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Original Research Article

Effects of essential oils on performance, egg quality, nutrient digestibility and yolk fatty acid profile in laying hens

Xuemei Ding*, Yang Yu, Zhuowei Su, Keying Zhang

Institute of Animal Nutrition, Sichuan Agricultural University, Ya'an 625014, China

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ABSTRACT

The study was conducted to investigate the effect of essential oils on performance, egg quality, nutrient digestibility and yolk fatty acid profile in laying hens. A total of 960 *Lohmann* laying hens aged 53 weeks were enrolled, under 4 different treatment diets supplemented with 0, 50, 100 and 150 mg/kg essential oils (Enviva EO, Dupont Nutrition Biosciences ApS, Denmark), respectively. Each treatment was replicated 8 times with 30 birds each. Birds were fed dietary treatment diets for 12 weeks (54 to 65 weeks). For data recording and analysis, a 12-week period was divided into 3 periods of 4 weeks' duration each: period 1 (54 to 57 weeks), period 2 (58 to 61 weeks), and period 3 (62 to 65 weeks). For the diet supplemented with Enviva EO, hen-day egg production and the feed conversion ratio (FCR) were significantly improved ($P < 0.05$) at weeks 58 to 61, and the eggshell thickness was significantly increased ($P < 0.05$) at week 65. However, egg production, egg weight, feed intake, FCR and other egg quality parameters (albumen height, Haugh unit, egg yolk color and eggshell strength) were not affected by the dietary treatment. In addition, compared with the control diet, protein digestibility in the 100 mg/kg Enviva EO treatment group was significantly increased ($P < 0.05$), and fat digestibility in the 100 and 150 mg/kg Enviva EO treatment groups was significantly decreased ($P < 0.05$), but Enviva EO had no effect on energy apparent digestibility. Saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) gradually decreased and polyunsaturated fatty acid (PUFA) increased with Enviva EO supplementation, but the difference was not significant. The data suggested that the supplementation of essential oils (Enviva EO) in laying hen diet did not show a significant positive effect on performance and yolk fatty acid composition but it tended to increase eggshell thickness and protein digestibility, especially at the dose of 50 mg/kg.

1. Introduction

Essential oils (EOs) are obtained from plant materials (flowers, herbs, leaves, roots, etc.), which are complex mixture of various components, such as terpenes, aldehydes, esters, alcohols and other chemical molecules. Essential oils have been employed in animal diets for their antimicrobial (Lee et al., 2004), antibacterial (Srinivasan, 2004), antioxidant (Placha et al., 2010) and digestive stimulant properties (Platel and Srinivasan, 2004). Over the past decade, EOs have been regarded as the possible antibiotic-substitute for animals.

There have been a number of studies about the use of EOs on broilers chickens. Çabuk et al. (2006) reported dietary supplementation of essential oil mixture significantly increased egg production and improved feed conversion ratio (FCR) compared with control. Otherwise, Amad et al. (2011) reported that the apparent ileal digestibility of crude ash, crude protein, crude fat, calcium, and phosphorus showed a linear increase related to the increase of phytogenic feed additive in the diet. On the other hand, research on laying hens found that diet supplemented with EOs of thyme, sage, and rosemary (Bölükbasi et al., 2008) and essential oil mixture (EOM, Çabuk et al., 2006) improved performance, immune response, and eggshell quality of laying hens. And, Olgun (2016) also reported that egg weight, egg mass and eggshell thickness were positively affected by EOM supplementation. On the contrary,

* Corresponding author.
E-mail address: dingxuemei0306@163.com (X. Ding).
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research also showed that the different dietary levels of EOM had no significant effect on performance parameters, damaged eggs, eggshell weight (Olgun and Yildiz, 2014). However, these previous studies on the EOs in the layers were mainly focused on the production performance, egg quality and nutrient digestibility, and the results are inconsistent.

Lipid composition of chicken eggs is a primary area of consumer concern due to the relationship between dietary lipids and the development of coronary heart disease (Simopoulos and Salem, 1992). Feeding strategies may change the fatty acid composition in eggs (Yi et al., 2014). Essential oils have beneficial influence on lipid metabolism (Acamovic and Brooker, 2005). Since there have been no reports about the effect of yolk diet supplemented with EOs on the fatty acid composition, the study was planned to evaluate the effect of EOs (Enviva EO) on performance, egg quality, nutrient digestibility and yolk fatty acid profile in laying hens.

