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Original Article

Effect of Plant Essential Oil on Growth Performance and Immune Function During Rearing Period in Laying Hens

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ABSTRACT

The effects of plant essential oil (PEO) on the production performance and immune function of laying hens were evaluated to provide theoretical basis for promoting the natural plant extracts. Eight thousand 1-day-old healthy laying hens were randomly divided into a control group or PEO group, with four replicates per treatment and 1000 hens per replicate. The PEO diet was supplemented with 3g/kg plant extract. Diets were fed for 56 days. The tibia length and keel length were detected on an empty stomach at the end of the trial. Blood samples were collected on the 28th and 56th days to detect the level of C3, C4, IL-1, IL-2, IL-17 and immunoglobulin in the serum. The results showed that, compared with the control, PEO supplementation significantly increased the weight gain rate (WGR) at the 2nd, 4th, 5th and 7th week (p<0.05), and decreased the WGR at the 3rd and 6th week. The tibial length was significantly increased at the 3rd, 5th, 6th and 7th week (p<0.01), and also the keel length at the 5th and 7th week in PEO group. The concentration of IgG and IgM also significantly influenced with PEO supplementation, but there was no significant difference in the complements, C3 and C4, and the IL levels between days 28 and 56. Moreover, no significant difference was observed in body weight and immune organ on day 56. Therefore, we conclude that the addition of PEO could improve the production performance and immune function in laying hens.

INTRODUCTION

Plant essential oil (PEO), as a feed additive extract that has been favored in recent years (Brenes *et al*., 2010), and have been conducted in many studies. It was reported that plant essential oils, probiotics and enzymes can be used as healthy substitutes for antibiotics, which can improve the balance of intestinal microbiome and maintain the intestinal homeostasis (Patterson *et al*., 2003; Vondruskova *et al*., 2010). In livestock sector, PEO was initially added in the diet as a flavor enhancer to induce animal feeding, which in turn can improve the feeding efficiency and growth performance (Franz *et al*., 2010). In the process of layer breeding, the growth level of the brooding period is directly related to the laying performance and production performance of the whole life. Therefore, how to develop and utilize plant essential oils to improve the growth performance of laying hens is the key technology urgently needed in the laying hen breeding industry at present.

With the deepening of the research, it was found that essential oil (EO) also has a wide range of physiological functions, such as sterilization, antioxidation, anti-inflammation, anti-viral action and so on (Suntres *et al*., 2015). Because of its rich and varied natural properties, PEO has a broad application prospect as a substitute for antibiotics in the field of animal production. Studies have shown

that the addition of 40mg/kg oregano and lavender leaf and lavender mixed essential oil or 500mg/kg oregano essential oil in broilers diets can effectively reduce the discharge of coccidian eggs in feces and reduce the degree of mucosal damage caused by coccidia infection (Bozkurt *et al*., 2014; Mohiti-Asli *et al*., 2015). When garlic and thyme essential oil were added to the diet of laying hens, the egg weight and yolk color increased significantly, which could enhance the production performance (Ghasemi *et al*., 2010). In addition, the dietary supplementation of plant essential oil could improve the immune function of animals (Li *et al*., 2012; Awaad *et al*., 2014). In the process of layer breeding, the growth level of the brooding period is directly related to the laying performance and production performance of the whole life. therefore, how to develop and utilize plant essential oils to improve the growth performance of laying hens is the key technology urgently needed in the laying hen breeding industry at present. In the present study, plant essential oils have attracted much attention in animal husbandry production. However, there are few reports on the effect of plant essential oils on the growth performance and immune function in laying hens. Therefore, in this study, we evaluated the effect of plant essential oils on the growth performance and immune function of laying hens. This study provides data support and theoretical basis for the application of plant essential oils in layer production.

MATERIALS AND METHODS

Ethics statement

All animal procedures performed in this study were reviewed, approved, and supervised by the Animal Ethics committee of Shandong Academy of Agricultural Science (Permit No.:2018412).

Plant essential oil

Coated plant essential oil products were provided by the Institute of Animal Husbandry and Veterinary Medicine, Shandong Academy of Agricultural Sciences. The effective components are the mixture of flavonoids and linalool, with approximately the content of flavonoids ≥ 12%, and linalool ≥ 10%.

