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Effects of adding tea tree oil on growth performance, immune function, and intestinal function of broilers

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ABSTRACT The aim of this study was to investigate the effects of adding tea tree oil (**TTO**) in the basal diet on growth performance, immune function, and intestinal function in broilers. This study utilized 1,650 one-day-old broilers with good health and similar body weight. Subjects were randomized into 5 groups with 6 replicates each: the control group (**CON**, basal diet), positive control group (**PCG**, basal diet + 100 mg/kg oregano oil in diet), low-dose TTO group (**TTO-L**, 50 mg/kg TTO added in the basal diet), medium-dose TTO group (**TTO-M**, 100 mg/kg TTO added in the basal diet), and high-dose TTO group (**TTO-H**, 200 mg/kg TTO added in the basal diet). The whole test period lasted 28 d. The results showed that the broilers fed with TTO supplemented diet had significantly higher body weight and average daily gain (**ADG**) ($P = 0.013$), and had a lower feed conversion ratio (**F/G**) ($P = 0.010$) throughout the trial period. The index of thymus in TTO-M increased significantly compared to CON ($P = 0.015$) on d 28. On d 14 and 28, C3, IFN-γ, TNF-α, and IL-2 levels in TTO-L serum were significantly increased ($P < 0.001$); the 3 test groups supplemented with TTO had significantly higher titers of avian influenza H9 subtype in their serum ($P < 0.05$). Tea tree oil supplement in the diet also had a positive and significant effect on the intestinal morphology of broilers throughout the experiment ($P < 0.05$). These results indicate that TTO has the ability to promote broiler growth, regulate immunity, and improve intestinal morphology. The proposed dosage of adding 50 mg/kg in broiler basal diets provides a theoretical basis for its subsequent use in livestock feeds.

Key words: tea tree oil (TTO), growth performance, immune function, intestinal integrity, broiler

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INTRODUCTION

The excessive utilization of antibiotics in livestock has contributed to a distressing surge in multidrug-resistant bacterial infections. This rise in resistance has caused severe health complications, environmental contamination, and has posed significant challenges to the sustainable development of the farming industry. Moreover, it presents a direct threat to public health safety (Cully, 2014) and compromises food security (Onishchenko et al., 2012; Chen et al., 2020). Appropriate use of antibiotics and prohibition of growth-promoting antibiotics in the livestock industry are current maneuvers to prevent antibiotic abuse in many countries. China has also explicitly proposed to withdraw all pharmaceutical feed additives from livestock production in 2020. Therefore, the urgency to explore safe and efficient alternatives to antibiotics has grown exponentially. Against this backdrop, an array of researchers had put forth various alternatives to chemical medicines in livestock farming. These alternatives encompass the use of herbal medicines (Huang et al., 2021), enzymes (Woyengo et al., 2019), acidifiers (Ateya et al., 2019; Roofchaei et al., 2019), and natural plant extracts (Khalaji et al., 2011). Tea tree oil (**TTO**) is a pure natural plant oil extracted from the stems and leaves of *Melaleuca alternifoli* (Bo et al., 2023) by steam distillation, which is a colorless to light yellow liquid with a distinctive aromatic odor. Tea tree oil contains a variety of active ingredients, mainly 4-terpineol, 1,8-eudesmanol, and α-pinoresinol (Bekhof et al., 2023).

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Tea tree oil has been shown to possess several beneficial properties, including anti-inflammatory (Hart et al., 2000), antiviral (Romeo et al., 2022), antitumor (Clark et al., 2021), and antioxidant (Liu et al., 2022a) activities. This has drawn significant attention to its potential use as a replacement for antibiotics.

The chemical stability of TTO makes it possible to become a feed additive (Vo et al., 2021). In a previous study conducted by Yang et al. (2022), supplement of TTO in the daily diet for fattening pigs improves immunity, antioxidant capacity, and growth performance; furthermore, the nutritional value and antioxidant capacity of pork are improved. It has been reported (Dong et al., 2019) that supplement of 150 mg/kg TTO in daily feed improves intestinal mucosal immunity significantly in weaning pigs compared to the use of antibiotics. Similar benefits have been reported in poultry medicine, which revealed that adding TTO in daily feed improves growth performance, cecum microflora, immunity, and antioxidant capacity in Partridge Shank chickens (Qu et al., 2019). However, information regarding how TTO affects intestinal morphology, immunity, and growth performance in Fengyan black bone chickens (*Ayam Cemani*) remains scant. The aim of this study is to investigate the effects of TTO supplementation on immunity, intestinal morphology and growth performance in Fengyan black bone chickens.