2. Materials and methods

2.1. Experimental birds, diet and management

The animal experiment was conducted in accordance with the principles of Animal Care and Use Committee of Sichuan Agricultural University (Ya'an, China). A total of 960 *Lohmann* laying hens aged 53 weeks were enrolled in the study, and diets were supplemented with EOs (Enviva EO, Dupont Nutrition Biosciences ApS, Denmark) at the concentrations of 0, 50, 100 and 150 mg/kg, respectively were provided for 12 weeks (54 to 65 weeks). Enviva EO was a commercial product including thyoml 13.5% and cinnamaldehyde 4.5% as the active components. Eight replicates were set for each treatment with 30 birds in each replicate.

All hens were housed in an environmentally controlled house with temperature maintained at approximately 24 °C. The house had controlled ventilation and lighting (16L:8D). All hens were supplied with diet and water for *ad libitum* consumption.

The hens were fed diets in mash form during the experiment. The basal diet was formulated using maize and soybean meal with composition and nutrient levels in line with Agricultural Trade Standardization of China (NY/T33-2004) (Table 1). For experimental diets, experimental diet of the maximum and minimum concentration was first mixed separately and then mixed together for the preparation of subsequent experiments diets. All diets contained a standard dose of Phyzyme XP phytase (500 FTU/kg feed, Dupont Nutrition Biosciences ApS, Denmark).

Table 1
Ingredients and chemical composition of the basal diet (dry matter basis).

Item	Content
Ingredient, %	
Corn	54.5
Wheat	10.0
Soybean meal	16.85
Rapeseed meal	4.0
Rice bran meal	3.5
Corn protein powder	0.60
Soybean oil	0.25
Limestone	8.4
Dicalcium phosphate	0.62
Lysine-HCl (70%)	0.15
DL-Methionine	0.09
Salt (NaCl)	0.4
Choline chloride	0.1
Vitamin premix[1]	0.03
Mineral premix[2]	0.5
Phyzyme XP 5000G	0.01
Total	100
Chemical composition[3], %	
ME, MJ/kg	11.0
Crude protein	15.3
Calcium	3.8
Available phosphorus	0.35
Lysine	0.75
Methionine	0.35
Methionine + Cystine	0.54
Threonine	0.58
Tryptophan	0.19

[1] Provided per kilogram of diet: retinyl acetate, 3.1 mg; cholecalciferol, 0.0375 mg; DL-α-tocopheryl acetate, 7.5 mg; thiamin, 0.6 mg; riboflavin, 4.8 mg; pyridoxine hydrochloride, 1.5 mg; cyanocobalamin, 0.009 mg; calcium-D-pantothenate, 7.5 mg; folic acid, 0.15 mg; niacin, 20 mg.
[2] Provided per kilogram of diet: copper ($CuSO_4 \cdot 5H_2O$), 6 mg; iron ($FeSO_4 \cdot H_2O$), 60 mg; zinc ($ZnSO_4 \cdot H_2O$), 80 mg; manganese ($MnSO_4 \cdot H_2O$), 60 mg; selenium ($NaSeO_3$), 0.3 mg; iodine (KI), 0.35 mg.
[3] The value of crude protein was analyzed and the value of metabolizable energy (ME) was calculated, others were calculated values.

2.2. Performance parameters

Mortality was recorded daily. Eggs were collected daily and the egg production percentage was expressed on a hen-day basis during period 1 (54 to 57 weeks), period 2 (58 to 61 weeks), and period 3 (62 to 65 weeks) and overall study intervals. Egg weight was recorded daily throughout the experimental period. Feed intake was recorded and the FCR was calculated for each period.

2.3. Egg quality parameters

To determine egg quality indices in every 4-week period, 24 eggs were randomly collected per treatment (3 eggs per replicate) to determine albumen height, Haugh units, yolk color, eggshell thickness and eggshell strength. Albumen height, Haugh units and yolk color were measured by EMT-5200 (Robotmation, Japan). Eggshell thickness was measured at 3 different sites (the upper and lower end, and middle) by using a micrometer screw gauge. An average of 3 thickness values measured from each egg was used to describe eggshell thickness. Eggshell strength was determined by EFR-01 (ORKA, Israel).

2.4. Nutrient digestibility

At the end of experiment, a total of 32 healthy laying hens (1 bird randomly selected from each replicate) were placed in metabolic cages, which were used for collecting excreta. Excreta samples of each hen were collected and immediately stored at −20 °C. During collection, care was taken to avoid contamination from feathers, feed, and foreign materials. Samples of the feed and excreta were analyzed for moisture, protein, and other extract, as described by the AOAC International (2000). The gross energy was determined using adiabatic bomb calorimetry (Parr Instrument Company, IL, USA).