Instruments and equipment

The following instruments and kits were used in this study: Enzyme label instrument (ELX800), Bio-Tek Baote Company; Desktop centrifuge (TGL-16G), Shanghai Anting Scientific Instrument Factory; YQX- II anaerobic incubator, Shanghai Kanghua Biochemical Instrument Factory; Chicken Immunoglobulin A (IgA), Immunoglobulin G (IgG), and Immunoglobulin M (IgM) ELISA Kits from Jiyinmei company.

Experimental animal

Eight thousand 1-day-old Hailan brown laying hens were randomly divided into two groups (control group or PEO group), Each group had 4 repetitions,1000 repetitions. The PEO group was supplemented with 0.3% coated plant essential oil in the basal diet. Under conventional conditions, the feeding conditions, feeding methods and feed amount of each group were equal. The trial period was 56 days, with a pre-trial period of 5 days.

Time and place of trial

The experiment was carried out in Huasheng Jifu laying hens farm in Haiyang City from April 24 to June 30, 2018.

Determination of production performance

The body weight of the hens was measured every weekend (on days 7, 14, 21, 28, 35, 42, 49), after fasting for 12 hours, and the average weekly weight gain rate (WGR) of repeated laying hens were calculated. The tibia length (TL), and keel length (KL) were detected every weekend.

Immune organ index

At the end of the experiment (on day 56), 56 chickens in each group were randomly selected, followed by fasting for 12 hours with free drinking water, and weighed and slaughtered. Thymus, spleen, bursa of Fabricius and liver, were completely dissected out and wiped out fat and other tissues and sucked the surface liquid. The fresh weight was measured by electronic balance, and the immune organ index was calculated.

Immune organ index = immune organ fresh weight (g) / premortem fasting live weight (kg).

Immunological factors

On the 28th and 56th day, 20 chickens were randomly selected from each group, and 5mL of blood was collected from the brachial vein (medial wing vein). The level of immunoglobulin G (IgG), immunoglobulin M (IgM), interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-17(IL-17), Complement C3(C3) and Complement C4(C4) was detected in serum, with ELISA kit (Wuhan Genmei Technology Co., Ltd.), according to the instructions.

Statistical Analysis

Experimental data were analyzed by One-way analysis of variance (ANOVA) using the SPSS (2008) statistical software (Ver.16.0 for windows, SPSS Inc., Chicago, IL) with a pen (cage) as an experimental unit. Differences among treatments were examined using the Tukey-Kramer's multiple range tests, which were considered significant when the P-value was less than 0.05. The means and standard errors of means (SEM) were also presented.

RESULTS

Production performance during brooding stage

The data of WGR showed that except for the 21th, 28th and 42nd day, the weight gain rate in PEO group during other days was significantly higher than that of the control group (p<0.05). The average weekly growth rate of the experimental group was 41.84%, and that of the control group was 41.14%. There was no significant difference in tibial length between the 7th, 14th and 28th day, but the tibial length of the experimental group was significantly higher than that of the control group at the 21th, 35th, 42nd and 49th day. The keel length of the experimental group was significantly higher than that of the control group at the 35th and 49th day, but there was no significant difference during the other days (Table 1).

Table 1 – Production performance during brooding period.

Item		2d	14d	21d	28d	35d	42d	49d
WGR %	PEO	-	77.09±3.88^{a}	52.51±4.63^{b}	34.93±2.88^{a}	41.05±3.08^{a}	22.64±9.58^{a}	22.81±9.45^{a}
	Control	-	74.83±5.21^{b}	60.01±7.12^{a}	34.68±2.00^{a}	31.85±3.11^{b}	26.94±7.08^{a}	18.51±5.76^{b}
TL (mm)	PEO	36.65±0.94^{a}	44.86±1.32^{a}	52.69 ±1.59^{a}	58.92 ±4.61^{a}	67.88±2.01^{a}	75.92±2.17^{a}	80.53±2.62^{a}
	Control	36.58±0.88^{a}	44.19 ±1.77^{a}	52.22 ±1.59^{b}	58.52±1.91^{a}	67.23±1.96^{b}	74.78±1.69^{b}	79.15±2.84^{b}
KL (mm)	PEO	-	-	46.51 ±1.80^{a}	50.92±1.91^{a}	58.23±1.95^{a}	62.55 ± 3.73^{a}	71.10±3.78^{a}
	Control	-	-	46.36 ±1.96^{a}	50.28±2.42^{a}	57.30±2.79^{b}	63.14 ± 2.69^{a}	69.41±3.75^{b}

^{a,b} Means in the same line with different letters were significantly different (p<0.05). Data represented mean ± SEM; n = 60 per group. WGR=weight gain rate, TL=tibial length, KL=keel length.

