Effects of Macromolecular Protein and Peptide Content in Fermented Soybean Meal on Small Intestinal Villus Structure in Piglets (Postprint)

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Abstract

This experiment was conducted to investigate the effects of macromolecular protein and peptide contents in soybean meal on the intestinal villus structure of piglets. Gel filtration chromatography was employed to analyze the macromolecular protein and peptide contents in soybean meal. Forty weaned piglets were selected and randomly assigned to four treatments: dehulled soybean meal, fermented soybean meal C, fermented soybean meal E, and animal protein. The experimental period lasted for 5 weeks. The results demonstrated that the macromolecular protein content in dehulled soybean meal accounted for approximately 80%, whereas in fermented soybean meal it was approximately 50%, even decreasing to 24%; the peptide content in fermented soybean meal was approximately 19%, compared to only 2.7% in dehulled soybean meal. Compared with the dehulled soybean meal treatment, the fermented soybean meal C treatment significantly increased duodenal villus height ($P<0.05$); the fermented soybean meal E and C treatments significantly decreased duodenal and jejunal crypt depth ($P<0.05$); and the fermented soybean meal E and C treatments significantly elevated the villus height/crypt depth ratio in both duodenum and jejunum ($P<0.05$). These findings indicate that the macromolecular protein content in soybean meal is negatively correlated with the villus height/crypt depth ratio in the small intestine; fermented soybean meal can be utilized in weaned piglet diets and effectively improves the intestinal villus structure of weaned piglets.

Full Text

Effects of Macromolecular Protein and Peptide Content in Fermented Soybean Meal on Intestinal Villus Structure in Piglets

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Abstract: This study investigated the effects of macromolecular protein and peptide content in fermented soybean meal on the intestinal villus structure of piglets. Gel filtration chromatography (GFC) was used to analyze the macromolecular protein and peptide content in soybean meal. Forty weaned piglets were randomly allocated to four treatments: dehulled soybean meal, fermented soybean meal C, fermented soybean meal E, and animal protein. The experiment lasted for five weeks. The results showed that macromolecular protein accounted for approximately 80% of protein content in dehulled soybean meal, but only about 50% in fermented soybean meal, with some samples dropping to 24%. Peptide content in fermented soybean meal was approximately 19%, compared to just 2.7% in dehulled soybean meal. Compared with the dehulled soybean meal treatment, fermented soybean meal C significantly increased duodenal villus height ($P<0.05$), while both fermented soybean meal E and C significantly decreased crypt depth in the duodenum and jejunum ($P<0.05$). Both fermented soybean meal treatments also significantly increased the villus height-to-crypt depth ratio (V/C) in the duodenum and jejunum ($P<0.05$). These findings indicate that macromolecular protein content in soybean meal is negatively correlated with the V/C ratio in the small intestine, and that fermented soybean meal can be effectively used in weaned piglet diets to improve small intestinal villus structure.

Keywords: fermented soybean meal; gel filtration chromatography; intestinal villus structure

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In recent years, the rapid development of animal husbandry has led to increasing demand for feed ingredients, particularly protein sources. However, animal-derived protein resources are scarce, expensive, and pose safety concerns, prompting a shift toward plant-based protein sources such as fermented soybean meal. Research has shown that fermentation reduces anti-nutritional factors in soybean meal and degrades some macromolecular proteins into peptides and amino acids, thereby mitigating adverse effects on the intestinal villus structure of weaned piglets. Some macromolecular proteins are even degraded into more readily absorbable small peptides. The differential content of macromolecular proteins and peptides represents a key distinction between dehulled and fermented soybean meals. This study selected two types of fermented soybean meal, using dehulled soybean meal and animal protein as controls, to analyze

macromolecular protein and peptide content via gel filtration chromatography (GFC) and examine their effects on intestinal villus height, crypt depth, and the V/C ratio. The objective was to provide a data foundation and scientific basis for the widespread application of fermented soybean meal in feed formulations.

1.1 Analysis of Soybean Meal Protein Content by GFC

Materials: Dehulled soybean meal, fermented soybean meal C, fermented soybean meal E, and other soybean products were collected from Shenyang Boeing Feed Co., Ltd. The main instruments included an Agilent 1260 high-performance liquid chromatograph and an FD-1100 freeze dryer. Standard reagents such as bovine serum albumin and cytochrome c were purchased from Beijing Putian Tongchuang Biological Technology Co., Ltd.

