Effect of Dietary Basil Essential Oil Supplementation on Performance and Egg Quality of Laying Hens

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Abstract

This study aimed at investigating the effect of dietary supplementation with basil essential oil (BEO, *Ocimum basilicum* L.) on laying performance and egg quality of laying hens. A total of 30 ISA Brown laying hens, 25 weeks of age, were allocated to three experimental groups/treatments, with 10 hens per group. They were housed in 5 cages (replicates) per treatment, with 2 hens per cage, and were fed a fixed amount of feed (mixed-form concentrated feed mixture, 120 g/hen/day). Basil essential oil (BEO) was added to the feed at inclusion levels of 0, 1000, and 2000 mg/kg for treatments BEO0 (control), BEO1000, and BEO2000, respectively. The trial lasted 8 weeks (56 days). Across the experimental period, basil essential oil did not affect feed intake, egg production (0.953 eggs/hen/day), abnormal egg production, egg weight, egg mass, or feed conversion (2.10 g feed/g egg mass) ($P>0.05$). However, higher dietary levels up to 2000 mg/kg reduced shell weight (6.23 g vs. 5.85 g), shell thickness (0.39 mm vs. 0.35 mm), yolk diameter, and albumen diameter ($P<0.05$), while improving, albumen index (16.14 vs. 19.84, $P<0.001$), and Haugh units (104.29 vs. 110.46, $P=0.001$). Other egg quality characteristics were not affected ($P>0.05$).

Keywords: egg characteristics, egg yield, feed ingredient, *Ocimum basilicum*.

1. Introduction

Poultry farming is one of the most important and dynamic sectors of animal production. It is independent of the country's soil and climatic conditions and is characterized by a relatively low cost of production [1]. Growing consumer concern about the possible health risks and environmental effects caused by the excessive use of antibiotics as growth promoters in animal production led to their ban in animal nutrition within the European Union in 2006. This measure aimed to minimize the transmission and spread of resistant bacteria through the food chain. However, industries involved in the animal-product production chain, including producers, processors, distributors, and traders, are continuously seeking effective, safe, and economically viable substances with similar properties. As a result, natural feed supplements derived from plants, such as essential oils (EO), have been investigated as alternatives in animal production for improving performance parameters and the quality characteristics of products such as meat, milk, and eggs [2].

Essential oils are complex mixtures composed mainly of terpenoids and various low-molecular-weight aliphatic hydrocarbons, such as phenols and aromatic aldehydes. The use of essential oils in the diet of farm animals can enhance the digestion of nutrients, stimulate the circulatory system, exert antioxidant activity, reduce the levels of pathogenic bacteria, decrease the production of fermentation products such as ammonia and biogenic amines, improve the absorption of essential nutrients in the intestine, and consequently support the gut-associated immune system [2].

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Basil (family *Lamiaceae*) is an herb that probably originates from the tropical and subtropical regions of India. It is used in traditional Indian medicine for the treatment of various infections, skin and liver disorders, colds, coughs, fever, and malaria [3]. Basil is an excellent source of β-carotene, magnesium, iron, and calcium. Recent research has also reported the hypoglycaemic and hypolipidemic activity of an aqueous extract of basil species, including *Ocimum sanctum* L. and *O. basilicum* L. [4,5]. This activity is based on the inhibition of the enzyme β-glucosidase, which prevents the breakdown of starch and sucrose and, consequently, glucose absorption, resulting in reduced blood sugar levels [6]. Testing of the essential oil of *O. basilicum* L. showed that its high eugenol content inhibited blood cell aggregation, classifying basil among natural anticoagulants [7].

The aim of this study was to investigate the effect of dietary basil essential oil (*Ocimum basilicum* L.) on the productive traits of laying hens and the quality characteristics of eggs.

2. Materials and methods

2.1 Basil essential oil

Basil essential oil (BEO) was obtained from a local company specialised in the cultivation, production, and processing of aromatic and medicinal plants (Dioscurides, Kozani, Greece).

