# Effects of replacing fish meal with fermented silkworm pupa and soybean meal powder on growth performance, serum biochemical indices, and organ indices in weaned piglets: Postprint

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## Abstract

This experiment aimed to investigate the effects of replacing fish meal with fermented silkworm pupa-soybean meal powder on growth performance, serum biochemical indices, and organ indices of weaned piglets, and to determine the appropriate replacement level. One hundred twenty healthy "Duroc × Landrace × Yorkshire" three-way crossbred piglets, $(28\$\pm1)days old at weaning with an initial body weight of (7.64\pm\$0.67)$ kg, were selected and allocated into 4 groups with 3 replicates per group and 10 piglets per replicate. The control group was fed a basal diet, while the three experimental groups had 25%, 50%, and 100% of the fish meal in the basal diet replaced with fermented silkworm pupa-soybean meal powder, respectively. The pre-experimental period lasted 3 days, and the formal experimental period lasted 21 days. The results showed: 1) No significant differences were observed in overall average daily gain, average daily feed intake (ADFI), and feed-to-gain ratio among all groups ($P>0.05$); however, the ADFI of the control group in week 1 was significantly higher than that of the 25% replacement group ($P<0.05$), and the ADFI of both the 50% and 100% replacement groups in week 2 was significantly higher than that of the 25% replacement group ($P<0.05$). 2) The serum total cholesterol content of the 25% replacement group was significantly higher than that of the control group ($P<0.05$); the serum high-density lipoprotein cholesterol content of the 25% replacement group was significantly higher than that of the control group and the 50% replacement group ($P<0.05$); the serum apolipoprotein B content of the 25% replacement group was significantly higher than that of the 50% replacement group ($P<0.05$); and the serum total protein content of the control group was significantly higher than that of the 50% and 100% replacement groups

(P<0.05). 3) No significant differences were found in diarrhea rate, organ indices, or serum immunoglobulin content among all groups (P>0.05). Based on comprehensive evaluation of all indices, fermented silkworm pupa-soybean meal powder can replace 25% of fish meal in the diet without affecting the growth performance of weaned piglets.

## Full Text

# Effects of Replacement of Fish Meal by Fermented Silkworm Pupa and Soybean Meal on Growth Performance, Serum Biochemical Indexes and Organ Indexes of Weaned Piglets

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### Abstract

This study investigated the effects of replacing fish meal with fermented silkworm pupa and soybean meal on growth performance, serum biochemical indexes, and organ indexes of weaned piglets, and determined the appropriate replacement level. One hundred and twenty healthy Duroc × Landrace × Large crossbred piglets weaned at (28$±1)*daysofagewithaninitialbodyweightof*(7.64±$0.67) kg were selected and divided into 4 groups with 3 replicates per group and 10 piglets per replicate. Piglets in the control group were fed a basal diet, while those in the three experimental groups were fed diets in which 25%, 50%, and 100% of the fish meal in the basal diet was replaced with fermented silkworm pupa and soybean meal. The experiment consisted of a 3-day preliminary period followed by a 21-day formal trial period. The results showed that: (1) No significant differences were observed among groups in average daily gain (ADG), average daily feed intake (ADFI), or feed-to-gain ratio (F/G) throughout the entire experimental period (P>0.05). However, the ADFI of the control group during week 1 was significantly higher than that of the 25% replacement group (P<0.05), while the ADFI of the 50% and 100% replacement groups during week 2 was significantly higher than that of the 25% replacement group (P<0.05). (2) The serum total cholesterol content of the 25% replacement group was significantly higher than that of the control group (P<0.05), and the serum high-density lipoprotein cholesterol content of the 25% replacement group was significantly higher than that of both the control and 50% replacement groups (P<0.05). The serum apolipoprotein B content of the 25% replacement group was significantly higher than that of the 50% replacement group (P<0.05), while the serum total protein content of the control group was significantly higher than that of the 50% and 100% replacement groups (P<0.05). (3) No significant differences were observed among groups

in diarrhea rate, organ indexes, or serum immunoglobulin content (P>0.05). Based on comprehensive evaluation of all indicators, fermented silkworm pupa and soybean meal can replace 25% of fish meal in the diet without affecting the growth performance of weaned piglets.