2.5. Fatty acid profile of egg yolk

Fatty acids of the egg yolk were quantified by using gas chromatography. Fatty acid methyl esters were separated using a GC-9A gas chromatograph equipped with a flame ionization detector and a silica capillary column. Nitrogen was used as the carrier gas with a flow rate of 35 mL/min. The pressure of hydrogen and air was set at 0.5 kg/cm. The temperature of the injector and detector was maintained at 250 °C. Fatty acid methyl esters were identified

based on comparison of retention times and standards. The fatty acid composition was expressed as the percentage of the total fatty acids.

2.6. Statistical analysis

Firstly, all data were recorded and sorted by excel. Then, data were analyzed by one-way ANOVA with Statistical Analysis System (SAS, 2003, version 9.3; SAS Inst. Inc., Cary, NC). When the ANOVA showed statistical significance, Duncan's multiple range test was conducted. $P < 0.05$ indicated significant difference.

3. Results

3.1. Egg production

The effect of Enviva EO on laying hen performance is shown in Table 2. Hen-day egg production and the FCR were significantly improved ($P < 0.05$) in 58 to 61 weeks. However, during a 12-week feeding trial, egg production, egg weight, feed intake and FCR were not affected by the dietary treatment.

3.2. Egg quality parameters

The effect of Enviva EO on egg quality is listed in Table 3. Supplementation of Enviva EO significantly increased the eggshell thickness ($P < 0.05$) at week 65. Dietary supplementations had no effect on eggshell thickness ($P > 0.05$) in the earlier laying period of hens. There was no significant difference in albumen height, Haugh unit, egg yolk color and eggshell strength among the different dietary treatments ($P > 0.05$).

3.3. Energy and nutrient apparent digestibility

There was no significant difference in energy apparent digestibility among the different treatment groups (Table 4). Compared with the control diet, protein digestibility in the Enviva EO (100 mg/kg) treatment group was significantly increased ($P < 0.05$), however, fat digestibility in the Enviva EO supplemented groups (100 and 150 mg/kg) was significantly decreased ($P < 0.05$).

Table 2
Effects of supplementation Enviva EO on laying performance of laying hens.[1]

Item	Enviva EO supplementation, mg/kg				SEM	*P*-value
	0	50	100	150		
Hen-day egg production, %						
54 to 57 wk	81.55	80.79	82.86	83.33	1.426	0.577
58 to 61 wk	75.49[a]	79.55[b]	80.34[b]	81.25[b]	1.094	0.012
62 to 65 wk	74.02	77.87	78.30	78.08	1.402	0.128
54 to 65 wk	77.03	79.40	80.52	80.90	1.201	0.128
Egg weight, g						
54 to 57 wk	62.24	62.32	62.58	62.91	0.331	0.498
58 to 61 wk	62.98	63.63	63.09	63.67	0.357	0.412
62 to 65 wk	63.11	62.97	63.04	63.30	0.305	0.878
54 to 65 wk	62.76	62.97	62.90	63.29	0.298	0.641
Feed intake, g/(bird·d)						
54 to 57 wk	127.8	126.7	127.2	129.1	1.507	0.712
58 to 61 wk	118.2	119.7	121.0	121.3	1.202	0.266
62 to 65 wk	115.1	115.2	115.1	117.9	1.241	0.306
54 to 65 wk	120.4	120.5	121.2	122.8	1.198	0.483
Feed conversion ratio						
54 to 57 wk	2.521	2.524	2.465	2.474	0.045	0.706
58 to 61 wk	2.496[a]	2.377[b]	2.401[b]	2.356[b]	0.027	0.008
62 to 65 wk	2.471	2.357	2.342	2.397	0.037	0.096
54 to 65 wk	2.496	2.420	2.404	2.409	0.033	0.096

[a,b] indicated the difference within a row was significant ($P < 0.05$).
[1] Means of 8 replicates (30 laying hens each).