2.2 Diet formulation, husbandry, sample collection and egg quality

In an 8-week experiment at the poultry experimental facility of the International Hellenic University (Thessaloniki, Greece; 40°39′ N, 22°48′ E), 30 laying hens (ISA Brown, ISA, 2021) [8], 25 weeks of age at the onset of the experiment, were used in total. The house had 30 identical cages equipped with similar troughs for feed and water. There were 5 replicate cages of three dietary treatments namely BEO0, BEO1000, and BEO2000 randomly allocated in the house. There were 2 hens per cage, 10 per treatment. At the onset of the experiment, initial body weight (BW) was similar between treatments, at 1,844.06 ± 145.03 g. All birds used in the experiment were cared for according to the European Union directive on animal welfare for scientific purposes [9]. The BEO was added to the basal diet at inclusion levels of 0, 1,000 and 2,000 mg/kg for treatments BEO0 (control), BEO1000, and BEO2000, respectively. Formulation of diets was done according to nutrient requirements of laying hens as provided by the classic reference for poultry nutrition of the US National Research Council [10]. The basal diet was common for all treatments and its compositional and calculated analysis is presented in Table 1. Addition of BEO was performed once every 5 days period and diets were kept in opaque polyethylene bags under cool and dry environment until fed to birds. Bags were hermetically sealed to minimize evaporation of volatile compounds.

During the experimental period, conventional management procedures were employed, light was provided for 16 h per day, ambient temperature was controlled, and birds were fed at 120 g/hen/day and watered ad libitum. The quantity of feed provided to layers was set at that level in order to meet hens' nutrients needs and guarantee that the provided feed quantity as well as the feed additive will be consumed. Hens' BW (g) was measured at the start (day 1) and end (day 56) of the study on a cage basis. Health/mortality, daily feed intake (FI, g), egg production (eggs/hen/day), cracked, thin-shelled and shell-less eggs (eggs/hen/day), egg weight (g), egg mass (g/hen/day) and feed conversion ratio (FCR, g feed/g egg mass) were recorded daily [11-14].

Furthermore, in one (1) egg per replicate (5 eggs/treatment) collected randomly at the 14th, 28th, 42nd and 56th day of the experimental period, external and internal egg qualities (20 eggs/treatment) were evaluated [11-16]. For the external egg quality, the following 5 parameters were determined: egg weight, egg length, egg diameter, eggshell weight, and eggshell thickness. For the internal egg quality, the following 8 parameters were determined: yolk colour, yolk and albumen height, yolk and albumen diameter, yolk weight, yolk and albumen pH. Afterwards, the following 6 parameters were calculated: eggshell area, egg shape index, yolk index, albumen index, Haugh Units [17], and albumen weight.

2.3 Statistical analysis

The effect of treatment was included in the statistical model. Cage was the observational unit. Results are presented as means, standard error of the mean (SEM), and statistical significance was declared at $P<0.05$. The statistical model was analysed using the SPSS statistical package [18].

3. Results and discussion

All hens remained healthy until the end of the experiment without altering their behaviour. During the experimental period, no differences ($P > 0.05$) occurred among BEO treatments in feed intake, egg production, production of cracked, thin-shelled and shell-less eggs, egg weight and egg mass (Table 2). The feed conversion ratio remained stable across all treatments, indicating that the addition of basil essential oil did not affect the dietary efficiency of the hens. In contrast, a mixed herbal extract, containing basil seed, rue (Ruta graveolens) and coriander, improved hen-day egg production, egg mass and feed intake at 4 mL/L drinking water, although this response cannot be attributed specifically to basil because it was not tested alone [19]. Hammershøj et al. [20] found that basil-containing forage did not significantly affect feed intake, forage intake, or laying rate. In addition, Nworgu et al. [21] reported that basil leaf supplementation significantly improved feed conversion ratio and advanced the onset of lay in growing pullets. In laying quail, basil essential oil at 300–600 mg/kg improved egg production performance, egg mass, feed conversion ratio, and shell percentage [22], suggesting a potential productive benefit of basil-derived additives in laying poultry, although direct evidence in laying hens remains limited.

Table 1. Ingredient and chemical composition of laying hens' basal diet.

