Effect of 25-hydroxyvitamin D and essential oil complex on productive performance, egg quality, and uterus antioxidant capacity of laying hens

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ABSTRACT This study was conducted to determine the effect of 25-hydroxyvitamin D (**HDV**) and essential oils (**EO**) on the uterus antioxidant capacity, egg quality, and eggshell ultrastructure in laying hens. A total of 400 48-wk-old Lohmann laying hens were randomly allocated into 2 groups and fed a basal diet (control) or a basal diet supplemented with a combination of 69 μg/kg HDV and EO (including 200 mg/kg thymol and 50 mg/kg carvacrol) for 12 wk. There are 10 replicates of 20 hens each. Compared with the control, dietary HDV+EO supplementation improved ($P < 0.05$) egg production rate, feed efficiency, eggshell thickness and strength, and decreased ($P < 0.05$) the translucent egg score. Ultrastructural changes indicated that dietary HDV+EO supplementation decreased ($P < 0.05$) mammillary knob width, mammillary thickness and the proportion of mammillary thickness, and increased ($P < 0.05$) the proportion of effective thickness and total thickness of the eggshells compared with the control. Supplementation with HDV+EO complex led to higher serum HDV concentration and increased antioxidant capacity in the uterus, indicated by higher ($P < 0.05$) antioxidant enzyme activities (catalase [**CAT**], total antioxidant capacity [**T-AOC**], and glutathione S-transferases [**GST**]) and lower malondialdehyde (**MDA**) content. Therefore, dietary HDV and EO complex (including thymol and carvacrol) supplementation can improve the productive performance and the eggshell quality in laying hens, and the improving effect on eggshell quality may through enhancing eggshell ultrastructure and antioxidant capacity of uterus.

Key words: 25-hydroxyvitamin D, essential oil, translucent eggshell, ultrastructure, laying hen

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INTRODUCTION

The eggshell quality is one of the most important properties of eggs (Fathi et al., 2007; Nys et al., 2011), as it provides physical protection and nutrients to the developing embryo and prevents microbial contamination of the egg contents. Eggshell problems such as broken eggs, dirty eggs, shell-less eggs, mottled shells eggs (dark spotted eggs), deformed eggs, etc. have seriously affected the development of the shell-egg industry. Although many factors are involved in the eggshell problems (such as, disease, stress, bird age, mycotoxins, poor nutrition, etc.), strategies to improve the hens' health status (gastrointestinal and reproductive system health) are reported to improve eggshell quality. The ultrastructure of eggshell consists of 5 layers, including the shell membranes (**SM**), the mammillary knob layer (**ML**), the palisade layer, the vertical crystal layer, and the cuticle (Arias et al.,1993; Nys et al.,1999; Gautron et al., 2021). It was reported that the average size, shape, and orientation of calcite crystals in the mammillary and palisade layers contribute to quality the eggshells (Ahmed et al., 2005; Fathi et al., 2007; Dunn et al., 2012; Zhang et al., 2017a, 2017b). So, it is necessary to study the influence of nutrition strategy on the ultrastructure of eggshell.

Vitamin D is generally involved in the calcium and phosphorus and maintains optimal Ca and P homeostasis for bone health and development (Lamberg-Allardt, 2006). The 25-hydroxyvitamin D (**HDV**) is an active metabolite of vitamin D_3, which has been shown to have better bioactivity than vitamin D

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(Vignale et al., 2015). In our previous study, we found that dietary administration of 69 μg/kg HDV improved the egg quality, tibia quality, and intestinal health of layers under high stocking density (Wang et al., 2020; Wang et al., 2021); however, whether HDV can alleviate the eggshell ultrastructure is not clear.