Table 3
Effects of supplementation Enviva EO on egg quality parameter of laying hens.[1]

Item	Enviva EO supplementation, mg/kg				SEM	*P*-value
	0	50	100	150		
Albumen height, mm						
57 wk	7.29	7.34	7.19	7.27	0.189	0.866
61 wk	7.13	7.26	7.19	7.11	0.247	0.591
65 wk	7.26	7.03	6.84	7.27	0.278	0.783
Haugh unit						
57 wk	83.55	84.21	82.6	83.45	1.303	0.896
61 wk	82.26	82.76	82.26	82.24	1.714	0.871
65 wk	84.14	82.01	80.31	82.94	1.994	0.712
Egg yolk color						
57 wk	7.66	8.14	7.93	7.89	0.151	0.346
61 wk	8.74	8.89	8.87	8.54	0.108	0.410
65 wk	9.03	9.24	9.14	8.90	0.102	0.254
Eggshell strength, kg/cm^2						
57 wk	4.65	4.26	4.11	4.37	0.204	0.653
61 wk	4.45	4.17	4.73	4.87	0.195	0.878
65 wk	4.11	4.12	4.35	4.20	0.159	0.494
Eggshell thickness, mm						
57 wk	0.25	0.23	0.24	0.22	0.009	0.107
61 wk	0.26	0.25	0.26	0.28	0.009	0.136
65 wk	0.24[b]	0.27[a]	0.24[b]	0.28[a]	0.009	0.014

[a,b] indicated the difference within a row was significant ($P < 0.05$).
[1] Means of 24 eggs per treatment (3 eggs per replicate).

Table 4
Effects of supplementation Enviva EO on energy and nutrient digestibility (%) of laying hens.[1]

Item	Enviva EO supplementation, mg/kg				SEM	*P*-value
	0	50	100	150		
Energy	82.16	82.68	82.10	81.81	0.459	0.608
Crude protein	45.68[b]	52.38[a,b]	54.51[a]	46.76[b]	2.475	0.037
Crude fat	81.92[a]	82.52[a]	73.33[b]	75.56[b]	1.705	0.001

[a,b] indicated the difference within a row was significant ($P < 0.05$).
[1] Means of 8 replicate (1 hen each).

3.4. Yolk fatty acid profile

The mean value of the percentage of yolk fatty acids in different dietary groups is shown in Table 5. No significant influence on the fatty acid composition of yolk was observed with or without supplementation of Enviva EO. Numerically, the saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) values were gradually decreased and the polyunsaturated fatty acid (PUFA) was increased with the gradual increase in concentration of Enviva EO in the diets of laying hen, but the difference was statistically not significant.

4. Discussion

Studies reported beneficial effects of EOM supplementation in layer's diet on laying performance (Özek et al., 2011; Bozkurt et al., 2012). However, in the present study, hen-day egg production and the FCR were significantly improved in weeks 58 to 61, however, during a 12-week feeding trial (birds aged 54 to 65 weeks), egg production, egg weight, feed intake and FCR were not affected with or without dietary supplementation of EOs. This result was consistent with previous reports which showed no significant difference in egg production, egg weight, feed intake and the FCR when laying hens were given diet supplemented with EOs or EOM (Özek et al., 2011; Bozkurt et al., 2012; Florou-Paneri et al., 2005; Bölükbasi et al., 2010). Bozkurt et al. (2012) indicated that EOM supplementation in the laying hen diet significantly increased the egg production rate and egg weight, but the egg mass, feed consumption, and FCR were not affected. However, Çabuk et al. (2006,

Table 5
Effects of supplementation Enviva EO on fatty acid composition (%) of egg yolk.[1]

Item	Enviva EO supplementation, mg/kg				SEM	*P*-value
	0	50	100	150		
Myristic acid (C14:0)	3.220	3.085	3.599	3.844	0.369	0.1807
Myristoleic acid (C14:1)	0.050	0.054	0.058	0.064	0.012	0.7759
Pentadecanoic acid (C15:0)	0.036	0.034	0.036	0.043	0.010	0.1857
Palmitic acid (C16:0)	27.71	26.98	26.89	27.12	0.401	0.3562
Palmitoleic acid (C16:1)	3.103	2.943	3.071	3.352	0.193	0.6338
Stearic acid (C18:0)	9.638	9.704	9.480	9.279	0.229	0.2809
Elaidic acid (C18:1t)	1.929	1.840	1.932	1.916	0.051	0.4070
Oleic acid (C18:1c)	40.95	41.87	41.22	39.92	0.768	0.1379
Linoleic acid (C18:2 n-6)	11.53	11.45	11.64	11.55	0.440	0.8281
β-Linolenic acid (C18:3 n-6)	0.059	0.091	0.061	0.043	0.027	0.8050
α-Linolenic acid (C18:3 n-3)	0.085	0.031	0.078	0.116	0.046	0.1678
Cis-11,14,17-Eicosatrienoic (C20:3 n-3)	0.029	0.044	0.223	0.025	0.109	0.9725
Arachidonic acid (C20:4 n-6)	1.119	1.191	1.153	2.140	0.320	0.5222
Docosahexaenoic acid (C22:6 n-3)	0.326	0.500	0.420	0.371	0.116	0.2407
SFA	40.60	39.80	40.00	40.28	0.460	0.8325
MUFA	46.03	46.71	46.28	45.24	0.640	0.2809
PUFA	13.17	13.30	13.49	14.22	0.503	0.1807

SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid.
[1] Means represent 16 eggs per treatment of 2 eggs per replicate.

