Effects of Spirulina on Growth Performance, Immune Function, and Fecal Microbiota in Piglets: Postprint

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Abstract

To explore spirulina as a feed resource, this study investigated the effects of spirulina alone and in combination with a probiotic containing Bacillus subtilis and lactic acid bacteria on growth performance, immune function, and fecal microbiota in piglets. Using a 2×2 factorial design, 80 weaned piglets (average body weight (8.85±0.20) kg) negative for foot-and-mouth disease (FMD) maternal antibodies were randomly allocated to four groups: control, spirulina (0.5% supplementation), probiotic (0.5 kg/t supplementation), and spirulina+probiotic (0.5% spirulina + 0.5 kg/t probiotic), with 5 replicates per group (4 pigs/replicate). Diets were provided in powder form. The experiment lasted 28 days. On day 8, pigs were vaccinated against FMDV serotypes O and A, and serum and fresh fecal samples were collected on days 7 and 28. The results showed: 1) Spirulina significantly increased average daily gain ($P < 0.05$) and reduced feed-to-gain ratio ($P < 0.05$) in piglets. 2) Spirulina had no significant effect on O-type and A-type FMD antibody titers on days 7 and 28 ($P>0.05$). 3) On day 28, spirulina extremely significantly increased serum immunoglobulin G (IgG), immunoglobulin M (IgM), and complement 3 concentrations ($P < 0.01$), but had no significant effect on serum complement 4 concentration ($P>0.05$). 4) Spirulina had no significant effect on Lactobacillus and Escherichia coli counts in piglet feces on days 7 and 28 ($P>0.05$). Except for IgG, IgM, and complement 3 concentrations on day 28, there was no significant interaction between spirulina and probiotics ($P>0.05$). These results indicate that dietary supplementation with 0.5% spirulina during FMD vaccination can promote piglet growth, and its combination with probiotics can enhance humoral immune function.

Full Text

Effects of Spirulina on Growth Performance, Immune Function and Fecal Flora of Piglets

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Abstract

To explore Spirulina as a feed resource, this study investigated the effects of Spirulina alone and in combination with probiotics (*Bacillus subtilis* and *Lactobacillus*) on growth performance, immune function and fecal flora of piglets. Following a 2×2 factorial design, eighty weaned piglets with average body weight of (8.85±0.20) kg and negative foot-and-mouth disease (FMD) maternal antibodies were randomly assigned to four groups: control group, Spirulina group (0.5% Spirulina supplementation), probiotics group (0.5 kg/t probiotics supplementation), and Spirulina-probiotics group (0.5% Spirulina + 0.5 kg/t probiotics). Each group comprised 5 replicates with 4 piglets per replicate, and diets were fed in powder form for 28 days. On day 8, piglets were vaccinated with O-type and A-type FMD vaccines, while serum and fresh feces were collected on days 7 and 28. The results showed: (1) Spirulina significantly increased average daily gain and decreased feed-to-gain ratio ($P<0.05$). (2) Spirulina had no significant effect on O-type and A-type FMD antibody titers on either day 7 or day 28 ($P>0.05$). (3) On day 28, Spirulina extremely significantly increased serum immunoglobulin G (IgG), immunoglobulin M (IgM) and complement 3 (C3) contents ($P<0.01$), but did not affect complement 4 ($P>0.05$). (4) Spirulina showed no significant influence on fecal *Lactobacillus* or *E. coli* counts on days 7 or 28 ($P>0.05$). Except for serum IgG, IgM and C3 contents on day 28, no interactions between Spirulina and probiotics were observed ($P>0.05$). These findings indicate that dietary supplementation with 0.5% Spirulina during FMD vaccination can promote piglet growth, and its combination with probiotics can enhance humoral immune performance.

Keywords: Spirulina; probiotics; piglets; foot and mouth disease; interaction

Introduction

As living standards continue to improve, the demand for "green food for humans and green feed for animals" has grown increasingly prominent. Spirulina (SPI) is a photoautotrophic microorganism rich in protein, polysaccharides, linoleic acid, docosahexaenoic acid (DHA), vitamins, pigments and minerals. Recognized by the World Health Organization and the Food and Agriculture Organization of the United Nations as "the most ideal food for humanity in the 21st century," SPI also contains physiologically active substances such as Spirulina polysaccharides, phycocyanin, -carotene, linoleic acid and inositol that exhibit antimicrobial, antioxidant and antiviral properties, making it widely consumed as a functional food. Recent advances in cultivating SPI using wastewater have substantially reduced production costs, drawing increased attention to its potential as a feed ingredient or additive.

