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Effects of Oregano Oil on Growth Performance, Serum Hormone Indices, and Insulin-like Growth Factor-I Content in Small Intestinal Tissue of Weaned Piglets (Postprint)

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Abstract

This experiment was conducted to investigate the effects of dietary oregano oil supplementation on growth performance, serum hormone indices, and insulin-like growth factor-I (IGF-I) content in small intestinal tissues of weaned piglets. One hundred and twenty 25-day-old weaned “Large White × Landrace” cross-bred piglets with similar body weight were selected and randomly allocated into 4 groups, with 3 replicates per group and 10 piglets per replicate. The control group was fed a basal diet, while the three experimental groups were fed the basal diet supplemented with 300, 500, and 800 mg/kg oregano oil, respectively. The pre-trial period lasted for 5 days, and the formal trial period lasted for 30 days. The results showed that, compared with the control group, the three experimental groups exhibited extremely significantly increased average daily feed intake (ADFI) during days 1-30 ($P < 0.01$), significantly or extremely significantly increased average daily gain (ADG) ($P < 0.05$ or $P < 0.01$), and extremely significantly reduced diarrhea rate ($P < 0.01$); the serum IGF-I content on day 15 was significantly increased ($P < 0.05$), and the serum IGF-I content on day 30 was extremely significantly increased ($P < 0.01$) in weaned piglets of the three experimental groups; the IGF-I content in the duodenum and ileum of weaned piglets in the three experimental groups was extremely significantly increased ($P < 0.01$). It can be concluded that dietary oregano oil supplementation can increase the IGF-I content in serum and small intestinal tissues, thereby promoting the growth of weaned piglets.

Full Text

Abstract

This experiment was conducted to investigate the effects of dietary oregano essential oil (OEO) supplementation on growth performance, serum hormone indices, and insulin-like growth factor-I (IGF-I) content in small intestine tissues of weaned piglets. One hundred and twenty 25-day-old weaned “Large White × Landrace” crossbred piglets with similar body weight were randomly allocated into four groups, with three replicates per group and ten piglets per replicate. The control group received a basal diet, while the three experimental groups received the basal diet supplemented with 300, 500, or 800 mg/kg OEO, respectively. A 5-day pre-trial period was followed by a 30-day experimental period. The results demonstrated that compared with the control group, all three experimental groups exhibited highly significant increases in average daily feed intake (ADFI) during days 1-30 ($P < 0.01$), significant or highly significant increases in average daily gain (ADG) ($P < 0.05$ or $P < 0.01$), and highly significant reductions in diarrhea rate ($P < 0.01$). Serum IGF-I content on day 15 was significantly elevated in all experimental groups ($P < 0.05$), while serum IGF-I content on day 30 showed highly significant increases ($P < 0.01$). Additionally, IGF-I content in the duodenum and ileum was highly significantly increased in all experimental groups ($P < 0.01$). These findings indicate that dietary OEO supplementation can enhance IGF-I levels in both serum and small intestine tissues, thereby promoting the growth of weaned piglets.

Keywords: oregano essential oil; weaned piglets; growth performance; hormone

Introduction

Early weaning often leads to malabsorption, reduced growth performance, diarrhea, and even mortality in piglets, causing significant economic losses to the swine industry [1]. Feed antibiotics have been widely used in weaned piglet diets due to their pronounced growth-promoting, disease-resistant, and anti-diarrheal effects. However, concerns over antibiotic abuse contributing to bacterial resistance and potential hazards to human health and the environment prompted the European Union to ban antibiotic growth promoters in feed in 2006. Consequently, identifying alternatives to growth-promoting antibiotics has become a major focus in livestock production. Plant extracts have attracted considerable attention from researchers worldwide due to their natural, efficient, and residue-free characteristics [2].

