Effects of essential oil/palygorskite composite on performance, egg quality, plasma biochemistry, oxidation status, immune response and intestinal morphology of laying hens

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ABSTRACT The current study aimed to assess the effects of different levels of essential oil/palygorskite composite (**EO-PGS**) supplementation on performance, egg quality, oxidative status, immunity and intestinal morphology of laying hens. A total of 480 laying hens aged 65 wk were randomly assigned into 4 groups (6 replicates of 20 hens each). Hens were fed the basal diet supplemented with 0 (control diet), 0.5, 0.75 or 1.0 g/kg EO-PGS for 56 d. Data were analyzed by One-way ANOVA. Results showed that birds fed with diet supplemented with EO-PGS had increased the egg production ($P < 0.05$) more than birds fed with control diet. The yolk index and shell thickness were increased in 0.75 and 1.0 g/kg EO-PGS groups at d56 ($P < 0.05$). There was no significant difference in plasma biochemical parameters among all groups. Compared with the control group, supplementation of EO-PGS increased the immunoglobulin-G and interleukin-2 levels in plasma ($P < 0.05$). The total antioxidant capacity in plasma and liver, the plasma catalase concentration, the activity of total superoxide dismutase in the liver and the activity of glutathione peroxidase in the spleen were increased in the EO-PGS groups ($P < 0.05$). The concentration of malondialdehyde in the liver was decreased with the increasing level of EO-PGS ($P < 0.05$). The crypt depth of ileum and duodenum of birds fed with EO-PGS supplemented diet had a tendency to decrease ($0.05 < P < 0.1$) and the villus height to crypt depth ratio of ileum increased ($P < 0.05$), compared with birds fed with control diet. In summary, EO-PGS supplementation improved the egg production, enhanced antioxidation and immune functions, and ameliorated egg quality and intestinal morphology of laying hens, and a level of 0.75 g/kg EO-PGS was recommended in laying hens diets.

Key words: essential oil, EO-PGS, laying hen, performance, intestinal morphology

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INTRODUCTION

Antibiotics have been used in animal production for many years to promote growth performance and prevent disease. However, with the long-term abuse of antibiotics, the problem of drug residue and resistance issues emerged (Han et al., 2020). Dietary antibiotics have been banned in China, 2020. Moreover, with the rapid development of Chinese poultry husbandry, people's dietary concepts have gradually paid attention to health. Therefore, it is a new task to develop safe and reliable alternatives to antibiotics in the field of poultry husbandry and feed production.

Essential oils (**EOs**) are a volatile and aromatic oily liquid extracted from plants, and are complex mixture of various components (such as terpenes, aldehydes, esters, and other chemical molecules), and with proven biological functions as antioxidants, antibacterial and immune regulators (Ding et al., 2017). In recent years, herbs or products containing plant extract, EOs or main components of EOs have been evaluated and introduced as suitable and safe alternatives for antibiotic growth promoters that are already used in practice (Perricone et al., 2015). Active substances including thymol, cinnamaldehyde, and carvacrol have been widely applied to livestock and poultry husbandry (Attia et al., 2016). However, some characteristics severely limit the application of EO, such as low water solubility, high volatility and poor heat resistance (Engel et al., 2017; Yildiz et al., 2018). Therefore, it is necessary to improve the stability of essential oils for more advanced application.

Palygorskite is a chain layered structured clay and has been approved to be used as a carrier in industrial and

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animal nutrition fields because of its strong adsorption capacity, large specific surface area and catalytic properties (Su et al., 2018). Moreover, palygorskite powder has shown an excellent effect on intestinal function and health and been approved to be used as food additive in China (Du et al., 2019). Lei et al. (2017) reported that the compound of modified palygorskite and EOs shows good antibacterial properties and has good thermal stability and acid resistance. However, there are few articles to report the application effects of EO-PGS in the livestock and poultry industry. Recently, a novel kind of composite EO-PGS using modified palygorskite as the carrier of essential oil was prepared by ion exchange process. The purpose of this study was to evaluate the influences of different EO-PGS levels on performance, egg quality, oxidative status, immunity, and intestinal morphology of laying hens.