2014) and Bölükbasi et al. (2008) found that the FCR was improved when the birds were fed EOM diet. Bozkurt et al. (2009) found that EOM supplementation at 24 and 48 mg/kg in diet did not affect egg production and egg weight of broiler breeders from 20 to 46 weeks of age. This variability in result might be related to the dose of EOs, plant extra or multiple components in the EOs and its utilization in broilers and layers.

In our study, supplementation of Enviva EO significantly increased the eggshell thickness at week 12, consistent with the results of Bozkurt et al. (2012) who reported that supplementation of EOM increased egg shell weight, egg shell thickness, and shell breaking strength. Panda et al. (2003) reported better results obtained for eggshell quality variables could be partly attributed to the fact that thymol influenced metabolic activity of the beneficial bacteria colonies within the layers' intestine, which positively influenced the mineral absorption rate, especially that of Ca^{2+} and Mg^{2+}. However, the mechanism of in-feed EOs achieving better eggshell quality is not completely clear. There was no significant difference in albumen height, Haugh unit, egg yolk color and eggshell strength among the different dietary treatments. Florou-Paneri et al. (2005) using oregano EOs in laying hen diet at the dose of 50 and 100 mg/kg also reported that there were no significant effects on egg shape index, Haugh unit, egg yolk color, yolk height and eggshell thickness. Similar results were reported in laying hens fed EOs diet of thyme and rosemary (Bölükbasi et al., 2008). However, Özek et al. (2011) reported that supplementation of EOM in diet significantly increased albumen height and Haugh unit score. Bozkurt et al. (2012) indicated that supplementation of EOM into the laying hen diet did not significantly alter yolk weight, albumen height, and Haugh unit, but significantly decreased relative albumen weight.

Essential oils affected gut functions by stimulating digestive secretions and enhancing enzyme activity (Manzanilla et al., 2004). In our study, the supplementation of 100 mg/kg Enviva EO significantly increased protein digestibility, but no effect was observed on energy apparent digestibility. Consistent with the results of our study, Amerah et al. (2011) reported that EOs supplementation in the wheat diet of broilers significantly improved apparent ileal nitrogen digestibility with no effect on apparent ileal energy digestibility. However, Hernandez, et al. (2004) reported that there was no effect of EOs extracts from oregano, cinnamon or pepper on crude protein digestibility in starter feed of broilers. It is interesting to note that in present study, layer birds fed diets supplemented with 100 and 150 mg/kg Enviva EO resulted in a decrease in digestibility of fat. Author has no suitable explanation for this as only few studies have been conducted on effects of EOs on the digestibility of nutrients in laying hens. However, Jamroz et al. (2005) demonstrated that adding plant extract in broilers diets increased pancreatic and intestinal lipase activity. The results showed that the addition of EOs in laying hens diet may increase the digestibility of fat. Therefore, further studies will be required to explore the effects of EOs on the nutrient digestibility in the laying hens.

The lipid metabolism in serum and liver of poultry was affected by supplementation EOs in diet (Bölükbaşi et al., 2010; Abdel-Wareth, 2016). To our knowledge, there have been a few reports about the effect of EOs on the fatty acid composition of yolk. In the present study, different treatments did not significantly influence the yolk fatty acid composition. Galobart et al. (2001) found that supplementation of rosemary extract in layer diet had no effects on fatty acid composition of egg yolk of laying hens. However, Bölükbaşi et al. (2010) revealed that the proportion of DHA and the n-3 proportion of egg yolk were significantly increased by dietary bergamot oils. This might be stem from amount of EOs and the source of EOs.

5. Conclusion

In conclusion, supplementation of EOs in laying hen diet did not show a significant positive effect on performance and yolk fatty acid composition but it tended to increase eggshell thickness and protein digestibility. Overall in nutshell, Enviva EO at the low dose of 50 mg/kg in layer diets may be beneficial and recommended.

Conflict of interest statement

The authors declare that they have no competing interest.

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