**Key words:** fermented silkworm pupa and soybean meal; weaned piglets; growth performance; serum biochemical index; organ index

## Introduction

Silkworm pupae are by-products of the silk industry, containing over 50% crude protein and approximately 20% crude fat primarily composed of unsaturated fatty acids, along with various bioactive components such as vitamins, polysaccharides, antimicrobial peptides, and lysozyme. These components have been shown to enhance immunity, lower blood glucose and pressure, protect the liver, exhibit anti-tumor effects, and promote wound healing [1-2]. In recent years, silkworm pupae have been applied in livestock, poultry, aquatic animals, and fur-bearing animals with good feeding results. Ji et al. [3] reported that silkworm pupa protein meal could replace 50% of fish meal in carp fry feed without significantly affecting growth performance. Rangacharyulu et al. [4] demonstrated that complete replacement of fish meal with fermented silkworm pupa protein meal significantly improved fish survival rate and yield. Qadri et al. [5] also indicated that silkworm pupa protein meal could replace fish meal in broiler diets without affecting growth performance. However, silkworm pupae produce strong odor substances through self-secretion and during storage and transportation, which can leave residual odors in animal products. Additionally, their high chitin content is difficult for animals to digest and absorb. Therefore, direct addition of silkworm pupae to animal diets affects feeding efficiency. To address this, our research team has long been engaged in solid-state fermentation technology for silkworm pupae, which solves the problems of odor and poor digestibility. The resulting fermented silkworm pupa and soybean meal product has achieved good feeding effects in mink and aquaculture. Consequently, this study further applied fermented silkworm pupa and soybean meal to weaned piglet diets, evaluating its potential to replace expensive fish meal by examining growth performance, organ indexes, and serum biochemical indicators, thereby providing a new pathway for developing novel protein feed sources and high-value feed utilization of silkworm pupae.

## Materials and Methods

### 1.1 Experimental Materials

Fermented silkworm pupa and soybean meal was prepared by mixing silkworm pupa powder and soybean meal at a 3:2 ratio, supplementing with 0.6% molasses, 0.3% ammonium sulfate, and 0.03% potassium dihydrogen phosphate to provide adequate nutrients, and inoculating with a 5% total inoculum of *Saccharomyces cerevisiae*, *Lactobacillus*, and *Bacillus subtilis* at a ratio of 1:1:2. The material

moisture was adjusted to approximately 50% and cultured at (32$±$2)°C for 56 hours before being dried at 100°C and pulverized. The measured nutritional composition was: crude protein 51.60%, crude fat 20.75%, crude fiber 2.46%, crude ash 5.44%, calcium 0.27%, total phosphorus 1.44%, lysine 2.43%, methionine 1.17%, and total amino acids 44.84%.

### 1.2 Experimental Design and Diet Composition

The experiment utilized 120 healthy Duroc × Landrace × Large crossbred piglets weaned at $(28\$\pm1)days of age with an initial body weight of (7.64\pm\$0.67)$ kg. Based on the principle of consistent body weight and gender ratio among groups, the piglets were divided into 4 groups with 3 replicates per group and 10 piglets per replicate. The control group was fed a basal diet, while the three experimental groups were fed diets in which 25%, 50%, and 100% of the fish meal in the basal diet was replaced with fermented silkworm pupa and soybean meal. Diets were formulated according to NRC (1998) *Nutrient Requirements of Swine*. All experimental diets were in powder form, and diet composition and nutrient levels are shown in Table 1 . The preliminary feeding period was 3 days, and the formal trial period was 21 days.

### 1.3 Feeding Management

The experiment was managed by dedicated personnel with feeding five times daily at 08:00, 11:00, 14:30, 17:30, and 21:00. Piglets had free access to feed and water, with the criterion that slight feed residue remained in the trough before the next morning's feeding. Daily observations were made of each piglet's mental status and feeding behavior, and daily feed intake and diarrhea frequency were recorded for each replicate. During the trial period, conventional feeding methods and immunization procedures of the pig farm were followed.

### 1.4 Measurement Methods

**1.4.1 Growth Performance Measurement** At the beginning and end of the formal experiment, all piglets in each pen were weighed individually in the early morning after overnight fasting to record initial and final body weights. Feed intake was recorded by pen (replicate) to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G).

