Effect of The Tea Tree Oil on Meat Quality, Antioxidant Status, and Serum Biochemical Indices in Finishing Pigs

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Research

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Abstract

Background: The increase use of antibiotics continues to posse a threat to public health due to the increasing concern of antibiotic residue. Tea tree oil [TTO] is an extract of the Australian plant *Melaleuca alternifolia* with anti-inflammatory and anti-oxidant properties. However, there is little information on TTO supplementation on the diet of finishing pigs. Hence, the aim of the present study was to investigate the effect of TTO supplemented diets on serum biochemical indices, fatty acid composition, the mRNA expression of genes, and antioxidant capability in finishing pigs.

Results: Our results showed that TTO supplementation increased ($P < 0.05$) the mRNA expression of insulin-like growth factors -I (IGF-I), growth acceleration hormone [GH] and heart fatty acid binding protein (H-FABP), while the mRNA expression of myostatin gene (MSTN) and calpain-1 (CAST) decreased by TTO supplementation, compared with control group. In addition, TTO supplementation increased ($P < 0.05$) serum alkaline phosphatase (ALP), immunoglobulin G (IgG), and IgM levels but decreased ($P < 0.05$) serum aspartate transaminase (AST) concentration, relative to controls. The TTO supplementation increased ($P < 0.05$) C18:2n6t concentration and decreased ($P < 0.05$) C12:0 and C16:0 concentration, relative to control group. Dietary supplementation with TTO decreased ($P < 0.05$) malondialdehyde (MDA) and increased ($P < 0.05$) glutathione peroxidase (GSH-Px) activity in serum. Compared with control group, TTO supplementation at 200mg/kg increased ($P < 0.05$) GSH-Px activity in LD muscle.

Conclusions: These results indicated that TTO supplementation could improve serum biochemical indices, fatty acid contents, the relative mRNA of expression, and antioxidant capacity of finishing pigs. Therefore, TTO has potential positive effects as a feed additive in pigs industry.

Introduction

Antibiotics have been used in livestock to improve feed efficiency, prevent diseases, and increase animal production since the 1950s [1]. In recent decades, with improvement in living standards, consumers are increasing their demand for quality meat products, which provides a healthy balance of nutrients [2–4]. The increase use of antibiotics continues to posse an environmental risk and a threat to public health due to the increasing concern of antibiotic residue [5]. Therefore, an effective alternative to antibiotics, especially from plant, in pigs production are vital for improving meat quality and human health.

Essential oils extracted from aromatic plants are potential alternative to antibiotics [6]. Tea tree oil (TTO), an essential oil derived from the Australian plant *Melaleuca alternifolia*, contains more than one hundred different compounds, which are mainly monoterpenes and their derivatives. The main components of TTO include terpinene-4-ol, γ-terpinene, α-terpinene, 1,8-cineole, and α-terpineol [7]. Zhan et al [8] demonstrated that supplement of TTO could alleviate inflammatory response in bovine mammary epithelial cells (BMEC) exposed to *Staphylococcus aureus*. Previous studies have focused on antibacterial activity and anti-inflammatory properties [9]. In addition, TTO could activate the antioxidant signaling pathway [10], thus increasing antioxidant capacity [11] and ameliorate oxidative damage in

animals [12]. Based on TTO anti-inflammatory and antioxidant properties, numerous research on TTO have also been investigated in animals. TTO can enhance growth performance, meat quality, antioxidant status, and anti-inflammatory function in animals [13–14]. Previous research showed that TTO supplementation could improve the growth performance, promote liver and thymus development, and ameliorate intestinal mucosal immunity by activating Notch2 signaling pathway in weaning pigs [11, 15]. Since dietary lipids are associated with the emergence of coronary heart disease, the lipid content of pork is another concern for consumers. Although it has been demonstrated that a positive effect exist between essential oils and body lipid metabolism [16], however, there is very limited information regarding diets supplemented with TTO on pork fatty acids profile in finishing pigs.

Therefore, in the present study, we investigated the effects of TTO supplementation on the serum biochemical indices, fatty acid composition, the mRNA expression of genes, and antioxidant capability of finishing pigs.

