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Effect of mineral mix-enriched essential oil supplementation on egg production, antibody titer, physical, and chemical egg quality, and yolk fatty acid profile of laying hens

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ABSTRACT

This study aimed to determine the effect of mineral mix–enriched essential oil (MMEO) on egg production, antibody titer, egg quality, and yolk fatty acid of laying hens. A total of 1680 Lohman Brown laying hens, aged 31 weeks, were randomly divided into two dietary treatment groups. Each group consisted of 6 replicates, with 140 hens per treatment. The hens were fed either a basal diet (CON) or a diet supplemented with 0.25% mineral mix–enriched essential oil (MMEO) for a period of 6 weeks. Egg production and feed efficiency were calculated on every week. On days 21 and 42 of treatments, two eggs per replicate were randomly collected to determine the physical and chemical quality. The antioxidant activity and fatty acid profile of one egg per replicate were analysed at the end of week 6. The results showed that dietary treatment with MMEO increased egg production and feed efficiency compared to the control group. The inclusion increased yolk weight at week 3. However, MMEO supplementation did not affect the physical quality of eggs at week 6. In addition, dietary MMEO supplementation increased the antioxidant activity of yolk at week 6. On contrast, feeding MMEO did not affect the fatty acid profile of yolk. In conclusion, supplementation of mineral mix–enriched essential oil improved laying hens' productivity and antioxidant activity of yolk.

Introduction

The ban on the use of antibiotic growth promoters (AGPs) in poultry diets in the European Union since 2006 has driven research towards exploring alternative additives to maintain the health and productivity of laying hens. Various studies have investigated substitutes for AGPs, such as probiotics, enzymes, and natural plant-derived products, to enhance production performance, egg quality, and overall health in laying hens (Khan *et al*., 2011; Xiang *et al*., 2019; Hanif *et al*., 2023;). The ban on AGPs has resulted in increased research focusing on the development of effective antibiotic alternatives, with a specific emphasis on natural additives due to their environmentally friendly nature and diverse biological activities (Wen *et al*., 2019). As consumer demand for antibiotic-free products rises, the poultry industry is increasingly investing in research to identify viable alternatives to AGPs that can ensure optimal growth, egg quality, and immune function in laying hens (Mohammed *et al*., 2021).

The mineral combination has been shown to enhance egg production in laying hens through various mechanisms. Studies have indicated that supplementing with specific mineral blends can improve laying performance, mineral deposition, and reduce mineral excretion in hens. Additionally, incorporating organic trace minerals in diets has been linked to increased egg production rates, enhanced egg quality, and improved bone mineralization in laying hens (Liao *et al*., 2023). Mineral mixes have been proposed as a potential alternative to antibiotics in laying hen diets (Gadde *et al*., 2017). Mineral mixes offer various benefits, such as improved growth performance and feed efficiency in poultry. The antibacterial properties of minerals like clay and metals have been demonstrated to disrupt essential bacterial functions and inhibit bacterial growth (Williams *et al*., 2011). Furthermore, the antibacterial mechanism of medicinal clay, as investigated by Morrison *et al*. (2016), presented a geochemical approach that could effectively address antibiotic resistance, offering a promising strategy to tackle bacterial challenges in poultry production.

In addition, the use of essential oil combinations in the diet of laying hens has been shown to enhance egg production through various mechanisms. Essential oils, such as those derived from oregano, thyme, and rosemary, possess antimicrobial, antioxidant, and anti-inflammatory properties that can positively influence the health and productivity of hens (Zhao *et al*., 2021). The investigation examined the productive performance, egg quality characteristics, and blood parameters of laying hens reared under cold stress conditions in relation to the individual and combined effects of peppermint essential oil, thyme essential oil, or their combination (Akbari *et al*., 2016). The potential benefits of essential oil combinations in enhancing egg production and overall performance in laying hens through various mechanisms, including effects on the jejunum, duodenum, and other intestinal parameters (He *et al*., 2017). The combination of minerals mixed with essential oil has good potential to improve egg production, hens' health, and egg quality. No studies have evaluated the effect of mineral mix-enriched essential oil supplementation on productivity, antibody titer, and egg quality of laying hens. This study aimed to determine the effect of essential oil supplementation enriched with mineral mixture on egg production, titer antibody, physical, and chemical egg quality, and yolk fatty acid profile of laying hens.