Immune Organ Index during brooding stage

Our research results show that there was no statistically significant difference in the body weight (BW), spleen index (SI), thymus index (TI) and bursa index (BI) between the PEO group and the control group. The liver index (LI) in the PEO group was significantly higher than that in the control group (Table 2).

Table 2 – Immune Organ Index during brooding stage.

Item	BW (kg)	SI	TI	LI	BI
PEO	0.750±0.059^{a}	2.65±0.11^{a}	3.66±0.21^{a}	25.11±0.56^{a}	3.76±0.22^{a}
Control	0.757±0.056^{a}	2.67±0.10^{a}	4.07±0.14^{a}	21.45±0.36^{b}	4.01±0.17^{a}
p value	0.6823	0.8808	0.1153	<.0001	0.0503

^{a,b} Means in the same line with different letters were significantly different (P<0.05). Data represented mean ± SEM; n = 30. BW=body weight, SI=spleen index,TI=thymus index, LI= liver index, BI=bursa index.

Detection of serum immunoglobulin concentration

The results of serum immunoglobulin showed that the concentrations of IgG and IgM in PEO group were significantly higher than that of the control group at 28 days (p=0.0020, p<0.01). Whereas, the IgG concentration in the serum of PEO group was significantly lower than that of the control group at 56 days (p=0.0018, p<0.01). There was no significant difference in the serum IgM concentration between the two groups. The highest content of immunoglobulin appeared in PEO group during the whole experimental period (Table 3).

Table 3 – The immunoglobulin concentration in serum.

Item	28d		56d	
	IgG(μg/ml)	IgM(ng/ml)	IgG(μg/ml)	IgM(ng/ml)
PEO	19.43±0.41^{a}	408.51±12.46^{a}	14.65±0.33^{a}	339.67±8.52^{a}
Control	17.62±0.38^{b}	347.11±8.11^{b}	16.45±0.48^{b}	359.52±10.03^{a}
p value	0.0020	<.0001	0.0018	0.1361

^{a,b} Means in the same line with different letters were significantly different (p<0.05). Data represented mean ± SEM; n = 30 per group.

Detection of Serum complement concentration

The quantitative results of serum complement components showed (Table 4) that there was no significant difference in the concentration of complement components C3 and C4 between the PEO group and the control group on the 28th day (p>0.05). On the 56th day, the complement C3 in the PEO group was significantly higher than that in the control group (p=0.0457, p<0.05), but the complement C4 in the experimental group was higher than that in the control group at the early and late stages, even though it was not statistically significant (p>0.05).

Table 4 – Effect of PEO supplementation on the serum complement concentrations of laying hens at 28d and 56d.

Item	28d		56d	
	C3 (pg/ml)	C4 (pg/ml)	C3 (pg/ml)	C4 (pg/ml)
PEO	2830.32±61.29[a]	807.52±24.91[a]	3540.5±90.16[a]	783.20±20.38[a]
Control	2717.47±61.90[a]	769.27±21.57[a]	3307.7 ±72.13[b]	738.84±20.86[a]
p value	0.2054	0.2655	0.0457	0.1445

[a,b] Means in the same line with different letters were significantly different (p<0.05). Data represented mean ± SEM; n = 30 per group.

Serum interleukin levels

The interleukin levels showed that there was no significant difference in the levels of IL-1, IL-2 and IL-17 between the two groups on the 28th day and the 56th day, but the IL levels of PEO group were slightly higher than those of the control group on these days, even though the difference was not statistically significant (p>0.05) (Table 5).

Table 5 – Effect of PEO supplementation on serum interleukin levels in laying hens at 28d and 58d.