Material And Methods

Animals and experimental design

A total of 64 finishing pigs (Duroc × Landrace × Yorkshire) with an average initial body weight (BW) of 68.13 ± 0.46 kg were randomly divided into 4 treatment groups. Each treatment group contained 16 pigs raised in 4 pens, each with 4 pigs (2 male and 2 female). The 4 treatment groups included the control treatment (CON, feed with only basal diets) and the low-, middle- and high-level TTO-supplemented diets (LTO, MTO and HTO, feed with the basal diets supplemented with 100 mg/kg, 200 mg/kg and 300 mg/kg TTO (net content of TTO), respectively). In addition, the feed supplemented with TTO was formulated every day to reduce TTO oxidation. The experiment was conducted in Jinzhu Agricultural Development Company, Taicang, Suzhou province, China on June 2016.

The TTO utilized was the Australian tea tree oil powder type provided by Chen Fang Biotechnology Company, Wuxi, China. The TTO was purified and processed by the company so that the constituents, such as 4-terpineol, were not completely the same as other TTOs (the details are proprietary of the company). Furthermore, TTO was absorbed in microcrystalline cellulose (the net content of the TTO is 20%).

The composition of the basal diet is illustrated in Table 1. Two samples were analyzed for the measured values, and the mean value calculated as shown in Table 1. The basal diet (Table 1) was formulated to meet or exceed the nutrient requirements recommended by NRC (1998) nutrient requirements. All pigs were fed ad libitum and had free access to water. The experiment lasted 56 days. All pigs were fed ad libitum and had free access to water. At the end of the experiment, three pen were randomly selected from each treatment, from which two pigs were selected per treatment pen, given a total of 6 pigs (3 male and 3 female) for the collection of sample data.

The mRNA expression analysis by real-time PCR

The total RNA was isolated from the liver, LD muscle, and back-fat using TRIzol (Takara, Code No. RR036A). The RT reaction mixtures contained 1 μg total RNA and 5× PrimeScript RT Master Mix in a final volume of 20 μL. The RT reactions were performed for 15 min at 37°C. Reverse transcriptase was inactivated by heating to 85°C for 5 s. qRT-PCR was performed with a SYBR® Premix Ex Taq™ II Kit (Takara, Code Nos. RR820A and RR420A). The qRT-PCR included an initial denaturation at 95°C for 30 s, followed by 40 cycles at 95°C for 5 s and 60°C for 30 s. The primers used are listed in Table 1. The relative expression of target genes was normalized to that of GAPDH and calculated using the $2^{-\Delta\Delta CT}$ method.

Serum biochemical indices

Total serum levels of immunoglobulin IgG, IgA and IgM (g/L) were measured by commercially available ELISA kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were analyzed by automatic biochemical analyzer.

Measurement of serum, liver, and LD muscle antioxidant

Approximately 0.3 g of liver and LD muscle samples were homogenized (1:9, wt/vol) with ice-cold 154 mmol/L sodium chloride solution, and centrifuged at 3000 r/min for 15 min at 4 ℃. The supernatant was then collected and stored for liver and LD muscle antioxidant analysis. The total protein concentration, malondialdehyde (MDA), total superoxide dismutase (T-SOD) activity, Catalase (CAT) activity, and glutathione peroxidase (GSH-PX) activity were measured using diagnostic kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, P. R. China) according to the manufacturer's instructions.

Fatty acid composition of longissimus muscle

Fatty acid content was determined according to GB/5413.27–2010. A 4 g thick section of longissimus muscle was removed from the center (in the region of the 10th rib) of a boneless pork loin. The sample was trimmed free of all subcutaneous fat and epimysial connective tissue. All the samples were immediately frozen at -80°C until fatty acid analysis.

