

## Effects of Plant Essential Oils on Animal Growth and Immune Function and Their Mechanisms of Action Postprint

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### Abstract

Plant essential oils are secondary metabolites synthesized in certain plants, exhibiting antioxidant, anti-inflammatory, and antimicrobial properties. Studies have demonstrated that plant essential oils can promote animal production, enhance systemic and intestinal immune function and antioxidant capacity, and modulate the balance of intestinal microecology in animals. Therefore, this article provides a comprehensive review of the effects of plant essential oils on promoting animal growth and improving immunity, along with their underlying mechanisms.

### Full Text

## Effects of Essential Oils on Animal Growth and Immunity and Their Mechanisms of Action

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**Abstract:** Essential oils are secondary metabolites produced by certain plants that possess antioxidant, anti-inflammatory, and antimicrobial properties. Research has demonstrated that essential oils can promote animal production, enhance systemic and intestinal immune function, improve antioxidant capacity, and regulate intestinal microecological balance. Therefore, this article reviews the effects of essential oils on promoting animal growth and enhancing immunity, along with their underlying mechanisms.

**Keywords:** essential oil; animal; growth; immunity; influence; mechanism of action

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In intensive animal production, livestock are vulnerable to various stressors, including pathogenic microorganisms and antinutritional factors in feed, which trigger stress responses leading to oxidative damage in the digestive tract, inflammatory responses, and intestinal diseases such as diarrhea, ultimately resulting in growth retardation or even death. Therefore, enhancing antioxidant function, intestinal health, and immunity is crucial for ensuring healthy animal growth. Essential oils (EO) are plant secondary metabolites synthesized in various plant organs with characteristic volatility. Recent studies have revealed that essential oils possess antioxidant, anti-inflammatory, and growth-promoting properties. Dietary supplementation with essential oils has been shown to improve antioxidant capacity in mouse kidneys and broiler chicken jejunum, enhance immune function in rainbow trout (*Oncorhynchus mykiss*), reduce diarrhea in piglets, and promote animal growth. This article reviews the digestion, absorption, and metabolism of essential oils in animals and summarizes their mechanisms for promoting growth and enhancing immunity.

## 1. Types, Absorption, and Metabolism of Essential Oils

### 1.1 Types

Essential oils are important plant secondary metabolites with small molecular weights, distinctive aromatic odors, oily liquid consistency at room temperature, and volatility. Over 3,000 essential oils are known, with complex compositions typically comprising dozens of compounds. They primarily consist of four basic components: terpenes, aromatic derivatives, aliphatic compounds, and nitrogen/sulfur-containing compounds. Several major essential oils currently under investigation are presented in Table 1 .

### 1.2 Absorption Pathways and Metabolites

Limited studies have shown that essential oils are rapidly absorbed through both the gastrointestinal tract and skin, metabolized quickly, and excreted primarily via urine within 24 hours through renal metabolism. Research indicates that plasma levels of thymol, carvacrol, and eugenol in piglets, as well as liver and kidney concentrations of *d*-limonene in rats, peak within 2 hours of consuming essential oil-supplemented diets. Further studies demonstrate that dietary thymol and carvacrol are mainly absorbed in the anterior small intestine of weaned piglets. Although essential oils are rapidly absorbed in the anterior gastrointestinal tract and quickly eliminated, microencapsulated carvacrol shows less than 20% degradation in simulated gastric fluid within 2 hours, while release exceeds 60% in simulated intestinal fluid within 2 hours and is nearly complete within 6 hours. These results suggest that while free essential oils are quickly

absorbed in the anterior gastrointestinal tract, encapsulated forms release in the posterior tract. Additionally, essential oils are rapidly absorbed through mammalian skin. Studies in humans and rodents show that plasma essential oil concentrations peak 10 minutes after dermal application, with absorption amounts dependent on essential oil concentration, skin characteristics, contact duration, and contact area.

Different essential oils exhibit varying metabolite profiles across animal species (Table 2 ). In rats, thymol and carvacrol are metabolized and excreted as sulfates, glucuronides, or in their unaltered form. In rabbits and humans, thymol metabolites appear as sulfates or thymohydroquinone. Trans-cinnamaldehyde metabolizes to hippuric acid and mercapturic acid in mice, while anethole in rats and mice is conjugated with sulfate, glucuronic acid, glycine, and glutathione.