Item	28d			56d		
	IL-1 (pg/ml)	IL-2 (pg/ml)	IL-17 (pg/ml)	IL-1 (pg/ml)	IL-2 (pg/ml)	IL-17 (pg/ml)
PEO	108.37±2.67[a]	164.66±5.11[a]	80.25±6.10[a]	106.56±2.46[a]	164.06±3.75[a]	80.37±1.85[a]
Control	103.39±2.43[a]	164.34±4.02[a]	79.79±4.73[a]	110.51±2.44[a]	160.61±4.96[a]	75.53±1.72[a]
p value	0.1764	0.9607	0.8912	0.2668	0.5745	0.0722

[a,b] Means in the same line with different letters were significantly different (p<0.05). Data represented mean ± SEM; n = 30 per group.

Serum anti-viral antibody levels

The specific anti-viral antibody assays against H9 Influenza virus and Newcastle disease virus (NDV) showed that (Table 6) the level of H9 subtype (H9) of Influenza virus antibody in the test group was significantly higher (p<0.05) than that in the control group on the 28th and 56th day, but there was no significant difference in the Newcastle disease virus (NDV) antibody level between the test group and the control group on the 28th and 56th day (p>0.05).

Table 6 – Serum anti-viral antibody levels of H9 Influenza virus and Newcastle disease virus (NDV).

Item	28d		56d	
	ND	H9	ND	H9
PEO	6.15± 0.31[a]	2.54± 0.36[a]	8.41± 0.13[a]	10.12± 0.17[a]
Control	5.95± 0.38[a]	1.54± 0.28[b]	8.50± 0.15[a]	9.42 ± 0.29[b]
p value	0.6913	0.0313	0.6475	0.0410

[a,b] Means in the same line with different letters were significantly different (p<0.05). Data represented mean ± SEM; n = 100 per group.

DISCUSSION

Production performance

Natural plant extracts have been showing promising effects to promote the growth of poultry (Jamroz *et al*., 2005; Windisch *et al*., 2008), and the addition of flavonoids to the diet could improve the growth performance of broilers (Starčević *et al*., 2014). Ouyang *et al.* (2016) found that alfalfa extract supplemented with 15mg flavonoids/kg feed had a positive effect on the average daily gain and feed conversion rate of broilers. Chen *et al.'s* (2016) study showed that dietary supplementation of 0.3g/kg alfalfa extract (81% flavonoids) could promote the growth of geese, increased feed intake and nitrogen utilization efficiency, and significantly increased the strength and weight of tibia, thus reduced the incidence of leg diseases.

Some previous studies have shown that the use of plant essential oil in poultry breeding could

increase feed intake and efficiency, increase the secretion of digestive enzymes, and promote intestinal microecological balance (Jang *et al*., 2008; Lee *et al*., 2003; Liolios *et al*., 2009). This study proved that the addition of plant essential oils to the diet can improve the growth performance of laying hens, which may be due to the fact that plant essential oils can promote the secretion of digestive enzymes and other enzymes, and then increase the absorption of nutrients in the diet. To achieve the effect of promoting growth. This is consistent with reports of Fardos *et al*. (2019), where the addition of plant essential oils to the diet showed considerable improvement in the body weight and growth rate of chickens. These marked variations in growth rate weight gain factors are mediated by and flavonoids in plant essential oils, which can affect and influence the intestinal morphology and promote intestinal mucosal absorption (Awad *et al.,* 2011; Viveros *et al.* 2011). Other studies have shown that flavonoids in the form of glycosides play a key role in plant growth and development by regulating the homeostasis of growth hormones (Yin *et al.* 2014; Kuhn *et al.* 2016). To sum up, plant essential oils has a significant effect on improving the production performance of laying hens and has a broad prospect in its breeding capacity. The development and utilization of plant essential oils are of great significance to promoting the poultry industry with healthy development of laying hens.

