

Effects of Flaxseed Oil on Intestinal Mucosal Structure and Immune Cells in LPS-Challenged Weaned Piglets (Postprint)

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Abstract

This experiment aimed to investigate the effects of flaxseed oil on intestinal mucosal structure and immune cells in LPS-challenged weaned piglets. Twenty-four (9.15 ± 0.90) kg Duroc×Landrace×Large White weaned piglets were selected and randomly divided into 4 groups with 6 replicates per group and 1 pig per replicate. The experiment adopted a 2×2 factorial design, with the main factors including: 1) dietary treatment (5% corn oil or 5% flaxseed oil); 2) immune challenge (saline or LPS injection). Diets supplemented with 5% corn oil or 5% flaxseed oil were fed for 21 days. On day 21 of the experiment, half of the pigs in each group received an intraperitoneal injection of 100 g/kg BW LPS, while the other half received an equal volume of saline. Four hours after LPS or saline injection, the piglets were anesthetized and slaughtered, and intestinal samples were collected for analysis. The results showed: 1) LPS challenge significantly decreased villus height in the jejunum and ileum and crypt depth in the jejunum ($P < 0.05$), significantly increased lamina propria cell density and neutrophil count in the jejunum ($P < 0.05$), and tended to decrease intraepithelial lymphocyte count in the jejunum and goblet cell count in the ileum ($P < 0.10$); 2) LPS challenge had no significant effect on villus height in the jejunum and ileum of piglets in the flaxseed oil group ($P > 0.05$); dietary supplementation with 5% flaxseed oil tended to increase Peyer's patch cell density in the ileum ($P < 0.10$), and under LPS challenge, significantly decreased lamina propria cell density in the jejunum ($P < 0.05$) and tended to increase intraepithelial lymphocyte count in the jejunum ($P < 0.10$). These results suggest that 5% flaxseed oil can, to a certain extent, maintain small intestinal mucosal structure and immune function in LPS-challenged weaned piglets.

Full Text

Effects of Flaxseed Oil on Intestinal Mucosa Structure and Immune Cells of Weaned Piglets after Lipopolysaccharide Challenge

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Abstract: This study investigated the effects of flaxseed oil on intestinal mucosa structure and immune cells in weaned piglets challenged with lipopolysaccharide (LPS). Twenty-four Duroc × Landrace × Yorkshire weaned piglets with an average body weight of (9.15 ± 0.90) kg were randomly assigned to four groups, with six replicates per group and one pig per replicate. The experiment employed a 2×2 factorial design, with the main factors being: 1) dietary treatment (5% corn oil or 5% flaxseed oil) and 2) immune challenge (saline or LPS injection). After 21 days of feeding diets supplemented with 5% corn oil or 5% flaxseed oil, half of the pigs in each group were intraperitoneally injected with 100 g/kg BW LPS, while the other half received an equivalent volume of saline. Four hours post-injection, the piglets were anesthetized and slaughtered, and intestinal samples were collected for analysis. The results showed that: 1) LPS challenge significantly reduced villus height in the jejunum and ileum and crypt depth in the jejunum ($P < 0.05$), significantly increased lamina propria cell density and neutrophil count in the jejunum ($P < 0.05$), and tended to decrease intraepithelial lymphocyte count in the jejunum and goblet cell count in the ileum ($P < 0.10$). 2) LPS challenge did not significantly affect villus height in the jejunum and ileum of piglets in the flaxseed oil group ($P > 0.05$). Dietary supplementation with 5% flaxseed oil tended to increase Peyer's patch cell density in the ileum ($P < 0.10$) and, under LPS challenge, significantly reduced lamina propria cell density in the jejunum ($P < 0.05$) while tending to increase intraepithelial lymphocyte count in the jejunum ($P < 0.10$). These findings suggest that 5% flaxseed oil can partially maintain small intestinal mucosal structure and immune function in LPS-challenged weaned piglets.

Keywords: flaxseed oil; weaned piglets; lipopolysaccharide; intestinal mucosal immune cells

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Introduction

The intestine serves as the primary digestive organ and the largest defense barrier in the body, effectively protecting against invasion by harmful substances

such as bacteria, viruses, and foreign proteins. Early weaning, heat stress, bacteria, viruses, and foreign proteins can damage the intestinal mucosal barrier function, leading to increased intestinal permeability and allowing harmful substances to cross the mucosa into the circulatory system, thereby damaging tissues and organs and ultimately causing slow growth or even death in piglets. Therefore, nutritional modulation to improve intestinal mucosal structure and function is crucial for maintaining gut health.