Materials and methods

Study location and ethical clearance

The experiment was conducted at PT. Sentra Gemilang Mulia, Layer Farm in Agrodadi Village, Sedayu Subdistrict, Bantul District, Special Region of Yogyakarta Province, Indonesia, and obtained prior consent from the Ethical Commission of the Faculty of Veterinary Medicine at Universitas Gadjah Mada, Indonesia, under reference number 98/EC-FKH/Eks/2023. The physical quality of eggs was analysed at the Laboratory of Poultry Science, Faculty of Animal Science, Universitas Gadjah Mada,

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Indonesia. Egg chemical quality was assessed at the Laboratory of Feed Technology, Universitas Gadjah Mada. Analysis of antibody titer levels was carried out at the Balai Besar Veteriner Wates, Kulonprogo, Indonesia. Quantification of fatty acid profile was conducted at the Integrated Research and Testing Laboratory, Universitas Gadjah Mada, Indonesia.

Hens, diet, and housing

A total of 1680 Lohman Brown hens, aged 31 weeks (BW: 1.70±0.11 kg, HDA: 81.2%), were randomly divided into two groups, each containing six replicates of 140 hens. The first group was fed a basal diet (CON), while the second group was administered a basal diet supplemented with 0.25% mineral mix-enriched essential oil (Twin Booster Unggas® provided by PT Agromix Lestari, Yogyakarta, Indonesia) (MMEO) for 31-37 weeks (a 6-week treatment). The experimental diet was formulated to meet the nutritional requirements of laying hens and included commercial high-protein concentrate, maize, rice bran, premix, and vitamin mix (Table 1). Hens were housed in bamboo-based layer cages with a 16-hour light and 8-hour dark cycle, simulating summer conditions.

Table 1. Ingredients and nutrient composition of experimental diets.

Feed Ingredient (%)	CON	MMEO
Maize	49.36	49.24
High protein concentrate[1]	36.43	36.33
Rice bran	12.85	12.82
Premix[2]	1.11	1.1
Vitamin mix[3]	0.26	0.25
Mineral mix-essential oil[4]	0	0.25
Total	100	100
Chemical composition		
Dry matter (%)	88.1	88.6
Organic matter (%)	83.7	83.6
Gross energy (Kcal/kg)	3525	3528
Crude protein (%)	18	18.3
Crude fiber (%)	4.5	4
Extract ether (%)	6.1	5.8
Nitrogen-free extract (%)	55.1	54.4
Ash (%)	16.4	16.4
Calcium (%)	3.4	3.1
Phosphor (%)	0.38	0.41

[1]Commercial high protein concentrate contained (per kg): moisture 120 g, ash 350 g, crude protein 360 g, extract ether 30 g, crude fiber 80 g, calcium 90 g, phosphor 5 g, methionine 8 g, lysine 17 g, threonine 11 g, tryptophan 3.4 g; Concentrate ingredients: corn gluten meal, rice bran, soybean meal, distillers dried grains with solubles, meat bone meal, palm oil, and essential amino acid

[2]Premix contained (per 10 kg): Vitamin A 12.000.000 UI, Vitamin D3 2.000.000 UI, Vitamin E 15.000 mg, Vitamin B1 2.400 mg, Vitamin B2 5.000 mg, Vitamin B6 4.000 mg, Vitamin B12 9.000 mg, Ca-d-Panthothenate 50.000 mg, Nicotinic acid 50.000 mg, Folic acid 50 mg, Methionine 30.000 mg, lysine 30.000 mg, Manganese 50.000 mg, Zinc 30.000 mg, Iron 24.000 mg, Copper 10.000 mg, Cobalt 500 mg, Selenium 400 mg and filler.

[3]Vitamin mix contained (per tons): vitamin A 5.000.000 IU, Vitamin D3 1.000.000 IU, Vitamin E 7.500 IU, Vitamin K 1.530 mg, Vitamin B1 800 mg, Vitamin B2 3.000 mg, Vitamin B6 800 mg, Vitamin B12 10.000 mg, Vitamin C 5.000 mg, Ca-d-Panthothenate 5.000 mg, Niacin 7.530 mg, Folic acid 140 mg, Choline chloride 100.000 mg, DL-Methionine 100.000 mg, Copper 2.200 mg, Cobalt 240 mg, Ferros 23.400 mg, Iodium 1.200 mg, Mangan 40.800 mg, Zinc 30.000 mg.