MATERIALS AND METHODS

Experimental Design, Animals, and Diets

The use of animals and procedures was approved by the Experimental Animal Ethics Committee of Sichuan Agricultural University. The TTO used in this study was obtained from Tianjin Jiaxinuo Company (LOT: 20190112; Tianjin, China) and had a concentration of 10%. Tea tree oil composition is dominated by terpinen-4-ol, γ-terpinene and α-terpinene, which account for about 70% of the total. P-cymene, 1, 4-p-menthadiene, α-pinene-ol, and α-pinene account for about 15% of the total. Oregano oil was purchased from Shandong Luci Veterinary Medicine Co., Ltd. (LOT: 20190407; Shandong, China) with a concentration of 10%. One thousand six hundred fifty, 1-day-old Fengyan black bone chickens (*Ayam Cemani*, Sichuan, China) purchased from Luzhou Yikang Selenium-enriched Ecological Animal Husbandry Co., Ltd. (Sichuan, China) were used in this study. Subjects were in good health with similar body weight and randomly divided to 5 groups with 6 replicates for each group: the control group (**CON**, basal diet), positive control group (**PCG**, 100 mg/kg oregano oil added in basal diet), low-dose TTO group (**TTO-L**, 50 mg/kg TTO added in the basal diet), medium-dose TTO group (**TTO-M**, 100 mg/kg TTO added in the basal diet), and high-dose TTO group (**TTO-H**, 200 mg/kg TTO added in the basal diet). The study lasted for 28 d. The cages and the utensils were cleaned and fumigated with formaldehyde for 1 wk before the experiment. The ambient temperature was set at 32°C to 35°C on the first day of the study for 1-day-old chickens. The ambient temperature was gradually decreased by 2°C to 3°C weekly until it dropped to about 26°C on the fourth week of study. For the first 14 d of the study, a 24-h light/dark cycle was maintained, after which the cycle was adjusted to 20 h of light and 4 h of darkness. All subjects were raised in disinfected cages with adequate ventilation. Water was offered ad libitum. The immunization protocols were carried out following standard procedures throughout the study. The compositions and nutrient facts of the basal diet used in this study are shown in Table 1. Chickens in each group were weighed and recorded on the first day of the experiment. The feed intake of each group was measured and recorded throughout the study. Chickens were weighed after 12 h of feeding at the last day of the experiment (d 28). As shown by Ruan et al. (2023), the average daily gain (**ADG**), the average daily feed intake (**ADFI**), and the feed conversion ratio (**F/G**) were calculated according to the following equations.

$$\text{ADFI (g/d)} = \text{total stage feed intake (g)} / \text{number of feeding days (d)}$$

$$\text{ADG (g/d)} = \text{stage weight gain (g)} / \text{number of feeding days (d)}$$

$$\text{F/G} = \text{average daily feed intake (g)} / \text{average daily weight gain (g)}$$

Sample Collection

For each group, 12 subjects (2 from each replicate) were randomly chosen and blood samples were collected

Table 1. Composition and nutrient levels of the basal diet (%, air-dry basis).

Ingredients	1−28 d
Corn	56.5
Soybean meal	35.31
Oil blends	2.8
Stonewash	1.31
Calcium hydrogen phosphate	1.26
Salt	1.26
Vitamin−mineral premixes[1]	0.3
Choline chloride, 50%	1
Lysine	0.02
Methionine	0.24
Total	100.00
Nutritional content[2]	
Metabolic energy, MJ/kg	12.13
Crude protein	20.5
Lysine	1.15
Methionine + cysteine	0.81
Calcium	0.9
Total phosphorus	0.57
Soybean meal	35.31

[1]The premix provided the following per kg of the diet: Cu 8 mg; Fe 90 mg; Zn 50 mg; Mn 80 mg; I 0.30 mg; Se 0.15 mg; vitamin A 10,000 IU; vitamin D3 2,100 IU; vitamin E 10 mg; vitamin K3 0.6 mg; vitamin B1 2.0 mg; vitamin B2 4.0mg; vitamin B12 0.01 mg; nicotinic acid 30.0 mg; folic acid 0.6 mg; biotin 0.15 mg; D-pantothenic acid 11 mg.

