising sustainable organic agricultural tool. A total of 360 Lohmann Brown hens, aged 54 weeks, divided into three different treatment diets, were supplemented with 0 (T1), 40 (T2) and 80 mg/kg (T3) of *M. alternifolia* essential oil, respectively. Experimental treatments were replicated four times within 30 birds each. The experiment lasted for a total of 56 days (55 to 62 weeks of hens age). A 56-day experimental had two timetable periods of 28 days each: period 1 (55 to 58 weeks of hen age) and period 2 (59 to 62 weeks of hen age). For compound feed supplemented with *M. alternifolia* essential oil, daily egg production and the efficiency of nutrient utilization (FCR) was improved significantly ($p < 0.05$) until the end of week 58, with a significant ($p < 0.05$) increase in the thickness of eggshell, as well as egg production ($p < 0.05$). However, egg mass, feed consumption, FCR and albumen height, Haugh unit, and eggshell strength did not show any significant ($p > 0.05$) differences influenced by essential oil feed supplementation. Lower concentrations of saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA), and higher concentrations of polyunsaturated fatty acid (PUFA), were recorded with *M. alternifolia* essential oil supplementation, but without significant ($p > 0.05$) differences. At the end of the experiment, the obtained results showed that the addition of *M. alternifolia* essential oil to hen nutrition had a positive effect on production parameters and eggs fatty acid profile, with increased eggshell thickness ($p < 0.05$).

Keywords: medicinal plants; sustainable; poultry; organic; eggs; fatty acids; agriculture

1. Introduction

Nowadays, modern organic agriculture has been described as production in a variety of organic production systems, which is often lower than in other traditional production systems. In poultry production, there is also a direct negative effect of the small scale regarding sustainable organic poultry production [1]. Organic agriculture helps to make farming sustainable, safe, and environmentally friendly, and essential oils and botanicals as a natural remedy serve that purpose.

For more than a hundred years, *M. alternifolia* has been used as a natural remedy all over the world, and primarily in Australia. Nowadays, bioactive ingredients in *M. alternifolia* are used in the manufacture of homeopathic drugs, and thus in pharmaceuticals and agriculture, with a growing interest in Europe, Japan and the United States [1]. Myrtle family *Myrtaceae*, represents the most important and known species for the industrialization of *M. alternifolia* oil because of its beneficial properties [2]. For the distillation of essential oils, flowers, herbs, leaves, roots, and another plant parts could be successfully used. *M. alternifolia* essential oil is a mixture of terpenes, aldehydes, esters, alcohols, and other chemical molecules, and therefore has been used in poultry nutrition for its antimicrobial, antibacterial, antioxidant and digestive stimulant properties. In the last twenty years, essential oils have been ignored as a possible natural remedy or natural antibiotic alternative for poultry [3]. However, they are recently gaining spotlight in the scientific community [4,5]. *M. alternifolia* oil, because of its antibiotic properties, is widely used as an antiseptic tool, often is used to minimize inflammation and can be very useful in fungal infection treatments [2]. *M. alternifolia* oil, due to its toxicity, should not be used in animal nutrition in high concentrations. Deaths regarding the higher dose of consumed *M. alternifolia* oil were not recorded in the known literature. The aforementioned statement was confirmed in the research of Hammer et al. [2]. However, in the right concentrations, *M. alternifolia* oil addition in broiler chickens diet increases body weight gain by 7% and improve nutrient utilization by 6% when compared with results of chickens that were not fed rations with the supplementation of *M. alternifolia* [6]. Olgun [7] research has shown that eggshell thickness was positively affected by essential oil addition to hens' diet, as well as other quality parameters of eggs, while Nikolova and Kocevski [8] in their research have shown its influence on the technological aspects of eggshell quality. In the research of Nadia et al. [9], usage of essential oil increased egg production and improved feed utilization, when the results of experimental treatments were compared with control treatment. Investigations with hens have shown that the dietary addition of thyme, sage, and rosemary essential oil [10] and essential oil mixture [6] increase hens' production, and increases immunity and eggshell quality, in line with the results obtained by Spasevski et al. [11], with the usage of powder form of marigold, paprika, and carrot in hens' diet. Due to the link between dietary lipids and developing coronary heart disease, the lipid composition of table eggs is the primary consumer concern, which is the same regarding dairy products [12]. It has been proven that adequate and well-balanced compound feeds for hens can improve the fatty acid profile of the eggs. Although the essential oils have a positive effect on the body metabolism of lipids [13], there have been no studies reported, or very few, on the effect of *M. alternifolia* dietary essential oil addition on egg fatty acids profile in hens.