[4]Mineral mix-enriched essential oil (Twin Booster Unggas®) contained (per kg): Calcium 200 g; Sodium 88,1 g; Phosphor 24.6 g; Feron 12.6 g; Magnesium 2.0 g, Zinc 1.8 g; Manganese 1.4 g; Kalium 725 mg; Copper 721 mg; Sulphur 144 mg; Cobalt 58.3 mg; Selenium 182 µg; and blend essential oils (*Cocos nucifera, Cymbopogon citrates*, Eucalyptus globules, Gardenia jasminoides, Gummi Boswellii, Gummi Myrrha, Herba origani, Pine oil, Carrot seed oil, and Paprika extracts)

Antibody titer assay

One hen per replicate group was blood collected for antibody titer analysis. Antibody titer was tested using the haemagglutination inhibition test (HI Test) for Newcastle Disease (ND) and Avian Influenza (AI) viruses. Blood was taken from the brachial vein using a syringe inserted under the pronator musculus tendon, and then the needle was directed to the brachial vein. Blood samples were then centrifuged at 2500 rpm for 10 minutes to separate serum. After serum was separated, it was transferred into a microtube and inactivated in a 56ºC water bath for 30 minutes and the sample was stored in the refrigerator until examined.

A microplate and isotonic PBS (0.01 M), pH 7.0-7.7, were prepared for the antibody titer assay. Red blood cells (erythrocytes) were selected from poultry blood samples and diluted to the same volume. Before employing red blood cells as a 1% (v/v) suspension, they were rinsed three times in PBS. Each test was conducted with both positive and negative control antigens and antisera. A total of 0.025 ml PBS was put into each microplate well and a total of 0.025 ml serum was put into the first well. Two-fold dilutions were made from 0.025 ml of serum volume in all wells. 4 HAU (haemagglutination unit) of virus/antigen was added into the microplate. The antibody titer in wells was observed for the inhibition of haemagglutination. HI antibody titer was calculated based on the highest dilution of antibody that was still able to inhibit haemagglutination. The antibody titer was positive if there was haemagglutination inhibition at a serum dilution of ≥1/16 (≥ 24 or log2 4) or more against antigen 4 HAU.

Physical egg quality assay

Two egg per replicate was collected on days 21 and 42 to assess physical egg quality. The parameters measured included egg weight, shape index, specific gravity, shell strength, shell thickness, shell weight, albumen index, albumen weight, yolk index, yolk weight, yolk color, and Haugh unit (HU). Eggshell strength was measured with an egg pressure gauge (FHK, Fujihira Industry Co., Ltd., Japan). Eggshell thickness was measured using a digital thickness gauge (TKG 002, Soha, China). The egg, eggshell, yolk and albumen were weighed using a digital scale (PT. Arta Joil Tappa, Indonesia). The egg shape index was determined by dividing the vertical diameter by the horizontal diameter using the Egg Size Measurement Instrument (FHK, Fujihira Industry Co., Ltd., Japan). Egg volume was determined using a 1000 ml glass measuring cylinder (PT. Iwaki Glass Indonesia, Indonesia). Egg-specific gravity was calculated by dividing the egg's weight by its volume. Yolk color was assessed with the Roche yolk color fan scores (RYCF; F. Hoffman-La Roche, Switzerland). The Haugh unit (HU) was calculated using the specified equation (Haugh, 1937): Haugh Unit= 100×Log (albumen height−1.7×egg weight+7.6).

Chemical egg quality assay

On days 21 and 42, one egg per replicate was collected for chemical quality analysis. The eggs were cracked open, and the yolk was extracted for examination. Proximate analysis was conducted to determine the moisture, crude protein, crude fat, and ash content of the yolk, following the AOAC guidelines by Hortwitz and Latimer (2005).