[2]The nutrient levels are calculated values.

from the basilic vein. Blood samples were centrifuged at 4,000 rpm for 10 min at 4°C to harvest serum. Serum was frozen at −20°C for further analysis. Approximately 2 cm of the duodenum, jejunum, and median ileum were collected. Samples were gently rinsed with ice-cold phosphate-buffered saline (**PBS**; pH 7.4) and transferred in 4% paraformaldehyde solution for histological examination. Immune organs, including bursa, thymus, and spleen were collected and weighed for calculation of relative immune organ weight (immune organ index) by the following equation: immune organ index (g/kg) = weight of immune organs (g)/body weight (kg) (Hussein et al., 2023).

Antibody Titer

The stored serum was used for Newcastle disease (**ND**) and avian influenza H9 subtype (H9) antibody titers test in the serum by hemagglutination inhibition (**HI**) test (Spackman and Sitaras, 2020). The antibody titers were expressed on a log2 scale in the results.

Detection of Serum Complements and Cytokines

Following other researchers (Engvall and Perlmann, 1971; Liu et al., 2016; Tabatabaei and Ahmed, 2022), serum levels of complement proteins (C3 and C4) and cytokines (IFN-γ, TNF-α, and IL-2) were determined by enzyme-linked immunosorbent assay (**ELISA**) in strict accordance with the kit purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Intestinal Morphological changes

To facilitate embedding, the preserved intestinal samples were rinsed in running water for 3 h and subsequently transferred to ethanol for dehydration. Embedded tissues were dissected by a microtome (Thermo Fisher Scientific HM325, Waltham, MA) and stained with hematoxylin and eosin. The histological changes of the duodenum, jejunum, and ileum were observed by a CX22 light microscope (OLMPUS, Tokyo, Japan) (Stenzinger et al., 2022). Three fields were selected for each slide, which contained 8 to 10 intact villi in each view. A Leica MC170HD camera system (Germany) was used for recording images, and Image-Pro plus 4.5 image analysis software (America) was used to determine the villi height, crypt depth, and intestinal wall thickness. For the calculation of the villi−crypt ratio, the average of the aforementioned numbers was utilized.

Statistical Analysis

One-way analysis of variance (**ANOVA**) was used to analyze the experimental data between groups using SPSS 18.0 statistical software (SPSS Inc., Chicago, IL). Duncan's multiple range test was used to determine the differences among treatment groups. Data are shown as mean values with standard error of the total mean (**SEM**). For all tests, $P < 0.05$ was considered a significant difference.

RESULTS

Growth Performance

The effects of TTO supplement on growth performance are shown in Table 2. The body weight of chickens increased significantly ($P = 0.013$) among all TTO supplement groups compared to CON on d 28; however, there were no significant changes of body weight compared to PCG throughout the study. ADG among all TTO supplement groups increased significantly ($P = 0.027$) from d 14 compared to CON. Overall, ADG among all TTO groups regardless the dosage increased significantly ($P = 0.013$) throughout the study. No significant changes were noticed regarding ADFI among all groups during the study. However, F/G started to decrease significantly ($P = 0.033$) from d 14 in all treatment groups compared to CON. The overall F/G in all treatment groups were significantly lower ($P = 0.01$) than CON throughout the study.

Table 2. Effects of TTO supplement to growth performance.

Items	AGE	CON	PCG	TTO-L	TTO-M	TTO-H	SEM	*P*-valve
BW	D 14	142.40	143.89	141.3	146.36	146.91	3.28	0.379
(g)	D 28	265.04[b]	280.51[a]	283.04[a]	288.90[a]	291.26[a]	7.33	0.013
ADG	D 1-14	7.24	7.37	7.24	7.56	7.62	0.24	0.376
(g)	D 14-28	8.76[b]	9.76	10.12[a]	10.18[a]	10.31[a]	0.49	0.027
	D 1-28	8.00[b]	8.56[a]	8.68[a]	8.87[a]	8.96[a]	0.27	0.013
ADFI	D 1-14	13.67	13.78	13.53	13.97	14.25	0.39	0.421
(g)	D 14-28	26.83	27.18	26.79	26.75	27.23	0.48	0.771
	D 1-28	20.25	20.48	20.16	20.36	20.74	0.30	0.372
F/G	D 1-14	1.93	1.87	1.87	1.86	1.88	0.07	0.890
(%)	D 14-28	3.09[a]	2.83	2.65[b]	2.64[b]	2.65[b]	0.16	0.033
	D 1-28	2.56[A]	2.41	2.30[B]	2.28[B]	2.30[B]	0.08	0.010

Abbreviations: ADFI, average daily food intake; ADG, average daily gain; BW, body weight; F/G, feed to gain ratio.

