Effects of Tea Tree Oil on Antioxidant Indices in Serum, Liver, and Intestinal Mucosa of Weaned Piglets: Postprint

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Date: 2018-12-24T00:00:00+00:00

Abstract

This study aimed to investigate the effects of tea tree oil on antioxidant indices in serum, liver, and intestinal mucosa of weaned piglets. A total of 120 healthy three-way crossbred weaned piglets (Duroc × Landrace × Large White) with similar body weight [(6.73±0.12) kg] at 21 days of age were selected and randomly allocated into 5 groups, with 6 replicates per group and 4 piglets per replicate. The five groups were: control group (CON group, fed basal diet), antibiotic group [ANT group, fed basal diet + 200 mg/kg colistin sulfate (10%) + 75 mg/kg chlortetracycline (15%)], low tea tree oil group (LTO group, fed basal diet + 50 mg/kg tea tree oil), medium tea tree oil group (MTO group, fed basal diet + 100 mg/kg tea tree oil), and high tea tree oil group (HTO group, fed basal diet + 150 mg/kg tea tree oil). The experimental period lasted 21 days. The results showed: 1) Serum total antioxidant capacity (T-AOC) in the HTO group was significantly higher than that in the LTO and MTO groups ($P<0.05$), serum superoxide dismutase (SOD) activity in the MTO group was significantly higher than that in the HTO, CON, and ANT groups ($P<0.05$), and serum hydrogen peroxide (H2O2) content in the LTO group was significantly lower than that in the MTO, HTO, and CON groups ($P<0.05$). 2) Compared with the CON and ANT groups, liver T-AOC in the LTO, MTO, and HTO groups was significantly increased ($P<0.05$), liver glutathione peroxidase (GSH-Px) activity and H2O2 content in the LTO and MTO groups were significantly increased ($P<0.05$), and liver reduced glutathione (GSH) content in the HTO group was significantly increased ($P<0.05$); compared with the CON group, liver SOD activity in the LTO and MTO groups was significantly increased ($P<0.05$). 3) Jejunal mucosal GSH-Px and SOD activities in the HTO group were significantly higher than those in the ANT group ($P<0.05$), and jejunal mucosal H2O2 content in the LTO, MTO, HTO, and ANT groups was significantly lower than that in the CON group ($P<0.05$). 4) Ileal mucosal

SOD activity and GSH content in the HTO group were significantly higher than those in the CON group (P<0.05), and ileal mucosal malondialdehyde (MDA) content in the LTO, MTO, HTO, and ANT groups was significantly lower than that in the CON group (P<0.05). In summary, tea tree oil can enhance antioxidant enzyme activities in serum, liver, and intestinal mucosa of weaned piglets, reduce H2O2 content in serum and jejunal mucosa, thereby improving the overall antioxidant function of weaned piglets, with overall effects superior to antibiotics, and the recommended supplementation level is 100 mg/kg.

Full Text

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Abstract

This experiment was conducted to investigate the effects of tea tree oil on antioxidant indices in serum, liver, and intestinal mucosa of weaned piglets. One hundred and twenty healthy "Duroc × Landrace × Yorkshire" crossbred piglets, 21 days old with similar body weight [(6.73±0.12) kg], were selected and randomly assigned to five groups with six replicates per group and four piglets per replicate. The five groups were: control group (CON, fed a basal diet), antibiotic group [ANT, fed the basal diet + 200 mg/kg colistin sulfate (10%) + 75 mg/kg chlortetracycline (15%)], low tea tree oil group (LTO, fed the basal diet + 50 mg/kg tea tree oil), medium tea tree oil group (MTO, fed the basal diet + 100 mg/kg tea tree oil), and high tea tree oil group (HTO, fed the basal diet + 150 mg/kg tea tree oil). The experimental period lasted 21 days.

The results showed: (1) Serum total antioxidant capacity (T-AOC) in the HTO group was significantly higher than in the LTO and MTO groups (P<0.05), serum superoxide dismutase (SOD) activity in the MTO group was significantly higher than in the HTO, CON, and ANT groups (P<0.05), and serum hydrogen peroxide (H O) content in the LTO group was significantly lower than in the MTO, HTO, and CON groups (P<0.05). (2) Compared with the CON and ANT groups, liver T-AOC in the LTO, MTO, and HTO groups was significantly increased (P<0.05), liver glutathione peroxidase (GSH-Px) activity and H O content in the LTO and MTO groups were significantly increased (P<0.05), and liver reduced glutathione (GSH) content in the HTO group was significantly increased (P<0.05). Compared with the CON group, liver SOD activity in the LTO and MTO groups was significantly increased (P<0.05). (3) Jejunum mucosa GSH-Px and SOD activities in the HTO group were significantly higher

than in the ANT group (P<0.05), and jejunum mucosa H O content in the LTO, MTO, HTO, and ANT groups was significantly lower than in the CON group (P<0.05). (4) Ileum mucosa SOD activity and GSH content in the HTO group were significantly higher than in the CON group (P<0.05), and ileum mucosa malondialdehyde (MDA) content in the LTO, MTO, HTO, and ANT groups was significantly lower than in the CON group (P<0.05).