Antioxidant activity assay

Yolk samples from eggs collected on days 21 and 42 (one egg per replicate) were analysed for antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. For the extraction process, 5 ml of methanol solvent were combined with 0.2 grammes of yolk and vortexed for a single minute. The mixture was then centrifuged at 3000 rpm for 10 minutes (Eppendorf 5804R, Germany). A 0.2 ml portion of the supernatant was combined with 2.8 ml of 0.1 mM DPPH and kept in a dark area for 1 hour. The absorbance was determined at 515 nm using UV-Vis spectrophotometers (Thermo Fisher Genesys 10s UV-Vis, USA) against a methanol blank at a wavelength of 515 nm. Scavenging activity was expressed as the percentage inhibition (I%), calculated using the following equation. I%= (Abs. blank – Abs. sample/Abs. blank) × 100.

Fatty acid profile assay

One egg per replicate was collected on days 21 and 42 for yolk fatty acid profile analysis. A test tube containing a 5 g yolk sample was filled with 10 ml of 37% HCl. The mixture was heated to 80°C for three hours. After cooling, the solution was extracted using a 1:1 blend of 25 ml diethyl ether and petroleum ether. The mixture was vortexed, and the upper layer (oil) was separated by evaporating it in a water bath with N_2 gas. A 0.5 ml portion of the oil was placed in a small test tube and sealed. Then, 1.5 ml of sodium methoxide solution was added. The solution was heated to 60°C for 10 minutes with shaking. After cooling, 2 ml of boron trifluoride methanol was added and heated again at 60°C for 10 minutes, followed by cooling. The mixture was extracted with 1 ml of heptane and 1 ml of saturated NaCl. The top layer was transferred into a GC vial, with 1 µl being used for analysis.

Fatty acid profile was determined using a gas chromatograph (Agilent 7890B autosampler Series, Agilent Technologies, Palo Alto, CA, USA) equipped with an HP-88 capillary column (100 m×0.3 µm×0.2 µm, Agilent Technologies, Palo Alto, CA, USA). The analysis was performed using gas chromatography-mass spectrometry (GC-MS). The temperature program was set as follows: it started at 100°C for 5 minutes, then increased to 240°C at a rate of 4°C per minute. Ultrahigh-purity helium was used as the carrier gas at a flow rate of 30 mL/min. The temperatures of the injector, interface, and ion source were set to 280°C, 260°C, and 240°C, respectively.

Statistical analysis

The response parameters evaluated in this study encompassed antibody titer, physical and chemical egg quality, antioxidant activity, and the yolk fatty acid profile. The data collected were analysed using a T-Test design with SPSS software, version 26 (SPSS Inc., Chicago, IL).

Results

Egg production and antibody titer

This study showed feeding MMEO increased egg production (Fig. 1). In addition, the treatment group showed a lower FCR than the control group (Fig. 2). However, supplementation of MMEO did not affect AI and ND antibody titer of laying hens (P>0.05) (Table 2).

Table 2. Effect of Mineral mix-enriched essential oil supplementation on titer antibody of laying hens at 6-week treatment

Antibody titer	CON	MMEO	SEM	p-value
AI (HI)	1676	1902	108	0.34
ND (HI)	256	311	37	0.49

CON: basal diet, MMEO: basal diet mixed with a Mineral mix-enriched essential oil 0.25%; SEM: standard error of the mean; p-value: indicates that the values are significantly different; AI: Avian influenza; ND: Newcastle disease .

Physical and chemical egg quality

The results of the egg physical quality assay showed that supplementation of MMEO increased the yolk index at week 3 (P=0.04, Table 3). However, MMEO supplementation did not affect the physical quality of eggs at week 6 (P>0.05). There is no difference between treatment and control groups on all chemical egg quality variables (P>0.05, Table 4).

Antioxidant activity and fatty acid profile

The results of MMEO supplementation on antioxidant activity and fatty acid profile of yolk are presented in Table 5. Supplementation of MMEO increased the antioxidant activity of yolk (P<0.05). Nevertheless, feeding MMEO did not affect the fatty acid profile of yolk (P>0.05). Classification of yolk unsaturated fatty acids based on n-3, n-6, and n-9 also showed no difference between treatment and control.

Table 3. Effect of mineral mix-enriched essential oil supplementation on physical egg quality.