CON, the control group (basal diet), PCG, positive control group (basal diet+100mg/kg oregano oil), TTO-L (basal diet + 50 mg/kg tea tree oil), TTO-M (basal diet + 100 mg/kg tea tree oil), and TTO-H (basal diet + 200 mg/kg tea tree oil).

[a,b]Different alphabets indicate a difference, $P \leq 0.05$.

[A,B]Different alphabets indicate significant difference, $P \leq 0.05$.

Table 3. Effects of TTO supplement to immune organ index.

AGE	Items	CON	PCG	TTO-L	TTO-M	TTO-H	SEM	*P*-valve
D 14	Spleen index	1.71	1.30	1.66	1.22	1.69	0.23	0.088
	Thymus index	3.82	5.07	4.12	4.47	5.14	0.59	0.118
	Bursal index	2.53	3.04	2.94	2.97	3.14	0.25	0.155
	Spleen index	1.41	1.09	1.32	1.08	1.21	0.14	0.092
D 28	Thymus index	2.95^{b}	3.47^{b}	3.67^{b}	4.47^{a}	3.33^{b}	0.43	0.015
	Bursal index	1.90	1.70	1.77	2.33	2.29	0.31	0.139

CON, the control group (basal diet), PCG, positive control group (basal diet + 100 mg/kg oregano oil), TTO-L (basal diet + 50 mg/kg tea tree oil), TTO-M (basal diet + 100 mg/kg tea tree oil), and TTO-H (basal diet + 200 mg/kg tea tree oil).
^{a,b}Different alphabets indicate a difference, $P \leq 0.05$.

Immune Organ Index

The effects of TTO supplement on the immune organ index are shown in Table 3. The index of thymus in TTO-M increased significantly compared to CON ($P = 0.015$) on d 28. However, the indices of spleen, thymus, and bursal in other treatment groups did not show significant differences compared to CON. There were no significant changes of immune organ indices in all TTO supplement groups compared to PCG.

Serum Complements and Cytokines

Table 4 shows the effect of TTO supplement on serum complements and cytokines. Compared with other groups, serum C3 levels of TTO-L were significantly different ($P < 0.001$); however, C4 levels did increase significantly compared to CON ($P < 0.001$), except for TTO-L values at d 14. Compared with other groups, the levels of IFN-γ, TNF-α, and IL-2 in TTO-L were significantly increased on d 14 and 28 ($P < 0.001$).

ND and H9 Antibody Titers

The effects of TTO supplement to antibody titers of ND and H9 are shown in Table 5. No significant changes were noticed on the titers of ND among groups; however, H9 titers in TTO-M and TTO-H increased significantly ($P = 0.009$) compared to CON on d 14. Furthermore, H9 titers increased significantly ($P < 0.001$) in all treatment groups including PCG compared to CON on d 28.

Intestinal Morphology Test Results

The effects of TTO supplement on intestinal morphological changes are shown in Table 6. The villus height of duodenum, jejunum, and ileum varied but overall increased in height in all TTO treatment groups, while the crypt depth was overall decreased in all TTO treatment groups. The villi−crypt ratio increased significantly on both d 14 ($P < 0.001$) and d 28 ($P = 0.004$) in all TTO supplement groups compared to CON, except for jejunum on d 28.

Table 4. Effects of TTO supplement to serum complements and cytokines.