MATERIALS AND METHODS

Ethical Statement

All experimental and sample collection procedures were carried out as per the Chinese guidelines for animal welfare and approved by the Institutional Animal Care and Use Committee of Hunan Agricultural University.

Essential Oil/Palygorskite Composite

Essential oil/palygorskite composite (**EO-PGS**) was provided by Jiangsu Sinitic Biological Technology Co., Ltd (Jiangsu, China). Plant essential oils were loaded onto palygorskite by modifying palygorskite, regulating charge, assembling antimicrobial factor and other processes. The main components were as follows: palygorskite>70% and EOs (the main ingredients are carvacrol and thymol) >15%, and the other mineral elements<15%.

Birds, Diets, and Management

A total of 480 healthy Lohmann laying hens with similar weight at 65 wk of age were obtained from Xianglan Commercial Company and randomly divided into 4 groups (6 replicates of 20 birds each).

The dietary treatments were basal diet (control) and basal diet supplemented with 0.5, 0.75 or 1.0 g/kg EO-PGS. The basal diet was formulated in accordance with the NRC (1994) to meet the nutrient requirements of laying hens (Table 1).

Before the experiment started, the hens were fed with a basal diet for 1 wk to adapt to their environment. The hens were raised in a wire cage with 3 ladders and 4 hens were raised in a cage which was randomly distributed in the shed. Hens were allowed free access to feed and water throughout the experiment. The total experimental period was 56 d. All the procedures were conducted according to the Chinese guidelines for animal welfare and the standards of the College of Animal Science and Technology, Hunan Agricultural University.

Table 1. Formulation and calculated composition of the basal diet (as-fed basis).

Ingredient	Content (%)	Calculated composition	Nutrient levels (%)
Corn	67	ME (MJ/kg)	11.44
Soybean meal	22.28	CP (%)	16.50
Limestone	8.72	Lys (%)	0.83
$CaHCO_3$	1.00	Met+- +Cys (%)	0.57
Premix[1]	1.00	Ca (%)	3.61
Total	100.00	Available P (%)	0.36

[1]The premix provided the following per kg of the diet: vitamin A (transretinyl acetare), 7,500 IU; vitamin B_1 (thiamine mononitrate), 2 mg; Vitamin B_2, 4.98 mg; Vitamin B_6 (pyridoxine hydrochloride), 4.98 mg; Vitamin B_{12} (cobalamine), 0.02 mg; vitamin D_3 (D-activated animal sterol), 3 000 IU; vitamin E (all-rac-α-tocopherol acetate), 20 IU; vitamin K_3 (menadione dimethylpyrimidinol bisulfite), 2 mg; nicotinic acid, 30 mg; pantothenic acid, 15 mg; folic acid, 0.8 mg; biotin, 0.2 mg; Fe (from ferrous sulfate), 75 mg; Cu (from copper sulfate), 10 mg; Se (from sodium selenite), 0.3 mg; Zn (from zinc sulfate), 70 mg; Mn (from manganese sulfate), 60 mg; I (from potassium iodide), 1 mg; Ca, 1 g; NaCl, 3.5 g.The experimental diet was the same basal diet supplemented with 0.5, 0.75, 1.0 g of EO-PGS/kg of the basal diet.

Sample Collection

Egg quality was measured on 5 eggs collected randomly from each replicate at d 28 and d 56.

At the end of the trial period, after a 12-h fast, 2 hens were randomly selected from each replicate. Blood samples (around 5 mL) were collected from the wing vein in heparinized tubes. Samples were then centrifuged at 3000 × g at 4°C for 10 min and stored in 1.5 mL centrifugal tubes at -20°C for further analysis.

Subsequently, one hen per replicate was randomly selected to be euthanized by cervical dislocation and necropsied after blood sampling. The liver and spleen were dissected free from vessels, and frozen until analyzed for oxidation status. The small intestine was divided into duodenum, jejunum and ileum. Intestinal segments of 3 cm were removed from the medial portion, cleaned thoroughly with 0.9% saline to remove the contents and fixed in 10% formalin solution for intestinal tissue fixation and morphology measurements.