Oregano essential oil (OEO) is a volatile oil extracted from the natural plant *Origanum vulgare* L., composed of various aromatic compounds with carvacrol and thymol as the primary active components [3]. *In vitro* studies have demonstrated that OEO possesses antimicrobial [4-6] and antioxidant activities [6-9]. Animal trials have shown that dietary OEO or its active ingredients can in-

crease average daily gain, effectively reduce diarrhea rates, and improve growth performance in weaned piglets [10-14]. Animal growth is primarily regulated by the hypothalamic-pituitary-liver axis, yet few studies have investigated the effects of OEO on serum hormone indices in weaned piglets. Therefore, this study aimed to examine the effects of dietary OEO supplementation on growth performance and serum levels of growth hormone (GH), triiodothyronine (T3), thyroxine (T4), and IGF-I, as well as IGF-I content in small intestine tissues, to explore the potential mechanisms underlying OEO's growth-promoting and health-promoting effects and provide a theoretical basis for its application in weaned piglets.

1.1 Experimental Materials

The OEO used was a semi-coated 10% premix (main components: 5% carvacrol and 5% thymol; coating material: light calcium carbonate), purchased from Fujian Zhong Nong Mu Biological Pharmaceutical Co., Ltd.

1.2 Experimental Animals and Methods

The trial was conducted at Youlike Agriculture and Animal Husbandry Development Co., Ltd. in Jiangkou Town, Putian City, Fujian Province. One hundred and twenty 25-day-old "Large White × Landrace" crossbred piglets with an average body weight of (7.19 ± 0.26) kg and similar parity (3-6 litters) were selected and randomly divided into four groups according to the principle of equal gender distribution (half male and half female) and similar body weight. Each group consisted of three replicates with ten piglets per replicate. The control group received a basal diet, while the three experimental groups received the basal diet supplemented with 300, 500, or 800 mg/kg OEO, respectively. The pre-trial period lasted for 5 days, followed by a 30-day experimental period. Feeding management and immunization procedures followed the routine protocols of the farm.

1.3 Experimental Diets

The composition and nutrient levels of the basal diet are presented in Table 1. The premix provided the following per kilogram of diet: VA 15,000 IU, VD 8,000 IU, VE 60 IU, VK 2.5 IU, VB1 30 mg, VB2 10 mg, VB6 30 mg, VB12 30 mg, nicotinic acid 260 mg, D-pantothenic acid 250 mg, folic acid 1.2 mg, choline chloride 500 mg, biotin 1.4 mg, Cu (as copper sulfate) 50 mg, Fe (as ferrous sulfate) 120 mg, Mn (as manganese sulfate) 40 mg, Zn (as zinc sulfate) 80 mg, I (as potassium iodide) 0.6 mg, Se (as sodium selenite) 0.3 mg, lysine 2.3 g, methionine 0.8 g, threonine 0.7 g, and tryptophan 0.5 g. Digestible energy (DE) and available phosphorus (AP) were calculated values, while other nutrients were measured values.

1.4.1 Growth Performance

During the experimental period, daily feed intake was recorded to calculate average daily feed intake (ADFI) for days 1-15, 16-30, and 1-30. All piglets were weighed individually on an empty stomach on days 1, 15, and 30 to calculate average daily gain (ADG) for each period. Feed-to-gain ratio (F/G) was calculated as ADFI/ADG for days 1-15, 16-30, and 1-30. Piglets were observed daily for mental status and fecal condition, and the number of diarrheic piglets was recorded to calculate diarrhea rate for each period using the formula: Diarrhea rate (%) = [(number of diarrheic piglets × days of diarrhea) / (total number of piglets × trial days)] × 100.

1.4.2 Serum Hormone Indices

On days 15 and 30, three piglets of average body weight were randomly selected from each replicate and blood samples (4-5 mL) were collected from the anterior vena cava into 10 mL centrifuge tubes. After serum separation, samples were centrifuged at 3,000 r/min for 10 minutes, and the serum was collected and stored at -20°C for subsequent analysis. Serum T3, T4, GH, and IGF-I concentrations were determined by enzyme-linked immunosorbent assay (ELISA) using kits purchased from Beijing Huaying Biotechnology Institute. The assay procedures followed the kit instructions, and measurements were performed using a microplate reader (BIO-RAD, USA).