GFC Procedure: Chromatographic conditions were analyzed according to . Standard retention times (t) were recorded to generate a standard curve. The peptide content distribution in soybean meal samples was calculated by combining sample peak areas (S) with the standard curve equation.

Standard Analysis Results: Standard names and retention times are presented in . When the retention time was approximately 10 minutes, all three types of soybean meal showed significant absorption peaks (FIGURE:1, FIGURE:2, FIGURE:3). As shown in , proteins with molecular weights around 37 kDa accounted for approximately 80% of protein content in dehulled soybean meal, but only 50% in fermented soybean meal, with some samples decreasing to 24%. Conversely, peptide content in fermented soybean meal reached 19%, compared to merely 2.7% in dehulled soybean meal.

1.2 Experimental Design

Based on the GFC analysis results, three products were selected with descending macromolecular protein content: dehulled soybean meal, fermented soybean meal E, and fermented soybean meal C. Forty crossbred piglets (Duroc × Landrace × Large White) weaned at 28 days with an average weight of 10 kg were randomly divided into four treatments with three replicates each (three piglets per replicate, except one replicate with four piglets). The dehulled soybean meal treatment served as the negative control (plant protein source: dehulled soybean meal). Fermented soybean meal C and E treatments were experimental groups (plant protein sources: fermented soybean meal C and E, respectively). The animal protein treatment served as the positive control. Diets were formulated according to NRC (1998) standards for piglets and nursery pigs, combined with production practices, using isoprotein replacement based on the total protein content of dehulled soybean meal . The experiment lasted five weeks with ad libitum feeding and routine management.

1.3 Measurement Indicators and Methods

During the experimental period, daily feed intake and diarrhea rate (defined as feces with paste-like or watery consistency) were recorded. At the end of the trial, piglets were weighed to calculate average daily feed intake (ADFI), average daily gain (ADG), feed-to-gain ratio (F/G), and diarrhea rate. Calculations were as follows: ADG = (final average body weight - initial average body weight) / experimental days; ADFI = (total initial feed amount - remaining feed amount at trial end) / experimental days; F/G = ADFI / ADG; Diarrhea rate (%) = (number of piglet diarrhea episodes / experimental days) × 100. At the conclusion of the experiment, two piglets per treatment were randomly selected for slaughter. Five-centimeter segments of duodenum, jejunum, and ileum were excised, rinsed with phosphate buffer, and 1–2 cm middle sections were fixed in 10% formalin solution for paraffin section preparation and imaging.

1.4 Data Analysis

Experimental data were initially organized using Excel 2007, followed by one-way ANOVA using SPSS 18.0 software. Significant differences were further analyzed using LSD multiple comparison tests, with $P<0.05$ as the significance threshold. Results are expressed as "mean ± standard deviation."

2 Results

2.1 Effects of Experimental Treatments on Growth Performance of Piglets

As shown in , experimental treatments influenced ADFI, ADG, and diarrhea rate, but had no significant effect on feed-to-gain ratio ($P>0.05$). Compared with the dehulled soybean meal treatment, fermented soybean meal E and C significantly increased ADFI and ADG ($P<0.05$) while significantly reducing diarrhea rate ($P<0.05$).

2.2 Effects of Experimental Treatments on Intestinal Villus Height of Piglets

Experimental treatments did not significantly affect ileal villus height ($P>0.05$) . Compared with dehulled soybean meal, fermented soybean meal C significantly increased duodenal villus height ($P<0.05$), while fermented soybean meal E showed no significant difference ($P>0.05$). Compared with animal protein, fermented soybean meal E significantly decreased duodenal villus height ($P<0.05$), whereas fermented soybean meal C showed no significant difference.

2.3 Effects of Experimental Treatments on Small Intestinal Crypt Depth of Piglets

Dehulled soybean meal treatment resulted in the greatest crypt depth, followed by fermented soybean meal E and C treatments, with animal protein treatment

showing the smallest crypt depth . Compared with dehulled soybean meal, fermented soybean meal E and C significantly decreased crypt depth in the duodenum and jejunum ($P<0.05$), but not in the ileum ($P>0.05$). Compared with animal protein, fermented soybean meal E and C significantly increased duodenal crypt depth ($P<0.05$).