Therefore, this research aimed to evaluate the effects of *M. alternifolia* essential oil in hen nutrition on productive performance results, table egg quality, and egg fatty acid profile.

2. Materials and Methods

Ethical Approval: Biological experiment with laying hens was approved by the University EC board and performed following the EU legislation and principle of the Three Rs within Directive 2010/63/EU.

Animals and Experimental Design: The experiment with laying hens was conducted under the principles of the European Union Strategy for the Protection and Welfare of Animals. A total of 360 Lohmann Brown hens aged 54 weeks were divided into three different treatment diets supplemented with 0, 40 and 80 mg/kg of *M. alternifolia* essential oil (Planet Fresh d.o.o., Montenegro), respectively. *M. alternifolia* was provided, including Terpinen-4-ol 40.0%, γ-Terpinene 23.0% and α-Terpinene

10.4% as the active components. Each treatment was replicated four times with 30 hens in each. Hens were in an environmentally controlled experimental facility with a constant temperature of 22 °C. Environmental conditions in the facility were in line with hybrid specifications. Hens were provided with a diet of 110 g of feed/hen/day, while water of an average temperature of 20 °C was provided ad libitum. Compound feed in mash form was used for hen nutrition during the experimental period. The basal feed was balanced according to the hybrid recommendation with and without the *on top* addition of *M. alternifolia* essential oils in the mixtures. The nutritive value of the used diets is given in Table 1.

Table 1. Nutritive value of the basal diet.

Nutrients*	Content,%
Crude protein	15.5
Crude fat	4.6
Calcium	3.8
Phosphorus (available)	0.37
L-Lysine	0.79
Methionine + Cystine	0.54
Threonine	0.60
Tryptophan	0.18
Metabolizable Energy, MJ/kg	11.0

*The value of crude protein, crude fat and calcium was analyzed and the value of metabolizable energy was calculated.

Sample Preparation and Eggs Quality Assessment: Eggs were gathered every day and the egg production was recorded for both period 1 (55 to 58 weeks of hens age), and period 2 (59 to 62 weeks of hens age), and for the complete periods of research. During the experiment, egg mass was registered daily. Consumption of feed was registered daily, and the feed conversion ratio was calculated for different production periods. Every 28 days in a row, 12 eggs per treatment were randomly gathered: three eggs per each replicate to measure albumen height, calculate Haugh units, and measure eggshell thickness and eggshell strength. Albumen height measurement was performed with a digital caliper, while Haugh units were calculated (1):

$$\begin{gathered} \mathrm{HU} = 100 \,\mathrm{Log}\,(\mathrm{h} - 1.7\,\mathrm{w}^{0.37} + 7.6) \\ \mathrm{HU} = \text{Haugh unit; h = albumen height (mm) and w = egg weight (g)} \end{gathered} \tag{1}$$

Eggshell thickness was measured at the three points of measurements, top end, bottom end and center, with a micrometer screw gauge. An average of three thickness values from each egg has been used to define the thickness of the eggshell. A test machine, Instron, was used to measure the break strength of uncracked eggs. An egg was laid down on a continually raised load until it broke down. The point of egg break under load is considered as the measured strength of the egg.