Performance and Egg Quality

To calculate egg production and feed conversation ratio, egg production and egg weight were recorded daily each replicate and feed consumption was recorded weekly by replicate.

Haugh unit (**HU**) and yolk color were determined with a digital egg tester (EA-01, ORKA Co. Ltd., Israel). Egg length, egg width, yolk width, and yolk height were measured by using electronic digital caliper (SH14100025, Shanghai, China). The egg shape index is egg length divided by egg width and the yolk index is yolk height divided by yolk width. Shell thickness was determined by an eggshell thickness tester (NFN380, FHK, Bunkyo-ku, Tokyo, Japan) and shell strength was measured by an egg force reader (EFR-01, ORKA Food Technology Ltd).

Plasma Indices

Activities of glutathione peroxidase (**GSH-PX**), catalase (**CAT**), and total superoxide dismutase (**T-SOD**) and concentration of malondialdehyde (**MDA**) and total antioxidant capacity (**T-AOC**) were assayed with commercial radioimmunoassay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) with microplate reader (Multiskan GO, Thermo Fisher Scientific, Waltham, MA) according to the instruction of manufacturer. Concentrations of immunoglobulin A (**IgA**), immunoglobulin G (**IgG**), immunoglobulin M (**IgM**), tumor necrosis factor-alpha (**TNF-α**) and interleukin-2 (**IL-2**) levels were measured by ELISA kit(CSB-E11232Ch, CSB-EQ027259Ch, CSB-E16200C, CSB-E11231Ch, CSB-E06755Ch, Cusabio Biotech Co., Ltd, Wuhan, Hubei, China) according to the instructions of the manufacturer. Concentrations of total protein (**TP**), total cholesterol (**TC**), calcium (**Ca**), urea acid (**UA**), albumin (**ALB**), triglyceride (**TG**) and glucose (**GLU**), and activities of alkaline phosphatase (**ALP**), aspartate aminotransferase (**AST**) and alanine aminotransferase (**ALT**) in plasma were measured by Mindray automatic analyzer(BS-300,S Shenzhen Mindray Bio-Medical Electronics Co., Ltd, Shenzhen, Guangdong, China) according to the commercial kits (Shenzhen Mindray Bio-Medical Electronics Co., Ltd, Shenzhen, China).

Liver and Spleen Oxidation Status

The fresh weighed samples from liver and spleen were mixed homogeneously (T10 BS25, IKA, Baden-Wurttemberg, Germany) at a ratio of 1 g sample to 9 mL of ice-cold saline in a 12 mL centrifuge tube and centrifuged at 1500 × g at 4°C for 10 min. The supernatant was used for analyses of T-SOD, GSH-PX, MDA, CAT and T-AOC. Concentration of protein in the supernatant of the liver and spleen homogenate was measured by using an assay kit (A045-2, Nanjing Jiancheng Bioengineering Institute).

Intestinal Morphology

Formalin-fixed intestinal tissues were processed, dehydrated, embedded in paraffin wax, sectioned at 3 μm and stained, using the hematoxylin and eosin method. Histological sections were examined with Villus height (**VH**), villus width (**VW**), and crypt depth (**CD**). Morphological measurements were performed on 10 villi chosen from each segment, using an image processing and analyzing system (version 6.0, Olympus IX51 inverted microscope, Olympus Optical Co., Ltd., Tokyo, Japan). The villus height-to-crypt depth ratio (**VH/CD**) was calculated subsequently.

Statistical Analysis

The results were statistically analyzed by one-way ANOVA. Significant differences for the means between treatments were determined with Duncan's Multiple Range Test. The results were expressed as arithmetic mean and SEM. Significance was declared at $P < 0.05$, while tendency was considered at $0.05 \leq P \leq 0.10$. Statistical analyses were carried out using the SPSS version 19.0 (SPSS Inc., Chicago, IL).