Several studies have demonstrated that essential oils (**EOs**) may improve laying performance and health status by its anti-inflammatory, anthelmintic, antimicrobial, and antioxidant properties as well as stimulation of digestive secretions and immune modulation. Thymol is a main constituent of commonly used EOs, such as oregano and thyme oils (Bassolé and Juliani, 2012). Carvacrol is a constituent of several medicinal plants, such as thyme, black cumin, and oregano (Alagawany et al., 2015). Carvacrol and thymol can inhibit the growth of both gram-positive and gram-negative bacteria. These compounds have antifungal and antibiofilm effects. Thymol and carvacrol can also be applied as an alternative antimicrobial agent against antibiotic-resistant pathogenic bacteria. It has been shown that dietary supplementation of thymol and carvacrol can improve the eggshell quality of laying hens (Ding et al., 2017; Ghanima et al., 2020), however, the mechanism has not been elucidated, also no literature was found on the effect of combination of HDV and EO in laying hens.

Therefore, the aim of the present study was to investigate the effects of HDV and essential oil complex on the uterus antioxidant capacity, egg quality, and eggshell ultrastructure in laying hens.

Table 1. Composition and nutrient level of basal diet (as-fed basis).

Item, %	Amount
Corn	58.78
Wheat bran	3.87
Soybean oil	1.50
Soybean meal (CP, 43%)	15.24
Corn gluten (CP, 60%)	5.00
Corn DDGS	5.00
Calcium carbonate (granular)	4.30
Calcium carbonate (powder)	4.30
Calcium hydrophosphate (powder)	1.04
NaCl	0.25
$NaHCO_3$	0.10
L-Lysine HCl	0.16
DL-methionine	0.01
Choline chloride, 60%	0.10
Vitamin premix[1]	0.20
Mineral premix[2]	0.15
Total	100.00
Calculated energy content, %	
ME[3], kcal/kg	2650
Analyzed nutrient concentration, %	
Crude protein	15.74
Calcium	3.57
Total phosphate	0.65
Lysine	0.62
Methionine	0.35

[1]Provided per kilogram of diets: vitamin A 9950 IU, vitamin B_1 37.7 mg, vitamin B_2 12 mg, D-pantothenate 18.2 mg, vitamin B_6 7.55 mg, vitamin B_{12} 0.5 mg, vitamin D_3 5000 IU, vitamin E 70 IU, VK_3 4.47 mg, Biotin 4 mg, vitamin C 195 mg, niacin acid 70.35 mg.

[2]Provided per kilogram of diets: Cu (as copper sulfate) 9.6 mg, Fe (as ferrous sulfate) 64 mg, Mn (as manganese sulfate) 121.5 mg, Zn (as zinc sulfate) 57 mg, I (as potassium iodide) 0.60 mg, Se (as sodium selenite) 0.36 mg.

[3]Calculated according to NRC (1994).

MATERIALS AND METHODS

Birds, Diets, and Management

The Animal Care and Use Committee of Sichuan Agricultural approved this study. A total of 400 48-wk-old Lohmann laying hens were fed a basal diet for 2 wk and then randomly allocated into 2 groups that were fed a basal diet (control) or a basal diet supplemented with a combination of 69 μg/kg HDV and EO (including 200 mg/kg thymol and 50 mg/kg carvacrol) complex for 12 wk. Each dietary treatment had 10 replicates with 20 hens each. The composition and nutrient concentrations of the basal diet (mash form) were listed in Table 1, and the 69 μg/kg HDV and 250 mg/kg EO were premixed with 1 kg corn to make a premix prior to addition. The HDV was obtained from DSM (DSM Nutritional Products Inc., Shanghai, China) with the 99.5% of purity, while the thymol and carvacrol were purchased from Sigma (St. Louis, MO). The EO was firstly mixed with its own carrier (30% silica and 50% dextrin) and then with wheat bran (1:10; w:w) prior to diet mixing. The experimental diets were prepared every week to minimize the loss of bioactive compounds in feeds. The blended mash feeds were packed in separate labeled high-density polyethylene bags with inner liner to avoid any loss of EO compounds in feeds. Layers (LD: 4 hens per cage; HD: 6 layers per cage) in each replicate were raised in 8 adjacent cages (45 cm width × 50 cm length × 45 cm height). Room environment was controlled at 22°C by a daily lighting schedule of 16 h light and 8 h dark. Birds were allowed ad libitum access to water and feed.