Table Egg Yolk Fatty Acids Analyses: Egg yolk fatty acids profile was analyzed by the gas chromatography technique. First, lipids were extracted from egg yolk. The extraction of lipids from the yolk was performed according to the Folch extraction method. Extracted lipids with 14% (w/w) boron trifluoride–methanol solution were used for the preparation of fatty acid methyl esters. Upon obtaining the samples, analyzes by GC were conducted on an Agilent 7890A chromatograph (Agilent Technologies, USA) with an FID, auto-injection module for liquid, equipped with fused silica capillary column (Supelco SP-2560 Capillary GC Column 100 m × 0.25 mm, d = 0.20 µm). As a gas carrier, helium of 99.9997 vol % purity, with a flow rate of 1.5 mL/min and with 1.092 bar pressure was used. The fatty acid profile of egg yolk was determined based on retention times and standard comparison. The fatty acid profile was shown as the % of the total fatty acids.

Statistical Analyses: The data obtained in the experiment were analyzed by one-way analysis of variance within statistical software Statistica 13. When the analysis of variance showed statistical significance, Duncan's multiple range test was used. The significant difference was registered at $p < 0.05$.

3. Results and Discussion

The effect of *M. alternifolia* essential oil on hen productive performance results is given in Table 2. During the 56 days of the experimental trial, only egg production showed significant ($p < 0.05$) results, while egg weight, feed intake and feed conversion ratio did not record any significant ($p > 0.05$) influence of diet supplementation with *M. alternifolia* essential oils. Feed conversion ratio and hen-day egg production were significantly improved ($p < 0.05$) in weeks 59 to 62 of the experimental period.

Table 2. Influence of *M. alternifolia* essential oil on productive performance results of hens.

Parameter	Dietary Treatments with *M. alternifolia* Essential Oil, mg/kg			SEM	*p*-Value
	0 (T1)	40 (T2)	80 (T3)		
Feed intake, g/(hen/day)					
55 to 58 wk	123.0	123.2	124.1	1.354	0.489
59 to 62 wk	120.5	120.5	121.2	1.198	0.483
55 to 62 wk	122.0	121.9	122.7	1.076	0.382
Feed conversion ratio					
55 to 58 wk	2.51	2.45	2.43	0.042	0.672
59 to 62 wk	2.49^{a}	2.36^{b}	2.40^{b}	0.025	0.004
55 to 62 wk	2.50	2.41	2.41	0.027	0.081
Hen-day egg production, %					
55 to 58 wk	78.5	80.2	81.6	1.353	0.622
59 to 62 wk	77.0^{b}	79.5^{a}	80.5^{a}	1.076	0.034
55 to 62 wk	77.8^{b}	79.9^{b}	81.1^{a}	1.098	0.016
Egg weight, g					
55 to 58 wk	62.6	63.0	63.0	0.319	0.558
59 to 62 wk	62.8	62.9	62.7	0.322	0.631
55 to 62 wk	62.7	63.0	62.9	0.297	0.529

^{a,b} indicated the difference within a row was significant ($p < 0.05$).

Generally, it is assumed that essential oils of herbs and spices may have a positive influence, and might improve the feed consumption, due to their aromatic properties [14,15]. Bozkurt et al. [16] compared four herbs (thyme, oregano, rosemary, turmeric) at an inclusion level of 5 or 10 g/kg diet in a study with 28-week old laying hens for 12 weeks. They found no differences in feed intake among the treated groups and did not observe any differences when comparing the treatments to the untreated control group, which is in agreement with our research. Similar results were obtained when garlic was included in diets fed to laying hens in the concentrations of 20, 40, 60, 80 or 100 g/kg diet for hens of nearly the same age (27–28 weeks), but for only 5–6 weeks [17]. However, Abdo et al. [18] found that the average feed intake was significantly reduced for hens which were fed either 10 to 50 g/kg of green tea leaves or 5 to 25 mg/kg of green tea essential oil when compared to an unsupplemented control treatment. In our research, feed conversion ratio and hen–day egg production were increased ($p < 0.05$) in weeks 59 to 62, however, during a total of 56 days of feeding, trial egg production record significant ($p < 0.05$) results, while dietary addition of essential oils did not show significant ($p > 0.05$) effects regarding egg weight, feed consumption and feed conversion ratio, in agreement with other research [9,11,16,19]. The essential oils used as "production stimulators" for hens were primarily introduced in their daily diet to increase the utilization of the limit-fed diet and, in turn, improve the production of eggs. Some studies showed positive effects on production results, such as egg production rate, as was the case in our research, and the egg mass, whereas some other studies demonstrated no effect of essential oil addition [20].