Longissimus muscle were placed in 30 ml beakers, which were then placed into vacuum flasks attached to the manifold of a Labconco freeze-dryer (LyoQUEST-55, USA). After freeze-dried, 0.5 g of LD muscle was weighed and placed in 15 mg spiral glass tube. 5.0ml toluene and 6.0ml 10% acetyl chloromethanol was added and the tube filled with nitrogen to ensure total air removal within the tube. The mixture was homogenized, placed it in a water bath at 80℃ for 2 hours, and vibrated once every 20 minutes. The mixture was taken out and cooled to room temperature. The cooled liquid was transferred to a 50 mL centrifuge tube, and the centrifuge tube was cleaned with sodium carbonate solution and transferred to a 50 mL another centrifuge tube. The glass tube was shaken and mixed, centrifuged at 5000 r/min for 5min, and the supernatant was measured by meteorological chromatograph; equipped with a 100-m capillary column (0.25-mm i.d.; Model 2560 fused-silica capillary column, Supelco Inc., Bellefonte, PA) and helium as the carrier gas at 1 ml/min (1:30 split ratio). Oven temperature was maintained at 140°C

for 5 min, increased at 4°C/min to 240°C for 15min, whereas injector temperatures were maintained at 260°C and detector temperatures were maintained at 280°C.

Statistical analysis.

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test for post-hoc correction for multiple comparisons of treatment means using the SPSS 16.0 software (SPSS Inc.; Chicago, IL, USA). The *P* values are represented in the figures as follows: statistical significance was set at $P < 0.05$.

Results

Relative gene expression of growth performance of finishing pigs

The effect of TTO on the relative gene expression of growth performance is shown in Table 2. Relative to CON pigs, MTO and HTO supplementation increased ($P < 0.05$) the mRNA expression of GH and IGF-I in LD muscle, the mRNA expression of IGF-I was up-regulating ($P < 0.05$) with TTO supplementation in liver.

Immune response

The effect of TTO on the immune response is shown in Table 3. To investigate TTO immunomodulation effects were quantified in serum 28 and 56 days using immunonephelometry. On day 28, the diets supplemented with TTO increased ($P < 0.05$) IgG and IgM, but the levels of IgA did not alter ($P > 0.05$), compared with control group. On day 56, TTO supplemented diets had increased ($P < 0.05$) levels of IgG, unlike the levels of IgA and IgM which did not alter, compared to control.

Serum biochemical indexes

The effect of TTO on serum biochemical indexes of finishing pigs are illustrated in table 4. In 28 days, TTO supplementation increased ($P < 0.05$) serum ALP levels and decreased ($P < 0.05$) serum AST concentration, relative to controls. In 56 days, serum AST concentration was decreasing ($P < 0.05$) with TTO supplementation, compared with controls.

Fatty acid composition in LD muscle

The effect of TTO on fatty acid composition and content in LD muscle of finishing pigs are summarized in Table 5. The concentration of C12:0 and C16:0 was decreased ($P < 0.05$) in MTO compared with control group. Diets supplemented with TTO had increased ($P < 0.05$) C18:2n6t concentration, relative to control group.

The genes expression of H-FABP, CAPN1, CAST, and HAL in LD muscle

The effects of TTO supplementation on the genes expression of H-FABP, CAPN1, CAST, and HAL in LD muscle in finishing pigs are showed in Table 7. The mRNA expression of H-FABP and CAPN1 increased

by supplementation with 200mg/kg TTO, compared with control group.

Antioxidant status

As shown in Table 8, MTO and HTO supplementation decreased ($P < 0.05$) MDA and increased ($P < 0.05$) GSH-Px activity in serum, GSH-Px activity was increased with 200mg/kg TTO supplementation in LD muscle, compared with control groups.