RESULTS

Performance and Egg Quality

Results of performance and egg quality are shown in Table 2. Compared with the control group, the laying rate of the 0.75 and 1.0 g/kg EO-PGS groups was significantly increased ($P < 0.05$). Dietary supplementation

Table 2. Effects of EO-PGS on performance and egg quality of laying hens.[1]

Items	EO-PGS supplemental level/(g/kg) 0	0.5	0.75	1.0	*SEM*	*P*-value
Performance						
Egg production/%	87.40^{b}	89.74^{ab}	90.95^{a}	91.37^{a}	0.57	0.039
Average daily feed intake/g	113.69	111.43	111.57	112.61	0.59	0.519
Feed conversation ratio	2.03	1.98	1.95	1.95	0.02	0.381
Egg quality						
D 28						
Egg shape index	1.32	1.33	1.33	1.31	0.02	0.092
Shell strength, kgf	4.45	4.33	4.41	4.48	0.06	0.851
Egg weight, g	63.38	62.97	63.61	63.41	0.37	0.933
Yolk color	5.37	5.23	5.17	5.03	0.09	0.629
Haugh unit	76.13	73.32	71.61	71.49	0.89	0.297
Yolk index	0.39	0.38	0.38	0.38	<0.01	0.698
Shell thickness, mm	0.29	0.29	0.30	0.30	<0.01	0.141
D 56						
Egg shape index	1.31	1.32	1.33	1.34	<0.01	0.301
Shell strength, kgf	4.11	4.02	4.08	4.35	0.08	0.525
Egg weight, g	64.23	63.90	65.14	65.19	0.37	0.521
Yolk color	5.77	6.43	6.20	6.13	0.14	0.384
Haugh unit	76.77	74.71	77.99	76.88	0.98	0.892
Yolk index	0.40^{b}	0.44^{a}	0.44^{a}	0.44^{a}	<0.01	0.002
Shell thickness, mm	0.33^{c}	0.34^{bc}	0.35^{b}	0.38^{a}	<0.01	<0.01

[1]Means represent 6 replicates per treatment with 20 hens per replicate.

with EO-PGS had no effects on egg quality of laying hens at d28 ($P > 0.05$). The yolk index and shell thickness were significantly increased in 0.75 and 1.0 g/kg EO-PGS groups at d56. There were no differences in feed intake and feed conversation ratio between the experimental groups and control group.

Plasma Biochemistry

As shown in Table 3, there were no noticeable differences in the plasma concentrations of biochemistry indices among all groups.

Immunoglobulin and Cytokine Concentrations

It has been shown that the plasma concentrations of IgG and IL-2 were significantly increased with dietary EO-PGS of 0.75 and 1.0 g/kg, compared with the control group ($P < 0.05$) (Table 4).

Oxidation Status

As is shown in Table 5, the activity of CAT and T-AOC in plasma and the activity of GSH-PX in spleen were significantly increased with dietary supplementation of 1.0 g/kg EO-PGS compared with the control group. Dietary supplementation with 0.75 g/kg EO-PGS significantly enhanced the activities of T-SOD and T-AOC in liver ($P < 0.05$). The concentration of MDA in liver was significantly decreased by 52.03, 58.78, and 64.86% with 0.5, 0.75, and 1.0 g/kg EO-PGS supplementation, respectively ($P < 0.05$).

Intestinal Morphology

The results of gut morphology are presented in Table 6. There were no statistically significant effects on villus height and villus width among all groups. Compared with the control group, the crypt depth of duodenum and ileum in experimental groups had a tendency to decrease ($P > 0.05$), and the villus height to crypt depth ratio of ileum was significantly increased in 0.75 g/kg EO-PGS group ($P < 0.05$).