1.4.3 IGF-I Content in Small Intestine Tissues

At the end of the trial, one piglet was randomly selected from each replicate and slaughtered. Segments of duodenum, jejunum, and ileum were collected, placed in cryovials, and stored at -80°C. IGF-I content in small intestine tissues was measured by ELISA.

1.5 Data Processing and Statistical Analysis

Data were analyzed using SPSS 17.0 software with one-way ANOVA, and multiple comparisons were performed using the LSD method. Results are expressed as “mean ± standard deviation.” Differences were considered significant at $P < 0.05$ and highly significant at $P < 0.01$.

Results

2.1 Effects of OEO on Growth Performance of Weaned Piglets

As shown in Table 2, compared with the control group, during days 1-15, ADFI in the three experimental groups increased by 7.86% ($P < 0.05$), 6.31% ($P < 0.05$), and 6.40% ($P < 0.05$), respectively; ADG increased by 12.64% ($P < 0.05$), 14.15% ($P < 0.01$), and 10.61% ($P < 0.05$), respectively; and diarrhea rate decreased by 61.40% ($P < 0.01$), 61.40% ($P < 0.01$), and 58.33% ($P < 0.01$), respectively. During days 16-30, ADFI increased by 5.89% ($P > 0.05$), 8.40% ($P < 0.05$), and

9.55% ($P < 0.01$), respectively, while diarrhea rate decreased by 62.39% ($P < 0.01$), 65.75% ($P < 0.01$), and 66.67% ($P < 0.01$), respectively. Over the entire experimental period (days 1-30), ADFI increased by 6.94% ($P < 0.01$), 8.16% ($P < 0.01$), and 8.95% ($P < 0.01$), respectively; ADG increased by 6.72% ($P < 0.05$), 8.75% ($P < 0.01$), and 9.17% ($P < 0.01$), respectively; and diarrhea rate decreased by 57.14% ($P < 0.01$), 67.94% ($P < 0.01$), and 68.98% ($P < 0.01$), respectively.

2.2 Effects of OEO on Serum Hormone Indices of Weaned Piglets

As presented in Table 3, compared with the control group, on day 15, serum GH concentration increased by 9.35% ($P > 0.05$), 16.15% ($P < 0.05$), and 17.56% ($P < 0.01$) in the three experimental groups, respectively, while serum IGF-I concentration increased by 12.31% ($P < 0.05$), 17.91% ($P < 0.05$), and 16.06% ($P < 0.05$), respectively. No significant differences were observed in serum T3 or T4 concentrations among groups ($P > 0.05$). On day 30, serum T4 concentration increased by 7.63% ($P > 0.05$), 13.67% ($P < 0.05$), and 18.54% ($P < 0.01$), respectively, and serum IGF-I concentration increased by 23.62% ($P < 0.01$), 29.43% ($P < 0.01$), and 27.58% ($P < 0.01$), respectively. No significant differences were found in serum GH or T3 concentrations ($P > 0.05$).

2.3 Effects of OEO on IGF-I Content in Small Intestine Tissues of Weaned Piglets

As shown in Table 4, compared with the control group, duodenal IGF-I content increased by 22.97% ($P < 0.01$), 48.45% ($P < 0.01$), and 55.21% ($P < 0.01$) in the three experimental groups, respectively. Jejunal IGF-I content increased by 0.56% ($P > 0.05$), 7.65% ($P > 0.05$), and 29.90% ($P < 0.01$), respectively. Ileal IGF-I content increased by 22.97% ($P < 0.01$), 30.51% ($P < 0.01$), and 29.71% ($P < 0.01$), respectively.

Discussion

3.1 Effects of OEO on Growth Performance of Weaned Piglets

Previous studies have shown that supplementing weaned piglet diets with 300 mg/kg of 10% OEO premix can significantly improve ADG and feed conversion ratio while reducing diarrhea rate [10,13]. Yao et al. [14] reported that dietary supplementation with 100, 200, or 300 mg/kg of 20% microencapsulated OEO improved growth performance, while 400 mg/kg significantly reduced feed conversion ratio. Wang et al. [12] evaluated four antibiotic alternatives (oregano extract, *Picrasma*-licorice extract, plant essential oil complex, and antimicrobial peptides) and found that 1,000 mg/kg oregano extract produced the best results, increasing ADG by 21.55%, ADFI by 2.97%, improving feed conversion, and effectively reducing diarrhea rate by 26.46%. The present study demonstrated that dietary OEO supplementation at various levels improved ADFI and ADG while reducing diarrhea rate throughout the trial period, consistent with previous findings. This effect may be attributed to the mild aromatic scent