Discussion

Dietary supplementation with antibiotics improve feed efficiency and prevent diseases that has been widely used in pigs production [17-19]. However, many consumer are concerned about the public health threat posed by antibiotic residues in animal products [20-21]. In addition, supplementation with antibiotic in diet has been fully or partially banned in several countries [22]. Consequently, the development of alternative, thus reducing antibiotics residues is imperative to improve meat quality and human health. Many essential oil extracted from some plants contain secondary metabolites that exhibits antioxidant, promotes growth performance, and shows anti-inflammatory roles [22-24]. TTO extract from the leaves of *M. alternifolia* exhibits anti-inflammatory, antioxidant, and anti-bacteria properties [22]. Dong et al [15] reported that TTO has the potential to replace the usage of antibiotics in weanling pigs. In our current study, dietary supplementation with TTO improve meat quality by increasing intramuscular fat content and tenderness [14]. Meat is a primary source of essential fatty acids to human health [25]. It has been shown that a positive effects exist between essential oil and lipid metabolism [16], however, there is limited information in the existing literature regarding the effect of TTO on fatty acids profile in finishing pigs. Hence, the present study investigated the effect of TTO on growth performance, serum profile, fatty acid composition, mRNA expression of genes, and antioxidant capability in finishing pigs.

Relative gene expression of growth performance of finishing pigs

GH-IGF axis components play important roles in regulating growth in finishing pigs. GH is a vital gene regulating the growth and development of pigs. Skarp et al [26] reported that GH can promote protein synthesis, while the degradation rate of protein was not affected. IGF-I can stimulate cell proliferation, differentiation, and other cellular functions in different kinds of tissues [27]. IGF-I level is a valuable tool for estimating growth rate and is positively correlated with growth rate in cattle and pigs [27]. The positive correlation between the expression of IGF-I in the liver and growth performance has been demonstrated [28]. Our results demonstrated that TTO supplementation increase the mRNA expression of GH and IGF-I in liver and LD muscle, which indicate that protein metabolism might be improved due to TTO supplementation. In addition, the IGF-I and IGF-II have similar functions, which can promote growth performance. In this study, the expression of IGF-II was not affected in the liver, LD muscle, and back fat tissues by supplementation with TTO, which is consistent with the study that IGF-II plays a vital role in embryonic development rather than in postnatal development [29]. MSTN, a member of the transforming growth factor type b (TGFβ) super-family, is a negative regulator of muscle growth [30]. This research

showed that the mRNA expression of MSTN was decreased in liver and LD muscle by TTO supplementation. The increased expression of GH and IGF-I as well as decreased expression of MSTN by supplementing with TTO may serve as another evidence to explain the improved growth performance in finishing pigs.

Serum profile

The ability of TTO supplemented diet to mount an innate immune response is well documented [8]. Serum IgA, IgG, and IgM levels shows a positive correlation with body immunity. IgG is the major immunoglobulin that can protect animals against infections by microorganisms [31]. Our result showed that supplementation with TTO increased the serum IgG and IgM concentration. Based on these research, it indicated that supplementation with TTO could enhance the immune response in finishing pigs.

The liver is a digestive gland which secretes bile, participates in the metabolism of protein, sugars, and fat and has important functions, including detoxification and provision of immunity [32]. The ALT and AST are clinically useful in evaluating acute hepatocellular injury in viral hepatitis [33-34]. The levels of ALT and AST activities are negatively correlated with the health of hepatocytes. The biologically active compounds in TTO also influence liver metabolism, as lower activity of AST was determined in blood plasma, which agrees with the present that dietary supplementation with TTO increased the liver weight, indicating improve liver health originating from TTO supplementation.

Fatty acid composition

Meat is a primary source of fat in human diets. It has been reported that saturated fatty acid (SFA) may increase cholesterol level and the risk of cardiovascular diseases [35-36]. Previous experiments conducted on TTO supplementation mainly focused on its anti-inflammatory and anti-oxidative ability. Although the essential oils have a positive effect on body lipid metabolism [16]. The concentration of fatty acid can influence the meat quality and meat products [37]. However, limited studies have been conducted to evaluate the effect of TTO supplementation on the fatty acid composition of pork. Therefore, we hypothesized that dietary supplementation with TTO could increase the polyunsaturated fatty acid (PUFA) and SFA concentration in meat. The mass consumption of SFA has been associated with increased risk of obesity and inception of other related diseases, conversely UFA, especially PUFA, reduced the risk of coronary heart disease [38]. Fatty acid composition positively correlates with meat quality by determining the nutritional value of muscle and oxidative stability. The level of fatty acid saturation can affect the degree of fat firmness, consequently influencing the quality and acceptability of meat products [37]. Therefore, appropriate levels of SFA and PUFA should be maintained to ensure superior meat quality. In the current study, diets supplemented with TTO had decreased SFA (lauric acid (C:12:0) and palmitic acid (C:16:0) and increased PUFA (mainly methyl linolelaidate (C18:2n6t) levels in meat, which confirmed our hypothesis. H-FABP is involved in intracellular targeting of fatty acids and facilitates the transport of fatty acids from the membrane to the sites of fatty acid oxidation or esterification into TAG or phospholipids [39-40]. H-FABP, first discovered in the heart, has been associated with the intramuscular fat content of pigs, meanwhile the mRNA expression of H-FABP affect