2.4 Effects of Experimental Treatments on Small Intestinal V/C Ratio of Piglets

Compared with dehulled soybean meal, fermented soybean meal E and C significantly increased the V/C ratio in the duodenum and jejunum ($P<0.05$), while animal protein significantly increased the V/C ratio in all three intestinal segments ($P<0.05$) .

3 Discussion

3.1 Effects of Experimental Treatments on Growth Performance of Piglets

The beneficial effects of fermented soybean meal on piglet growth performance can be attributed to two main factors: first, reduced anti-nutritional factor content diminishes anti-nutritional effects and lessens harm to physiological functions; second, increased content of bioactive substances such as small molecular proteins, peptides, and amino acids enhances digestibility and absorption, promoting animal growth. In this study, fermented soybean meal C increased ADFI and ADG by 5.06% and 5.29%, respectively, while reducing diarrhea rate by over 19.27%, demonstrating that fermented soybean meal treatment improves piglet growth performance.

3.2 Analysis of Soybean Meal Protein Content by GFC

GFC technology separates proteins based on molecular size using porous gel particles as the chromatographic medium, with larger molecules eluting first, followed by medium-sized and small molecules. Separated proteins are detected sequentially by a UV detector, forming chromatographic peaks. System integration yields percentage content of each molecular weight fraction. In this study, macromolecular proteins (average molecular weights approximately 38, 37, and 35 kDa) accounted for 79.54%, 51.10%, and 24.27% of protein content in dehulled soybean meal, fermented soybean meal E, and fermented soybean meal C, respectively. Peptide content (average molecular weight ranges of 372–125 u, 572–157 u, and 601–198 u, respectively) was 2.74%, 13.27%, and 19.28%. Both fermented soybean meals had lower macromolecular protein content and higher peptide content compared with dehulled soybean meal.

3.3 Effects of Experimental Treatments on Intestinal Villus Structure of Piglets

The integrity of small intestinal structure and function is prerequisite for effective nutrient absorption. Villus height, crypt depth, and their ratio are important indicators of intestinal functional status. Villus height reflects nutrient absorption capacity, and decreased height indicates reduced absorptive function. Shallower crypt depth suggests healthy intestinal cell growth, while the V/C ratio comprehensively reflects intestinal functional status and digestive enzyme activity. In young animals with immature intestinal development, some dietary macromolecular proteins can enter blood and lymphatic tissues intact, stimulating production of immunoglobulin A (IgA) and immunoglobulin E (IgE). These antibodies form antigen-antibody complexes that trigger immune responses and cause immune damage, reducing villus height and enzyme secretion. Additionally, activated immune effector cells and macrophages can cause intestinal tissue damage through cytokine secretion or direct target cell attack, resulting in villus atrophy and increased crypt depth.

Plant protein sources contain numerous anti-nutritional factors, including trypsin inhibitors, phytic acid, and antigenic proteins, with soy antigen proteins causing the most severe intestinal damage in young animals. Fermented soybean meal effectively improves small intestinal villus structure, with effects positively correlated with inclusion level. Previous studies reported that fermented soybean meal increased intestinal villus height by over 2.24% and decreased crypt depth by over 13.27% in piglets. In this study, compared with dehulled soybean meal, animal protein treatment increased villus height, decreased crypt depth, and increased the V/C ratio, indicating beneficial effects on intestinal villus structure. Fermented soybean meal C increased duodenal villus height by 10.96%, decreased duodenal and jejunal crypt depth by 16.38% and 20.63%, respectively, and increased V/C ratios in the duodenum, jejunum, and ileum by 32.05%, 31.76%, and 18.42%, respectively. Fermented soybean meal E decreased duodenal and jejunal crypt depth by 13.93% and 18.03%, respectively, and increased V/C ratios in the duodenum and jejunum by 18.59% and 24.71%, respectively. These results demonstrate that fermented soybean meal can increase villus height, reduce crypt depth, and improve the V/C ratio, thereby effectively enhancing small intestinal villus structure in piglets.

Macromolecular protein content in soybean meal is negatively correlated with the small intestinal V/C ratio. Compared with dehulled soybean meal, fermented soybean meal contains lower macromolecular protein and higher peptide content, which effectively increases villus height, reduces crypt depth, and improves the V/C ratio, ultimately enhancing intestinal villus structure and promoting growth performance in weaned piglets.

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