

Effects of Dietary Supplementation with Functional Compound Additive on Growth Performance and Immune Function in Growing Pigs (Postprint)

Authors: Wu Lijuan, Mao Xiangbing, Yu Bing, He Jun, Huang Zhiqing, Yu Jie, Zheng Ping, Luo Junqiu, Chen Daiwen

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Abstract

This study aimed to investigate the effects of dietary supplementation with a functional compound additive on growth performance and immune function in growing pigs. Twenty healthy “Duroc × Landrace × Large White” three-way crossbred growing pigs with an average body weight of (21.06±1.98) kg were selected and randomly divided into 2 groups according to the principle of similar body weight: a normal diet group (control group) and a 0.1% functional compound additive diet group (additive group), with 10 replicates per group and 1 pig per replicate. The experimental period lasted 14 days. The results showed that, compared with the control group, dietary supplementation with the functional compound additive significantly increased average daily gain (ADG) ($P<0.05$), extremely significantly increased serum interleukin-10 (IL-10) content ($P<0.01$), significantly or extremely significantly decreased serum interleukin-1 (IL-1) and urea nitrogen (UN) contents ($P<0.05$ or $P<0.01$), and significantly or extremely significantly decreased the relative mRNA expression levels of nuclear factor- κ B (NF- κ B), tumor necrosis factor receptor-associated factor 6 (TRAF6), and interleukin-1 receptor-associated kinase 1 (IRAK1) associated with the Toll-like receptor (TLR) signaling pathway in blood leukocytes ($P<0.05$ or $P<0.01$). In conclusion, dietary supplementation with the functional compound additive can improve growth performance in growing pigs, which may be related to its ability to enhance protein utilization and regulate immune function.

Full Text

Effects of Dietary Functional Compound Additive Supplementation on Growth Performance and Immune Function of Growing Pigs

WU Lijuan, MAO Xiangbing, YU Bing, HE Jun, HUANG Zhiqing, YU Jie, ZHENG Ping, LUO Junqiu, CHEN Daiwen*

Institute of Animal Nutrition, Sichuan Agricultural University, Chengdu 611130, China

Abstract: This study investigated the effects of dietary functional compound additive supplementation on growth performance and immune function in growing pigs. Twenty healthy Duroc \times Landrace \times Large White crossbred growing pigs with an average initial body weight of (21.06 ± 1.98) kg were randomly allocated into two groups according to similar body weight: a basal diet group (control group) and a basal diet supplemented with 0.1% functional compound additive group (additive group). Each group comprised 10 replicates with one pig per replicate. The experimental period lasted 14 days. The results showed that compared with the control group, dietary supplementation with the functional compound additive significantly increased average daily gain (ADG) ($P < 0.05$), significantly elevated serum interleukin-10 (IL-10) content ($P < 0.01$), and significantly reduced serum interleukin-1 (IL-1) and urea nitrogen (UN) contents ($P < 0.05$ or $P < 0.01$). Additionally, the additive significantly or extremely significantly decreased the relative mRNA expression of nuclear factor B (NF- κ B), tumor necrosis factor receptor-associated factor 6 (TRAF6), and interleukin-1 receptor-associated kinase 1 (IRAK1) in peripheral blood leukocytes ($P < 0.05$ or $P < 0.01$). In conclusion, dietary supplementation with the functional compound additive can improve growth performance in growing pigs, likely by enhancing protein utilization and modulating immune function.