AGE	Items	CON	PCG	TTO-L	TTO-M	TTO-H	SEM	*P*-valve
D 14	C3 (mg/L)	167.62^{B}	175.03^{B}	214.37^{A}	172.66^{B}	167.87^{B}	5.64	<0.001
	C4 (mg/L)	48.66	50.84	53.45	53.56	49.57	2.18	0.132
	IFN-γ (pg/mL)	12.73^{B}	13.04^{B}	18.89^{A}	13.24^{B}	13.48^{B}	0.97	<0.001
	TNF-α (pg/mL)	13.74^{B}	13.42^{B}	17.60^{A}	12.85^{B}	13.38^{B}	0.48	<0.001
	IL-2 (pg/mL)	83.76^{B}	92.09^{B}	110.43^{A}	84.49^{B}	84.62^{B}	5.03	<0.001
D 28	C3 (mg/L)	83.57^{B}	86.26^{B}	152.90^{A}	88.07^{B}	86.00^{B}	2.38	<0.001
	C4 (mg/L)	20.17^{B}	21.28^{B}	38.97^{A}	21.33^{B}	20.67^{B}	1.60	<0.001
	IFN-γ (pg/mL)	8.33^{C}	10.42^{B}	16.78^{A}	8.21^{C}	8.61^{C}	0.59	<0.001
	TNF-α (pg/mL)	8.24^{BD}	9.56^{B}	16.05^{A}	8.45^{B}	6.58^{CD}	0.87	<0.001
	IL-2 (pg/mL)	35.00^{B}	36.66^{B}	63.02^{A}	35.90^{B}	37.74^{B}	2.63	<0.001

CON, the control group (basal diet), PCG, positive control group (basal diet + 100 mg/kg oregano oil), TTO-L (basal diet + 50 mg/kg tea tree oil), TTO-M (basal diet + 100 mg/kg tea tree oil), and TTO-H (basal diet + 200 mg/kg tea tree oil).
$^{A\text{-}D}$Different alphabets indicate significant difference, $P \leq 0.01$.

Table 5. Effects of TTO supplement to antibody titers.

Items	AGE	CON	PCG	TTO-L	TTO-M	TTO-H	SEM	*P*-valve
ND	D 14	5.20	5.30	5.70	5.50	5.80	0.38	0.462
	D 28	5.30	5.40	5.70	6.80	6.50	0.79	0.234
H9	D 14	4.00^{B}	4.80^{AB}	4.20^{B}	5.40^{A}	5.40^{A}	0.47	0.009
	D 28	6.00^{B}	7.90^{A}	8.00^{A}	8.00^{A}	8.20^{A}	0.52	<0.001

CON, the control group (basal diet), PCG, positive control group (basal diet + 100 mg/kg oregano oil), TTO-L (basal diet + 50 mg/kg tea tree oil), TTO-M (basal diet + 100 mg/kg tea tree oil), and TTO-H (basal diet + 200mg/kg tea tree oil).
$^{A, B}$Different alphabets indicate significant difference, $P \leq 0.01$.

Table 6. Effects of TTO supplement on intestinal morphological changes.

Items	Intestine	AGE	CON	PCG	TTO-L	TTO-M	TTO-H	SEM	*P*-valve
Villus height (μm)	Duodenum	D 14	912.97^{C}	821.09^{D}	999.12^{B}	1072.12^{A}	954.47^{C}	21.02	<0.001
		D 28	754.88^{b}	758.48^{b}	791.72^{ab}	781.95^{ab}	819.37^{a}	20.36	0.025
	Jejunum	D 14	546.12^{C}	669.54^{B}	645.31B	762.89^{A}	559.36^{C}	29.48	<0.001
		D 28	412.28	437.64	450.51	436.41	416.83	13.78	0.050
	Ileum	D 14	332.56^{D}	350.80^{CD}	403.71^{B}	365.34^{C}	447.58^{A}	12.82	<0.001
		D 28	282.41^{B}	329.05^{A}	283.56^{B}	289.44^{B}	347.55^{A}	7.90	<0.001
Crypt depth (μm)	Duodenum	D 14	230.87^{A}	187.64^{C}	212.87^{B}	204.55^{B}	184.28^{C}	7.68	<0.001
		D 28	136.17^{A}	124.63^{B}	116.40^{C}	130.51^{AB}	130.97^{AB}	4.06	0.001
	Jejunum	D 14	222.54^{A}	179.58^{B}	220.46^{A}	172.24^{B}	132.10^{C}	8.67	<0.001
		D 28	95.95	93.10	89.67	94.83	91.77	3.12	0.313
	Ileum	D 14	142.16^{A}	108.93^{CD}	136.11^{AB}	95.45^{D}	118.66^{BC}	9.82	<0.001
		D 28	92.08^{A}	77.76^{C}	68.22^{D}	86.38^{B}	81.54^{C}	2.31	<0.001
Villus height: crypt depth (μm: μm)	Duodenum	D 14	3.97^{C}	4.38^{B}	4.70^{B}	5.28^{A}	5.19^{A}	0.19	<0.001
		D 28	5.54^{C}	6.10^{BC}	6.83^{A}	6.03^{BC}	6.28^{AB}	0.30	0.004
	Jejunum	D 14	2.47^{C}	3.74^{B}	2.94^{C}	4.50^{A}	4.29^{AB}	0.30	<0.001
		D 28	4.51	4.79	4.96	4.54	4.66	0.23	0.293
	Ileum	D 14	2.38^{C}	3.24^{B}	3.05^{B}	3.86^{A}	3.84^{A}	0.27	<0.001
		D 28	3.07^{B}	4.25^{A}	4.16^{A}	3.35^{B}	4.28^{A}	0.17	<0.001

