Effects of Nutritional Compound Additive Feeding Patterns on Immune Function and Classical Swine Fever Antibody Titer in Growing Pigs (Postprint)

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Abstract

This experiment aimed to investigate the effects of feeding patterns of nutritional compound additives on immune function and classical swine fever (CSF) antibody titer in growing pigs. Twenty-four healthy "Duroc × Landrace × Yorkshire" growing pigs with a body weight of (40.5$±$2.3) kg were selected and randomly divided into 4 groups according to the principle of similar body weight and consistent gender, with 6 replicates per group and 1 pig per replicate. On day 7 of the experiment, CSF vaccine was administered; the 7 days before vaccination were designated as the early experimental period, and the 28 days after vaccination as the late experimental period, with a total experimental period of 35 days. The control group was fed a basal diet, while the experimental groups were supplemented with 0.1% nutritional compound additive in the basal diet during the early period, late period, and entire period, respectively. The results showed that: compared with the control group, feeding nutritional compound additive during the entire period significantly increased serum CSF antibody titer in growing pigs on day 21 of the experiment ($P<0.05$); the entire period group had significantly higher serum CSF antibody levels in growing pigs on day 28 than the control and early period groups ($P<0.05$). Compared with the control group, the late period and entire period groups significantly decreased whole blood CD3+ count in growing pigs on day 21 of the experiment ($P<0.05$); the entire period group had significantly lower whole blood CD3+ count on day 35 than the other groups ($P<0.05$). Compared with the control group, the entire period group significantly increased serum immunoglobulin content in growing pigs on days 21 and 35 ($P<0.05$); the early period or entire period groups significantly increased serum superoxide dismutase activity and total antioxidant capacity in growing pigs ($P<0.05$), while the entire pe-

riod group significantly decreased serum malondialdehyde content (P<0.05). In conclusion, different feeding patterns of nutritional compound additive had differential effects on CSF antibody levels, immunoglobulin content, and systemic antioxidant capacity in growing pigs, with feeding during the entire period showing the best efficacy.

Full Text

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Abstract: This experiment investigated the effects of nutritional compound additive feeding patterns on immune function and classical swine fever (CSF) antibody titer in growing pigs. Twenty-four healthy Duroc × Landrace × Yorkshire growing pigs with an initial body weight of (40.5$±$2.3) kg were randomly allocated to 4 groups according to similar body weight and gender, with 6 replicates per group and 1 pig per replicate. On day 7 of the experiment, pigs were vaccinated against CSF. The 7 days before vaccination were defined as the early stage, while the 28 days after vaccination constituted the late stage, making the total experimental period 35 days. The control group was fed a basal diet, while experimental groups received the basal diet supplemented with 0.1% nutritional compound additive during the early stage, late stage, or entire stage. The results showed that feeding the additive throughout the entire stage significantly increased serum CSF antibody titer on day 21 compared with the control group (P<0.05). On day 28, the entire-stage group exhibited significantly higher serum CSF antibody levels than both the control group and early-stage group (P<0.05). Compared with the control group, the late-stage and entire-stage groups significantly reduced whole blood CD3+ counts on day 21 (P<0.05), with the entire-stage group showing the lowest CD3+ counts on day 35 (P<0.05). The entire-stage group significantly elevated serum immunoglobulin contents on days 21 and 35 (P<0.05), while the early-stage and entire-stage groups significantly increased serum superoxide dismutase activity and total antioxidant capacity (P<0.05), and the entire-stage group significantly decreased serum malondialdehyde content (P<0.05). In conclusion, different feeding patterns of nutritional compound additive differentially affect CSF antibody levels, immunoglobulin content, and antioxidant capacity in growing pigs, with continuous feeding throughout the entire period showing the optimal effect.

Keywords: nutritional compound additive; antibody titer; immune function; classical swine fever; growing pigs

Introduction

In recent years, severe infectious diseases have frequently occurred in swine populations, posing serious threats to pig health. Vaccination represents one of the most effective methods for controlling infectious diseases, yet immunization failure and low vaccine antibody titers commonly occur in production practice, directly compromising disease control efficacy and hindering the development of the pig industry. Research has demonstrated a direct relationship between nutrition and vaccine efficacy, as nutrition not only influences immune system development and maturation but also because antibody production consumes substantial nutrients including proteins, vitamins, and trace elements [1]. Therefore, nutritional modulation to improve animal immune function can effectively enhance vaccine efficacy. Previous studies have shown that supplementation with Astragalus polysaccharide or vitamin E individually improved serum antibody levels and strengthened immune function in weaned piglets under vaccination [2-3]. Selenium yeast, folic acid, and biotin can affect immune function by influencing immune organ development and lymphocyte differentiation [4-5]. While numerous studies have investigated the effects of individual additives, research on nutritional compound additives and novel additive combinations remains relatively limited. Our research group has previously identified optimal formulations and supplementation levels for a nutritional compound additive [6], but the appropriate feeding methods and patterns require further investigation. This study therefore aimed to evaluate how different feeding patterns of a nutritional compound additive affect immune function and CSF vaccine antibody titers in growing pigs, providing scientific guidance for practical application.