Table 3. Effects of EO-PGS on plasma biochemical of laying hens.[1,2]

Items	EO-PGS supplemental level/(g/kg) 0	0.5	0.75	1.0	SEM	P-value
AST, U/L	159.80	166.05	172.76	167.26	5.04	0.850
ALT, U/L	29.79	38.00	31.63	22.03	2.79	0.239
ALP, U/L	507.67	467.41	460.55	487.87	26.99	0.932
UA, mmol/L	219.26	157.55	202.32	181.93	13.08	0.370
TP, g/L	55.64	50.50	54.29	56.32	1.24	0.350
ALB, g/L	11.84	10.65	9.75	9.79	0.34	0.101
TG, mmol/L	9.69	9.91	9.00	7.33	0.38	0.069
TC, mmol/L	3.14	2.90	3.30	2.89	0.15	0.736
Ca, mmol/L	5.37	5.89	6.16	5.78	0.20	0.580
GLU, mmol/L	12.34	12.27	11.96	12.31	0.16	0.841

[1]Means represent 6 replicates per treatment with 20 hens per replicate.
[2]ALB, albumin; ALP, alkaline phosphatase; ALT, glutamic-pyruvic transaminase; AST, glutamic oxalacetic transaminase; GLU, glucose; TC, total cholesterol; TG, triglyceride; TP, total protein; UA, urea.

Table 4. Effects of EO-PGS on plasma immunoglobulin and cytokine concentration of laying hens.[1,2]

Items	EO-PGS supplemental level/(g/kg) 0	0.5	0.75	1.0	SEM	P-value
IgA, μg/mL	35.62	32.66	35.33	37.02	0.89	0.385
IgG, μg/mL	387.39[b]	388.53[b]	442.28[a]	457.17[a]	8.39	<0.001
IgM, μg/mL	32.01	31.51	31.24	33.86	0.52	0.283
IL-2, ng/L	123.60[b]	126.14[b]	149.01[a]	147.24[a]	2.89	<0.001
TNF-α, ng/L	22.90	19.66	20.61	21.98	0.55	0.157

[1]Means differences between this group and the control group was different at $P < 0.05$.
[2]IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; IL-2, interleukin-2; TNF-α, tumor necrosis factor.

DISCUSSION

Laying Performance

The results of the present study revealed that dietary EO-PGS supplementation increased egg production of laying hens during the whole period of the experiment. These results are in agreement with previous studies (Torki et al., 2015; Reshadi et al., 2020), who reported dietary supplementation with oregano essential oil or cinnamon essential oil can enhance egg production and feed conversation ratio of laying hens. The positive effects of dietary EO supplementation on production were predicted due to the pivotal role of EO in nutrient metabolism. The effective components of EO could balance gut microbial ecosystem and stimulate the secretion of digestive enzymes, thereby improving production performance parameters of poultry (Alagawany et al., 2021; Youssef et al., 2021). However, it was reported that dietary 1 to 2% dried and ground thyme leaves supplementation did not affect feed intake and egg performance of laying hens (Yalin et al., 2020). The inconsistent results may be ascribed to several factors, including the different supplementation dosages and sources, the age of animals and animal species, etc.

Egg Quality

The shell thickness and strength are important indicators to evaluate the egg shell quality. Egg shell quality is an important concern in the commercial poultry industry, influencing the storage and transportation of eggs. Olgun (2016) observed that dietary essential oil mixture, composed with 5 totally different essential oils (thyme oil, black cumin oil, funnel oil, anise oil, and rosemary oil), increased eggshell thickness without differences in egg shell strength. The results of the current study showed that EO-PGS in diet has no effects on egg quality on d 28 of the experiment, while the egg shell thickness at d 56 of the experiment significantly increased. Ca was one of the dominant elements in the eggshell. Extending eggshell formation time and elevating blood Ca content is beneficial for Ca deposition and eggshell

Table 5. Effects of EO-PGS on plasma, liver and spleen antioxidant indexes of laying hens.[1,2]