Variables	CON	MMEO	SEM	*p-value*
Week 3				
Egg weight (g)	60.8	61.2	0.26	0.39
Egg shape index	77.1	76.9	0.23	0.58
Egg specific gravity (g/ml)	1.1	1.1	0.01	0.08
Eggshell strength (mPa)	0.39	0.4	0.01	0.31
Eggshell thickness (mm)	0.34	0.34	0	0.86
Eggshell weight (g)	6.5	6.6	0.06	0.25
Albumen index	0.14	0.14	0	0.78
Albumen weight (g)	40.1	40	0.27	0.75
Yolk index	0.43	0.44	0	0.32
Yolk weight (g)	14.2	14.7	0.12	0.04
Yolk color	10.1	10.4	0.16	0.26
Haugh unit	95.9	97	0.67	0.43
Week 6				
Egg weight (g)	61	61.4	0.26	0.38
Egg shape index	77.3	77.1	0.24	0.79
Egg specific gravity (g/ml)	1.1	1.1	0.01	0.13
Eggshell strength (mPa)	0.4	0.41	0.01	0.47
Eggshell thickness (mm)	0.34	0.35	0	0.31
Eggshell weight (g)	6.8	6.9	0.07	0.48
Albumen index	0.14	0.14	0	0.96
Albumen weight (g)	39.9	40	0.24	0.91
Yolk index	0.43	0.43	0	0.29
Yolk weight (g)	14.3	14.6	0.12	0.21
Yolk color	10.6	11.1	0.14	0.12
Haugh unit	96.9	97	0.76	0.92

CON: basal diet, MMEO: basal diet mixed with a mineral mix-enriched essential oil 0.25%; SEM: standard error of the mean, p-value: indicates that the values are significantly different

Table 4. Effect of mineral mix-enriched essential oil supplementation on chemical egg yolk quality

Variables	CON	MMEO	SEM	*p-value*
Week 3				
Dry matter (%)	50.2	50.3	0.61	0.96
Organic matter (% fresh)	47.3	47.7	0.61	0.7
Protein (% fresh)	15	14.9	0.07	0.44
Fat (% fresh)	28.4	28.5	0.3	0.82
Ash (% fresh)	2.9	2.6	0.08	0.1
Week 6				
Dry matter (%)	52.5	53.3	0.18	0.31
Organic matter (% fresh)	50	50.7	0.18	0.28
Protein (% fresh)	16.2	16.4	0.15	0.18
Fat (% fresh)	30.1	30.6	0.25	0.11
Ash (% fresh)	2.5	2.6	0.05	0.67

CON: basal diet, MMEO: basal diet mixed with a mineral mix-enriched essential oil 0.25%; SEM: standard error of the mean, p-value: indicates that the values are significantly different

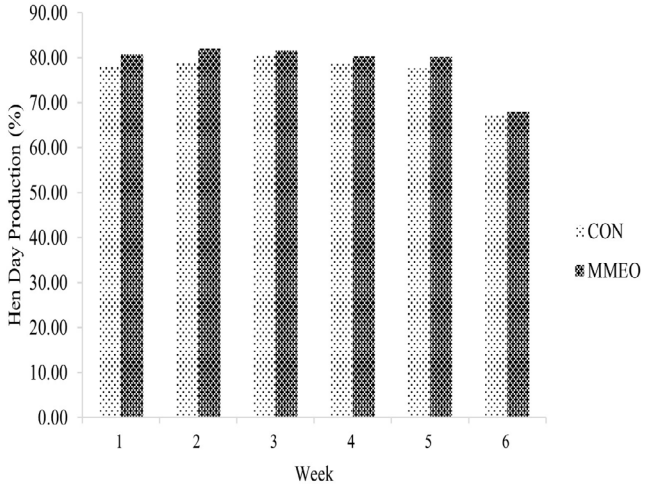

Fig. 1. Effect of Mineral mixed-enriched essential oil supplementation on hen day production.

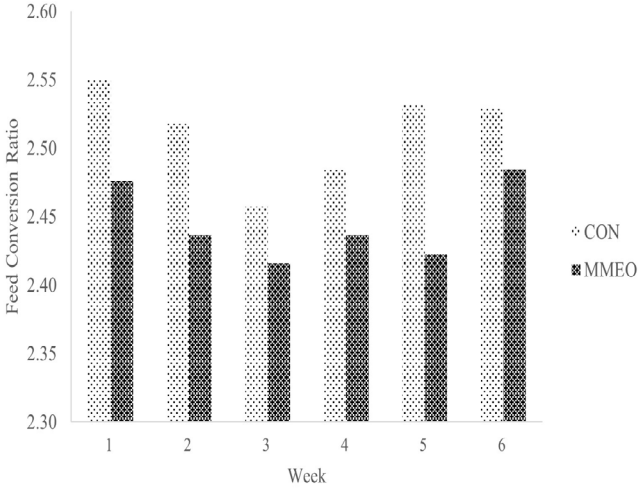

Fig. 2. Effect of Mineral mixed-enriched essential oil supplementation on feed conversion ratio.