of OEO, as the olfactory nerve is the only cranial nerve directly connected to the brain, which can promote the secretion of brain-gut peptides, saliva, and gastric juice, thereby increasing feed intake [18]. Additionally, studies have shown that the growth-promoting effects of plant essential oils are closely associated with improved gut microbiota, stimulated digestive enzyme secretion, enhanced intestinal structure, better feed utilization, and improved immune function [19-20].

3.2 Effects of OEO on Serum Hormone Indices of Weaned Piglets

Animal growth is primarily regulated by the hypothalamic-pituitary-liver axis. GH and IGF-I are the most representative indicators of nutritional and growth status in this axis, directly promoting cell growth, increasing amino acid uptake, enhancing protein and RNA synthesis, and stimulating muscle growth. They also cooperate with other growth factors to promote the proliferation and differentiation of muscle cells, osteoblasts, adipocytes, and glial cells. Due to the pulsatile release of GH, it is difficult to establish a direct relationship between GH and growth characteristics; however, IGF-I levels are relatively stable and can reflect animal growth status [21], making it an important indicator of growth rate. Studies have shown that serum IGF-I concentration positively correlates with pig body weight and weight gain [22]. GH plays a crucial role in promoting protein deposition in piglets, while T3 and T4 are important hormones that enhance metabolic efficiency and can synergize with GH to promote piglet growth and development [23].

Xu et al. [24] suggested that the growth-promoting effect of *Atractylodes* polysaccharide in piglets may be achieved by promoting GH and IGF-I synthesis and secretion, enhancing anabolic metabolism, while simultaneously promoting T3 and T4 synthesis and secretion to mobilize and accelerate fat oxidation and decomposition. Hua et al. [25] reported that inulin significantly increased free triiodothyronine (FT3), free thyroxine (FT4), GH, and IGF-I concentrations in weaned piglets. Cheng et al. [26] found that Chaizhu Kangji granules (containing saikosaponins, *Atractylodes* polysaccharide, and total glucosides of peony) promoted piglet growth by reducing serum cortisol (COR) levels and increasing serum GH, IGF-I, T3, and T4 concentrations. Liu et al. [27] demonstrated that allixin improved growth performance by increasing serum FT3 and IGF-I concentrations. Huang et al. [28] showed that dietary *Ginkgo biloba* extract improved growth performance by increasing serum GH, T3, and T4 concentrations to promote protein synthesis. The present study found that dietary OEO supplementation increased relevant hormone concentrations, particularly serum IGF-I, suggesting that OEO may promote piglet growth by stimulating IGF-I secretion.

3.3 Effects of OEO on IGF-I Content in Small Intestine Tissues of Weaned Piglets

Research has shown that IGF-I can regulate intestinal development and plays a vital role in piglet gut maturation [29]. The present study found that dietary OEO supplementation increased IGF-I content in small intestine tissues. Furthermore, our previous laboratory studies have demonstrated that compared with the control group, OEO supplementation increased intestinal villus length, decreased crypt depth, increased the villus length/crypt depth ratio, and up-regulated the mRNA expression of tight junction proteins zonula occludens-1 (ZO-1), Claudin, and Occludin. These results indicate that dietary OEO supplementation can increase intestinal IGF-I content, promote intestinal epithelial cell proliferation and differentiation, improve small intestinal mucosal morphology, enhance intestinal development, and consequently improve nutrient absorption and piglet growth. Further research is needed to determine whether OEO can influence IGF-I mRNA expression in intestinal and hepatic tissues.

Conclusion

Dietary supplementation with oregano essential oil improves average daily gain and average daily feed intake, reduces diarrhea rate, and increases IGF-I content in both serum and small intestine tissues of weaned piglets.

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