Immunity

A large number of studies have reported that plant essential oils have extensive potential antibacterial activity. So it is speculated that the level of immune response will be improved after feeding plant essential oils in the brooding period of laying hens. The thymus, spleen and bursa of Fabricius are important immune organs of poultry and are the main sites that secrete many kinds of immune activity factors (Fan *et al.*, 2013), and their status is closely related to immune function. Ravis *et al*. (1988) have reported that the relative weight of immune organs can be used to evaluate their immune status, and a larger weight of immune organs usually represents a stronger immune function to some extent. Therefore, the immune organ index is often used to evaluate the immunity level of poultry (Heckert *et al*., 2002). The liver is one of the main organs to regulate the metabolism of the body. It can secrete a variety of metabolic enzymes and is also the main organ of detoxification and immune defense in the body (Chao *et al.,* 2019). This study confirmed that the addition of plant essential oils to the diet could significantly increase the liver index of the experimental group, indicating that plant essential oils may regulate the production of immune coping factors through the liver, and then achieve the goal of regulating immune function. IgG and IgM are important factors that affect the immune response to many microbes and prevent infections (Zhao *et al*., 2017). From the increase of IgG and IgM content in brooding hens in this study, it can be seen that adding plant essential oils to the diet can promote the secretion of immunoglobulin, thus protect the body from harmful bacteria and improve the immunity and disease resistance of the body. These results are similar with Özek. Özek *et al. (*2019), state that adding mixed essential oils (oregano essential oil, lauryl essential oil, sage essential oil, myrtle essential oil, fennel essential oil, citrus peel essential oil) to 52-day-old layers' diets could increase the antibody levels of Newcastle disease virus and infectious bursal virus in blood. Hao *et al.* (2018) adding 20mg/kg Daidzein to breeder chickens could increase serum IgA and IgG levels and serum NDV antibody titer in 21-day-old broilers. It was believed that the addition of plant essential oil to the diet can promote the secretion of immunoglobulin and improve the antibody titer.

The complement system is an efficient amplification system of immune activity in the body, which plays a very important role in the defense response and immune regulation of the body against microbial infections. At the same time, it can also mediate the traumatic immunopathological reactions. In recent years it has been found that the complement has a regulatory effect on immunity. In terms of immune complex, the complement can prevent the precipitation and dissolution of immune complex and can accelerate the clearance of immune complex. In addition, the complement also has a series of regulatory effects on the cellular immunity and humoral immunity. The complement system includes many complement components. C3 and C4 are inherently important components of the complement system, which exists in body fluids to participate in the complement activation cascade. C3 is mainly synthesized by the liver, but can also be synthesized by macrophages, and C4 is synthesized exclusively by macrophages (book). Our results showed that the addition of plant essential oil to the diet of laying hens could increase the concentration of complement components C3 and C4 in blood, which indicated that the plant essential oil could promote the immune function of laying hens (Wang *et al*., 2001).

Cytokines also play an important role in the process of immune regulation, such as IL-1, IL-2, IL-6 and TNF, which can act on the hypothalamus, pituitary, adrenal gland and gonads. It has a variety of activities such as immune regulation, anti-inflammation and so on (Parkin *et al*., 2001). Interleukin-1 (IL-1) is also known as lymphocyte activating factor (LAF), B cell activating factor (BAF), thymocyte proliferation factor (TPF) and so on. At present, it is believed that the main role of IL-1 is to mediate inflammatory response, regulation of immune response, elicit anti-tumor effect and so on. IL-1 plays a wide range of regulatory roles in immune response. It can induce lymphocytes to release lymphokines, enhance the antigen binding ability of T cells, and enhance the activity of natural killer cells (NK cells). However, IL-1 alone does not have any stimulating effect on NK cells. Only IL-1 plus IL-2 or interferon, or both, can significantly enhance the activity of NK cells and could promote B cells to proliferate and differentiate into antibody-producing cells (Kuby *et al*., 1997). Interleukin 2 (IL-2) can induce T cell proliferation and maintain the continuous growth of T cells *in vitro*, so it was once called T cell growth factor (TCGF). This study found that adding plant essential oil to the diet of laying hens can increase the concentration of IL-1 and IL-2 in serum, although the difference is not statistically significant, it may plays an important role in the antibody mediated immune response by stimulating B lymphocytes, which also confirms the fact that the addition of plant essential oil can improve the level of antibody.

CONCLUSION

We concluded that 0.3% coated PEO supplementation in diet could enhance immunity, improve antibody levels, and promote the growth performance in laying hens, which indicates a great potential utilization value of PEO.

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