**1.4.2 Diarrhea Rate Measurement** During the experiment, the anus of each piglet was examined daily at regular intervals. Diarrhea was defined as feces that were pasty or watery and visibly adhered to the piglet' s anus, with observation of whether the anus was red and swollen. Records were maintained, and diarrhea rate was calculated as the percentage of piglets with diarrhea in each pen relative to the total number of piglets in that pen. Diarrhea rate (%) = [Total number of diarrhea episodes per replicate / (Total number of piglets × Experimental days)] × 100.

**1.4.3 Serum Biochemical Index Measurement** At the end of the experiment, one piglet close to the average body weight was selected from each replicate (3 piglets per group) for blood collection. Ten milliliters of blood was collected from the anterior vena cava, allowed to clot at room temperature, and then centrifuged at 10,000 r/min for 1 minute at 4°C. The serum supernatant was collected, aliquoted, and stored at -20°C. An automatic biochemical analyzer was used to determine serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH), as well as contents of total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), apolipoprotein A1 (ApoA1), apolipoprotein B (ApoB), total protein (TP), albumin (ALB), prealbumin (Pa), retinol-binding protein (RBP), free fatty acids (FFA), urea (UREA), creatinine (CREA), triiodothyronine (T3), and thyroxine (T4). Immunoglobulin G (IgG) and immunoglobulin M (IgM) contents were measured using commercial IgG and IgM assay kits.

**1.4.4 Organ Index Measurement** At the end of the experiment, piglets were slaughtered after blood collection from the anterior vena cava. The thoracic cavity was opened to remove the thymus and spleen, with attached fat stripped away and connective tissue removed. The heart, liver, and kidneys were also removed and blood was blotted dry before immediate weighing. Organ index (g/kg) = Organ weight (g) / Body weight (kg).

### 1.5 Data Processing and Analysis

Experimental data were organized using Excel 2007 and analyzed for significant differences using SPSS 17.0 software. Multiple comparisons were performed using LSD and S-N-K methods. Results are expressed as mean ± standard deviation, with $P<0.05$ as the criterion for statistical significance.

## Results

### 2.1 Effects of Replacement of Fish Meal by Fermented Silkworm Pupa and Soybean Meal on Growth Performance of Weaned Piglets

As shown in Table 2 , no significant differences were observed among groups in initial weight, final weight, ADG, ADFI, or F/G of weaned piglets ($P>0.05$). However, the ADFI of the control group during week 1 was significantly higher than that of the 25% replacement group ($P<0.05$), though it showed no significant difference from the 50% and 100% replacement groups ($P>0.05$). The ADFI of experimental groups during weeks 2 and 3 showed no significant difference from the control group ($P>0.05$), but the ADFI of the 50% and 100% replacement groups during week 2 was significantly higher than that of the 25% replacement group ($P<0.05$).

### 2.2 Effects of Replacement of Fish Meal by Fermented Silkworm Pupa and Soybean Meal on Diarrhea Rate of Weaned Piglets

As shown in Table 3 , the diarrhea rate of weaned piglets during week 1 was numerically higher than during weeks 2 and 3. No significant differences were observed among groups in diarrhea rate during week 1, week 2, week 3, or the entire 21-day period ($P>0.05$).

### 2.3 Effects of Replacement of Fish Meal by Fermented Silkworm Pupa and Soybean Meal on Serum Conventional Biochemical Indexes of Weaned Piglets

As shown in Table 6 , the serum TP content of the control group was significantly higher than that of the 50% and 100% replacement groups ($P<0.05$), while no significant differences were observed in serum TP content among the three experimental groups ($P>0.05$). No significant differences were observed among groups in serum ALB, Pa, RBP, FFA, UREA, CREA, T3, or T4 contents ($P>0.05$).

### 2.4 Effects of Replacement of Fish Meal by Fermented Silkworm Pupa and Soybean Meal on Serum Immunoglobulin Contents of Weaned Piglets

As shown in Table 7 , no significant differences were observed among groups in serum IgM or IgG contents ($P>0.05$). Preliminary judgment suggests that under these experimental conditions, replacement of fish meal with fermented silkworm pupa and soybean meal had no significant effect on humoral immune function in piglets.

### 2.5 Effects of Replacement of Fish Meal by Fermented Silkworm Pupa and Soybean Meal on Organ Indexes of Weaned Piglets

As shown in Table 8 , no significant differences were observed among groups in thymus index, spleen index, heart index, liver index, or kidney index ($P>0.05$).