Materials and Methods

Experimental Design

Twenty-four healthy Duroc × Landrace × Yorkshire growing pigs with an initial body weight of (40.5$±$2.3) kg were randomly allocated to 4 groups according to similar body weight and gender, with 6 replicates per group and 1 pig per replicate. On day 7 of the experiment, all pigs were vaccinated against CSF. The experimental period was divided into two stages: the early stage (7 days before vaccination) and the late stage (28 days after vaccination), with the total trial lasting 35 days. The nutritional compound additive feeding patterns are shown in .

Experimental Diets

The basal diet was a corn-soybean meal type formulated according to the NRC (2012) nutrient requirements for 25-50 kg growing pigs, with composition and nutrient levels shown in . The nutritional compound additive was supplemented at 0.1%. The composition and content of the nutritional compound additive (per kg of complete feed) were: selenium yeast 0.5 mg/kg, Astragalus polysaccharide 300 mg/kg, biotin 0.1 mg/kg, folic acid 0.02 mg/kg, and vitamin C 100 mg/kg.

Animal Management

A 4-day pre-trial adaptation period was provided, during which pigs received the basal diet. All pigs were housed individually in metabolism cages, fed three times daily with ad libitum access to feed and water. Feed was provided in small amounts with frequent additions, maintaining slight residual amounts in the trough. Pens were cleaned and disinfected daily, with adequate ventilation and dryness maintained.

Sample Collection and Measurements

Blood samples (3-5 mL) were collected from the anterior vena cava at the start of the formal experiment and on days 7, 14, 21, 28, and 35 for antibody titer determination. On days 21 and 35, 10 mL blood samples were collected; serum was harvested by centrifugation at 3,000 rpm for 15 minutes, with additional samples collected in anticoagulant tubes for lymphocyte subset detection.

Classical Swine Fever Antibody Determination Serum CSF antibody levels were measured by enzyme-linked immunosorbent assay (ELISA) at the start and on days 7, 14, 21, 28, and 35, following the manufacturer's instructions for the CSF virus antibody detection kit (IDEXX, USA). Antibody titer was expressed as blocking rate:

$$\text{Blocking rate } (\%) = 100 \times \frac{\text{OD}_{\text{N450}} - \text{OD}_{\text{TEST450}}}{\text{OD}_{\text{N450}}}$$

where ODTEST450 is the mean optical density of test samples and ODN450 is the mean optical density of negative controls.

T Lymphocyte Subset Determination On days 21 and 35, 2 mL of anticoagulated whole blood was analyzed by flow cytometry to determine T lymphocyte subsets CD3+, CD4+, CD8+ counts and CD4+/CD8+ ratio.

Immunoglobulin Determination Serum immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM) contents were measured on days 21 and 35 using a double-antibody two-step sandwich ELISA method with kits from Wuhan GeneMay Biological Technology Co., Ltd.

Antioxidant Index Determination Serum samples collected on days 21 and 35 were aliquoted into 500 L EP tubes and stored at -20°C. Serum total antioxidant capacity (T-AOC), malondialdehyde (MDA) content, and superoxide dismutase (SOD) activity were determined using kits from Nanjing Jiancheng Bioengineering Institute, following the manufacturer' s instructions strictly.

Statistical Analysis

Data were organized using Excel 2007 and analyzed by one-way ANOVA using SPSS 17.0 software. Duncan' s multiple comparison test was used for post-hoc analysis. Results are expressed as means ± standard deviation. Differences were considered significant at $P<0.05$ and non-significant at $P>0.05$.

Results

Effects of Nutritional Compound Additive Feeding Patterns on Serum CSF Antibody Titer

As shown in , all groups developed high CSF antibody levels after vaccination, with substantial production beginning on day 21, which was significantly higher than at the start, day 7, and day 14 ($P<0.05$). Compared with the control group, the entire-stage group significantly increased serum CSF antibody titer on day 21 ($P<0.05$). On day 28, the entire-stage group exhibited significantly higher serum CSF antibody titer than both the control group and early-stage group ($P<0.05$).

Effects of Nutritional Compound Additive Feeding Patterns on Blood Lymphocyte Subsets

As shown in , compared with the control group, the late-stage and entire-stage groups significantly reduced whole blood CD3+ counts on day 21 ($P<0.05$). On day 35, the entire-stage group showed significantly lower CD3+ counts than all other groups ($P<0.05$), with a decreasing trend in CD4+/CD8+ ratio ($P>0.05$). This suggests that the nutritional compound additive affected the percentages of helper and suppressor T cells in whole blood, reducing CD3+ counts and potentially compromising cellular immune function while increasing disease susceptibility. No significant differences were observed among groups in CD4+, CD8+ counts, or CD4+/CD8+ ratio ($P>0.05$).