In conclusion, dietary tea tree oil supplementation can improve antioxidant enzyme activities in serum, liver, and intestinal mucosa, reduce H O content in serum and jejunum mucosa, and thereby enhance the overall antioxidant function of weaned piglets. The overall effect was superior to antibiotics, with a recommended supplementation level of 100 mg/kg.

Keywords: tea tree oil; antibiotics; weaned piglets; antioxidant enzymes; hydrogen peroxide

Introduction

Weaning stress in piglets leads to diarrhea, growth retardation, and immune stress, while simultaneously causing substantial oxidative stress that disrupts the dynamic balance of free radicals in the body. Reactive oxygen species (ROS) produced during oxidative stress, primarily including superoxide anions (O), hydroxyl ions (OH), and hydrogen peroxide (H O), are major causes of DNA damage and can disrupt the balance of cell proliferation, apoptosis, and death. ROS can also attack amino acids such as proline, arginine, lysine, and threonine, inducing protein inactivation, and attack biological membranes, causing lipid peroxidation. Oxidative stress from weaning is not only associated with DNA damage, protein damage, and lipid peroxidation but is also closely related to immune function, as ROS can induce inflammatory diseases such as enteritis, diabetes, and atherosclerosis. Furthermore, oxidative stress can damage intestinal mucosal barrier function and cause oxidative damage to tissues and organs such as the liver.

In recent years, nutritional regulation has become a research hotspot for alleviating weaning stress in piglets. Tea tree oil (TTO) is an aromatic essential oil distilled from fresh branches and leaves of *Melaleuca alternifolia* (family Myrtaceae, genus *Melaleuca*). It possesses broad-spectrum antimicrobial, antitumor, anxiolytic, and immune-enhancing properties. Our previous research demonstrated that dietary supplementation with appropriate amounts of tea tree oil could promote growth, reduce diarrhea, enhance liver and thymus development, and regulate lipid metabolism in weaned piglets, with 100 mg/kg showing optimal effects and potential as an antibiotic alternative. Tea tree oil also exhibits positive antioxidant effects, as studies have shown that certain components can bind with free radicals to scavenge them, thereby slowing aging and treating diseases. Building on our previous work, this study further investigated the effects of tea tree oil on antioxidant indices in serum, liver, and intestinal mucosa of weaned

piglets compared with antibiotics, providing a theoretical basis for developing new non-polluting feed additives.

Materials and Methods

1.1 Experimental Materials

The tea tree oil used was a powdered feed additive provided by Wuxi Chenfang Biotechnology Co., Ltd., with main components: terpinen-4-ol >60%, *p*-cymene 5%-10%, cineole 2%-10%, -terpineol >3%, -terpinene <10%, and -pinene <0.5%.

1.2 Experimental Design and Management

One hundred and twenty healthy "Duroc × Landrace × Yorkshire" crossbred piglets, 21 days old with similar body weight [(6.73±0.12) kg], were selected and randomly assigned to five groups using a single-factor design, with six replicates per group and four piglets per replicate (half male and half female). The groups were: control group (CON, fed a basal diet), antibiotic group [ANT, fed the basal diet + 200 mg/kg colistin sulfate (10%) + 75 mg/kg chlortetracycline (15%)], low tea tree oil group (LTO, fed the basal diet + 50 mg/kg tea tree oil), medium tea tree oil group (MTO, fed the basal diet + 100 mg/kg tea tree oil), and high tea tree oil group (HTO, fed the basal diet + 150 mg/kg tea tree oil). The experiment lasted 21 days. The basal diet was formulated according to NRC (2012) nutrient requirements for piglets and production practices, with composition and nutrient levels shown in Table 1 .

All experimental pigs were housed in the same barn, with each replicate separated in identical pens under consistent management conditions. No immunizations were administered during the trial, and no mortality occurred. The experiment was conducted in June 2016 at Taicang Jinzhu Pig Farm. At that time, the Ministry of Agriculture had not yet issued regulations prohibiting colistin sulfate as a feed additive. This study aimed to investigate feed additives that could replace antibiotics such as colistin sulfate, hence the inclusion of an antibiotic group. All pigs fed antibiotics were used solely for research purposes.

1.3 Detection Indicators

Twenty-four hours before the end of the experiment, blood samples were collected after fasting. On the final day, one piglet per replicate was weighed and slaughtered to collect blood for serum separation, liver tissue, and intestinal mucosa from jejunum and ileum after rinsing intestinal contents. Antioxidant indices in serum, liver, jejunum mucosa, and ileum mucosa were measured.