	Basal diet
Ingredient composition (g/kg, as mixed)	
Corn grain, ground	588
Soybean meal (440 g/kg CP)	240
Wheat bran	35
Limestone	90
Monocalcium phosphate	20
Salt	2
Vitamin mineral premix[1]	25
Chemical composition[2] (g/kg, as fed)	
Metabolizable energy (kcal/kg)	2550
Dry matter	903
Crude protein (CP)	161
Crude fat	25.3
Crude fiber	33.6
Ash	159.4
Calcium	39.6
Available Phosphorus	7.5
Sodium	2.5
Linoleic acid	14.5
Arginine	10.1
Lysine	8.5
Threonine	6.0
Methionine + cystine	6.4
Tryptophan	2.2

[1]Premix contained 196 g/kg Ca, 95 g/kg P, 60 g/kg Na, 40 g/kg D,L-methionine and 10 g/kg lysine and supplied/kg of diet: 10,000 IU vitamin A; 2 mg vitamin B_1; 5 mg vitamin B_2; 20 mg niacin; 10 mg pantothenic Ca; 3 mg vitamin B_6; 0.06 mg biotin; 1 mg folic acid; 0.02 mg vitamin B_{12}; 3,500 IU vitamin D_3; 25 mg vitamin E; 2 mg vitamin K_3; 3 mg Cu; 1.3 mg I; 30 mg Fe; 65 mg Mn; 0.10 mg Se; 30 mg Zn, 100 mg antioxidant, 500 FTU 3-phytase.
[2]All values were calculated from NRC (1994) values.

The majority of examined external and internal egg quality parameters did not differ between treatments ($P>0.05$) (Table 3). However, increasing dietary inclusion of basil essential oil resulted in a significant linear increase ($P<0.05$) in albumen height, albumen index, and Haugh unit score. Conversely, shell weight, shell thickness, yolk diameter, albumen diameter, and albumen weight decreased linearly ($P<0.05$) as the dietary level of basil essential oil increased.

Evidence from the scientific literature indicates that basil supplementation appears to exert

variable effects on egg quality, depending on the form of basil used and the inclusion level. Dietary basil essential oil improved internal egg quality, as indicated by increased albumen height, albumen index, and Haugh units, although shell weight and shell thickness decreased with increasing inclusion level [23]. In contrast, holy basil powder had no marked effect on external or internal egg-quality traits in semi-free-range laying hens [24], suggesting that the effect of basil on eggshell quality may depend on its form and inclusion level. Similarly, a mixed herbal extract containing basil seed, coriander, and Ruta graveolens did not significantly affect egg weight, albumen, yolk, or shell traits in White Leghorn layers, suggesting limited effects on egg quality when basil is included as part of a phytogenic mixture [19]. Overall, current evidence suggests that basil may improve albumen quality, whereas its effects on shell traits are inconsistent.

Table 2. Performance of laying hens

	Treatment[1, 2]			SEM	Significance level[3]	
	BEO0	BEO1000	BEO2000		Linear	Quadratic
Initial body weight (BW) (g)	1,761.0	1,891.2	1,880.0	40.065	0.246	0.419
Feed intake (g/hen/day)	119.44	119.68	119.53	0.109	0.750	0.431
Egg production – (eggs/hen/day)	0.94	0.96	0.96	0.008	0.598	0.632
Cracked, thin-shelled and shell-less eggs (eggs/hen/day)	0.01	0.01	0.01	0.004	0.406	0.295
Egg weight (g)	59.97	59.99	58.99	0.697	0.599	0.750
Egg mass (g/hen/day)	56.60	57.67	56.48	0.854	0.959	0.568
Feed efficiency (g feed/g egg mass)	2.12	2.08	2.12	0.032	0.924	0.587

[1]BEO0 = control treatment, BEO1000 = treatment with 1,000 mg basil essential oil (BEO)/kg, BEO2000 = treatment with 2,000 mg BEO/kg.
[2]Number of cages (replicates)/treatment = 5. Number of hens/cage (replicate) = 2.
[3]Numbers are probability values.