## 2. Effects on Animal Growth

### 2.1 Growth Promotion

Research demonstrates that essential oils such as thymol and carvacrol improve body weight gain (BWG) and specific growth rate (SGR) in rainbow trout (*Oncorhynchus mykiss*), channel catfish (*Ictalurus punctatus*), and Pacific white shrimp (*Litopenaeus vannamei*), while increasing average daily weight gain in piglets and broilers and improving feed utilization in rainbow trout, channel catfish, broilers, and piglets. The growth-promoting effects of essential oils may relate to enhanced feed intake and feed efficiency. Studies show that dietary supplementation with thymol and cinnamaldehyde increases feed intake in yellowtail tetra (*Astyanax altiparanae*) and piglets. While essential oils are generally considered to improve feed palatability through their aromatic properties, research in piglets indicates no clear preference for thymol-supplemented diets, with preference actually decreasing at thymol concentrations of 1,250 or 2,000 mg/kg. Therefore, whether essential oils consistently improve feed palatability requires further investigation.

### 2.2 Mechanisms of Growth Promotion

Essential oils likely promote animal growth by enhancing digestive and absorptive capacity. Animal growth is closely related to digestive capability, which can be reflected by digestive enzyme activity. Studies show that appropriate dietary levels of thymol and cinnamaldehyde increase intestinal trypsin and amylase activities in broilers. Intestinal growth and development are closely linked to digestive capacity. Research demonstrates that dietary oregano and citrus essential oils increase intestinal villus height in broilers while reducing villus shedding in Pacific white shrimp. These findings suggest that essential oils may enhance digestive and absorptive capacity by promoting intestinal growth and development. Tiihonen et al. found that dietary thymol and cinnamaldehyde increase the relative content of butyric acid in broiler ceca, while Tian et al. demonstrated that butyric acid can improve intestinal digestive enzyme activ-

ity and promote villus growth in grass carp. These results indicate that dietary essential oils may promote intestinal development by increasing butyric acid content, thereby enhancing digestive and absorptive capacity and ultimately promoting growth.

The intestinal microecosystem is a critical factor affecting digestive and absorptive capacity, with balanced intestinal microbiota contributing to improved animal digestion and absorption. Studies show that appropriate dietary levels of thymol and cinnamaldehyde increase intestinal *Lactobacillus* populations while decreasing *Escherichia coli* in piglets and chickens, while oregano and garlic essential oils reduce *Clostridium* and *Streptococcus* populations in broilers. These results demonstrate that essential oils can improve intestinal microbiota balance by promoting beneficial bacteria and inhibiting harmful bacteria, thereby ensuring optimal digestive and absorptive function.

In summary, essential oils may enhance digestive and absorptive capacity and promote animal growth by increasing intestinal digestive enzyme activity, promoting intestinal development, and maintaining intestinal microbiota balance.

### 3. Effects on Animal Immunity

#### 3.1 Immunomodulatory Effects

Animal growth is influenced by overall health status, with post-challenge survival rates reflecting disease resistance capacity. Research shows that dietary oregano essential oil reduces mortality in channel catfish challenged with *Aeromonas hydrophila*, indicating that essential oils can enhance disease resistance. Disease resistance is closely related to immune function, with immune substances such as complement, immunoglobulins, and cytokines playing crucial roles in immune responses and serving as indicators of immune status. Studies demonstrate that dietary essential oils increase total complement content and lysozyme activity in rainbow trout serum, while elevating immunoglobulin A (IgA), immunoglobulin G (IgG), complement 3 (C3), and complement 4 (C4) levels in piglet serum. These results confirm that essential oils can enhance animal immunity and promote overall health.

#### 3.2 Mechanisms of Immune Enhancement

**3.2.1 Immunomodulation Through Anti-Inflammatory Effects** Inflammatory responses are important immune reactions, but excessive inflammation can compromise animal health. Cytokines are the primary mediators of inflammation, including pro-inflammatory cytokines [such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ), and interleukin-6 (IL-6)] and anti-inflammatory cytokines [such as interleukin-10 (IL-10)], which are mainly secreted by immune cells. Research shows that dietary carvacrol and other essential oils alleviate intestinal inflammation in broilers and mice and reduce diarrhea indices in weaned piglets. Further studies reveal that thymol and cinnamic

acid can decrease TNF- $\alpha$  and IL-6 production in mouse mammary epithelial cells and mononuclear macrophages, respectively, while *Artemisia fukudo* essential oil reduces gene expression of IL-1 $\beta$ , TNF- $\alpha$ , and IL-6 in mouse macrophages. These findings indicate that essential oils can alleviate inflammatory responses by reducing pro-inflammatory cytokine levels, thereby promoting animal health.