For serum antioxidant indices, on day 21, one piglet per replicate (six per group, half male and half female) was selected for 10 mL blood collection from the anterior vena cava. After standing for 15 minutes, serum was obtained by centrifugation (3,000 r/min, 10 min), aliquoted, and stored at -20°C. Serum

total antioxidant capacity (T-AOC), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT) activities, and malondialdehyde (MDA), reduced glutathione (GSH), and H O contents were measured using kits from Nanjing Jiancheng Bioengineering Institute according to manufacturer instructions.

For liver antioxidant indices, liver samples were homogenized with physiological saline at 1:9 ratio (mass/volume), centrifuged (3,000 r/min, 10 min), and the supernatant was used to measure liver T-AOC, SOD, GSH-Px, CAT activities, and GSH, H O , MDA contents using the same kits.

For intestinal mucosa antioxidant indices, after measuring and weighing jejunum and ileum segments, intestinal contents were removed by rinsing with physiological saline. The segments were opened, surface moisture and impurities were absorbed with filter paper, and mucosa was scraped with a glass slide and stored at -80°C. Samples were homogenized with physiological saline at 1:9 ratio, centrifuged (3,000 r/min, 10 min), and the supernatant was used to measure T-AOC, SOD, GSH-Px, CAT activities, and GSH, H O , MDA contents in jejunum and ileum mucosa using the same kits.

1.4 Statistical Analysis

Experimental data were initially processed using Excel 2013, then analyzed by one-way ANOVA using SPSS 19.0 software. Duncan' s multiple comparison test was used for inter-group comparisons. Results are expressed as means, with $P<0.05$ indicating significant differences.

Results

2.1 Effects of Tea Tree Oil on Serum Antioxidant Indices of Weaned Piglets

As shown in Table 2 , serum T-AOC in the HTO group was significantly higher than in the LTO and MTO groups ($P<0.05$). Serum SOD activity in the MTO group was significantly higher than in the HTO, CON, and ANT groups ($P<0.05$). Serum H O content in the LTO group was significantly lower than in the MTO, HTO, and CON groups ($P<0.05$).

2.2 Effects of Tea Tree Oil on Liver Antioxidant Indices of Weaned Piglets

As shown in Table 3 , liver T-AOC in the LTO group was significantly higher than in the MTO group, and both LTO and MTO groups had significantly higher liver T-AOC than the CON and ANT groups ($P<0.05$). Liver GSH-Px activity in the LTO and MTO groups was significantly higher than in the HTO, CON, and ANT groups, while HTO group activity was significantly higher than in the ANT group ($P<0.05$). Liver SOD activity in the LTO and MTO groups was significantly higher than in the CON group ($P<0.05$). Liver CAT activity in

the LTO group was significantly higher than in the HTO group, and all tea tree oil groups had significantly higher CAT activity than the CON group (P<0.05). Liver GSH content in the HTO group was significantly higher than in the CON and ANT groups (P<0.05). Liver H O content in the LTO and MTO groups was significantly higher than in the CON, ANT, and HTO groups (P<0.05).

2.3 Effects of Tea Tree Oil on Jejunum Mucosa Antioxidant Indices of Weaned Piglets

As shown in Table 4 , jejunum mucosa GSH-Px and SOD activities in the HTO group were significantly higher than in the MTO and ANT groups (P<0.05). Jejunum mucosa H O content did not differ significantly among the LTO, MTO, and HTO groups (P>0.05) but was significantly lower than in the CON group (P<0.05).

2.4 Effects of Tea Tree Oil on Ileum Mucosa Antioxidant Indices of Weaned Piglets

As shown in Table 5 , ileum mucosa SOD activity and GSH content in the HTO group were significantly higher than in the CON group (P<0.05). Ileum mucosa MDA content did not differ significantly among the LTO, MTO, and HTO groups (P>0.05) but was significantly lower than in the CON group (P<0.05).

Discussion

3.1 Effects of Tea Tree Oil on Serum Antioxidant Indices of Weaned Piglets

Weaning causes oxidative stress in piglets, and excessive free radicals can induce lipid peroxidation and protein and DNA damage, resulting in diarrhea, growth retardation, and reduced immunity. Previous studies have shown that dietary supplementation with appropriate plant essential oil extracts can improve antioxidant function in weaned piglets. The antioxidant function of plant essential oils may originate from two factors: first, phenolic hydroxyl compounds in essential oils can inhibit free radical generation, directly scavenge free radicals, and enhance the body' s antioxidant system function, blocking oxidative damage at multiple stages; second, essential oils can mobilize or activate endogenous antioxidants to reach levels and activities needed by the body, thereby preventing and reducing free radical damage. Previous research indicates that tea tree oil is a natural antioxidant that can effectively scavenge the DPPH free radical. This study found that dietary antibiotic supplementation had no significant effect on serum SOD activity in piglets, while 100 mg/kg tea tree oil significantly increased serum SOD activity, enhancing the ability to scavenge O free radicals. Dietary supplementation with 50 mg/kg tea tree oil significantly reduced serum H O content. These findings are consistent with previous research, and the main antioxidant component in tea tree oil may be -terpinene. Additionally, antibiotics reduced H O content in weaned piglet serum, suggesting they also