CON, the control group (basal diet), PCG, positive control group (basal diet + 100 mg/kg oregano oil), TTO-L (basal diet + 50 mg/kg tea tree oil), TTO-M (basal diet + 100 mg/kg tea tree oil), and TTO-H (basal diet + 200 mg/kg tea tree oil).
^{a,b}Different alphabets indicate a difference, $P \leq 0.05$
$^{A\text{-}D}$Different alphabets indicate significant difference, $P \leq 0.01$.

DISCUSSION

Growth performance is an important indicator to assess animal growth and the efficacy of supplements in the poultry industry. Tea tree oil has been reported to have broad-spectrum antibacterial functions (Carson et al., 2006) as well as antioxidant properties (Souza et al., 2018), antitumor effects (Clark et al., 2021), and anti-inflammation activity (Shao et al., 2021). TTO is used extensively as a daily supplement for medical care and oral health in human medicine (Lee et al., 2017; Salvatori et al., 2017).

The results of this study showed that ADG increased significantly while F/G significantly decreased with supplement of TTO since 14th day of the study. Qu et al. (2019) found that TTO supplementation had a significant effect on ADG in Partridge Shank chickens. Therefore, these results indicate that the addition of TTO to the ration can promote broiler growth and increase the feed conversion ratio.

The complement system plays an important role to determine immunity and regulates immune response (Defendi et al., 2020). C3 is synthesized by the liver and macrophages, supporting nonspecific and specific immunity via different pathways (Zarantonello et al., 2023). C4 determines immunity in conjugation with C3 in the immune chain reactions, which is a key to trigger immune response for clearance of pathogens (Wang and Liu, 2021). Therefore, the performances of C3 and C4 are assessed concurrently in this study.

Serum levels of cytokines, such as IFN-γ, TNF-α, and IL-2, have been used as indicators to assess the strength of immunity (Kveler et al., 2018). IL-2 is a product of T lymphocytes, which enhance the activities of T cells (Hernandez et al., 2022) and promote B cells to produce antibodies (DiToro et al., 2018). IL-2 is also involved in different immune responses triggered by inflammation (Morita et al., 2015) or neoplasia (Mullard, 2021; Mullard, 2022). IFN-γ is a potent immunomodulatory molecule, which facilitates virus defense and enhances macrophage activity (Castro et al., 2018). It has been revealed that IFN-γ promotes maturation of cytotoxic T lymphocytes, proliferation of B cells, and production of antibodies (Ni and Lu, 2018). Nonetheless, TNF-α is a product of macrophages and is involved in the modulation of immunity (Moatti and Cohen, 2021), inflammatory responses (Sun et al., 2021), and antitumor processes (Ji et al., 2014). Because of the diversity of immune factors and the complexity of their relationships, the effects of essential oils on cytokines have been less studied. ELISA is a well-established method to assess the serum levels of complements and cytokine; therefore, it was used in this study for further understanding of the effects of TTO supplement to immunity.