Table 5. Effect of mineral mix-enriched essential oil supplementation on antioxidant activity and yolk fatty acid profile.

Variables	CON	MMEO	SEM	*p-value*
DPPH (inhibition %)	2.8	2.9	0.04	0.03
Butyric (C4:0)	0.32	0.2	0.05	0.25
Myristic (C14:0)	0.38	0.38	0	0.83
Heptadecanoic (C17:0)	3.5	3.4	0.12	0.61
Arachidic (C20:0)	11.9	12.2	0.22	0.45
Heneicosanoic (C21:0)	0.2	0.2	0.01	0.92
Palmitoleic (C16:1)	28.4	28.8	0.18	0.28
Cis-9-oleic (C18:1 n9c)	11.3	11	0.17	0.43
Linolelaidic (C18:2 n6t)	37.8	37.9	0.23	0.78
Cis-11-eicosenoic (C20:1 n9)	0.15	0.15	0	0.91
Cis-11,14-eicosadienoic (C20:2)	0.33	0.29	0.01	0.38
Cis-11,14,17-eicosatrienoic (C20:3 n3)	0.33	0.32	0.01	0.94
Erucic (C22:1 n9)	0.17	0.17	0.01	0.86
Cis-5,8,11,14-eicosatetraenoic (C20:4 n6)	4.1	4	0.08	0.52
Nervonic (C24:1 n9)	0.35	0.34	0.02	0.97
Cis-4,7,10,13,16,19-docosahexaenoic (C22:6 n3)	0.89	0.85	0.02	0.48
SFA	16.4	16.4	0.15	0.96
MUFA	40.3	40.4	0.17	0.8
PUFA	43.3	43.2	0.22	0.86
n-3	1.2	1.2	0.02	0.5
n-6	4.4	4.2	0.06	0.14
n-9	11.8	11.6	0.16	0.45
n-6/n-3	0.37	0.36	0.01	0.54
PUFA/SFA	2.6	2.6	0.03	0.95

CON: basal diet, MMEO: basal diet mixed with a mineral mix-enriched essential oil 0.25%; SEM: standard error of the mean, p-value: indicates that the values are significantly different

Discussion

In this study, productive laying hens supplemented with mixed mineral-enriched essential oil showed higher egg production and feed efficiency than the control group. Previous research showed supplementation of organic minerals mixture (Mn, Zn, and Cu) at doses of 0.5 g/kg and 1.0 g/kg increased egg production of laying hens (Saleh *et al*., 2020). In addition, supplementing hydroxy trace minerals (Cu, Zn, and Mn) increased egg production at 4-week treatments under tropical conditions (Palanisamy *et al*., 2023). In contrast, methionine chelates mineral supplements (Zn, Cu, Mn) did not affect the productivity of laying hens (Lim & Paik, 2003). Moreover, feeding organic minerals showed no effect on egg production of white layers (Fernandes *et al*., 2008). In another study, laying hens fed diets supplemented with organic trace minerals did not affect egg production and FCR (Stefanello *et al*., 2014). giving mineral premix feed up to 0.50% did not affect the performance of SAN laying hens (Untari *et al*., 2024). Research on essential oil mix supplementation in laying hens increases egg production of laying hens (Çabuk *et al*., 2006). Additionally, the addition of an essential oil mixture to the nutrition of laying hens resulted in an increase in the rate of egg production and the weight of eggs (Bozkurt *et al*., 2009). Feeding essential oil enhanced egg production of laying hens at week 2 treatment and increased protein digestibility (Ding *et al*., 2017). In the mineral-essential oil combination study, the hens fed a diet supplemented by Zinc and

cinnamon oil (individually or in the combined form) had lower FCR and higher egg production, egg weight, and egg mass (Torki *et al*., 2015). In contrast, laying hens fed selenium and oregano oil showed no difference between treatment and control groups (Reshadi *et al*., 2020).