Laying Performance and Sampling

The number of eggs and their weights were recorded daily. Feed consumption by each replicate was recorded weekly. Feed conversion ratio was calculated as the ratio of grams of total feed intake to grams of total egg weight. Egg production was expressed as an average day production. At end of 12 wk, a total of 30 eggs with the exception of unqualified eggs and broken eggs were randomly collected from each treatment and assessed for egg quality traits. Twenty layers (10 replicates/treatment, 2 hens/replicate) were sacrificed by CO_2 asphyxiation, the uterus tissue was taken and then stored at −20°C till antioxidant capacity analysis.

Egg Quality

Eggs (10 replicates/treatment, 3 eggs/replicate) were collected at the final day of the experiment to measure egg quality. Egg yolk color, albumen height, and Haugh

unit were evaluated using an egg multitester (EMT-7300, Robotmation Co. Ltd., Tokyo, Japan). Eggshell breaking strength was evaluated using an eggshell force gauge model II (Robotmation Co. Ltd., Tokyo, Japan). Eggshell thickness was measured on the large end, equatorial region, and small end, using an eggshell thickness gauge (Robotmation Co., Ltd., Tokyo, Japan). The egg yolk color and Haugh unit were evaluated using an egg multitester (EMT-7300, Robotmation Co. Ltd., Tokyo, Japan). Eggshell ratio was calculated as eggshell weight/egg weight × 100. The egg shape index was calculated as length of the egg divided by its width.

Translucent Eggshell Score

Eggs (10 replicates/treatment, 3 egg/replicate) were stored (temperature at 20 ± 2°C; comparative humility at 60 ± 5%) for 5 d before translucent eggshell score analysis. Translucent eggshell score ranged from 0 to 4 points, and the specific score standard is shown in Figure 1. The eggs were put on the lighter, and scored with the translucent area of the shell gradually increasing. All of this completed by one person and repeated 4 times at for one egg.

Scanning Electronic Microscopy of Eggshell Ultrastructure

After being stored for 5 d (temperature at 20 ± 2°C; relative humility at 60 ± 5%), eggs (10 replicates/treatment, 3 egg/replicate) were also collected for ultrastructure analysis according to the method described previously (Dunn et al., 2012; Zhang et al., 2017a, 2017b). Briefly, 2 pieces of each eggshell (~0.5 cm^2 from its equatorial section) were subjected to scanning electronic microscope (**SEM**; FEI Quanta 600, Thermo Fisher Scientific Ltd., Portland, OR). Before imaging, both inside and outside of each eggshell were washed with distilled water to remove dirt, and then each eggshell was dried overnight. The eggshell samples of each treatment were mounted on aluminum blocks, and coated with gold powder before undergoing SEM. The mammillary layer (**ML**) thickness, width of mammillary knob, and effective thickness (combined with palisade, vertical crystal, and cuticle sections, μm) were determined by the SEM ruler according to the model of Dunn et al., (2012). The mammillary thickness was taken as the length from the top of the membrane to the bottom of the palisade layer. The average width of the mammillary knobs was calculated as the length of the mammillary knobs/the number of the mammillary knobs. The total thickness referred to the combined effective and mammillary thickness. The effective and mammillary layers (%) are thickness of each layer relative to the total thickness. Each replicate had 6 eggs, and 2 samples were examined for each egg, with 2 images taken for each sample.

Calcium and Phosphorus Content in Eggshell

At the end of experiment, 3 eggs from each replicate were selected to determine the calcium (Ca) and phosphorus (P) contents of the eggshell. First, both the inside and outside of all the eggshells were washed with distilled water to remove dirt, and were dried at room temperature. Then the eggshells were crushed into powder. Eggshell powder from each eggshell to determine the content of calcium and phosphorus by ammonium metavanadate colorimetric and EDTA titration method respectively (AOAC, 1995).