The effect of *M. alternifolia* essential oil on egg quality is shown in Table 3. Supplementation of *M. alternifolia* essential oil significantly increased the eggshell thickness ($p < 0.05$) at the end of the experimental period of 56 days, in line with the research of Bozkurt et al. [21], who concluded that dietary addition of essential oil mixture increases the weight of eggshell, eggshell thickness, and strength of shell breaking, while dietary supplementations of *M. alternifolia* essential oil did not affect eggshell thickness ($p > 0.05$) at the beginning of the hen laying period in our study. No significant differences in albumen height, Haugh unit, and eggshell strength between the different dietary treatments T1 and T2 ($p > 0.05$), compared to control treatment T1, were recorded.

Table 3. Influence of *M. alternifolia* essential oil on egg quality parameters.

Parameter	Dietary Treatments with *M. alternifolia* Essential Oil, mg/kg			SEM	*p*-Value
	0 (T1)	40 (T2)	80 (T3)		
Albumen height					
58 wk	7.30	7.35	7.20	0.145	0.755
62 wk	7.20	7.30	7.20	0.321	0.562
Haugh unit					
58 wk	83.60	83.00	82.50	1.235	0.890
62 wk	82.30	82.30	82.30	1.899	0.869
Eggshell strength, kg/cm^2					
58 wk	4.70	4.30	4.20	0.257	0.693
62 wk	4.50	4.20	4.71	0.178	0.813
Eggshell thickness, mm					
58 wk	0.26	0.24	0.25	0.007	0.158
62 wk	0.24^b	0.28^a	0.27^a	0.007	0.031

^{a,b} indicated the difference within a row was significant ($p < 0.05$).

Bölükbaşi et al. [22] showed that the supplementation of bergamot essential oil at three inclusion levels (0.25, 0.50, 0.75 mL/kg) had no impact on the proportion of albumen and eggshell thickness of the eggs of hens at the age of 67 weeks. However, at any addition level, bergamot essential oil decreased eggshell percentage by 15% or more when compared with an untreated control group. The dietary addition of black cumin essential oil did not show a significant ($p > 0.05$) influence on shell weight when added in concentrations of 1; 2 or 3 mL/kg, to hens' diet, respectively. Similarly, essential oil of oregano in the amount of 50 or 100 mg/kg, did not show either positive or negative effects on yolk color score, Haugh unit or shell thickness when added to the feed at the age of 32 weeks [19], but when used in broiler chickens diet, oregano oil and powder form mixtures of medicinal plants, and have shown significant effects [4,23]. The Haugh unit value as the main measure of internal quality of eggs was not compromised by the dietary supplementation of bergamot essential oil [22], oregano essential oil [19], a mixture of essential oils [21], green tea leaves or essential oil [18], thyme, oregano, rosemary or turmeric powder [9], or a garlic–thyme combination [24], in agreement with our study with a dietary addition of *M. alternifolia* essential oil to hen nutrition in concentrations of 40 and 80 mg/kg, respectively.

The influence of *M. alternifolia* essential oil on the fatty acid profile of egg yolk of control and experimental treatments is given in Table 4. The addition of *M. alternifolia* essential oil in treatments T2 and T3 compared with control treatment, T1, did not show a significant ($p > 0.05$) influence on the fatty acid profile of the yolk. Lower concentrations of saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA), while higher concentrations of polyunsaturated fatty acid (PUFA), were recorded with *M. alternifolia* essential oil supplementation, but without significant ($p > 0.05$) differences.