Table 3. Egg quality of laying hens

	Treatment[1, 2, 3]			SEM	Significance level[4]	
	BEO0	BEO1000	BEO2000		Linear	Quadratic
External egg quality						
Egg weight (g)	59.97	59.99	58.99	0.519	0.448	0.647
Egg shell area (cm^2)	72.64	72.68	71.84	0.417	0.439	0.626
Egg shape index	79.28	79.05	80.43	0.356	0.189	0.290
Egg shell weight (g)	6.23	6.02	5.85	0.068	0.021	0.893
Egg shell thickness (mm)	0.39	0.36	0.35	0.006	0.001	0.429
Internal egg quality						
Yolk colour (15-point colour scale by using a Roche fan)	11.90	12.05	11.85	0.075	0.787	0.277
Yolk index	49.44	49.02	50.64	0.325	0.129	0.137
Yolk weight (g)	14.25	13.62	14.35	0.195	0.835	0.103
Yolk pH	5.80	5.83	5.93	0.028	0.084	0.622
Albumen index	16.14	17.69	19.84	0.439	<0.001	0.720
Haugh units	104.29	107.22	110.46	0.795	0.001	0.922
Albumen weight (g)	36.94	37.57	35.95	0.466	0.399	0.265
Albumen pH	7.75	7.90	7.90	0.037	0.106	0.300

[1] BEO0 = control treatment, BEO1000 = treatment with 1,000 mg basil essential oil (BEO)/kg, BEO2000 = treatment with 2,000 mg BEO/kg.
[2] Number of cages (replicates)/treatment = 5. Number of hens/cage (replicate) = 2.
[3] Number of eggs (replicates)/treatment/determination (14th, 28th, 42nd and 56th day) = 5.
[4] Numbers are probability values.

Regarding yolk colour, Zurak et al. [25] showed that dried basil leaves can act as a natural carotenoid source, increasing yolk carotenoid deposition and redness, particularly when the

'Dark Opal' cultivar was included, although the pigmentation response remained lower than that achieved with canthaxanthin.

4. Conclusion

The inclusion of basil essential oil in the diet of laying hens, at levels up to 2,000 mg/kg, did not affect productive performance and most egg-quality traits. Although shell weight and shell thickness were reduced in the supplemented treatments compared with the control no increase in cracked eggs was observed.