Flaxseed oil, mainly produced in Inner Mongolia, Shanxi, and Gansu provinces, is rich in α -linolenic acid (ALA). Research has shown that ALA can be converted into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are active components of fish oil. Numerous studies have found that EPA and DHA can effectively improve intestinal mucosal barrier function and exert anti-inflammatory effects in animals. However, the role of flaxseed oil in intestinal mucosal barrier function remains controversial. Therefore, this experiment used lipopolysaccharide (LPS) to establish an intestinal damage model and investigated whether flaxseed oil provides protective effects against LPS-induced intestinal mucosal structural and functional damage in piglets.

Materials and Methods

1.1 Experimental Animals and Design

Based on body weight $[(9.15 \pm 0.90) \text{ kg}]$, a completely randomized block design was used to allocate 24 Duroc \times Landrace \times Yorkshire crossbred weaned piglets into four groups, with six replicates per group and one pig per replicate. The experiment employed a 2×2 factorial design, with the main factors being: 1) dietary treatment (5% corn oil or 5% flaxseed oil) (corn oil purchased from Shandong Xiwang Food Co., Ltd.; flaxseed oil purchased from Gansu Longyu Sesame Oil Industry Co., Ltd.) and 2) immune challenge (saline or LPS injection) (LPS purchased from Sigma, E. coli serotype O55:B5). The four resulting groups were: 1) 5% corn oil + saline group (Group I); 2) 5% corn oil + LPS group (Group II); 3) 5% flaxseed oil + saline group (Group III); and 4) 5% flaxseed oil + LPS group (Group IV).

Piglets were fed the experimental diets supplemented with 5% corn oil or 5% flaxseed oil for 21 days. On day 21, piglets in Groups II and IV were intraperitoneally injected with 100 g/kg BW LPS, while those in Groups I and III received an equivalent volume of saline. The basal diet was formulated according to NRC (1998) standards, and its composition and nutrient levels are shown in Table 1. The fatty acid composition of the corn oil and flaxseed oil used in the experiment is presented in Table 2.

1.2 Sample Collection

LPS is a membrane component of Gram-negative bacteria with strong toxicity to animals. Studies have shown that LPS injection causes acute intestinal

morphological damage and collapse of intestinal barrier function within 3–6 hours. Therefore, this experiment selected the 4-hour post-injection time point for sampling. On the morning of day 21, four hours after saline or LPS injection, piglets were anesthetized via intramuscular injection of pentobarbital sodium (80 mg/kg BW) in the neck, then slaughtered. The middle segments of the jejunum and ileum were rapidly removed and fixed in 4% paraformaldehyde for hematoxylin-eosin (HE) staining.

1.3 Histological Analysis

1.3.1 Morphological Analysis Intestinal tissue sections were examined under an Olympus optical microscope at 10×10 magnification. Ten longest and intact villi were selected from each section to measure villus height (vertical height from villus tip to crypt opening) and crypt depth (distance from crypt base to crypt opening). The average values were calculated, and the villus height/crypt depth ratio was determined.

1.3.2 Intraepithelial Lymphocyte and Goblet Cell Counting Under an Olympus optical microscope at 40×10 magnification, six intact villi were selected from each section. Intestinal epithelial cells, intraepithelial lymphocytes, and goblet cells within the villi and crypts were counted, and average values were calculated to determine the intraepithelial lymphocyte/epithelial cell ratio and goblet cell/epithelial cell ratio.

1.3.3 Neutrophil Counting At 40×10 magnification, six lamina propria fields were selected from each section. The area of each field was measured, and neutrophils in the field were counted. The average value was calculated to determine neutrophil density in the section.

1.3.4 Lamina Propria Cell Counting At 40×10 magnification, six intact villi were selected from each section. The lamina propria area on each villus was measured, and cells within the lamina propria were counted. The average value was calculated to determine lamina propria cell density in the section.

1.3.5 Ileal Peyer' s Patch Cell Counting Ileal tissue sections were examined at 40×10 magnification. Six fields were selected from each section, the area of each field was measured, and Peyer' s patch cells in the field were counted. The average value was calculated to determine Peyer' s patch cell density in the section.

1.4 Statistical Analysis

Experimental data were analyzed using SPSS 17.0 software for two-way ANOVA. The model included main effects of diet treatment, immune challenge, and their interaction. When interactions were significant, Duncan' s multiple comparison

test was applied. Significance was declared at $P < 0.05$, and trends were noted at $P < 0.10$.

Results

2.1 Effects of Flaxseed Oil on Growth Performance of Weaned Piglets

As shown in Table 3, there were no significant differences in growth performance between piglets fed diets containing 5% corn oil or 5% flaxseed oil ($P > 0.05$).