Key words: growing pigs; functional compound additive; growth performance; immune function

*Corresponding author, professor, E-mail: dwchen@sicau.edu.cn

Introduction

In livestock production, antibiotics have been widely used as feed additives to combat stress, promote growth, and prevent diseases. However, the abuse of feed antibiotics has caused numerous social problems, including rising antimicrobial resistance and drug residues in animal products. Consequently, developing effective antibiotic alternatives has become a major research focus. Previous studies have demonstrated that dietary supplementation with vitamin D, vitamin C, vitamin E, and Astragalus polysaccharides can improve growth performance and immune function in livestock and poultry [1-4]. Since different additives exert

distinct effects, combining them into a compound additive may produce complementary and synergistic benefits. Molina et al. [5] reported that compound additives exhibit superior antioxidant and anti-inflammatory effects. Zhou et al. [6] found that combined supplementation of vitamin C and vitamin E in piglet diets produced synergistic effects. Zhu et al. [7] and Xu et al. [8] also demonstrated that dietary supplementation with a compound additive composed of vitamin C, vitamin E, tea polyphenols, lipoic acid, and lactic acid bacteria could effectively improve intestinal function and enhance piglet growth performance. This study aimed to investigate the effects of a self-developed functional compound additive (primarily containing vitamin C, vitamin E, vitamin D, Astragalus polysaccharide, and folic acid) on growth performance and immune function in growing pigs, thereby providing a scientific basis for its practical application.

1.1 Experimental Materials

The functional compound additive used in this experiment had the following composition and content: vitamin C 10.75%, folic acid 0.21%, vitamin A 0.67%, vitamin D 3.77%, vitamin E 6.00%, vitamin B2 0.56%, Astragalus polysaccharide 7.14%, sodium bicarbonate 10.10%, and glucose 60.80%.

1.2 Experimental Animals and Design

Twenty healthy Duroc \times Landrace \times Large White crossbred growing pigs with an initial body weight of (21.06 ± 1.98) kg were selected and randomly divided into two groups according to similar body weight: a control group fed the basal diet and an additive group fed the basal diet supplemented with 0.1% functional compound additive. Each group consisted of 10 replicates with one pig per replicate. The experimental period was 14 days.

1.3 Experimental Diets

The basal diet was a corn-soybean meal-based diet formulated according to the nutrient requirements for 25–50 kg growing pigs as recommended by NRC (2012). The composition and nutrient levels of the basal diet are presented in Table 1. The experimental diet was prepared by supplementing the basal diet with 0.1% functional compound additive, with the additive replacing an equal amount of corn.

1.4 Management

The experiment was conducted at the Teaching and Research Farm of the Institute of Animal Nutrition, Sichuan Agricultural University, from May 21 to June 4, 2016. Pigs were housed individually in metabolism cages with free access to water and were fed three times daily (08:00, 14:00, and 20:00). Feed was provided in small amounts with frequent additions, with slight remaining feed in the trough as the criterion. The pig house temperature was maintained at 27–

28°C, and relative humidity was controlled at approximately 70%. Pens were washed regularly to maintain ventilation, cleanliness, dryness, and hygiene.

1.5 Sample Collection

On day 15 of the experiment, all growing pigs were fasted and 10 mL of blood was collected from each pig. The blood samples were allowed to stand at room temperature for 45 min, then centrifuged at 3,000 r/min for 10 min to separate serum, which was stored at -20°C. Additionally, 3–5 mL of blood was slowly injected into EDTA-containing anticoagulant tubes, gently inverted to mix, and used for leukocyte isolation.

1.6.1 Growth Performance

Daily feed intake was recorded throughout the experiment to calculate average daily feed intake (ADFI). Body weight was measured on the morning of days 1 and 15 after fasting to calculate average daily gain (ADG) from days 1–14. Feed-to-gain ratio (F/G) was calculated from ADFI and ADG.

1.6.2 Serum Indices

Serum urea nitrogen (UN) content was determined using a kit from Nanjing Jiancheng Bioengineering Institute according to the manufacturer's instructions. Serum interleukin-1 (IL-1) and interleukin-10 (IL-10) contents were measured using enzyme-linked immunosorbent assay (ELISA) kits from Shanghai Nuoyuan Industrial Co., Ltd., following the manufacturer's protocols.