intramuscular fat content of pigs [41]. In the present study, diet supplemented with TTO increase the mRNA expression of H-FABP. It has been reported that mRNA expression of H-FABP was positively correlated with intramuscular fat content [42], which is in agreement with our results. Tenderness is another index of meat quality. The extent of protein proteolysis is the main factor to determine tenderness [43]. Calpains belong to the protease family and play a vital role in meat quality. Moreover, there is a positive association between calpain activity and meat quality [44]. It has been demonstrated that calpain 1 is mainly responsible for the degradation of myofibrillar protein in skeletal muscle [45]. The CAST act as calpain-specific endogenous inhibitor [46]. In the present study, supplementation with TTO increased the mRNA expression of CAPN1 but the expression of CAST was not affected in LD muscle.

Antioxidant status

Reactive oxygen species (ROS) are produced by cells during normal metabolism [47]. However, over-generation of ROS in pigs leads to oxidative stress, resulting in reduced immune function and decreased growth performance [48]. The antioxidant capacity of the host was evaluated by the determination of related enzymes inhibiting ROS formation, such as MAD, SOD, T-AOC, and GSH-Px. Broiler fed with essential oil in diet exhibited increased T-SOD and T-AOC levels, but down-regulation of MDA concentration has been reported [49-50]. Puvaca et al [51] reported that diet supplemented with TTO improve the anti-oxidative ability of laying hens by increasing SOD and GSH-Px activities. Supplementation of animal diets with essential oil to fed can improve the antioxidant status of the animal body and increase meat quality [52]. Consistent with previous studies, our result demonstrated that diet supplemented with TTO enhanced GSH-Px levels in serum and LD muscle and decreased MAD levels in serum. Improved oxidative status observed when TTO supplemented diets are fed might be due to its ingredients, such as α-terpinene, terpinen-4-ol, and γ-terpinene, which have enhancing antioxidant abilities which has been described in previous study [53-54]. It is hypothetical that supplementation with TTO may improve meat quality in part due to the appropriate levels of SFA and PUFA, the up- or down-regulation of genes associated with lipid and protein metabolism in the muscle, and improvement of anti-oxidation status.

Conclusions

Supplementation with TTO could improve the growth performance of finishing pigs, which was probably associated with activating the relative mRNA expression, improvement of the body immune and health, the appropriate levels of SFA and PUFA, and improvement of anti-oxidation status. Therefore, TTO has potential positive effects as a feed additive in pigs industry.

Abbreviations

TTO: Tea tree oil; BW: body weight; CON: control group; LTO: low-level TTO-supplemented treatments; MTO: middle-level TTO-supplemented treatments; HTO: hight-level TTO-supplemented treatments; IGF-I: insulin-like growth factors-I; IGF-II: growth factors-II; MSTN: myostatin; GH: growth acceleration hormone;

LD: longissimus dorsi; ALT: Alanine Transaminase; ALP: alkaline phosphatase; AST: aspartate transaminase; SFA: saturated fatty acid; PUFA: polyunsaturated fatty acid; H-FABP: heart fatty acid binding protein; CAPN1: Calpain-1; CAST: Calpastatin; HAL: Halothane; MDA: malondialdehyde; CAT: Catalase; T-SOD: total superoxide dismutase; GSH-Px: glutathione peroxidase;

Declarations

Authors' contributions

YTY performed experiment work, analyzed the data, and wrote the manuscript. FFF also performed experiment work. JMC revised the manuscript. ZK and Osmond Datsomor: Writing-Reviewing and Editing. ZXY and HYJ provide the support of TTO extract. Professor ZGQ contributes to the experimental idea.