Table 4. Influence of *M. alternifolia* essential oil on fatty acid profile of egg yolk, %.

Fatty Acids	Dietary Treatments with *M. alternifolia* Essential Oil, mg/kg			SEM	*p*-Value
	0 (T1)	40 (T2)	80 (T3)		
C14:0	3.3	3.1	3.6	0.322	0.1931
C16:0	27.0	26.9	26.9	0.425	0.3887
C16:1	3.2	3.0	3.1	0.220	0.6228
C18:0	9.6	9.7	9.5	0.351	0.2190
C18:1	41.0	42.0	41.2	0.834	0.1558
C18:2 n-6	11.6	11.4	11.6	0.381	0.8647
C18:3 n-6	0.06	0.09	0.06	0.045	0.8110
C18:3 n-3	0.09	0.03	0.08	0.062	0.1473
C20:3 n-3	0.03	0.05	0.23	0.098	0.9472
C22:6 n-3	0.3	0.5	0.42	0.187	0.2219
SFA	40.6	40.0	39.9	0.511	0.8732
MUFA	46.0	45.2	46.3	0.700	0.2965
PUFA	13.4	14.8	13.8	0.479	0.1632

In our study, different dietary treatments did not show significant ($p > 0.05$) influence on the egg yolk fatty acid profile. The research of Galobart et al. [10] showed that the dietary addition of rosemary essential oil in hen nutrition had no effects on the fatty acid composition of egg yolk. Besides that, the research of Bölükbaşi et al. [22] showed that the ratio of docosahexaenoic fatty acid (DHA) and the ratio of n-3 fatty acids of egg yolk were elevated by the dietary addition of bergamot essential oils. Other studies have shown changes in the lipid metabolism of poultry fed with the addition of essential oils [22,25]. To our knowledge, there have been no reports on the effect of *M. alternifolia* essential oil on the fatty acid composition of egg yolk, which makes this research a very valuable asset to the scientific community. The adequate ratio between n-6 and n-3 polyunsaturated fatty acids is a very important standard in healthy human nutrition. Being aware of n-3 polyunsaturated fatty acids' benefits and health-promoting effects, the nutritionists recommend a diet rich in n-3 fatty acids, as well as a lower ratio between n-6 and n-3 fatty acids, from the currently common 15–20:1 to 1–4:1. According to Simopoulos [26] and Kralik et al. [27], the ratio of n-6 and n-3 polyunsaturated fatty acids in egg yolk lipids less than 4:1 is considered to be beneficial to human health. This desirable ratio of n-6 and n-3 polyunsaturated fatty acids was highlighted by other authors as well [28,29].

4. Conclusions

Obtained results have shown that the addition of *M. alternifolia* essential oil in the hen diet has a significant positive effect on egg production, but did not show a significant effect on the yolk fatty acid profile, except increased eggshell thickness. Overall, *M. alternifolia* essential oil in a lower concentration of 40 mg/kg in may be beneficial and recommended for hen nutrition, but further investigation about *M. alternifolia* essential oil's influence on laying hens' nutrition and its effects and mode of action is more than necessary.