The effectiveness of essential oils blend was determined by the composition of the herb ingredients. In this study, lemongrass (*Cymbopogon citrates*) was included in the essential oil blend. Lemongrass has been researched to have ability as an anthelmintic agent (Rodenbücher *et al*., 2023; Tiwari *et al*., 2018). In addition, the essential oil blend is composed of Eucalyptus globules. The antibacterial properties of Eucalyptus globules essential oil have been extensively demonstrated in vitro against pathogens including *Pseudomonas aeruginosa*, *E. coli*, *Moraxella catarrhalis*, *Salmonella typhi*, and *Staphylococcus aureus* (Dhakad *et al*., 2018; Ghalem and Mohamed, 2008; Mekonnen *et al*., 2016). Furthermore, the beneficial impact of Eucalyptus globules essential oil on chicken productivity may be attributed to the bioactive compounds' capacity to stimulate the secretion of digestive and pancreatic enzymes (Hashemipour *et al*., 2013), improve gut morphology (Giannenas *et al*., 2018a), and improve immune function (Chowdhury *et al*., 2018). One of the ingredients in essential oil mixtures that is poisonous to several bacterial species is gardenia sp. The bactericidal activity (BA50) of *Campylobacter jejuni* ranges from 0.003 to 0.009, while *Listeria monocytogenes* has a BA50 of 0.057 to 0.092 (Friedman *et al*., 2002). *Boswellia serrata* is an additional component of the essential oils blend. The supplementation of chickens with *Boswellia serrata* resulted in improved production and efficiency due to the improved configuration of intestinal villi, gastric microflora, and overall health of the chickens (Al-Yasiry *et al*., 2017; Kiczorowska *et al*., 2016). *Boswellia* also promotes the thyroid gland's function, increasing the basal metabolic rate and upregulation of metabolism (Amer *et al*., 2023).

Another herb included in the essential oil blend is myrrh, which has been found to possess a variety of biological properties, including anti-inflammatory, antioxidant, anti-microbial, and anti-parasitic properties (Batiha *et al*., 2023). Myrrh emulsion has demonstrated significant antioxidant potential and protects against hepatic oxidative damage and immunotoxicity when exposed to lead acetate by downregulating LPO and activating immune and antioxidant defence mechanisms (Ashry *et al*., 2010). Herba Origani, the dried whole herb of *Origanum vulgare* L., which also composed the essential oil blend in this study. *Origanum vulgare* L. and its derivative products can be used to relieve stress, reduce oxidative stress; improve intestinal microbiota, and to improve egg productivity in laying hens. In addition, they have a pronounced antibacterial and anticoccidial effect, as well as help to improve the intestinal microbiota and thus contribute to improving the productivity of eggs in the rearing of laying hens (Ivanov & Bozakova, 2022). Pine oil is an essential oil constituent that has been researched to have antioxidant and antimicrobial properties (Dziedziński *et al*., 2021). The diversity and structure of the gut microbial composition, production performance, egg quality, and liver function of laying chickens can be enhanced by the supplementation of pine needles extract at 100 mg/kg (Guo *et al*., 2022). Furthermore, carrot seed oil exhibited antibacterial and antioxidant properties against *Staphylococcus aureus* and *Escherichia coli* (Ji *et al*., 2023). Paprika extract is also a source of antioxidants and antibacterials (Salamatullah *et al*., 2022). The carotenoids contained in paprika have a wide range of bioactive properties due to their distinctive structure, including anti-inflammatory and antioxidant properties (Hanif *et al*., 2024; Kabir *et al*., 2022).

Feeding dietary mineral mix-enriched essential oil didn't affect the antibody titer against AI and ND. Similar with Untari *et al*. (2024), the addition of mineral premix to layer feed showed no difference in antibody titer. Moreover, the antibody titer against AI and ND were not influenced by the dietary level of calcium, as demonstrated by Attia *et al*. (2020). Interestingly, the study conducted by Saleh *et al*. (2020) revealed that the supplementation of an organic mineral mixture had no impact on antibody titer against Newcastle disease (ND), but it did enhance antibody titer against avian influenza H9N1. Furthermore, the supplementation of plant essential oils by Gao *et al*. (2020), showed an increase in Influenza virus subtype H9 antibody levels but did not affect Newcastle disease virus (NDV) antibody levels during the rearing period. Tsiagbe *et al*. (1987) demonstrated that layers given a corn-soybean meal diet supplemented with Zn had higher levels of antibodies against sheep red blood cells (SRBC). Liu *et al*. (2020) reported Bacillus subtilis and essential oils supplementation improved the level of AIV-antibody. The levels of antibody titer in poultry vary widely, influenced by several factors such as virus distribution profile, bird breed, farm management, and vaccination (Gupta *et al*., 2021).