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Authors' contributions

YTY performed experiment work, analyzed the data, and wrote the manuscript. FFF also performed experiment work. JMC revised the manuscript. ZK and Osmond Datsomor: Writing-Reviewing and Editing. ZXY and HYJ provide the support of TTO extract. Professor ZGQ contributes to the experimental idea.

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Availability of data and materials

The data generated or analyzed during this study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The experiments were approved by the Institutional Animal Care and Use Committee of Yang Zhou University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table.1 composition and nutrient levels of the basal diets

Ingredient g/kg	Content
Corn	670
Soybean meal	250
Wheat bran	40
Pre-mixture[a]	40
Total	1000
Nutrition levels, g/kg	
Digestible energy, MJ/kg	13.36
CP[b]	162.5
Ca[b]	5.8
Total P[b]	3.8
Lys	8.6
Met	2.0
Thr	6.5
Trp	2.8

[a] The premix provided the following per kg of the diet: Fe (as ferrous sulfate) 80 mg, Cu (as copper sulfate) 15 mg, Zn (as zinc sulfate) 80 mg, Mn (as manganese sulfate) 5 mg, Se (as sodium selenite) 0.1 mg, I (as potassium iodide) 0.1 mg, VA 4 480 IU, VD3 500 IU, VE 20 IU, VK_3 2.20 mg, VB_1 1.80 mg, VB 2 2.20 mg, VB 6 1.50 mg, VB12 12 μg, folic acid 0.30 mg, biotin 0.05 mg, nicotinic acid 10 mg, calcium pantothenate 8 mg.

[b] CP, crude protein; Ca, calcium; P, phosphorus; Three samples were analyzed for the analyzed values and the mean value was calculated and showed.

Table 2 Effect of TTO on the genes expression of GH, IGF-I, IGF-II, and MSTN of finishing pigs (n=6).

		Treatment[1]					
Sites	Symbol	CON	LTO	MTO	HTO	SEM	*P*-value
Liver	GH	1.06	1.02	1.52	1.13	0.09	0.21
	IGF-I	1.03^{b}	2.98^{a}	4.25^{a}	3.06^{a}	0.33	0.002
	IGF-II	1.01	0.93	0.93	0.78	0.05	0.47
	MSTN	1.02^{a}	0.69^{b}	0.73^{b}	0.70^{b}	0.47	0.03
LD muscle	GH	1.02^{b}	1.17^{ab}	1.88^{a}	1.94^{a}	0.15	0.04
	IGF-I	1.00^{b}	1.11^{b}	1.72^{a}	1.24^{b}	0.07	<0.001
	IGF-II	1.02	1.29	1.03	0.98	0.66	0.36
	MSTN	1.00^{a}	0.72^{b}	0.73^{b}	0.74^{b}	0.04	0.007
Backfat	GH	1.01	1.10	1.32	1.26	0.08	0.48
	IGF-I	1.01	1.12	1.10	1.08	0.07	0.95
	IGF-II	1.01	1.04	1.20	0.96	0.07	0.70

$^{a, b}$ Means in the same row with different superscripts differ significantly for treatment effect. [1]CON (control), pigs receiving a control diet; LTO (low tea tree oil supplemented treatment), pigs receiving a control diet supplemented with 100 mg/kg tea tree oil; MTO (middle tea tree oil supplemented treatment), pigs receiving a control diet supplemented with 200mg/kg tea tree oil; HTO (high tea tree oil supplemented treatment), pigs receiving a control diet supplemented with 300 mg/kg tea tree oil; TTO, tea tree oil.

Table 3. Effects of tea tree oil on the serum immunity of finishing pigs (n=6).