The primary objectives of SPI supplementation in animal feed are to promote growth and enhance immunity. While SPI application in aquaculture has been proven to improve growth and immunity in fish and shrimp, its effects on swine production performance remain controversial, mainly concerning the efficacy differences between powder and pellet forms. Given that SPI contains antimicrobial and antiviral substances, its safety during vaccination periods remains unclear. Probiotics such as *Bacillus subtilis* and *Lactobacillus* are commonly used as green feed additives to promote growth, prevent diarrhea and enhance immunity in piglet diets. However, the compatibility and potential interactions between SPI and probiotics are poorly understood. Therefore, this study examined the effects of SPI alone and combined with probiotics containing *Bacillus subtilis* and *Lactobacillus* on growth performance, immune function and fecal flora in piglets vaccinated against foot-and-mouth disease (FMD), providing insights for feed resource development and healthy animal production.

Materials and Methods

1.2 Experimental Design

A 2×2 factorial design was employed, with eighty weaned piglets randomly allocated to four groups (5 replicates per group, 4 piglets per replicate) based on sex and body weight: control group (CG) fed basal diet, Spirulina group (SG) fed basal diet + 0.5% SPI, probiotics group (PG) fed basal diet + 0.5 kg/t probiotics, and Spirulina-probiotics group (SPG) fed basal diet + 0.5% SPI + 0.5 kg/t probiotics. The experimental period lasted 28 days.

1.3 Experimental Diets and Management

The basal diet was formulated as powder according to the Chinese Ministry of Agriculture standard "Feeding Standard of Swine" (NY/T 65—2004). Composition and nutrient levels are presented in Table 1 . All piglets were housed in the same facility with wire mesh separation between replicates, provided ad libitum access to feed and water, and managed under uniform procedures.

1.4 Measurements and Analytical Methods

All piglets were weighed individually at 08:00 on the first and last day of the trial after overnight fasting. Weekly feed intake was recorded per replicate to calculate initial weight (IW), final weight (FW), average daily gain (ADG), average daily feed intake (ADFI) and feed-to-gain ratio (F/G) (n=5). On day 7, two piglets per replicate were randomly selected for cardiac blood collection after fasting; serum was harvested by centrifugation at 3,000 r/min for subsequent analysis (n=10). On day 8, piglets were vaccinated with O-type and A-type FMD vaccines via intramuscular injection in the neck region at 1 mL per head. On day 28, the remaining two piglets per replicate underwent identical blood collection procedures (n=10). Fresh feces were collected from two randomly selected piglets per replicate at 12:00 on days 7 and 28, and *Lactobacillus* and *E. coli* counts were determined using MRS medium and eosin-methylene blue plate counting methods, respectively (n=10).

1.5 Statistical Analysis

All data were analyzed using the GLM procedure of SAS V9.1 software according to a two-factor model to calculate means and standard errors, and to evaluate the main effects of SPI, probiotics and their interactions. Duncan' s multiple range test was used for post-hoc comparisons. Differences were considered significant at $P<0.05$ and extremely significant at $P<0.01$. Results are expressed as means ± standard error.

Results

2.1 Effects of SPI on Growth Performance of Piglets

As shown in Table 2 , final weight, ADG and ADFI in the Spirulina, probiotics and Spirulina-probiotics groups were extremely significantly higher than those in the control group ($P<0.01$), while feed-to-gain ratio was significantly lower ($P<0.05$). No significant differences were observed among the three treatment groups ($P>0.05$). Both SPI and probiotics exerted significant effects on ADG and feed-to-gain ratio ($P<0.05$), with no significant interaction between them ($P>0.05$).

2.2 Effects of SPI on Antibody Titers of Piglets

Table 3 shows that neither SPI nor probiotics significantly affected O-type or A-type FMD antibody titers on days 7 or 28 ($P>0.05$), and no interaction was detected ($P>0.05$).

2.3 Effects of SPI on Serum Immunoglobulin Contents of Piglets

Regarding IgG, no significant differences were observed among groups on day 7 ($P>0.05$), whereas serum IgG contents on day 28 were extremely significantly higher in the Spirulina, probiotics and Spirulina-probiotics groups compared with the control group ($P<0.01$), with an extremely significant interaction between SPI and probiotics ($P<0.01$). For IgM, serum contents on both days 7 and 28 were extremely significantly higher in all three treatment groups versus the control group ($P<0.01$). For IgA, serum contents on day 7 were significantly higher in the Spirulina and Spirulina-probiotics groups compared with the control and probiotics groups ($P<0.05$), with no difference between the latter two ($P>0.05$). On day 28, IgA contents were significantly higher in all three treatment groups versus the control group ($P<0.05$), with no significant differences among them ($P>0.05$).

2.4 Effects of SPI on Serum Complement Contents of Piglets

As shown in Table 5 , serum C3 content showed no significant differences among groups on day 7 ($P>0.05$), but was extremely significantly lower in the control group than in the other three groups on day 28 ($P<0.01$), with a significant SPI $\times$ probiotics interaction ($P<0.05$). Serum C4 content did not differ significantly among groups on either day 7 or day 28 ($P>0.05$).