Serum 25-hydroxyvitamin D Content Determination

At the end of the study, blood samples (10 birds/treatment) were collected from the wing vein after 12 h

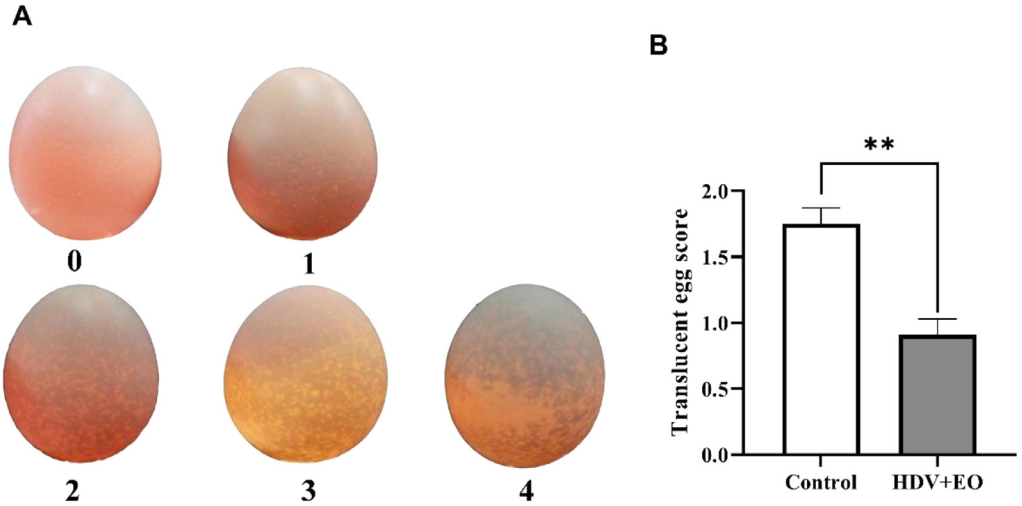

Figure 1. Translucent egg score standard and the effect of HDV+EO on the translucent area score of laying hens during 48 to 60 wk of age. (A) Grading and scoring of the translucent eggshell. 0 represents opaque egg; 1 to 5 represents the translucent areas level, with the scored increased, the translucent areas increased accordingly. (B) The effect of HDV+EO on the translucent egg score. **indicate $P < 0.05$. Abbreviations: EO, 200 mg/kg thymol + 50 mg/kg carvacrol; HVD, 25-hydroxyvitamin D.

of fasting. Serum samples were obtained from these blood samples at 4°C for 30 min and subsequent centrifugation at 1500 × g for 20 min. The serum was collected and stored at -80°C until further analysis. The serum HDV concentration was determined using mass spectrometry procedure (Heartland Assay, Ames, IA) according to previous study (Cai et al., 2020).

Antioxidant Enzyme Activity of Uterus

Enzyme activities of total superoxide dismutase (**T-SOD**) activity, total antioxidant capacity (**T-AOC**), catalase (**CAT**), glutathione peroxidase (**GPx**) and glutathione S-transferase (**GST**), and malondialdehyde (**MDA**) concentration in uterus (1 layer/replicate, 10 replicates/treatment) were analyzed by using commercial kits obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) according to the manufacturer's instructions.

Statistical Analysis

All experimental data were analyzed as one-way ANOVA with feed treatment as the main effect using GLM procedure of SAS 9.2 software (SAS Institute Inc., Cary, NC). Student t test was used to determine significant differences among means. The concentration of significance was assessed at $P \leq 0.05$.

RESULTS

Laying Performance

As shown in Table 2, supplementation of HDV+ EO complex improved egg laying rate, feed efficiency, and also had lower broken egg ratio than that of control treatment ($P < 0.05$). No effect of dietary treatment was found on egg weight and average daily feed intake in current study ($P > 0.05$).

Egg Quality and Eggshell Ultrastructure

There was no difference shown in eggshell relative weight, albumen height, Haugh unit, and yolk color ($P > 0.05$; Table 3). Compared with the control group, layers treated with HDV+EO complex improved ($P < 0.05$) strength and thickness of eggshell, and decreased ($P < 0.05$) the translucent egg score. As shown in Figure 2 and Table 4, dietary HDV+EO complex supplementation decreased ($P < 0.05$) mammillary knob width, mammillary thickness and the proportion of mammillary thickness, and increased ($P < 0.05$) the proportion of effective thickness and total thickness of the eggshells compared with the control.