References

1. Giannakopoulos, A.L., Tserveni-Gousi, A.S., Monogastric Animal Farming, Vol. B: Poultry Farming, Christina and Vasiliki Kordali OE, 2009, pp. 1–350.
2. Simitzis, P.E., Enrichment of animal diets with essential oils: a great perspective on improving animal performance and quality characteristics of the derived products, Medicines, 2017, 4, 35.
3. Gupta, S.K., Prakash, J., Srivastava, S., Validation of traditional claim of Tulsi Ocimum sanctum Linn. as a medicinal plant, Indian Journal of Experimental Biology, 2002, 40, 765-773.
4. Volpato, G.T., Damasceno, D.C., Calderon, I.M.P., Rudge, M.V.C., Review of Brazilian plants with proven hypoglycemic effect in the control of diabetes mellitus, Revista Brasileira de Plantas Medicinais, 2002, 4, 35-45.
5. Stanojevic, L.P., Marjanovic-Balaban, Z.R., Kalaba, V.D., Stanojevic, J.S., Cvetkovic, D.J., Cakic, M.D., Chemical composition, antioxidant and antimicrobial activity of basil (Ocimum basilicum L.) essential oil, Journal of Essential Oil Bearing Plants, 2017, 20, 1557–1569.
6. Wongsa, P., Chaiwarit, J., Zamaludien, A., In vitro screening of phenolic compounds, potential inhibition against α-amylase and α-glucosidase of culinary herbs in Thailand, Food Chemistry, 2012, 131, 964-971.
7. Tognolini, M., Barocelli, E., Ballabeni, V., Bruni, R., Bianchi, A., Chiavarini, M., Impicciatore, M., Comparative screening of plant essential oils: phenylpropanoid moiety as basic core for antiplatelet activity, Life Sciences, 2006, 78, 1419-1432.
8. ISA, 2021. ISA Brown: Commercial Management Guide. ISA, a Hendrix Genetics Company. Home page address: https://www.hendrix-isa.com/en/.
9. European Union (EU), Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, Official Journal of the European Union, 2010, L 276, 33-79.
10. National Research Council, Nutrient Requirements of Poultry, 9th revised ed., National Academy Press, Washington DC, 1994.
11. Bageridizaj, S., Pirmohammadi, R., Bampidis, V., Effects of dietary probiotics on performance, egg quality and yolk/serum cholesterol of laying hens, Journal of Animal and Veterinary Advances, 2006, 5, 1175-1180.
12. Jafari, M., Pirmohammadi, R., Bampidis, V., The use of dried tomato pulp in diets of laying hens, International Journal of Poultry Science, 2006, 5, 618-622.
13. Chalvatzi, S., Arsenos, G., Tserveni-Gousi, A., Fortomaris, P., Tolerance and efficacy study of palygorskite incorporation in the diet of laying hens, Applied Clay Science, 2014, 101, 643-647.
14. Kostogrys, R.B., Filipiak-Florkiewicz, A., Dereń, K., Drahun, A., Czyżyńska-Cichoń, I., Cieślik, E., Szymczyk, B., Franczyk-Żarów, M., Effect of dietary pomegranate seed oil on laying hen performance and physicochemical properties of eggs, Food Chemistry, 2017, 221, 1096-1103.
15. Hanusová, E., Hrnčár, C., Hanus, A., Oravcová, M., Effect of breed on some parameters of egg quality in laying hens, Acta Fytotechnica et Zootechnica, 2015, 18, 20-24.
16. Pappas, A. C., Charisi, A., Chatziantoniou, Ch.-M., Giamouri, E., Mitsiopoulou, C., Moschopoulos, V., Christodoulou, Ch., Papadomichelakis, G., Kotsampasi, B., Mitsopoulos, I. K., Tsiplakou, E., and Bampidis, V. A., Effects of dietary pomegranate seed oil addition to diets for laying hens on fatty acid profile of eggs, Animal Feed Science and Technology, 2023, 300, 115643.
17. Haugh, R. R., The Haugh unit for measuring egg quality, U.S. Egg and Poultry Magazine, 1937, 43, 552–555.
18. IBM Corp., IBM Statistical Package for the Social Sciences (SPSS) Statistics for Windows, Version 25.0, IBM Corp., Armonk, NY, USA, 2017.
19. Tessema, S., Girma, M., Yirgalem, M., Ameha, N., Zeryehun, T., Evaluation of Ruta graveolen, coriander and basil seed extracts as natural additives on productive performance, blood profiles and egg quality of White Leghorn layers, Journal of the Indonesian Tropical Animal Agriculture, 2023, 48, 196-207.
20. Hammershøj, M., Kidmose, U., Steenfeldt, S., Deposition of carotenoids in egg yolk by short-term supplement of coloured carrot varieties as forage material for egg-laying hens, Journal of the Science of Food and Agriculture, 2010, 90, 1163-1171.
21. Nworgu, F.C., Yekinni, S.O., Oduola, O.A., Effects of basil leaf (Ocimum gratissimum) supplement on performance and egg quality characteristics of growing pullets, Sustainable Agriculture Research, 2012, 1, 101-111.
22. Al-Shaheen, S.A., Abbas, R.J., Majeed, T.I., Effect of different levels of basil and peppermint as essential

oils on productive and physiological performance of two quail lines during egg production period, Annals of Forest Research, 2023, 66(1), 2526–2546.
23. Marušić Radovčić, N., Krog, L., Kljak, K., Medić, H., Effects of dietary basil supplementation on egg quality and functional properties in laying hens, In: Book of Abstracts of the XXth European Symposium on the Quality of Eggs and Egg Products and XXVIth European Symposium on the Quality of Poultry Meat, 2025, 71.
24. Nulla-ong, Y., and Maneewan, B., The red holy basil and duckweed powder supplementation in laying hens' diet in the semi-free-range system. Doctoral dissertation, Maejo University, 2021.
25. Zurak, D., Vidak, M., Carović-Stanko, K., Kiš, G., Kos, I., Janječić, Z., and Kljak, K., Basil leaves as a source of carotenoids for yolk pigmentation: Effect of cultivar on carotenoid profile, colour and oxidative stability. Proc. 14th CASEE Conf. "The Role of the Life Sciences Universities in the Green Transition of Central and Eastern Europe", 2024