Effects of Nutritional Compound Additive Feeding Patterns on Serum Immunoglobulin

As shown in , the late-stage and early-stage groups increased serum IgG, IgA, and IgM contents on days 21 and 35 compared with the control group, but differences were not significant ($P>0.05$). The entire-stage group significantly elevated serum IgG, IgA, and IgM contents on both days 21 and 35 compared with the control group ($P<0.05$).

Effects of Nutritional Compound Additive Feeding Patterns on Serum Antioxidant Capacity

As shown in , the late-stage and early-stage groups reduced serum MDA content on days 21 and 35 compared with the control group, but differences were not significant ($P>0.05$). The entire-stage group significantly decreased serum MDA content on day 21 ($P<0.05$). Compared with the control group, the early-stage group significantly increased serum SOD activity on day 21 ($P<0.05$), while the early-stage and entire-stage groups significantly elevated SOD activity on day 35 ($P<0.05$). Nutritional compound additive supplementation increased serum T-AOC on day 21, with the entire-stage group showing significant improvement ($P<0.05$).

Discussion

Vaccination is an effective method for disease control, yet the stimulation by foreign pathogens during immunization can trigger metabolic changes that affect immune efficiency. Classical swine fever remains a significant factor hindering modern pig industry development. Reducing stress responses and improving immune function to enhance vaccination efficiency represents the primary focus of this research. Previous studies have confirmed that herbal additives can serve as immune adjuvants to improve antiviral capacity, while trace elements and vitamins play unique roles in resisting environmental changes and pathogens [7-9]. This study simulated field CSF vaccination conditions in growing pigs. The results demonstrated that antibody titers in all groups increased significantly by 21 days post-vaccination, confirming effective vaccine response.

Humoral immunity constitutes a vital component of host defense, primarily mediated by antibodies whose serum levels reflect immune status. Rapid activation of the immune system to produce specific antibodies following vaccination is crucial for disease prevention. IgA serves as the first line of defense against pathogen invasion, working with IgG to resist bacterial, viral, and toxic infections. IgM appears earlier than IgG and effectively binds complement, playing an important role in early defense. Chen et al. [10] reported that dietary biotin supplementation at 0.3-0.5 mg/kg significantly increased serum IgG content in piglets. Hou [11] found that 0.05% Astragalus polysaccharide supplementation significantly elevated serum IgG in weaned piglets. Niu et al. [12] observed that 0.4 mg/kg organic selenium supplementation increased total antibodies, IgM, and IgG in broilers immunized with sheep red blood cell antigen. Supplementation with single or dual nutritional additives under immunization conditions has shown synergistic effects on antibody titers [13-14]. Dietary Astragalus polysaccharide significantly improved oil-adjuvanted vaccine antibody titers [15], while selenium supplementation stimulated immunoglobulin and antibody production, enhancing humoral immune function [16]. This study found that nutritional compound additive use increased serum immunoglobulin content in growing pigs, with continuous feeding showing the most pronounced effect. Notably, entire-stage feeding significantly improved CSF vaccine antibody levels in im-

munized pigs without affecting non-immunized pigs, suggesting the additive may function as an immune adjuvant. Interestingly, entire-stage feeding also reduced whole blood CD3+ counts with a decreasing trend in CD4+/CD8+ ratio, indicating that the additive primarily modulates immune function through humoral rather than cellular immunity.

Antioxidant capacity is closely related to animal health, with numerous studies reporting that nutritional additives promote antioxidant function. Astragalus polysaccharide reduced alanine aminotransferase and aspartate aminotransferase activities while enhancing SOD and glutathione activities in mouse liver [17]. Zeng et al. [18] demonstrated that vitamin C significantly increased T-AOC and decreased MDA content in broiler liver and serum. Folic acid promotes glutathione peroxidase production in serum and liver, reducing free radical generation and protecting cell membrane structure and function. In this study, entire-stage feeding significantly increased serum T-AOC and SOD activity while decreasing MDA content, likely attributable to antioxidant components including vitamin E, vitamin C, and selenium yeast in the compound additive. Furthermore, the effects on antioxidant capacity were primarily concentrated before immunization, with late-stage feeding showing limited though some beneficial effects, suggesting that continuous supplementation throughout the entire period is more advantageous for improving antioxidant capacity.

Conclusion

Feeding nutritional compound additive improved CSF antibody levels, immunoglobulin content, and antioxidant capacity in growing pigs. Different feeding patterns differentially affected CSF vaccine antibody titers and immune function, with continuous feeding throughout the entire experimental period demonstrating optimal efficacy under the conditions of this study.

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