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References

1. Puvača, N.; Čabarkapa, I.; Bursić, V.; Petrović, A.; Aćimović, M. Antimicrobial, antioxidant and acaricidal properties of tea tree (*Melaleuca alternifolia*). *J. Agron. Technol. Eng. Manag.* **2018**, *1*, 29–38.
2. Hammer, K.A.; Carson, C.F.; Riley, T.V.; Nielsen, J.B. A review of the toxicity of *Melaleuca alternifolia* (tea tree) oil. *Food Chem. Toxicol.* **2006**, *44*, 616–625. [CrossRef] [PubMed]
3. Puvača, N.; Stanaćev, V.; Glamočić, D.; Lević, J.; Perić, L.; Stanaćev, V.; Milić, D. Beneficial effects of phytoadditives in broiler nutrition. *Worlds Poult. Sci. J.* **2013**, *69*, 27–34. [CrossRef]
4. Popović, S.; Puvača, N.; Kostadinović, L.; Džinić, N.; Bošnjak, J.; Vasiljević, M.; Djuragic, O. Effects of dietary essential oils on productive performance, blood lipid profile, enzyme activity and immunological response of broiler chickens. *Eur. Poult. Sci.* **2016**, *80*. [CrossRef]
5. Puvača, N. Bioactive compounds in selected hot spices and medicinal plants. *J. Agron. Technol. Eng. Manag.* **2018**, *1*, 8–17.
6. Khattak, F.; Ronchi, A.; Castelli, P.; Sparks, N. Effects of natural blend of essential oil on growth performance, blood biochemistry, cecal morphology, and carcass quality of broiler chickens. *Poult. Sci.* **2014**, *93*, 132–137. [CrossRef]
7. Olgun, O. The effect of dietary essential oil mixture supplementation on performance, egg quality and bone characteristics in laying hens. *Ann. Anim. Sci.* **2016**, *16*, 1115–1125. [CrossRef]
8. Nikolova, N.; Kocevski, D. Quality of hen's eggshell in conditions of high temperatures. *J. Agron. Technol. Eng. Manag.* **2018**, *1*, 84–90.
9. 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. *Int. J. Poult. Sci.* **2008**, *7*, 134–150. [CrossRef]
10. Galobart, J.; Barroeta, A.C.; Baucells, M.D.; Codony, R.; Ternes, W. Effect of dietary supplementation with rosemary extract and α-tocopheryl acetate on lipid oxidation in eggs enriched with ω3-fatty acids. *Poult. Sci.* **2001**, *80*, 460–467. [CrossRef]
11. Spasevski, N.; Puvača, N.; Pezo, L.; Tasić, T.; Vukmirović, Đ.; Banjac, V.; Čolović, R.; Rakita, S.; Kokić, B.; Džinić, N. Optimisation of egg yolk colour using natural colourants. *Eur. Poult. Sci.* **2018**, *82*. [CrossRef]
12. Puvača, N.; Ljubojević Pelić, D.; Tomić, V.; Radišić, R.; Milanović, S.; Soleša, D.; Budakov, D.; Cara, M.; Bursić, V.; Petrović, A.; et al. Antimicrobial efficiency of medicinal plants and their influence on cheeses quality. *Mljekarstvo* **2020**, *70*, 3–12. [CrossRef]
13. Acamovic, T.; Brooker, J.D. Biochemistry of plant secondary metabolites and their effects in animals. *Proc. Nutr. Soc.* **2005**, *64*, 403–412. [CrossRef] [PubMed]
14. Puvača, N.; Kostadinović, L.; Popović, S.; Lević, J.; Ljubojević, D.; Tufarelli, V.; Jovanović, R.; Tasić, T.; Ikonić, P.; Lukač, D. Proximate composition, cholesterol concentration and lipid oxidation of meat from chickens fed dietary spice addition (*Allium sativum, Piper nigrum, Capsicum annuum*). *Anim. Prod. Sci.* **2016**, *56*, 1920–1927. [CrossRef]
15. Tashla, T.; Puvača, N.; Pelić Ljubojević, D.; Prodanović, R.; Bošković, J.; Ivanišević, D.; Jahić, M.; Mahmoud, O.; Giannenas, I.; Lević, J. Dietary medicinal plants enhance the chemical composition and quality of broiler chicken meat. *J. Hell. Vet. Med. Soc.* **2019**, *70*, 1823–1832. [CrossRef]
16. Bozkurt, M.; Hippenstiel, F.; Abdel-Wareth, A.A.A.; Kehraus, S.; Küçükyilmaz, K.; Südekum, K.-H. Effects of selected herbs and essential oils on performance, egg quality and some metabolic activities in laying hens—A review. *Eur. Poult. Sci.* **2010**, *78*, 15.
17. Chowdhury, S.R.; Chowdhury, S.D.; Smith, T.K. Effects of dietary garlic on cholesterol metabolism in laying hens. *Poult. Sci.* **2002**, *81*, 1856–1862. [CrossRef]
18. Abdo, Z.M.A.; Hassan, R.A.; El-Salam, A.A.; Helmy, S.A. Effect of adding green tea and its aqueous extract as natural antioxidants to laying hen diet on productive, reproductive performance and egg quality during storage and its content of cholesterol. *Egypt. Poult. Sci.* **2010**, *30*, 1121–1149.
19. Giannenas, I.; Koidis, A.; Botsoglou, E.; Dotas, V.; Mitsopoulos, I.; Florou-Paneri, P.; Nikolakakis, I. Hen performance and egg quality as affected by dietary oregano essential oil and alpha-tocopheryl acetate supplementation. *Int. J. Poult. Sci.* **2005**, *4*, 449–454.