2.2 Effects of Flaxseed Oil on Intestinal Mucosal Structure of LPS-Challenged Weaned Piglets

The effects of flaxseed oil on intestinal mucosal structure of LPS-challenged weaned piglets are shown in Figure 1 [Figure 1: see original paper] and Table 4. Figure 1 shows that LPS injection caused villus atrophy and epithelial shedding in the small intestine. Table 4 indicates that LPS challenge significantly reduced villus height in the jejunum and ileum and crypt depth in the jejunum of the corn oil group ($P < 0.05$), but did not significantly affect villus height in the jejunum and ileum of the flaxseed oil group ($P > 0.05$).

2.3 Effects of Flaxseed Oil on Intestinal Mucosal Immune Cells of LPS-Challenged Weaned Piglets

2.3.1 Intraepithelial Lymphocytes Intraepithelial lymphocytes are a unique cell population residing in the intestinal mucosal epithelium, characterized by large, round nuclei and scant, deeply stained cytoplasm (Figure 2 [Figure 2: see original paper]). Table 5 shows that LPS challenge tended to reduce intraepithelial lymphocyte count in the jejunum ($P < 0.10$). A trend for interaction between flaxseed oil and LPS was observed ($P < 0.10$), indicating that 5% flaxseed oil tended to alleviate the LPS-induced decrease in intraepithelial lymphocyte count in both the jejunum and ileum.

2.3.2 Neutrophils Neutrophils are immune cells derived from hematopoietic stem cells in bone marrow, with irregular morphology, polymorphonuclear lobulated nuclei, and purple-red cytoplasm (Figure 3 [Figure 3: see original paper]). Table 5 shows that LPS challenge significantly increased neutrophil count in the jejunum ($P < 0.05$). Flaxseed oil had no significant effect on neutrophil count in the jejunum or ileum ($P > 0.05$), and no interaction between flaxseed oil and LPS was observed for neutrophil count ($P > 0.10$).

2.3.3 Goblet Cells Goblet cells are secretory cells that secrete mucus and are interspersed among intestinal epithelial columnar cells, appearing columnar or conical in shape (Figure 2). Table 5 shows that LPS challenge tended to reduce goblet cell count in the ileum ($P < 0.10$). Flaxseed oil had no significant effect on goblet cell count in the jejunum or ileum ($P > 0.05$), and no interaction between flaxseed oil and LPS was observed for goblet cell count ($P > 0.10$).

2.3.4 Lamina Propria Cell Density The lamina propria contains numerous lymphocytes, goblet cells, and neutrophils (Figure 2). Table 6 shows that LPS challenge significantly increased lamina propria cell density in the jejunum ($P < 0.05$). A significant interaction between flaxseed oil and LPS was observed ($P < 0.05$), indicating that 5% flaxseed oil significantly alleviated the LPS-induced increase in lamina propria cell density in the jejunum.

2.3.5 Ileal Peyer' s Patch Cell Density Peyer' s patches are important components of the intestinal mucosal immune system, representing groups of lymphoid follicles in the small intestinal mucosa that serve as inductive sites for mucosal immunity. Table 6 shows that dietary supplementation with 5% flaxseed oil tended to increase Peyer' s patch cell density in the ileum ($P < 0.10$). LPS challenge did not significantly affect Peyer' s patch cell density in the ileum ($P > 0.05$), and no interaction between flaxseed oil and LPS was observed for this parameter ($P > 0.10$).

Discussion

The small intestine is not only the primary site for digestion and absorption but also the first barrier against harmful substances such as bacteria, viruses, and endogenous proteins, preventing their translocation across the intestinal mucosa and protecting animal health. Flaxseed oil is rich in ALA, which can be partially converted to EPA and DHA in vivo. Studies have shown that EPA and DHA can effectively improve intestinal mucosal barrier function in animals. Our previous research also found that fish oil (rich in EPA and DHA) protects the intestine by inhibiting production of intestinal inflammatory cytokines. However, the role of flaxseed oil in intestinal mucosal barrier function remains controversial. Therefore, we hypothesized that flaxseed oil might have similar effects to fish oil. This experiment established an intestinal damage model by intraperitoneal LPS injection in piglets to investigate the protective effects of dietary 5% flaxseed oil against LPS-induced intestinal structural damage.

Intestinal morphology is a primary indicator of small intestinal structural and functional integrity, including villus height, crypt depth, and their ratio. After intestinal mucosal damage, villi exhibit atrophy, shedding, and increased crypt depth, accompanied by decreased villus height/crypt depth ratio. In this study, LPS challenge significantly reduced intestinal villus height, indicating that LPS damaged intestinal mucosal structure, consistent with findings by Pi et al. and Touchette et al. Additionally, dietary 5% flaxseed oil showed significant interaction with LPS challenge, suggesting that flaxseed oil provides protective effects against LPS-induced intestinal damage. Similar to our results, Jonecova et al. reported that flaxseed oil alleviates intestinal morphological damage in piglets. However, dietary supplementation with 5% corn oil or 5% flaxseed oil did not significantly affect growth performance, likely because flaxseed oil protects intestinal morphology primarily under stress and inflammatory conditions, while not increasing feed intake under normal feeding conditions.

