

## Effects of Dietary Supplementation with Antibiotics and Plant Essential Oils on Sow Productive Performance, Immune Function, and Milk Composition: Postprint

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### Abstract

This study aimed to investigate the effects of dietary supplementation with tylosin tartrate (TT) and plant essential oil (PEO) on the reproductive performance, immune function, and milk composition of sows in late gestation. Twenty-four “Landrace × Large White” breeding sows of 3rd to 4th parity with similar body weight were randomly assigned to three groups: 1) control group: basal diet; 2) TT group: basal diet + 100 mg/kg TT; 3) PEO group: basal diet + 200 mg/kg PEO. Each group had 8 replicates with 1 sow per replicate. The results showed that dietary supplementation with TT and PEO could improve sow reproductive performance; compared with the control and TT groups, the stillbirth rate in the PEO group was reduced by 71.43% and 53.33% ( $P>0.05$ ), respectively, and the weaning survival rate was increased by 1.17% and 1.60% ( $P>0.05$ ), respectively; serum immunoglobulin (Ig) A content in sows from the PEO group on days 1, 14, and 21 postpartum and from the TT group on day 21 postpartum was significantly higher than that in the control group ( $P<0.05$ ); whey IgG and IgM contents in sows from the PEO group on day 14 postpartum were also significantly higher than those in the other two groups ( $P<0.05$ ), while their whey IgG content on day 21 postpartum was significantly higher than that in the control group ( $P<0.05$ ); milk fat content in sows from the PEO group on day 21 postpartum was significantly higher than that in the control group ( $P<0.05$ ). In conclusion, dietary supplementation with both TT and PEO can improve sow reproductive performance and immune function to varying degrees, but PEO demonstrates superior effects compared with TT and has the potential to replace TT in applications for gestating sows.

## Full Text

# Effects of Dietary Antibiotics and Plant Essential Oils on Production Performance, Immune Function and Milk Composition of Sows

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## Abstract

This study investigated the effects of dietary tylvalosin tartrate (TT) and plant essential oils (PEO) on production performance, immune function, and milk composition of sows during late gestation. Twenty-four Landrace × Yorkshire sows (parity 3-4) with similar body condition were randomly allocated to three groups: 1) control group fed a basal diet, 2) TT group fed the basal diet supplemented with 100 mg/kg TT, and 3) PEO group fed the basal diet supplemented with 200 mg/kg PEO. Each group comprised 8 replicates with one sow per replicate. The results demonstrated that dietary supplementation with TT and PEO improved sow production performance. Compared with the control and TT groups, the PEO group exhibited a 71.43% and 53.33% reduction in still-birth rate ( $P > 0.05$ ), respectively, and a 1.17% and 1.60% increase in weaning survival rate ( $P > 0.05$ ), respectively. Serum immunoglobulin (Ig) A concentrations in the PEO group on days 1, 14, and 21 postpartum, and in the TT group on day 21 postpartum, were significantly higher than those in the control group ( $P < 0.05$ ). The PEO group also showed significantly higher whey IgG and IgM concentrations on day 14 postpartum compared with the other two groups ( $P < 0.05$ ), and significantly elevated whey IgG content on day 21 postpartum relative to the control group ( $P < 0.05$ ). Additionally, milk fat content on day 21 postpartum was significantly higher in the PEO group than in the control group ( $P < 0.05$ ). In conclusion, dietary supplementation with both TT and PEO can improve sow production performance and immune function to varying degrees, but PEO demonstrates superior effects and shows potential as a safe alternative to TT in gestating sow diets.

**Keywords:** breeding sows; tylvalosin tartrate; plant essential oils

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## Introduction

Sow health has long been a critical factor affecting the development of the pig industry. Over the past decades, breeding sow production in China has faced nu-

merous disease threats that severely constrain reproductive potential and impact the sustainable development of pig production. The discovery and application of antibiotics have substantially mitigated these challenges. Previous reports indicate that low-dose antibiotic supplementation during the breeding period can increase litter size [1], and research by Xu et al. [2] found that dietary supplementation with tiamulin and chlortetracycline during the weaning-to-breeding and pre-to-postpartum periods effectively prevented reproductive tract infections during breeding and farrowing, thereby improving conception rates and embryo survival. However, the problems associated with extensive antibiotic use have become increasingly prominent, including bacterial resistance, drug residues, and host microbiota disruption, posing serious threats to livestock and human health [3]. In 2006, the European Union completely banned the use of antibiotic-containing drugs in livestock. Consequently, the search for safer and more effective alternatives has become one of the most urgent research priorities.