		Treatment[1]					
Item		CON	LTO	MTO	HTO	SEM	
	Time /d						P value
IgA/（μg/mL）	28	104.26	165.44	151.70	120.34	9.23	0.06
	56	114.90	123.58	116.85	126.10	4.68	0.83
IgG/（μg/mL）	28	384.90^{b}	490.47^{a}	575.80^{a}	463.71^{a}	18.61	<0.001
	56	273.04^{b}	371.49^{a}	407.97^{a}	366.82^{a}	17.24	0.03
IgM/（μg/mL）	28	82.05^{b}	116.96^{a}	122.48^{a}	89.44^{b}	5.14	0.003
	56	83.07	91.18	89.31	87.62	3.4	0.87

$^{a, b}$ Means in the same row with different superscripts differ significantly for treatment effect.

[1]CON (control), pigs receiving a control diet; LTO (low tea tree oil supplemented treatment), pigs receiving a control diet supplemented with 100 mg/kg tea tree oil; MTO (middle tea tree oil supplemented treatment), pigs receiving a control diet supplemented with 200mg/kg tea tree oil; HTO (high tea tree oil supplemented treatment), pigs receiving a control diet supplemented with 300 mg/kg tea tree oil; TTO, tea tree oil.

Table 4. Effects of tea tree oil on the serum enzyme of finishing pigs (n=6).

		Treatment[1]					
Item	Time /d	CON	LTO	MTO	HTO	SEM	
							P value
ALT/（U/L）	28	54.50	51.50	47.67	47.50	1.08	0.052
	56	50.91	56.17	51.33	54.33	1.58	0.63
AST/（U/L）	28	47.00^{a}	44.17^{ab}	40.83^{bc}	36.83^{c}	1.35	0.04
	56	49.00^{a}	54.67^{a}	39.50^{b}	36.00^{b}	2.08	0.001
ALP/（U/L）	28	70.66^{b}	73.17^{b}	93.33^{a}	85.93^{a}	2.52	<0.001
	56	130.00	148.50	143.17	138.00	4.90	0.61

$^{a, b, c}$ Means in the same row with different superscripts differ significantly for treatment effect.

[1]CON (control), pigs receiving a control diet; LTO (low tea tree oil supplemented treatment), pigs receiving a control diet supplemented with 100 mg/kg tea tree oil; MTO (middle tea tree oil supplemented

treatment), pigs receiving a control diet supplemented with 200mg/kg tea tree oil; HTO (high tea tree oil supplemented treatment), pigs receiving a control diet supplemented with 300 mg/kg tea tree oil; TTO, tea tree oil.

Table 6. Effects of TTO supplementation on fatty acids composition and content in Longissimus dorsi muscle in finishing pigs. (n=6)

	Treatment[1]					
Item	CON	LTO	MTO	HTO	SEM	P value
C10:0	0.12	0.09	0.12	0.14	0.01	0.14
C12:0	0.12[a]	0.10[ab]	0.07[b]	0.13[a]	0.04	0.02
C14:0	1.08	1.06	1.12	1.13	0.02	0.46
C16:0	22.77[a]	22.06[ab]	21.83[b]	22.07[ab]	0.13	0.04
C16:1	2.90	3.06	2.61	3.02	0.07	0.08
C18:0	9.37	9.97	9.40	11.34	0.31	0.08
C18:1	41.94	41.57	41.77	38.96	0.50	0.11
C18:2n6t	3.63[b]	4.06[a]	4.08[a]	4.06[a]	0.05	<0.001
C18:2n6c	12.70	12.27	13.26	12.26	0.18	0.16
C20:1	0.53	0.44	0.43	0.80	0.06	0.10
C20:4	1.94	1.96	1.93	1.92	0.10	0.99
C22:4	0.49	0.35	0.39	0.45	0.03	0.54
SFA[2]	33.47	33.29	32.54	34.81	0.31	0.06
MUFA[3]	45.37	45.07	44.82	42.78	0.47	0.20
PUFA[4]	18.77	18.64	19.66	18.70	0.18	0.17
PUFA/SFA	0.56[b]	0.56[b]	0.60[a]	0.54[b]	0.01	0.003

[a, b] Means in the same row with different superscripts differ significantly for treatment effect.