	EO-PGS supplemental level/ (g/kg)					
Items	0	0.5	0.75	1.0	*SEM*	*P-value*
Plasma						
T-AOC, U/mL	3.51[b]	4.47[b]	5.35[ab]	6.37[a]	0.37	0.024
CAT, U/mL	10.27[b]	14.82[b]	15.58[b]	29.93[a]	1.91	<0.01
MDA, nmol/mL	3.53	3.37	3.17	3.05	0.21	0.858
T-SOD, U/mL	84.22	85.43	83.95	86.69	0.95	0.737
GSH-Px, U/mL	2,456.71	2,332.61	2,277.27	2,391.12	47.72	0.335
Liver						
T-AOC, U/mg of prot	0.82[b]	1.00[ab]	1.20[a]	1.09[ab]	0.05	0.036
CAT, U/mg of prot	2.32	2.73	2.76	2.55	0.15	0.713
MDA, nmol/mg of prot	1.48[a]	0.71[b]	0.61[b]	0.52[b]	0.14	0.043
T-SOD, U/mg of prot	105.97[b]	102.4[b]	118.57[a]	101.42[b]	2.24	0.014
GSH-Px, U/mg of prot	219.09	198.10	221.91	206.28	5.43	0.388
Spleen						
T-AOC, U/mg of prot	1.02	1.16	1.45	1.41	0.09	0.314
CAT, U/mg of prot	2.97	3.73	4.87	4.96	0.38	0.188
MDA, nmol/mg of prot	1.37	1.31	1.14	1.14	0.10	0.743
T-SOD, U/mg of prot	85.01	106.58	97.63	107.39	4.96	0.365
GSH-Px, U/mg of prot	274.21[b]	315.70[ab]	412.20[ab]	454.50[a]	25.84	0.036

[1]In the same row, values with different small letter superscripts mean significant difference ($P < 0.05$), while with the same or no letter superscripts mean no significant difference ($P > 0.05$).
[2]T-AOC, total antioxidant capacity; CAT, catalase; MDA, malonaldehyde; T-SOD, total superoxide dismutase; GSH-Px, glutathione peroxidase.

formation. In a previous study, Olgun and Yildiz (2014) reported that supplementing diets with essential oils decreased excretion of minerals and improved bioavailability of Ca. Meanwhile, we found that adding EO-PGS increased the content of Ca in plasma, and speculated that the change could be a possible explanation for the improved eggshell thickness. The yolk index and Haugh unit are key indicators to assess the freshness of eggs. Our study revealed that dietary EO-PGS increased the yolk index, whereas it did not affect the HU. Parallel to our study, Liu et al. (2020) found that fed with 300 mg/kg EO (including 10% cinnamaldehyde and 5% thymol) improved the yolk index and had no effects on Haugh unit in laying hens. Similarly, Torki et al. (2021) reported that supplementation of the layer diet with 250 mg/kg lavandula augustifolia and 250 mg/kg Mentha spicata essential oils (singly and as combination) have no significant effects on egg index, yolk index, Haugh unit, and egg quality variables. However, Abo Ghanima et al. (2020) showed that rosemary and cinnamon supplementation (0.3 g/kg) decreased the egg yolk index for laying hens. According to these studies, we can speculate that different effects of supplemental essential oils on egg quality might stem from the amount and the source of essential oils.

Table 6. Effects of EO-PGS on intestinal morphology of laying hens.[1,2]

	EO-PGS supplemental level/ (g/kg)					
Items	0	0.5	0.75	1.0	SEM	*P*-value
Duodenum						
VH, μm	989.00	1069.20	1022.00	1062.17	30.97	0.849
CD, μm	283.00	217.20	244.50	251.67	8.68	0.069
VW, μm	202.33	174.40	163.50	163.17	11.23	0.569
VH/CD	3.49	4.92	4.28	4.29	0.19	0.099
Jejunum						
VH, μm	948.00	965.17	1139.17	1093.83	34.07	0.125
CD, μm	193.75	161.00	207.00	188.83	8.25	0.221
VW, μm	148.00	139.20	139.00	163.83	5.61	0.310
VH/CD	5.18	6.15	5.83	5.90	0.32	0.809
Ileum						
VH, μm	532.80	539.40	570.33	556.6	12.97	0.761
CD, μm	98.20	82.80	73.83	82.50	16.06	0.082
VW, μm	92.20	73.80	82.20	80.50	24.76	0.732
VH/CD	5.45[b]	6.69[ab]	7.97[a]	6.98[ab]	1.60	0.038

[1]Means represent 6 replicates per treatment with 1 hen per replicate.
[2]VH, villus height; CD, crypt depth; VW, villus width.