Table 2. Effect of HYD and EO on the productive performance of laying hens during 48 to 60 wk of age.

Item[1]	Control	HVD+EO	SE	*P*-value
Egg production rate, %	93.67	96.45	0.87	0.04
Egg weight, g	61.14	61.22	0.48	0.19
Average daily feed intake, g	114	116	1.24	0.76
FCR	2.10	1.96	0.03	0.02
Breaking egg ratio, %	2.57	0.45	0.09	<0.01

[1]Each mean represents 10 replicates per treatment, with 20 layers per replicate.Abbreviations: EO, 200 mg/kg thymol + 50 mg/kg carvacrol; HVD, 25-hydroxyvitamin D.

Table 3. Effect of HYD and EO on the egg characteristics of laying hens during 48 to 60 wk of age.

Item[1]		Control	HVD+EO	SEM	*P*-value
Eggshell color	L*	80.54	79.44	0.22	0.42
	a*	5.78	5.89	0.18	0.15
	b*	15.57	16.08	0.29	0.61
Eggshell thickness, mm^{-2}		41.05	41.54	1.24	0.87
Eggshell breaking strength, kg/cm^3		4.387	4.695	0.214	0.04
Eggshell relative weight, %		10.24	10.57	0.54	0.87
Egg shape index					
Albumen height		8.75	8.92	0.55	0.59
Haugh unit		89.24	90.05	1.04	0.28
Yolk color		7.89	7.65	0.47	0.66

[1]Each mean represents 10 replicates per treatment, with 3 eggs per replicate.Abbreviations: EO, 200 mg/kg thymol + 50 mg/kg carvacrol; HVD, 25-hydroxyvitamin D.

Serum 25-hydroxyvitamin D and Eggshell Calcium and Phosphorous content

The serum HDV concentration was increased in birds fed HDV+EO complex than the control group (Table 5; $P < 0.05$), while no difference was observed in total ash, Ca, and P content in eggshell among treatments ($P > 0.05$).

Antioxidant Capacity of Uterus

Compared with the control group, supplementation with HDV+EO complex led to higher antioxidant enzyme activities (SOD, T-AOC, and GST) and lower MDA content in the uterus ($P < 0.05$; Table 6). No effect was found on CAT and GPx activities between2 treatments.

DISCUSSION

Vitamin D and also its metabolite, 25-hydroxyvitamin D (HDV) are essential nutrients and are involved in maintaining calcium and phosphorus homeostasis, immune response, and muscle development (Morris et al., 2014; Vignale et al., 2015). Vitamin D_3 deficiency has been reported to reduce egg production (egg laying rate, egg weight, and FCR) and egg quality (eggshell quality) in several studies (Geng et al., 2018; Adhikari et al., 2020). Essential oils, including thymol, carvacrol, and cinnamaldehyde were reported to have beneficial effects on laying performance (Bozkurt et al., 2012; Ding et al., 2017). In the current study, we found that the administration of HDV and EO complex diet had improved laying performance (egg laying rate and FCR); however, we did not observe any difference when