Essential oils are volatile aromatic compounds composed of terpenes, aldehydes, esters, alcohols, and other chemical constituents. Research has demonstrated their potential as antibiotic alternatives [4]. Essential oils are classified into natural and synthetic types, with natural oils derived from plant secondary metabolites [5], commonly referred to as plant essential oils (PEO). PEOs have complex compositions and exert broad biological effects, including antimicrobial, anti-inflammatory, antioxidant, anti-spoilage, anticancer, and insecticidal activities [6]. In recent years, essential oils have been widely used in medicine, cosmetics, food, and other fields. Studies have shown that adding 0.01% essential oils to weaned piglet diets significantly improves average daily gain and tumor necrosis factor- (TNF-) levels [7]. Huang et al. [8] reported that dietary oregano oil improved feed conversion ratio, daily gain, and intestinal *Lactobacillus* and *Bifidobacterium* counts while reducing *Escherichia coli* numbers and enhancing disease resistance in broilers. Michiels et al. [9] demonstrated that garlic essential oil could inhibit gastrointestinal methanogenic bacteria activity and reduce methane emissions. However, few studies have investigated the application of PEO in gestating sows. Therefore, this study aimed to evaluate the potential of dietary TT and PEO to replace antibiotics in sow production by examining their effects on production performance, immune function, and milk composition.

## Materials and Methods

### 1.1 Experimental Materials

TT (20% active content) and PEO (main active components: 4.5% thymol and 13.5% cinnamaldehyde) were provided by Chengdu Hualuo Biotechnology Co., Ltd., China Animal Husbandry Industry Co., Ltd. Analytical equipment included porcine enzyme-linked immunosorbent assay (ELISA) kits (Beijing Chenglin Biotechnology Co., Ltd.), a microplate reader (Molecular Devices, SpectraMax M2, Shanghai, China), and a milk composition analyzer (LM61-MILKYWAY-CP2, Beijing Siptek Technology Co., Ltd.).

## 1.2 Experimental Design and Diets

The experiment was conducted at the Sichuan Lanyan Group breeding farm. Twenty-four Landrace × Yorkshire sows (parity 3-4) with similar body weight were randomly assigned to three groups (8 replicates per group, one sow per replicate). The trial period spanned from day 96 of gestation to weaning. Sows were housed individually and fed either a basal diet (control group), basal diet + 100 mg/kg TT (TT group), or basal diet + 200 mg/kg PEO (PEO group). Feed was provided three times daily at 08:00, 14:00, and 20:00. From the start of the experiment to one day before farrowing, sows were limit-fed (3.5 kg/d). No feed was provided on the day of farrowing, after which daily feed allowance was increased by 1 kg per day until ad libitum intake was achieved on day 5 postpartum.

Sow diets consisted of gestation and lactation formulas formulated according to NRC (2012) nutrient requirements for gestating and lactating sows .

## 1.3 Measurements

**1.3.1 Production Performance** At birth, total piglets born, live piglets, healthy piglets, stillbirth rate, and mummy rate were recorded for each sow. Piglets were weighed before colostrum intake. Cross-fostering was performed on day 2, with individual weights and litter sizes recorded. Tail docking, teeth clipping, iron supplementation, and health treatments were conducted on day 3. Castration was performed on day 7. At weaning on day 21, all piglets were weighed, litter sizes were recorded, and weaning survival rate and average daily gain were calculated. Five sows per group were weighed on the day of farrowing and at weaning to calculate lactation weight loss. Weaning-to-estrus interval was recorded after weaning. Daily feed intake during lactation was recorded, with feed remaining weighed on the weaning day to calculate average daily feed intake.

**1.3.2 Serum Immunoglobulins** Blood samples were collected via ear vein on days 1, 14, and 21 postpartum. Two tubes of blood were collected in anti-coagulant tubes, left to stand for 0.5 h, and centrifuged at 3,500 r/min for 10 min. The supernatant was carefully collected, aliquoted into 0.5 mL tubes, and stored at -20 °C. Serum samples were thawed on ice, mixed well, and analyzed for IgA, IgG, and IgM concentrations using porcine ELISA kits according to the manufacturer' s instructions.

**1.3.3 Whey Immunoglobulins** Following blood collection, prolactin was injected via ear vein to collect milk samples (20 mL each) on days 1, 14, and 21 postpartum, which were stored at -20 °C. A 5 mL aliquot of each milk sample was centrifuged at 8,000 r/min for 10 min at 4 °C. The whey supernatant was carefully collected into 0.5 mL tubes and stored at -80 °C. Whey samples were thawed on ice, mixed well, and analyzed for IgA, IgG, and IgM concentrations using porcine ELISA kits.

