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 experiment 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 Duroc × Landrace × Yorkshire crossbred weaned piglets with similar body weight [(6.73±0.12) kg] at 21 days of age were selected and randomly divided 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, hepatic T-AOC in the LTO, MTO, and HTO groups was significantly increased ($P<0.05$), hepatic glutathione peroxidase (GSH-Px) activity and H2O2 content in the LTO and MTO groups were significantly increased ($P<0.05$), and hepatic reduced glutathione (GSH) content in the HTO group was significantly increased ($P<0.05$); compared with the CON group, hepatic 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 conclusion, 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 (TTO) on antioxidant indices in the serum, liver, and intestinal mucosa of weaned piglets. A total of 120 healthy 21-day-old "Duroc × Landrace × Yorkshire" crossbred weaned piglets with similar body weight [(6.73±0.12) kg] were selected and randomly assigned to 5 groups, with 6 replicates per group and 4 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) The 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). Serum hydrogen peroxide (H O) 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 was significantly increased in the LTO, MTO, and HTO groups (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). Liver reduced glutathione (GSH) content in the HTO group was significantly increased (P<0.05). Compared with the CON group, liver SOD activity was significantly increased in the LTO and MTO groups (P<0.05). 3) Jejunum mucosal GSH-Px and SOD activities in the HTO group were significantly higher than those in the ANT group (P<0.05). Jejunum mucosal H O content in the

LTO, MTO, HTO, and ANT groups was significantly lower than that in the CON group (P<0.05). 4) Ileum mucosal SOD activity and GSH content in the HTO group were significantly higher than those in the CON group (P<0.05). Ileum 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 conclusion, dietary supplementation with tea tree oil 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 that of 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. Simultaneously, weaning induces significant oxidative stress responses that disrupt the dynamic balance of free radicals in the body. Reactive oxygen species (ROS) produced during oxidative stress, primarily including oxygen ions, 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. Additionally, ROS attack biological membranes, causing lipid peroxidation of cell membranes. Oxidative stress caused by weaning in piglets is not only associated with DNA damage, protein damage, and lipid peroxidation but is also closely related to immune function. ROS can induce inflammatory diseases such as enteritis, diabetes, and atherosclerosis. Oxidative stress also damages intestinal mucosal barrier function and causes 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*). Tea tree oil exhibits broad-spectrum antimicrobial, antitumor, anxiolytic, and immune-enhancing effects. Our laboratory's 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 to some extent, thereby partially replacing antibiotics, with 100 mg/kg showing optimal effects. Tea tree oil also demonstrates positive antioxidant activity. Studies have shown that certain components in tea tree oil can bind with free radicals, scavenging them to slow aging and treat diseases. Therefore, based on our previous research, this experiment further investigated the effects of tea tree oil on antioxidant indices in serum, liver, and intestinal mucosa of weaned piglets, comparing these effects with antibiotic supplementation to provide a theoretical foundation for developing new non-polluting feed additives.

1.1 Experimental Materials

The tea tree oil used in this experiment was a powdered feed additive provided by Wuxi Chenfang Biotechnology Co., Ltd. Its main components included: 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

A total of 120 healthy 21-day-old "Duroc × Landrace × Yorkshire" crossbred weaned piglets with similar body weight [(6.73±0.12) kg] were selected and randomly assigned to 5 groups using a single-factor experimental design. Each group had 6 replicates with 4 piglets per replicate (half male and half female). 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 basal diet was formulated according to NRC (2012) nutrient requirements for piglets and practical production conditions. Its composition and nutrient levels are shown in Table 1 .

The experimental pigs from all five groups were housed in the same barn, with each replicate separated into identical pens under consistent management conditions. The pigs were not vaccinated during the trial period, and no mortality occurred. It should be noted that the experiment was conducted in June 2016 at Taicang Jinzhu Pig Farm, before the Ministry of Agriculture issued regulations prohibiting the use of colistin sulfate as a feed additive. This experiment aimed to study feed additives that could replace antibiotics such as colistin sulfate, hence the inclusion of an antibiotic-supplemented control group. All pigs fed antibiotics were used solely for research purposes.

1.3 Detection Indices

Twenty-four hours before the end of the experiment, blood samples were collected after overnight fasting. On the final day, one piglet from each replicate was weighed and slaughtered. Blood was collected for serum separation, liver samples were harvested, and intestinal contents were washed and scraped to obtain jejunum and ileum mucosa for measurement of relevant antioxidant indices in serum, liver, jejunum mucosa, and ileum mucosa.

1.3.1 Serum Antioxidant Indices Detection On day 21, one piglet from each replicate (6 piglets per group, half male and half female) was selected for blood collection (10 mL) from the anterior vena cava. After standing for 15 minutes, the blood was centrifuged (3,000 r/min, 10 min) to obtain serum, which was aliquoted and stored at -20°C for later analysis. Serum total antioxidant capacity (T-AOC), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) activities, as well as malondialdehyde (MDA), reduced glutathione (GSH), and H O contents were measured using assay kits from Nanjing Jiancheng Bioengineering Institute according to the manufacturer' s instructions.

1.3.2 Liver Antioxidant Indices Detection Liver samples were homogenized with physiological saline at a 1:9 ratio (mass/volume). After thorough mixing, the homogenate was centrifuged (3,000 r/min, 10 min) to obtain the supernatant as enzyme extract. The supernatant was used to measure liver T-AOC, SOD, GSH-Px, and CAT activities, as well as GSH, H O , and MDA contents using assay kits from Nanjing Jiancheng Bioengineering Institute according to the manufacturer' s instructions.