[1]CON (control), pigs receiving a control diet; LTO (low tea tree oil supplemented treatment), pigs receiving a control diet supplemented with 100 mg/kg tea tree oil; MTO (middle tea tree oil supplemented treatment), pigs receiving a control diet supplemented with 200mg/kg tea tree oil; HTO (high tea tree oil supplemented treatment), pigs receiving a control diet supplemented with 300 mg/kg tea tree oil; TTO, tea tree oil.

[2]SFA = C10:0+C12:0+C14:0 + C16:0 + C17:0 + C18:0;

[3]MUFA = C16:1 + C18:1+ C20:1;

[4]PUFA = C18:2n6t + C18:2n6c + C20:4 + C22:4;

Table 7. Effects of TTO supplementation on the genes expression of H-FABP, CAPN1, CAST, and HAL in Longissimus dorsi muscle in finishing pigs. (n=6)

	Treatment[1]					
Symbol	Control	LTO	MTO	HTO	SEM	*P*-value
H-FABP	1.00^{b}	1.08^{b}	1.37^{a}	0.60^{c}	0.06	<0.001
CAPN1	1.00^{b}	0.96^{b}	1.29^{a}	1.34^{a}	0.05	0.001
CAST	1.01	1.01	1.10	1.20	0.09	0.89
HAL	1.00	1.09	1.14	1.06	0.09	0.96

[a, b, c] Means in the same row with different superscripts differ significantly for treatment effect.

[1] CON(control), pigs receiving a control diet; LTO (low tea tree oil supplemented treatment), pigs receiving a control diet supplemented with 100 mg/kg tea tree oil; MTO (middle tea tree oil supplemented treatment), pigs receiving a control diet supplemented with 200mg/kg tea tree oil; HTO (high tea tree oil supplemented treatment), pigs receiving a control diet supplemented with 300 mg/kg tea tree oil; TTO, tea tree oil.

Table 8. Effects of tea tree oil supplementation on antioxidant activity in finishing pigs (n=6).

		Treatment[1]					
Sites	Symbol	CON	LTO	MTO	HTO	SEM	*P*-value
Serum	MDA(nmol/ml)	2.97[a]	3.08[a]	1.99[b]	1.93[b]	0.17	0.014
	CAT(U/ml)	5.68	4.76	5.68	5.04	0.17	0.13
	T-SOD(U/ml)	114.52	114.84	120.52	126.57	4.02	0.71
	GSH-PX(U/ml)	879.02[b]	889.69[b]	963.49[a]	966.73[a]	10.83	<0.001
Liver	MDA(nmol/mg prot)	0.52	0.63	0.45	0.60	0.03	0.36
	CAT(U/mg prot)	40.39	49.53	54.08	40.38	3.2	0.37
	T-SOD(U/ mg prot)	136.41	136.76	146.51	149.15	7.82	0.92
	GSH-PX(U/mg prot)	239.95	239.90	244.12	232.23	6.06	0.92
LD muscle	MDA(nmol/mg prot)	0.32	0.42	0.35	0.31	0.01	0.09
	CAT(U/mg prot)	0.93	0.90	1.66	1.16	0.15	0.28
	T-SOD(U/mg prot)	12.36	16.47	15.26	13.94	0.83	0.33
	GSH-PX(U/mg prot)	11.85[b]	13.19[ab]	17.80[a]	10.45[b]	0.96	0.03

[a, b] Means in the same row with different superscripts differ significantly for treatment effect.

[1]CON (control), pigs receiving a control diet; LTO (low tea tree oil supplemented treatment), pigs receiving a control diet supplemented with 100 mg/kg tea tree oil; MTO (middle tea tree oil supplemented treatment), pigs receiving a control diet supplemented with 200mg/kg tea tree oil; HTO (high tea tree oil supplemented treatment), pigs receiving a control diet supplemented with 300 mg/kg tea tree oil; TTO, tea tree oil.

MDA = malondialdehyde; CAT = Catalase; T-SOD = total superoxide dismutase; GSH-Px = glutathione peroxidase.