1.6.3 Relative mRNA Expression of Leukocyte-Related Genes

1.6.3.1 Leukocyte Isolation

Leukocytes were isolated using the Animal Peripheral Blood Leukocyte Separation Kit from Tianjin Haoyang Biological Products Technology Co., Ltd. The procedure was as follows: (1) Add 3 mL of separation solution to a 15 mL centrifuge tube; (2) Slowly and carefully layer the blood sample onto the separation solution surface, then centrifuge at 400×g for 36 min at 18–20°C; (3) After centrifugation, four distinct layers form: the top pale yellow layer is diluted plasma, the second narrow white cloudy band contains primarily mononuclear cells, the third colorless transparent layer is the separation solution, and the bottom dark red precipitate is the erythrocyte layer; (4) Using a sterile Pasteur pipette, remove and discard the upper liquid layer, then carefully collect the separation solution layer, mononuclear cell layer, and erythrocyte layer into a new centrifuge tube; (5) Add cell washing solution, mix gently, centrifuge at 250×g for 10 min, discard the supernatant, remove erythrocyte contents and debris using erythrocyte lysis solution, and wash repeatedly with cell washing solution; (6) After washing, add 1 mL TRIzol, pipette repeatedly to mix, label, and store at -80°C.

1.6.3.2 Relative Gene Expression

Real-time quantitative PCR was used to detect the relative expression of genes related to the Toll-like receptor (TLR) signaling pathway in leukocytes, including nuclear factor B (NF- κ B), myeloid differentiation factor 88 (MyD88), mitogen-activated protein kinase (MAPK), tumor necrosis factor receptor-associated factor 6 (TRAF6), interleukin-1 receptor-associated kinase 1 (IRAK1), interleukin-1 receptor-associated kinase 4 (IRAK4), and I κ B kinase α (IKK- α). Primers were designed using Primer Premier 5.0 based on target gene sequences obtained from NCBI (Table 2) and synthesized by Chengdu Kangdi Biotechnology Co., Ltd. Total RNA was extracted from leukocytes according to the kit instructions. RNA was reverse-transcribed into cDNA using the PrimeScriptTM RT Reagents Kit (TaKaRa). Relative expression of target genes was quantified using SYBR Green PCR Mix with a 10 L reaction system. The thermal cycling program was: 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 60°C for 34 s, then 95°C for 15 s, 60°C for 1 min, and 95°C for 15 s. β -actin served as the reference gene, and relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

1.7 Data Processing and Analysis

All data were initially processed using Excel 2007 and analyzed by t-test using SPSS 17.0 software. All measurements were expressed as “mean \pm standard error” with each replicate as the statistical unit. Differences were considered significant at $P < 0.05$, extremely significant at $P < 0.01$, and a trend at $0.05 \leq P < 0.10$.

2.1 Effects of Dietary Functional Compound Additive on Growth Performance of Growing Pigs

As shown in Table 3 , dietary supplementation with the functional compound additive significantly increased ADG ($P < 0.05$) and tended to decrease F/G ($P = 0.09$) in growing pigs compared with the control group.

2.2 Effects of Dietary Functional Compound Additive on Serum UN Content of Growing Pigs

Table 4 shows that dietary functional compound additive supplementation significantly reduced serum UN content in growing pigs ($P < 0.05$).

2.3 Effects of Dietary Functional Compound Additive on Serum Cytokine Content of Growing Pigs

As presented in Table 5 , dietary functional compound additive supplementation extremely significantly decreased serum IL-1 content ($P < 0.01$) and extremely significantly increased serum IL-10 content ($P < 0.01$) in growing pigs.

2.4 Effects of Dietary Functional Compound Additive on Relative mRNA Expression of Genes in Leukocytes of Growing Pigs

Table 6 demonstrates that dietary functional compound additive supplementation significantly or extremely significantly decreased the relative mRNA expression of NF- κ B, TRAF6, and IRAK1 in leukocytes ($P < 0.05$ or $P < 0.01$), and tended to reduce the expression of IRAK4 ($P = 0.08$) and IKK α ($P = 0.05$).