1.3.3 Intestinal Mucosa Antioxidant Indices Detection After measuring and weighing the jejunum and ileum segments, intestinal contents were washed out with physiological saline. The intestinal segments were opened, contents removed, and surfaces dried with absorbent paper to remove water and other impurities. The mucosa was scraped with a glass slide and stored at -80°C. Samples were homogenized with physiological saline at a 1:9 ratio (mass/volume). After thorough mixing, the homogenate was centrifuged (3,000 r/min, 10 min) to obtain the supernatant for measurement of T-AOC, SOD, GSH-Px, and CAT activities, as well as GSH, H O , and MDA contents in jejunum and ileum mucosa using assay kits from Nanjing Jiancheng Bioengineering Institute according to the manufacturer' s instructions.

1.4 Statistical Analysis

Experimental data were initially processed using Excel 2013. One-way ANOVA was performed using SPSS 19.0 software, and Duncan' s multiple range test was used for pairwise comparisons among groups. Results are expressed as means, and $P<0.05$ was considered statistically significant.

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 that in the LTO and MTO groups ($P<0.05$). Serum SOD activity in the MTO group was significantly higher than that in the HTO, CON, and ANT groups ($P<0.05$). Serum H O content in the LTO group was significantly lower than that 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 that in the MTO group ($P<0.05$), 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 that in the HTO, CON, and ANT groups ($P<0.05$), while HTO group activity was significantly higher than that in the ANT group ($P<0.05$). Liver SOD activity in the LTO and MTO groups was significantly higher than that in the CON group ($P<0.05$). Liver CAT activity in the LTO group was significantly higher than that in the HTO group ($P<0.05$), and all TTO-supplemented groups (LTO, MTO, and HTO) had significantly higher CAT activity than the CON group ($P<0.05$). Liver GSH content in the HTO group was significantly higher than that in the CON and ANT groups ($P<0.05$). Liver H O content in the LTO and MTO groups was significantly higher than that 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 mucosal GSH-Px and SOD activities in the HTO group were significantly higher than those in the MTO and ANT groups ($P<0.05$). No significant differences in jejunum mucosal H O content were observed among the LTO, MTO, and HTO groups ($P>0.05$), but all three groups had significantly lower H O content than 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 mucosal SOD activity and GSH content in the HTO group were significantly higher than those in the CON group ($P<0.05$). No significant differences in ileum mucosal MDA content were observed among the LTO, MTO, and HTO groups ($P>0.05$), but all three groups had significantly lower MDA content than 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 damage to proteins and DNA, resulting in diarrhea, growth retardation, and reduced immunity. Previous studies have shown that dietary supplementation with appropriate amounts of plant essential oil extracts can improve antioxidant function in 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 own antioxidant system function, thereby blocking oxidative damage caused by free radicals at multiple stages; second, essential oils can mobilize or activate endogenous antioxidants to reach levels required by the body, thereby preventing and reducing free radical damage. Previous research has demonstrated that tea tree oil is a natural antioxidant that can effectively scavenge the free radical DPPH. This study found that dietary antibiotic supplementation had no significant effect on serum SOD activity in piglets, whereas dietary supplementation with 100 mg/kg tea tree oil significantly increased serum SOD activity, enhancing the ability to scavenge free radicals (O). Dietary supplementation with 50 mg/kg tea tree oil significantly reduced serum H O content. These findings are generally consistent with previous research, and the main antioxidant component in tea tree oil may be -terpinene. Additionally, antibiotics reduced H O content in serum of weaned piglets, suggesting that antibiotics also possess some antioxidant function. However, the issues of antibiotic resistance and environmental pollution are prominent, and prohibiting antibiotic use is an inevitable trend in animal husbandry development. Therefore, we do not recommend adding antibiotics to weaned piglet diets to alleviate weaning stress.

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 the endothelium, 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. Furthermore, liver immune function is also 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 GSH-Px and SOD activities in the liver, while 150 mg/kg tea tree oil significantly increased liver GSH content. These results indicate that tea tree oil can enhance the liver' s ability to eliminate O and H O by increasing antioxidant enzyme activities, thereby improving the antioxidant function of weaned piglets. Tea tree oil has antioxidant efficacy, with antioxidant strength of its components ranked as: -terpinene > -terpinolene > -terpinene. However, no studies on the antioxidant function of tea tree oil in the liver have been reported to date. 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 remains 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 and utilization. This study found that dietary supplementation with tea tree oil and antibiotics significantly reduced H O content in jejunum mucosa and MDA content in ileum mucosa. Dietary supplementation with 150 mg/kg tea tree oil resulted in significantly higher ileum mucosal SOD activity and GSH content compared to the CON group, and significantly higher jejunum mucosal GSH-Px and SOD activities compared to the ANT group. These results indicate that both tea tree oil and antibiotic supplementation can reduce H O content in the jejunum and alleviate lipid peroxidation in the ileum. Supplementation with 150 mg/kg tea tree oil resulted in stronger free radical scavenging capacity in the jejunum mucosa than antibiotic supplementation and significantly enhanced free radical scavenging capacity in the ileum mucosa. These findings are consistent with our laboratory' s previous research, which showed that dietary tea tree oil supplementation could 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 2 drops of tea tree oil on a handkerchief and holding it under the nose for 5 minutes can clear nasal congestion, suggesting that tea tree oil may improve mucosal antioxidant and immune function. Currently, tea tree oil is primarily used externally for skin care and anti-inflammatory/antimicrobial purposes, or as a food additive for flavoring. However, no studies have reported its use as a feed additive in livestock production or its effects on intestinal mucosal antioxidant function. Tea tree oil originates from Australia, and currently, 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 is expected to be applied as a feed additive in pig production to improve antioxidant stress capacity, reduce diarrhea, and alleviate weaning stress in piglets.

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. The recommended supplementation level is 100 mg/kg.

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