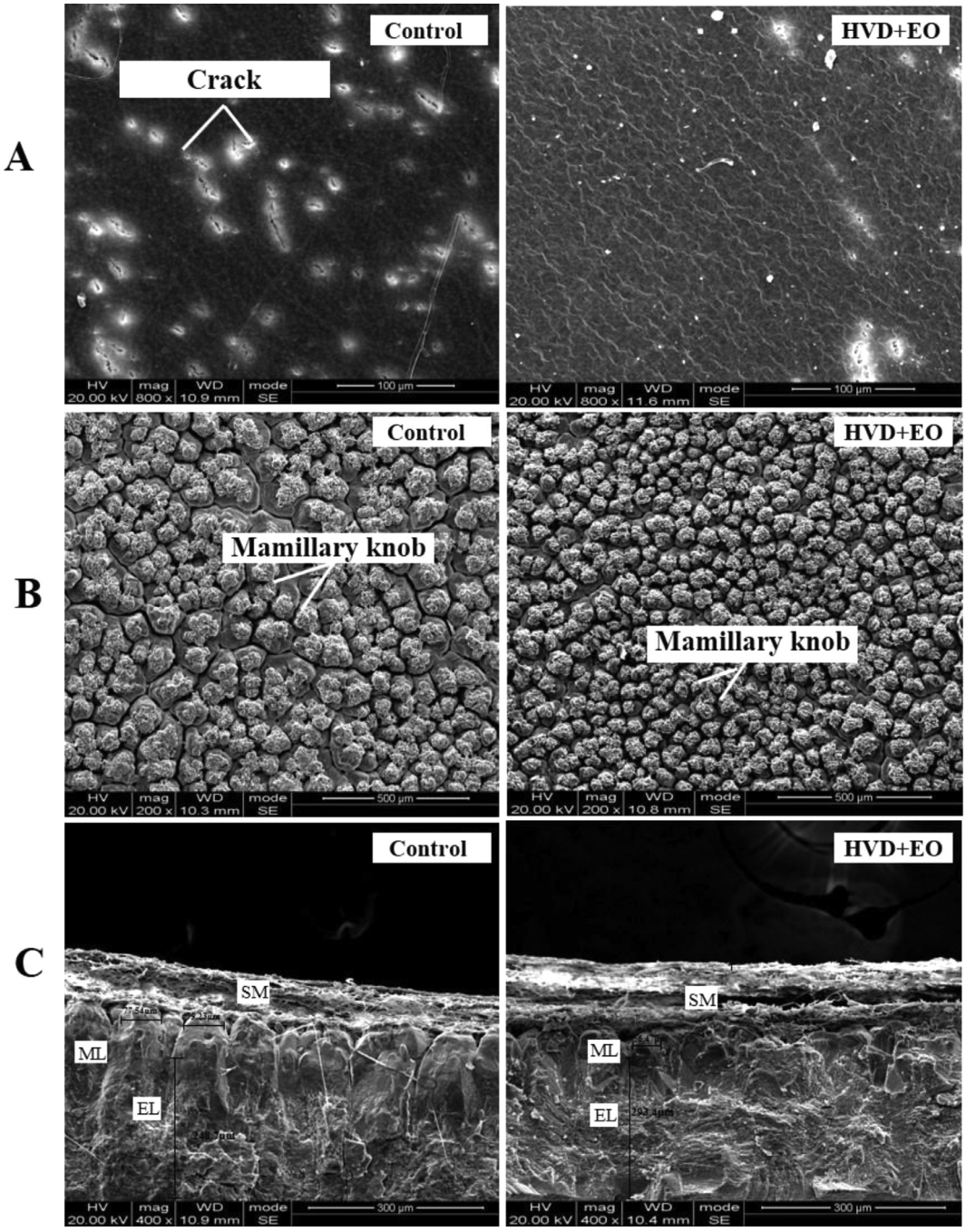

Figure 2. Scanning electron microscope images of the eggshell ultrastructure for laying hens after treating HDV and EO during 48 to 60 wk of age. (A) Eggshell appearance. (B) Mammillary knobs of the eggshell. (C) Thickness and width of the mammillary knobs and the effective thickness of the eggshell. Control: basal diet; HDV+EO: control diet + 69 μg/kg HDV+EO. Scale bar: 200 μm. Abbreviations: EL, effective layer; EO, 200 mg/kg thymol + 50 mg/kg carvacrol; HVD, 25-hydroxyvitamin D; ML, mammillary layer; SM, shell membrane.

Table 4. Effect of HYD and EO on eggshell ultrastructure of laying hens after 12 wk of treatment during 48 to 60 wk of age.