Plasma Biochemical Indices

Plasma biochemistry indices are indicators to reflect the healthy statue and the nutritional level of the animal body. Plasma TG determination is a routine item in lipid analysis and reflects the level of lipid metabolism. Researchers found that raised concentrations of TG might cause cardiovascular disease, acute pancreatitis, atherosclerosis, and other diseases (Nordestgaard and Varbo, 2014). Our study showed that dietary EO-PGS supplementation had a trend to decrease the concentration of TG and have virtually no effects on other plasma biochemistry indexes, which revealed that EO-PGS might improve lipid metabolism and be beneficial to body health.

Plasma Immunological Indices and Oxidation Statue

Immunoglobulin and cytokines are closely related to immune function, with a higher IgG concentration in blood indicating a better immune response. In our current study, diet with EO-PGS supplementation increased the concentration of IgG and IL-2. Consistent with our outcome, Basmacioglu-Malayoglu et al. (2014) also demonstrated that diet with oregano essential oil supplementation increased the proliferation of lymphocytes to mitogenic stimulus and the concentration of IgM and IgG. These increasing plasma immunoglobulins level may be owing to higher levels of B-cell proliferation, stimulating the immune system (Galal et al., 2015). Meanwhile, antioxidant status is highly related to immune system, which is considered as an important index of immune function. The activities of antioxidant enzymes and concentrations of oxidative products are essential to evaluate the oxidant statues of animals. In our present study, the inclusion of EO-PGS in the layer's diet led an increase in liver and spleen T-SOD and GSH-PX, as well as plasma and liver T-AOC contents. Zhang et al. (2021) also demonstrated that the inclusion of oregano essential oil in the birds' diet caused an increase in serum GSH-Px and SOD, as well as serum T-AOC contents, which is consistent with our finding. As well, we found EO-PGS addition decreased the level of MDA (the most important indicator of lipid peroxidation) in plasma, liver, and spleen. These results are similar to the results reported by Mousavi et al. (2017), who showed that the addition of 200 mg/kg essential oil mixture to a laying hen ration caused a decrease in plasma MDA concentration. Taken together, the results indicate that EO-PGS supplementation could benefit the immune function and oxidant statue of laying hens.

Intestinal Morphology

In poultry husbandry, the GI system has then major nutrient absorption capacity which credits GI system the important endocrine, metabolic, immunologic and barrier functions. Features of intestinal morphology, including villus height, villus width and crypt depth, are considered as important indicators and reflections of digestive and absorptive capacity in the gut (Qiao et al., 2015). It has been reported that dietary supplementation with oregano essential oil maintains the intestinal mucosal integrity by reducing the harmful bacteria and preventing adhesion to the epithelium, further improve nutrients absorption (Du et al., 2016; Mohiti-Asli and Ghanaatparast-Rashti, 2017). In our study, we found that EO-PGS supplementation had a tendency to decrease the crypt depth in duodenum and ileum and had higher VH and V/C in ileum duodenum and jejunum of laying hens compared with control diet. Our results were consistent with previous research of He et al. (2017), who observed a significant increase in the villus height and the V/C ratio in the duodenum of hens supplemented with 100 mg/kg oregano essential oil compared with negative control group. Similarly, Wang et al. (2019) also reported that supplementation of essential oil (containing minimum of 100 g/kg thymol) in laying hens' diet could improve morphometric parameters of the small intestine. These findings indicated that dietary EO-PGS enhanced the digestive and absorptive capacity of the intestinal mucous membrane.

CONCLUSIONS

Based on the results above, we conclude that dietary supplementation with EO-PGS improves performance of laying hens by enhancing immunity and antioxidant ability and modulating intestinal morphology, with a recommendation of a level of 0.75 g/kg EO-PGS in laying hens diet.

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DISCLOSURES

The authors declared that they have no conflicts of interest to this work. We declare that we do not have commercial or associative interest that represents a conflict of interest in connection with this work submitted.

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