20. Saki, A.A.; Aliarabi, H.; Hosseini Siyar, S.A.; Salari, J.; Hashemi, M. Effect of a phytogenic feed additive on performance, ovarian morphology, serum lipid parameters and egg sensory quality in laying hen. *Vet. Res. Forum* **2014**, *5*, 287–293.
21. Bozkurt, M.; Küçükyilmaz, K.; Catli, A.U.; Cinar, M.; Bintas, E.; Cöven, 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 conditions. *Poult. Sci.* **2012**, *91*, 1379–1386. [CrossRef] [PubMed]
22. Bölükbasi, S.C.; Erhan, M.K.; Ürüsan, H. The effects of supplementation of bergamot oil (*Citrus bergamia*) on egg production, egg quality, fatty acid composition of egg yolk in laying hens. *J. Poult. Sci.* **2010**, *47*, 163–169. [CrossRef]
23. Popović, S.; Kostadinović, L.; Đuragić, O.; Aćimović, M.; Čabarkapa, I.; Puvača, N.; Pelić Ljubojević, D. Influence of medicinal plants mixtures (*Artemisia absinthium*, *Thymus vulgaris*, *Menthae piperitae* and *Thymus serpyllum*) in broilers nutrition on biochemical blood status. *J. Agron. Technol. Eng. Manag.* **2018**, *1*, 91–98.
24. Ghasemi, R.; Zarei, M.; Torki, M. Adding medicinal herbs including garlic (*Allium sativum*) and thyme (*Thymus vulgaris*) to diet of laying hens and evaluating productive performance and egg quality characteristics. *Am. J. Anim. Vet. Sci.* **2010**, *5*, 151–154. [CrossRef]
25. Abdel-Wareth, A.A.A. Effect of dietary supplementation of thymol, synbiotic and their combination on performance, egg quality and serum metabolic profile of Hy-Line Brown hens. *Br. Poult. Sci.* **2016**, *57*, 114–122. [CrossRef]
26. Simopoulos, A.P. Human requirement for N-3 polyunsaturated fatty acids. *Poult. Sci.* **2000**, *79*, 961–970. [CrossRef]
27. Kralik, G.; Škrtić, Z.; Suchý, P.; Straková, E.; Gajčević, Z. Feeding fish oil and linseed oil to laying hens to increase the n-3 PUFA in egg yolk. *Acta Vet. Brno* **2008**, *77*, 561–568. [CrossRef]
28. Ebeid, T.A. The impact of incorporation of n-3 fatty acids into eggs on ovarian follicular development, immune response, antioxidative status and tibial bone characteristics in aged laying hens. *Animal* **2011**, *5*, 1554–1562. [CrossRef]
29. Puvača, N.; Lukač, D.; Ljubojević, D.; Stanaćev, V.; Beuković, M.; Kostadinović, L.; Plavša, N. Fatty acid composition and regression prediction of fatty acid concentration in edible chicken tissues. *Worlds Poult. Sci. J.* **2014**, *70*, 585–592. [CrossRef]