Item[1]	Control	HVD+EO	SEM	*P*-value
Mammillary knob width, μm	87.6	74.3	2.51	<0.01
Mammillary thickness, μm	91.3	72.4	3.45	0.03
Effective thickness, μm	266	283	4.60	0.01
Total thickness, μm	356	369	4.30	<0.01
Mammillary layer, %	25.65	19.62	0.79	<0.01
Effective layer, %	74.35	80.38	0.84	0.04

[1]Each mean represents 10 replicates per treatment, with 3 eggs per replicate.Abbreviations: EO, 200 mg/kg thymol + 50 mg/kg carvacrol; HVD, 25-hydroxyvitamin D.

HDV was added in the diet alone in our previous study (Wang et al., 2020), which is also in agreement with other studies (Keshavarz, 2003; Persia et al., 2013; Chen et al., 2020). The reason behind no effect of concentrations of HDV (or vitamin D) on laying performance parameters during our study may be due to inclusion of adequate concentrations of Ca, available P, and vitamin D_3 in the control diet. It has been indicated that suitable supplementation dosage of VD_3 for middle-phase laying hens is up to 3000 IU/kg to reach a maximal laying performance (Geng et al., 2018; Wen et al.,

Table 5. Effect of HYD and EO on the serum 25-hydroxyvitamin D concentration and calcium and phosphorus content in eggshell of laying hens during 48 to 60 wk of age.

Item[1]	Control	HVD+EO	SEM	*P*-value
Blood				
HVD, ng/mL	45.52	70.34	8.24	<0.01
Eggshell				
Ash, %	94.72	96.97	1.89	0.45
Ca, %	36.65	37.54	0.89	0.41
P, %	17.54	16.21	0.94	0.41

[1]Each mean represents 10 replicates per treatment, with 1 layer per replicate for blood sample and 3 eggs for eggshell quality.Abbreviations: EO, 200 mg/kg thymol + 50 mg/kg carvacrol; HVD, 25-hydroxyvitamin D.

Table 6. Effect of HYD and EO on the uterus antioxidant capacity of laying hens during 48 to 60 wk of age.

Item[1]	Control	HVD+EO	SEM	*P*-value
CAT, U/mg of protein	1.75	1.91	0.12	0.45
T-SOD, U/mg of protein	94.62	116.54	1.89	<0.01
T-AOC, U/mg of protein	37.65	45.54	1.44	<0.01
MDA	21.54	17.21	0.94	0.01
GST, U/mg of protein	123.56	211.29	35.23	0.03
GPx, U/mg of protein	235.4	221.2	18.42	0.89

[1]Each mean represents 10 replicates per treatment, with 1 layer per replicate.Abbreviations: CAT, catalase; EO, 200 mg/kg thymol + 50 mg/kg carvacrol; GPx, glutathione peroxidase; GST, glutathione S-transferase; HVD, 25-hydroxyvitamin D; MDA, malondialdehyde; T-SOD, total superoxide dismutase.

2019, Adhikari et al., 2020). Also evidences from other studies supported that the laying performance and egg quality were not affected by VD_3 supplementation when the dietary vitamin D is sufficient (Mattila et al., 2004; Chen et al., 2020). Obviously, the content of vitamin D_3 (up to 5,000 IU) in the diet is adequate for laying hens had already met the layers' requirements for performance in current study. All these may indicate this positive effect may result from or at least partially result from the usage of essential oils. Ghanima et al. (2020) have shown that thymol (300 mg/kg), carvacrol (300 mg/kg), and eugenol (300 mg/kg) augmented the egg production, egg weight and feed efficiency of laying hens from 28 to 28 wk of age under various housing system. Similarly, other studies have also revealed that dietary supplementation of EO mixture (including thymol and carvacrol) increased the egg weight and egg production rate of laying hens (Bozkurt et al., 2012; Ding et al., 2017). Since feed intake was not affected by the EOs and HDV addition, the improvements in egg production and feed efficiency may be attributed to increased dietary nutrients digestibility and the digestive capacity that induces the intestinal availability of other nutrients for the benefit of the body (Ding et al., 2017). Abdel-Wareth, (2016) and Ding et al. (2017) have reported that essential oils might improve the ovary functions and the nutrients digestibility in the intestine and consequently increase egg weight and egg mass in laying hens. Supportably, we found that the HDV concentration were improved in blood, which may indicate that supplementation of essential oils improves VD_3 utilization in laying hen.