**1.3.4 Milk Composition** A 10 mL frozen milk sample was slowly thawed on ice, transferred to a 20 mL beaker, and analyzed for fat, non-fat solids, protein, and lactose content using a milk composition analyzer.

#### 1.4 Statistical Analysis

Experimental data were initially processed using Excel 2010 and analyzed using one-way ANOVA in SPSS 21.0. Duncan' s multiple range test was used for post-hoc comparisons. Results are expressed as means  $\pm$  standard deviation. Differences were considered significant at  $P < 0.05$  and extremely significant at  $P < 0.01$ .

### Results

#### 2.1 Effects of TT and PEO on Sow Production Performance

As shown in Table 2 , the PEO group exhibited numerical increases in total piglets born, live piglets, and birth litter weight compared with the control and TT groups, though differences were not significant ( $P > 0.05$ ). The TT and PEO groups showed numerical improvements in healthy piglets, weaning weight, individual average daily gain, and sow average daily feed intake relative to the control group, with no significant differences among groups ( $P > 0.05$ ). Notably, the PEO group reduced stillbirth rate by 71.43% and 53.33% ( $P > 0.05$ ) and increased weaning survival rate by 1.17% and 1.60% ( $P > 0.05$ ) compared with the control and TT groups, respectively. The weaning-to-estrus interval was reduced by 0.42 and 0.29 days in the PEO group compared with the control and TT groups, respectively ( $P > 0.05$ ).

#### 2.2 Effects of TT and PEO on Serum Immunoglobulin Content

Table 3 shows that serum IgA concentrations in the PEO group on days 1, 14, and 21 postpartum were significantly higher than those in the control group ( $P < 0.05$ ), with no significant differences compared with the TT group ( $P > 0.05$ ). The TT group also exhibited significantly elevated serum IgA on day 21 postpartum compared with the control group ( $P < 0.05$ ). However, serum IgG concentrations in the control group on days 1 and 14 postpartum were significantly higher than those in the PEO and TT groups ( $P < 0.05$ ).

#### 2.3 Effects of TT and PEO on Whey Immunoglobulin Content

Table 4 indicates that whey IgA concentration in the control group on day 1 postpartum was significantly higher than in the other two groups ( $P < 0.05$ ), with no significant difference between the PEO and TT groups ( $P > 0.05$ ). The PEO group showed significantly higher whey IgG and IgM concentrations on day 14 postpartum compared with both the control and TT groups ( $P < 0.05$ ). Additionally, whey IgG concentration in the PEO group on day 21 postpartum was significantly higher than that in the control group ( $P < 0.05$ ).

## 2.4 Effects of TT and PEO on Milk Composition

Table 5 reveals no significant differences in milk composition among groups on day 1 postpartum ( $P > 0.05$ ). On day 14 postpartum, the TT group exhibited significantly higher non-fat solids, protein, and lactose content compared with the control group ( $P < 0.05$ ), though these did not differ significantly from the PEO group ( $P > 0.05$ ). The PEO group showed significantly higher milk fat content on day 21 postpartum compared with the control group ( $P < 0.05$ ).

## Discussion

Over the past decades, reproductive performance of breeding sows in China has remained relatively low. Although genetic improvement, nutritional regulation, and disease control have enhanced sow productivity, research indicates that increased litter size reduces piglet birth weight, which largely determines subsequent development. Sun et al. [11] reported that dietary chlortetracycline supplementation during breeding and lactation significantly increased litter size and live piglets while reducing lactation weight loss and slightly increasing birth weight. However, antibiotic misuse in livestock has exacerbated problems such as bacterial resistance, drug residues, and host microbiota disruption [3]. Ruan [12] and Wang et al. [13] noted that current antibiotic dosages in animal production far exceed adaptive levels, with most unabsorbed drugs excreted in feces as parent compounds or metabolites, causing substantial environmental harm. Studies show that essential oil active components can be completely absorbed through the gastric and intestinal walls and safely metabolized before excretion [14]. Furthermore, essential oil supplementation in gestating sow diets can increase litter size without significantly affecting piglet birth weight [15]. Our findings align with Zhong [16], showing that the PEO group had increased total piglets born, live piglets, and healthy piglets, along with reduced neonatal mortality (stillbirth and mummy rates).