2.5 Effects of SPI on Fecal Microflora of Piglets

Table 6 reveals that *Lactobacillus* counts showed no significant differences between the control and Spirulina groups on days 7 or 28 ($P>0.05$), but both were significantly lower than the probiotics and Spirulina-probiotics groups ($P<0.05$). Similarly, *E. coli* counts showed no significant differences between the control and Spirulina groups on either sampling day ($P>0.05$), but both were significantly higher than the probiotics and Spirulina-probiotics groups ($P<0.05$). For both bacterial groups, probiotics showed significant effects ($P<0.05$) while SPI did not ($P>0.05$), and no interaction was observed ($P>0.05$).

Discussion

3.1 Effects of SPI on Growth Performance of Piglets

The observed improvement in ADG and reduction in feed-to-gain ratio with SPI powder supplementation align with findings by Lü et al. but contradict those of Grinstead et al. Lü et al. reported that SPI wet-mash feeding promoted piglet growth, whereas Grinstead et al. found no significant effect when SPI replaced soybean meal in pelleted diets. These discrepancies likely stem from differences in diet type and SPI' s role as a protein substitute. The lack of significant interaction between SPI and probiotics on ADG and feed-to-gain ratio suggests that SPI' s nutritional or functional components do not have additive effects with *Bacillus subtilis* and *Lactobacillus* on growth promotion, which differs from Ramakrishnan et al.' s report of synergistic growth enhancement in carp. This variation may be attributed to species differences. Since *Bacillus subtilis* and *Lactobacillus* primarily function by modulating intestinal flora, these results indicate that SPI' s growth-promoting mechanism differs from that of probiotics. SPI contains balanced profiles of 17 amino acids, and its polysaccharides, linoleic acid, DHA and other nutrients all contribute to growth promotion, though the primary active components require further investigation.

3.2 Effects of SPI on Immune Performance of Piglets

Due to potential adverse effects of antiviral drugs on vaccines, their use is generally discouraged for four weeks before and after vaccination. Although SPI may contain antiviral and antimicrobial substances, its impact on FMD antibodies in pigs has not been reported. This study demonstrated that SPI did not negatively affect the production of A-type or O-type FMD antibodies either pre- or post-vaccination, indicating its compatibility with viral vaccines and supporting its application during piglet immunization. Daoud et al. reported that safe doses of SPI ethanol extract reduced the virulence of O-type, A-type and SAT2-type FMD viruses in mice, indirectly confirming SPI' s safety for use in piglets.

Since IgG, IgM and IgA are the primary antibodies involved in humoral immunity, their concentrations reflect the body' s humoral immune status. The increased serum IgG, IgM and IgA contents observed in this study suggest that SPI enhances immunity primarily through humoral mechanisms, consistent with Qureshi et al.' s findings in broilers. Complement comprises a group of immunologically relevant, enzymatically active globulins in serum that assist and supplement specific antibodies to mediate immunity and bacterial lysis. While SPI' s effects on porcine complement have not been reported, the increased serum C3 content observed post-FMD vaccination in this study corresponds with elevated IgG levels, suggesting that SPI may amplify humoral immune responses through the alternative pathway to modulate immune responses.

The significant interactions between SPI and probiotics on IgG, IgM and C3 contents after immunization warrant further investigation. The observed enhancement of serum IgG and C3 by probiotics aligns with findings by Zhu et al. Both *Bacillus subtilis* and SPI can stimulate immune cells including T cells, B cells and macrophages or induce mucosal immune responses, suggesting the interaction may involve cell-mediated or mucosal immunity.

3.3 Effects of SPI on Fecal Microflora of Piglets

The lack of SPI effect on intestinal microflora in this study contrasts with findings by Dou et al. However, this experiment involved normal piglets under conventional rearing conditions, whereas Dou et al. used SPI to treat diarrheal model mice. The absence of interaction between SPI and probiotics on piglet microflora also differs from Liang et al., who conducted in vitro assessments of SPI' s effects on different bacterial groups. These results indirectly confirm that SPI does not enhance growth performance or immune function through intestinal microflora modulation, indicating distinct mechanisms of action from probiotics.

Given that SPI contains numerous physiologically active substances with growth-promoting, antimicrobial, antiviral and antioxidant properties, interactions among these compounds may occur, necessitating individual investigation of their effects on piglet growth and immunity. Additionally, the cost implications of SPI supplementation must be considered. The food-grade SPI cultivated in spring water used in this study costs over 1,000 yuan/kg, increasing feed cost by more than 5.0 yuan/kg and substantially raising overall feeding expenses, which may limit practical application. However, as wastewater cultivation technology matures and SPI production costs decline, broader adoption of SPI-supplemented diets may become feasible.

Conclusions

1. Dietary supplementation with 0.5% SPI during FMD vaccination improves piglet growth performance without adversely affecting FMD antibody production.
2. SPI combined with probiotics containing *Bacillus subtilis* and *Lactobacillus* synergistically enhances humoral immune function in FMD-vaccinated piglets, though their mechanisms of action differ regarding growth promotion, immune modulation and intestinal microflora regulation.
3. Powder-form diets supplemented with SPI are recommended for piglet feeding.

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