The role of VD_3 in calcium and phosphorus metabolism is crucial for its well-documented involvement in bone and eggshell formation in laying hens (Lamberg-Allardt, 2006). We also observed that HDV and EO complex resulted in a lower broken egg ratio and higher eggshell quality (increased breaking strength and thickness of eggshell) in current study. Similarly, Geng et al. (2018) have reported that hens fed diets containing VD_3 at concentrations ranged from 1500 IU and 3000 IU/kg of diet had better eggshells than birds given 500 IU VD3/kg of diet. Translucent eggshell is a result of the transfer of moisture from the egg's content through the shell membrane and its accumulation in the eggshell, resulting in increased transmission of light (Solomon, 1991), and is also one of the main problems of eggshell quality. Translucent eggshell (black spot) may increase the incidence of microcracks in eggshells and can be easily penetrated by pathogens such as *Salmonella* (Chousalkar et al., 2010). At present study, we also observe that administration of HDV and EO reduced incidence of translucent egg. Ultrastructure is the fundamental structure of eggshell, and it plays in important role in determination of eggshell quality (Nys et al., 2004; Fathi et al., 2007; Guru and Dash, 2014). The eggshell is a highly ordered structure, and it include the shell membranes (inner and outer), mammillary layer, palisade layer, vertical crystal layer, and cuticle from the inside outward (Guru and Dash, 2014; Gautron et al., 2021). Studies also reported that translucent eggshell is caused by irregular mammillary knobs (Bain et al., 2006) and the changes in the mammillary layer and mammillary cores (Chousalkar et al., 2010) during the early phases of eggshell formation. In the present study, the dietary HDV+EO complex supplementation improved eggshell ultrastructure by increasing its effective thickness, decreasing mammillary, thickness and the proportion of mammillary thickness. It has evident that the thickness of EL was positively corresponded with eggshell strength (Fathi et al., 2007; Dunn et al., 2012). Therefore, we hypothesize that the increased strength and thickness caused by HDV+EO were mainly a consequence of the ameliorated shell ultrastructure. No study has been conducted to determine the effect of HDV and/or essential oils on translucent egg incidence and shell ultrastructure of laying hens. Further studies are needed to study the inner relationship between translucent egg incidence and nutrients.

The uterus of the hen oviduct was the main position for eggshell formation, and is believed to play a key role in establishing the texture of shell and its resulting mechanical properties (Brionne, et al., 2014; Gautron et al., 2021). We observed that combination of HDV and EO in the diet significantly increased the activity of SOD, T-AOC and GST, decreased MDA concentrations in uterus at current study. It has been demonstrated that both HDV and its active hormonal form ($1,25(OH)_2D_3$) are essential for physiological functions,

including damping down inflammation and oxidative stress (Nakai et al., 2014; Wimalawansa, 2019). In our previous study, we also found that HDV increased the T-AOC and decreased MDA content in small intestine of layers under high stocking density (Wang et al., 2021). On the other hand, it has demonstrated that natural plant supplements, such as resveratrol, hesperidin, genistein, thymol and carvacrol and herbs, can be used to enhance antioxidant defense mechanisms and reduce the intensity of oxidation processes to improve quality of poultry products (eggs and meat) (Alagawany et al., 2015; Ogenik et al., 2016). Similarly, Luna et al., (2010) and Hashemipour et al. (2013) have reported that feed supplementation with 150 to 200 mg/kg thymol and carvacrol enhanced SOD and GST activities and decreased MDA concentration in thigh and breast muscle of broilers.

CONCLUSION

Therefore, dietary HDV and EO complex (including thymol and carvacrol) supplementation can improve the productive performance and the eggshell quality in laying hens, and the improving effect on eggshell quality may through enhancing eggshell ultrastructure and antioxidant capacity of uterus.

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DISCLOSURES

No conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors for publication. I would like to declare on behalf of my co-authors that the work described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part. All the authors listed have been approved the manuscript that is enclosed.

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