Jin [17] reported that dietary essential oils can stimulate sow appetite and increase feed intake through taste enhancement. Research also indicates that sow feed intake substantially influences milk quality, milk yield, and weaning-to-estrus interval [18,19]. In our trial, the PEO group showed numerical improvements in average daily feed intake and weaning survival rate, along with reduced lactation weight loss and shorter weaning-to-estrus interval compared with the control group, though these differences were not significant—consistent with the findings of Zhong [16].

Serum immunoglobulins are proteins with antibody activity that specifically bind to corresponding antigens, serving as important indicators of immune status. Foroughi et al. [20] found that PEO has immunomodulatory effects. In our study, the PEO group exhibited significantly elevated serum IgA concentrations on days 1, 14, and 21 postpartum compared with the control group, corroborating the results of Li et al. [21]. However, both PEO and TT supplementation significantly reduced serum IgG concentrations on days 1 and

14 postpartum. Some studies report that dietary essential oils do not affect serum IgG and IgM concentrations [21,22], whereas Zhong et al. [23] found that 0.04% essential oil supplementation significantly increased serum IgG in breeding sows, though 0.02%, 0.04%, and 0.06% doses did not affect IgA or IgM. In our study, the control group had higher serum IgM on day 1 postpartum but the lowest values on days 14 and 21, differing from Allan et al. [15]. These discrepancies may be attributed to variations in dosage and composition of active ingredients, leading to differential effects on the organism. Additionally, research indicates that various PEOs can exert anti-inflammatory effects by inhibiting TNF- $\alpha$  production and blocking TNF- $\alpha$ -induced neutrophil adhesion [24]. PEOs have also been shown to accelerate intestinal villus epithelial cell renewal, stabilize gut microbiota, reduce pathogenic microorganisms, increase digestive mucus and bile acid production, and enhance pancreatic enzyme activity (lipase, amylase, protease), thereby improving feed digestibility and nutrient absorption [17,25]. These mechanisms may enhance sow health, reduce delayed immune responses, mitigate postpartum decreases in uterine leukocyte activity, and activate the postpartum immune system. Consequently, the increased number of live and healthy piglets, improved weaning survival rate, reduced lactation weight loss, and shortened weaning-to-estrus interval in the PEO group are likely attributable to enhanced anti-inflammatory capacity, increased feed intake, improved feed utilization, and strengthened immune function, collectively improving postpartum sow condition.

Milk is a vital and complex nutrient source for neonatal piglets, particularly colostrum. In addition to rich nutrients, colostrum contains numerous non-nutritive components that promote rapid intestinal growth in newborn piglets, such as IgA, IgG, IgM, and insulin-like growth factor-1 (IGF-1). During the first two days after birth, most colostral immune components can directly cross the intestinal wall to provide immune protection. Research shows that newborn piglets have low energy reserves and limited immune defense, making timely colostrum intake crucial [26]. Furthermore, milk composition during lactation plays a critical role in piglet survival and growth [15]. King'ori [27] reported that dietary essential oils can effectively improve milk quality and yield. Ji et al. [28] found that essential oil supplementation during gestation-lactation increased serum IGF-1 concentrations and significantly improved piglet growth rate and weaning weight. Zhong et al. [23] also demonstrated that improved milk feeding enhances offspring health and growth rate. In our study, the PEO group showed a numerical increase in piglet average daily gain and improved weaning survival rate by 1.17% and 1.60% compared with the control and TT groups, respectively. Similar reports indicate that dietary essential oils benefit piglet average daily gain [22], and Matysiak et al. [29] showed reduced piglet mortality during lactation. Additionally, essential oil supplementation has been reported to improve milk immunoglobulin content [30]. Our results demonstrate that the PEO group had significantly higher whey IgM on day 14 postpartum and whey IgG on days 14 and 21 postpartum compared with the control group. The PEO group also showed numerically higher non-fat solids, protein, and lactose

on days 1 and 14, and significantly elevated milk fat on day 21 postpartum. Zhong et al. [23] reported that 0.02% and 0.06% essential oil supplementation significantly increased lactose content on day 7 postpartum, while 0.02% and 0.04% doses increased whey IgG. However, Tan et al. [22] found that dietary essential oils did not affect milk composition or immunoglobulin content, and other studies reported increased lactose without effects on other components [29,31]. These findings suggest that dietary PEO supplementation (thymol + cinnamaldehyde) can effectively improve milk quality, particularly IgG content, thereby influencing piglet health during lactation.

## Conclusion

1. Dietary supplementation with TT and PEO can improve sow production performance and, to varying degrees, enhance immune function and milk bioactive component content.
2. Compared with TT, PEO demonstrates superior effects and can be safely applied in gestating sow diets.

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