possess some antioxidant function. However, antibiotic resistance and environmental pollution are significant concerns, and prohibiting antibiotic use is an inevitable trend in animal husbandry development. Therefore, antibiotics are not recommended for alleviating weaning stress in piglets.

3.2 Effects of Tea Tree Oil on Liver Antioxidant Indices of Weaned Piglets

The liver is the largest glandular organ in the body and plays important roles in nutrient metabolism, bile production, detoxification, coagulation, immunity, heat production, and water-electrolyte regulation. During oxidative stress, excessive oxygen radicals can cause lipid peroxidation damage to liver cell membranes, and if occurring in endothelial cells, can increase liver capillary permeability and cause microcirculation disorders. Weaning can induce oxidative stress in piglets, damaging liver tissue and organ function and affecting nutrient metabolism. Additionally, liver immune function is affected under oxidative stress conditions. This study found that dietary tea tree oil supplementation significantly enhanced liver T-AOC in weaned piglets, with effects superior to antibiotics. Dietary supplementation with 50 and 100 mg/kg tea tree oil significantly increased liver GSH-Px and SOD activities, while 150 mg/kg tea tree oil significantly increased liver GSH content. These results demonstrate that tea tree oil can enhance the ability to eliminate O and H O in weaned piglet liver by increasing antioxidant enzyme activities, thereby improving antioxidant function. Tea tree oil has antioxidant efficacy, with antioxidant strength of its components ranked as: -terpinene > -terpinolene > -terpinene. However, no previous studies have reported on the antioxidant function of tea tree oil in the liver. Additionally, this study found that low-level tea tree oil supplementation (50 and 100 mg/kg) increased liver H O content, the reason for which is unclear and requires further investigation.

3.3 Effects of Tea Tree Oil on Small Intestinal Mucosa Antioxidant Indices of Weaned Piglets

Studies have shown that early weaning has relatively weak effects on serum antioxidant status but more readily causes oxidative damage to the piglet intestine. Oxidative stress in weaned piglets primarily manifests as intestinal mucosal damage and reduced barrier function, leading to severe diarrhea and decreased nutrient digestibility. This study found that dietary tea tree oil and antibiotic supplementation significantly reduced jejunum mucosa H O content and ileum mucosa MDA content in weaned piglets. Dietary supplementation with 150 mg/kg tea tree oil resulted in significantly higher ileum mucosa SOD activity and GSH content compared with the CON group, and significantly higher jejunum mucosa GSH-Px and SOD activities compared with the ANT group. These results indicate that dietary tea tree oil and antibiotics can both reduce jejunum H O content and alleviate ileum lipid peroxidation in weaned piglets, with 150 mg/kg tea tree oil showing stronger free radical scavenging

ability in jejunum mucosa than antibiotics and significantly enhancing free radical scavenging capacity in ileum mucosa. These findings are consistent with our previous research showing that dietary tea tree oil supplementation can promote growth and reduce diarrhea in weaned piglets, possibly related to improved intestinal mucosal antioxidant function. Other studies have shown that tea tree oil can inhibit mucosal inflammation; placing two drops on a handkerchief and holding it under the nose for 5 minutes can clear nasal congestion, suggesting improved mucosal antioxidant and immune function. Currently, tea tree oil is mainly used externally for skin care, anti-inflammatory and antibacterial purposes, or as a food additive for flavoring. However, its application as a feed additive in livestock production and its effects on intestinal mucosal antioxidant function have not been reported. Tea tree oil originates from Australia, and large-scale cultivation and processing have begun in Chinese provinces such as Guangdong, Guangxi, and Yunnan, with annual production reaching dozens of tons in Guangxi alone. In the future, tea tree oil may be applied as a feed additive in pig production to improve antioxidant stress capacity, reduce diarrhea, and alleviate weaning stress.

Conclusion

Dietary supplementation with tea tree oil can improve antioxidant enzyme activities in serum, liver, and small intestinal mucosa, reduce H O content in serum and jejunum mucosa, and thereby enhance the overall antioxidant function of weaned piglets, with a recommended supplementation level of 100 mg/kg.

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Note: Figure translations are in progress. See original paper for figures.