In this study, supplementation of mineral mix enriched essential oil tended not to influence the physical quality of the eggs. Otherwise, the dietary supplementation of organic minerals enhanced shell thickness, shell weight, and albumen (Saleh *et al*., 2020). According to Stefanello *et al*. (2014), the addition of Zn, Mn, and Cu proteinates improved the quality of eggshells by increasing their strength and thickness, which resulted in a reduction of damaged eggs. In addition, Favero *et al*. (2013) determined that eggshell quality of broiler breeders was enhanced, particularly in terms of eggshell weight and thickness, by substituting inorganic sulphates of zinc, manganese, and copper with organic amino acid complexes of these microelements. Interestingly, dietary supplementation with Zn increased shell thickness, but cinnamon essential oil supplementation and combination with Zn did not affect egg quality (Torki *et al*., 2015). In another study, laying hens fed selenium and oregano oil didn't affect the physical egg quality (Reshadi *et al*., 2020). Furthermore, dietary minerals mixed-enriched essential oil showed similar chemical egg quality in all groups. In contrast, a study by Ogunwole *et al*. (2015) showed the inclusion of vitamin-mineral premixes altered the moisture, crude protein, and extract ether composition of eggs.

Interestingly, the inclusion of minerals mixed-enriched essential oil improved the antioxidant activity of yolk. Similarly, Arbabi-Motlagh *et al*. (2022) documented supplementation of zinc and selenium improves antioxidant activity of hen eggs fed with oxidised oil. Saleh *et al*. (2020) reported that dietary organic minerals decreased yolk malondialdehyde (MDA) concentration. MDA is an oxidation product of unsaturated fatty acids. These positive effects may be related to the antioxidant properties of trace minerals (Yatoo *et al*., 2013). In addition, a decrease in MDA level was detected in the egg yolk when EOs were added to the diet. This finding suggests that essential oils (EOs) have a reducing effect on the oxidation of lipids in the yolk (Rodjan *et al*., 2022). Previous study by Gholami-Ahangaran *et al*. (2022) and Giannenas *et al*. (2018b) who demonstrated that essential oils (EOs) consist of diverse bioactive components possessing antioxidant capabilities. These compounds can be absorbed in the intestines of laying hens and then reach the egg yolk through blood circulation. The process by which the antioxidant chemicals in the EOs are absorbed partially accounts for the reported antioxidant activity in the yolk in this investigation (Saleh *et al*., 2020). However, the feeding of dietary minerals mixed-enriched essential oil didn't affect the fatty acid profile of yolk. In a study conducted by Ding *et al*. (2017), it was shown that adding essential oil to the diet of laying hens did not have any impact on the fatty acid composition of the egg yolk. Nevertheless, the addition of a combination of plant extracts and copper to the diets of laying hens resulted in an augmentation of oleic acid and the overall content of monounsaturated fatty acids (MUFA) (Kaya *et al*., 2013). Chelate compounds-based mineral supplements in laying hen diet improved the total PUFA content and PUFA/SFA ratio in lipids of egg yolks (Ghasemi *et al*., 2022). Antioxidant activity may contribute to the fatty acid profile when tested at different storage times. Lipid oxidation will enhance as egg storage time increases. Antioxidant compounds in essential oils help maintain the stability of egg fatty acid oxidation (Yu *et al*., 2018).

Conclusion

The inclusion of mineral mix-enriched essential oil 0.25% in layer diet

improved egg production and feed efficiency. In addition, the treatment also increased the antioxidant activity of the yolk. Future research should evaluate the inclusion of mineral mix-enriched essential oils on the antioxidant activity and fatty acids of eggs at different storage times.

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Conflict of interest

The authors have no conflict of interest to declare.

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