Nuclear factor- $\kappa$ B (NF- $\kappa$ B) is a critical transcription factor regulating cytokine and other inflammation-related gene expression. Studies show that thymol and bergamot essential oil reduce NF- $\kappa$ B p65 phosphorylation levels in mouse mammary epithelial cells and macrophages, respectively, and can decrease pro-inflammatory cytokine production by inhibiting NF- $\kappa$ B activation. Under normal physiological conditions, NF- $\kappa$ B remains bound to its inhibitor (I $\kappa$ B) in the cytoplasm; however, upon stimulation, I $\kappa$ B undergoes phosphorylation and degradation, leading to NF- $\kappa$ B p65 phosphorylation and subsequent regulation of target gene expression. In HeLa cells, I $\kappa$ B kinase (IKK) activation phosphorylates I $\kappa$ B $\alpha$ , causing its dissociation from NF- $\kappa$ B and converting NF- $\kappa$ B to its active state. Research demonstrates that thymol reduces I $\kappa$ B $\alpha$  phosphorylation in mouse mammary epithelial cells and macrophages, while citral decreases IKK protein expression in mouse mononuclear macrophages. These findings suggest that essential oils may regulate inflammatory responses through the IKK/I $\kappa$ B $\alpha$ /NF- $\kappa$ B p65 signaling pathway, though specific mechanisms require further investigation.

Additionally, the mitogen-activated protein kinase (MAPK) family, comprising extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAPK, regulates cytokine production during inflammatory responses. Studies show that thymol reduces JNK, ERK, and p38 phosphorylation levels in mouse mammary epithelial cells during inflammation, while cinnamaldehyde decreases phosphorylation of these kinases in mouse macrophages, and bergamot essential oil reduces ERK and JNK phosphorylation in LPS-stimulated mouse macrophages. These results indicate that essential oils may also regulate pro-inflammatory cytokine production through MAPK signaling pathways, thereby alleviating inflammatory responses and enhancing immunity, though more systematic research is needed.

**3.2.2 Immunomodulation Through Antioxidant Function** Immune cell membranes contain abundant unsaturated fatty acids, making them highly sensitive to oxidative stress. Moreover, immune processes generate substantial reactive oxygen species (ROS), which can impair immune cell function and compromise overall immune function when present in excess. Malondialdehyde (MDA), an end product of lipid peroxidation, reflects the degree of oxidative damage, while increased non-enzymatic antioxidant content and antioxidant enzyme activity indicate enhanced antioxidant capacity. Studies show that thymol and carvacrol supplementation reduces MDA content in rainbow trout muscle and thiobarbituric acid reactive substances in broiler liver, thereby decreasing oxidative damage. These compounds also increase catalase (CAT) and glutathione

S-transferase (GST) activities in rainbow trout serum and muscle, elevate superoxide dismutase (SOD) and CAT activities in channel catfish serum, and enhance SOD, glutathione peroxidase (GPx), and GST activities in rat kidney, collectively strengthening enzymatic antioxidant capacity. However, Gowder et al. found that essential oils reduced non-enzymatic antioxidants (vitamins C and E) and CAT activity in rat kidney. This discrepancy may arise because the antioxidant activity of essential oils primarily depends on their abundant phenolic compounds, whose phenolic hydroxyl groups serve as hydrogen donors during the initial step of lipid peroxidation, thereby slowing hydroperoxide formation. Antioxidant enzyme activity is closely related to gene expression, which is regulated by the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling molecule. Studies show that *Schisandra* essential oil upregulates Nrf2 protein expression in muscle cells, suggesting that essential oils may regulate antioxidant enzyme gene expression through Nrf2. These results indicate that essential oils can reduce lipid peroxidation products, modulate non-enzymatic antioxidants, and potentially upregulate antioxidant enzyme activity via Nrf2-mediated gene expression, thereby reducing oxidative damage and enhancing immunity.

#### 4. Summary

In summary, essential oils promote animal growth by enhancing digestive and absorptive capacity and strengthening immune function. The improvement in digestive capacity is associated with promoted intestinal development and balanced intestinal microecology, while immune enhancement is related to increased immune substance content, alleviated inflammatory responses, and improved antioxidant capacity. As a green and safe feed additive, essential oils have been widely applied in production and show broad prospects. However, current research remains limited and lacks detailed mechanistic studies, necessitating more systematic and in-depth investigations.

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