The intestinal mucosal barrier comprises three components: the epithelial barrier, biological barrier, and immune barrier. The epithelial barrier consists of intact intestinal epithelial cells and tight junctions between them. The biological barrier is formed by normal microbiota in dynamic equilibrium. The immune barrier includes intestinal Peyer's patches, mucosal epithelial cells, lamina propria lymphocytes, and other immune cells. The integrity of these three barriers determines the overall integrity of intestinal mucosal barrier function, which is essential for maintaining gut health.

Intraepithelial lymphocytes are immune cells that suppress intestinal mucosal hypersensitivity reactions, neutralize harmful substances produced by bacteria and viruses, and secrete lymphokines, playing a critical role in intestinal mucosal immune barrier protection. Reduced intraepithelial lymphocyte count is often associated with damaged small intestinal morphology. This study showed that LPS challenge decreased intraepithelial lymphocyte count in the jejunum. Maeshima found that dietary fish oil alleviated nutrition-induced reduction of intraepithelial lymphocytes. Furthermore, n-3 polyunsaturated fatty acids promote proliferation and repair of intestinal mucosal epithelial cells and maintain intestinal mucosal integrity during inflammatory damage. Our study found that dietary 5% flaxseed oil tended to alleviate the LPS-induced decrease in intraepithelial lymphocyte count, possibly because ALA in flaxseed oil is converted to EPA and DHA, which promote proliferation and differentiation of intraepithelial lymphocytes, thereby protecting intestinal mucosal structural integrity and immune function.

Neutrophils are immune cells with both phagocytic and bactericidal functions and strong chemotactic activity. During inflammation, neutrophils accumulate at inflammatory sites and participate in inflammatory responses. In this study, LPS challenge increased neutrophil count in the jejunum, likely due to excessive production of inflammatory cytokines in the intestine after LPS stimulation. Studies have shown that flaxseed oil has anti-inflammatory effects, but our results showed no significant effect of flaxseed oil on neutrophil count, and the underlying mechanism remains unclear and warrants further investigation.

Goblet cells are secretory cells interspersed among intestinal mucosal epithelium that secrete glycoproteins to coat the epithelial surface, blocking damage to the intestinal mucosa by bacteria and viruses through adhesion while providing lubrication and protection. Rogers reported that bacteria, viruses, allergens, and oxygen free radicals can induce massive proliferation of goblet cells. This study showed that LPS challenge tended to reduce goblet cell count in the ileum, consistent with Ren's finding that LPS challenge decreases ileal goblet cell count, possibly due to LPS-induced damage to intestinal mucosal epithelium.

Peyer's patches contain numerous immune-active cells, mainly B cells, T cells, and antigen-presenting cells, which play important roles in inducing immune-active cells to migrate to intestinal mucosal effector sites (intestinal mucosal epithelium and lamina propria) to exert immune functions. This study showed that dietary 5% flaxseed oil tended to increase Peyer's patch cell density in the

ileum, possibly by promoting proliferation of Peyer' s patch immune cells and protecting the integrity of intestinal mucosal barrier function. This suggests that flaxseed oil can enhance intestinal mucosal immune barrier function to some extent. Additionally, during inflammation, Peyer' s patch cells proliferate massively. We found that flaxseed oil could also alleviate the LPS-induced increase in Peyer' s patch cells, indicating that flaxseed oil alleviates LPS-induced intestinal mucosal inflammatory responses and maintains mucosal immune function.

The lamina propria is the primary site of intestinal mucosal immune responses, containing numerous lymphocytes, dendritic cells, goblet cells, mast cells, macrophages, and neutrophils. In gastrointestinal mucosal tissue, lymphocytes are mainly concentrated in the lamina propria. Smith et al. found that earlier weaning age causes intestinal mucosal inflammation and increases lamina propria cell density. In this study, LPS challenge significantly increased lamina propria cell density in the jejunum, mainly due to inflammatory responses in the intestinal mucosa and damage to mucosal barrier function, leading to massive proliferation and differentiation of lymphocytes, consistent with findings by Li et al. Dietary 5% flaxseed oil significantly reduced lamina propria cell density in LPS-challenged weaned piglets, indicating that flaxseed oil can alleviate LPS-induced inflammatory responses and mitigate damage to mucosal immune function.

Conclusion

LPS challenge can damage intestinal mucosal morphology, structure, and immune function in piglets. Dietary supplementation with 5% flaxseed oil can partially maintain small intestinal mucosal structure and immune function in LPS-challenged weaned piglets.

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