Zhang et al. (2021a) found that on d 28, serum concentrations of TNF-α were significantly higher in pigs fed TTO diets than in pigs fed chrysomycin-containing diets, and INF-γ concentrations did not differ between dietary treatments. In addition, the levels of IL-2, TNF-α, and IFN-γ in the jejunal and ileal mucosa of the weaned piglets with TTO in the diet had a tendency to increase compared to the CON (Dong et al., 2019). In the present study, we found that C3 content, IFN-γ, TNF-α, and IL-2 levels of TTO-L were significantly higher than those of CON and PCG on d 14 and 28, except for C4 content on d 14, which was not statistically significant. Thus, it indicates that TTO has a beneficial effect on the immune function of chicks and provides a reference for its application in immune regulation.

We also found that supplement of TTO in the basal diet significantly increased the TTO-M thymus index. As the central immune organ (Ciriaco et al., 2003), the thymus is the site of lymphatic stem cell proliferation

and T cell differentiation, and is mainly involved in cellular immunity (Ehnert et al., 2021). It has been shown that (Zhang et al., 2021b) the immune organ index is often used as an indicator of immune function in broiler chicks and that an increase in the immune organ index indicates an increase in body immunity. Studies have shown that camellia sinensis (green tea) extract has a positive immunomodulatory effect on broiler chickens infected with coccidiosis (Abbas et al., 2017; Zhang et al., 2020). Thus, by adding TTO to the basic diet of chicks, the development of immune organs can be promoted, which is important for improving the survival and morbidity rate in poultry breeding.

Antibodies are proteins secreted by effector B cells and are mainly involved in the immune response of the body (Carter and Rajpal, 2022). This study showed that the H9 antibody titers in the sera of broiler chickens from all dose groups supplemented with TTO were significantly higher than those of CON on d 14 and 28. Therefore, the 3 aspects of the immune organ index, antibody titer, and serum immune factor can reflect that TTO improves the humoral immune function of the body. To a certain extent, TTO has improved the resistance and anti-infection level of chickens.

The intestinal mucosa is the first barrier that prevents the invasion of pathogens and foodborne antigens into the body. Its healthy morphological structure is a prerequisite for ensuring the digestion and absorption of nutrients and resisting the risk of diseases (Odenwald and Turner, 2017). The histomorphology of the small intestine is generally evaluated by histological determination of its villi and crypt (Liu et al., 2022b). The length of the villi in the small intestine is positively correlated with the absorption area of the small intestine. Additionally, higher villi create a larger contact area with intestinal contents, which is conducive to nutrient absorption. A smaller crypt value indicates a greater proportion of mature intestinal epithelial cells with stronger secretion and digestion functions, resulting in an enhanced capacity for nutrient absorption in the intestine (Xie et al., 2020).

In a study on the effect of supplementation with a mixture of glycerol monolaurate and oregano essential oil on broiler intestinal morphology, a linear, secondary, and tertiary highly significant increase in duodenal and ileal villus height was found in all oregano essential oil supplemented groups compared to the control group (Amer et al., 2021). The same test results were found in a study exploring the effect of essential oil/palygorskite composite on the intestinal morphology of laying hens (Cheng et al., 2022). Meanwhile, in exploring the effect of oregano essential oil on the intestinal morphology of fattening Sewa sheep, it was found that the V/C ratios in the ileum, duodenum and jejunum were significantly higher in the test group with the addition of oregano essential oil than in the control group (Sun et al., 2022). The results of this experimental study are consistent with those of previous studies. All intestinal morphometric indices were statistically significant compared to CON except jejunal villus length, crypt depth and V/C ratio, which were not significantly different among 3 test groups supplemented with TTO on d 28. In summary, TTO can well promote the growth and development of the duodenum, jejunum, and ileum of chicks, making it a potential growth promoter.

CONCLUSIONS

The addition of different doses of TTO to the diet significantly increased BW, ADG, and ADFI in chicks; reduced F/G; improved growth performance; and reduced feed loss compared to the basal diet. Serum complements, cytokine levels, and antibody levels were significantly increased in the test group, thus providing an advantage in improving immune function. Tea tree oil supplement had a significant enhancement effect on morphological development of duodenum, jejunum, and ileum. In conclusion, this study provides valuable data reference for understanding the regulation of growth performance, immune function, and intestinal morphology of Fengyan Black Bone Chickens by supplementing TTO in the basal diet. The trial results showed that the addition of 50 mg/kg TTO to the basal diet had the most significant impact in reducing breeding costs and improving overall economic efficiency.

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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.

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