3.1 Effects of Dietary Functional Compound Additive on Growth Performance and Serum Urea Nitrogen Content

The self-developed functional compound additive in this study contained vitamin C, vitamin E, and Astragalus polysaccharide, which have been shown to improve piglet growth performance and reduce mortality [9-11]. Consistently, our results showed that dietary supplementation with this functional compound additive significantly increased ADG and tended to decrease F/G in growing pigs. Serum UN content can indirectly reflect protein metabolism and amino acid balance in the body; decreased serum UN indicates enhanced amino acid utilization and deposition [12-13]. Harvey et al. [14] found that dietary vitamin E and C supplementation significantly reduced serum UN in growing pigs. Peng et al. [15] reported that Astragalus polysaccharide decreased serum UN and improved nitrogen balance in piglets. These findings align with our results, suggesting that the functional compound additive improved amino acid utilization in growing pigs. We can therefore speculate that the additive enhances growth performance by promoting protein deposition.

3.2 Effects of Dietary Functional Compound Additive on Serum Cytokine Content and TLR Signaling Pathway-Related Gene Expression in Leukocytes

Serum cytokines serve as indicators of systemic immune responses and reflect the overall inflammatory status [16]. IL-1, primarily produced by macrophages, participates in vascular endothelial activation, tissue damage, fever, lymphocyte activation, and acute-phase protein synthesis. IL-10 is a potent anti-inflammatory cytokine that promotes pre-B cell proliferation, inhibits T cell activation, induces immune tolerance, and suppresses pro-inflammatory cytokine synthesis. Cantorna et al. [17] demonstrated that vitamin D modulates T cell differentiation and IL-10 secretion, thereby exerting anti-inflammatory effects. Vitamin C can alleviate inflammatory damage by reducing pro-inflammatory cytokine TNF- α and increasing anti-inflammatory IL-10 [5]. Liao et al. [18] reported that dietary vitamin D decreased IL-2 and IL-6 levels in serum and intestinal contents of weaned piglets. Fischer et al. [19] found that vitamins C and E reduced IL-6 release in humans. Zhao et al. [4] showed that Astragalus polysaccharide downregulated IL-1 and IL-6 in colonic tissues of mice. Our results with the functional compound additive are consistent with these findings, showing significantly reduced pro-inflammatory IL-1 and increased anti-inflammatory IL-10, indicating that the additive improves immune status by modulating cytokine

secretion.

The TLR signaling pathway initiates innate immune responses and subsequently activates adaptive immunity. The MyD88-dependent TLR pathway involves recruitment of MyD88, which then recruits IRAK4, facilitating its binding to and phosphorylation of IRAK1. This activates TRAF6, leading to downstream activation of MAPK and NF- κ B [20]. NF- κ B is a classic inflammatory signaling pathway that induces pro-inflammatory cytokine secretion [21]. Previous studies on individual components of our functional compound additive demonstrated that vitamins D, C, and E effectively modulate the TLR pathway in various tissues. Vitamin D can regulate TLR signaling and inflammatory cytokine release [22-23]. In human leukocytes, vitamin C modulates inflammatory cytokine secretion via the MyD88 pathway [24]. Vitamin E ameliorates myocarditis by regulating the TLR pathway and NF- κ B in human cardiac tissue [25]. Our study examined TLR pathway-related gene expression in peripheral blood leukocytes and found that the functional compound additive tended to decrease MyD88 mRNA expression and significantly or extremely significantly reduced NF- κ B, TRAF6, and IRAK1 expression, with a trend toward decreased IRAK4 and IKK α expression. These results suggest that the self-developed functional compound additive modulates immune function, likely through regulation of the TLR signaling pathway.

In summary, dietary supplementation with the functional compound additive improves growth performance in growing pigs, possibly by enhancing amino acid utilization and modulating immune function through regulation of the TLR signaling pathway.

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