## Discussion

### 3.1 Effects of Replacement of Fish Meal by Fermented Silkworm Pupa and Soybean Meal on Growth Performance and Diarrhea Rate of Weaned Piglets

The results of this experiment indicate that isocaloric replacement of fish meal with fermented silkworm pupa powder in piglet diets had no significant effect on ADG, ADFI, F/G, or diarrhea rate throughout the entire experimental period. However, the ADFI of the 25% replacement group during week 1 was significantly reduced, and diarrhea rate increased markedly during this phase. This may be attributed to the underdeveloped digestive system of piglets post-weaning, the lower palatability of powder feed compared to pellet feed, and

weaning stress, which collectively caused the significant reduction in ADFI during week 1. Additionally, residual anti-nutritional factors in fermented silkworm pupa and soybean meal may have caused diarrhea, subsequently affecting feed intake. However, as the replacement level of fermented silkworm pupa and soybean meal increased, both diarrhea rate and ADFI tended to approach control group levels. This may be because silkworm pupae naturally contain antimicrobial peptides, lysozyme, and abundant vitamins and trace elements, or because fermentation converts silkworm pupa protein into functional bioactive peptides and chitin into chitosan, which have demonstrated effects in alleviating stress, exerting antibacterial and anti-inflammatory actions, enhancing digestive enzyme secretion and activity, improving nutrient digestion and absorption, and strengthening immune function [6-8]. When administered at sufficient levels, these substances exert effective biological activity.

Jia [9] investigated the effects of replacing fish meal in basal diets with *Bacillus*-fermented cottonseed protein on piglet growth performance and found no significant differences in ADFI, ADG, or F/G among groups after 28 days, consistent with the overall growth performance observed in this study. Chae et al. [10] also reported that fermented feed replacing fish meal maintained normal growth rates in piglets, aligning with our findings. However, Jeong et al. [11] used three types of microorganisms to produce fermented soybean meals that replaced 50% of fish meal in feed, and found that ADFI and F/G of weaned piglets in the later experimental period were significantly lower than in the control group, which contradicts our results. This discrepancy may be because our study utilized mixed fermentation of soybean meal and silkworm pupa powder with composite strains, achieving higher fermentation efficiency and improved nutritional value of the raw materials, while the rich nutrients and functional active substances in silkworm pupae more effectively promoted the growth and development of weaned piglets.

### 3.2 Effects of Replacement of Fish Meal by Fermented Silkworm Pupa and Soybean Meal on Serum Biochemical Indexes of Weaned Piglets

ALT, AST, ALP, and LDH are important indicators reflecting liver health and function. In this experiment, no significant differences were observed among experimental groups and the control group in serum ALT, AST, ALP, or LDH activities, or in liver index, indicating that fermented silkworm pupa and soybean meal had no adverse effect on liver health. Serum cholesterol, TG, HDL-C, and LDL-C are major components of blood lipids, while ApoA1 and ApoB are transport carriers for HDL-C and LDL-C, respectively. HDL-C is generally considered beneficial to health, whereas LDL-C is considered harmful. In this study, replacement of 25% fish meal with fermented silkworm pupa and soybean meal significantly increased serum HDL-C and TC contents, but 50% and 100% replacement levels had no significant effect, suggesting that 25% replacement may have certain regulatory effects on lipid metabolism in weaned piglets, though the mechanism requires further investigation. Serum TP, ALB, and UREA can

reflect protein synthesis and metabolism in the body. Increased serum TP and ALB contents and decreased serum UREA content indicate enhanced anabolic metabolism, whereas decreased serum TP and ALB contents suggest reduced dietary nutritional levels or digestibility and that animals are typically under stress [12]. Pa is synthesized by the liver, and serum Pa content can also reflect nutritional status and liver health, being associated with inflammatory responses [13]. The results showed that replacement of fish meal with fermented silkworm pupa and soybean meal had no significant effect on serum ALB, UREA, or Pa contents, but serum TP content in the 50% and 100% replacement groups was significantly lower than in the control group. This may be because residual anti-nutritional factors in fermented silkworm pupa and soybean meal at higher replacement levels affected protein digestion and absorption, or because the amino acid balance in fermented silkworm pupa and soybean meal still differs somewhat from that of fish meal. Retinol-binding protein transports vitamin A from the liver to target tissues, thereby mediating intracellular vitamin A transport and metabolism [14]. FFA are products of neutral fat hydrolysis; when glycogen stores are depleted, adipose tissue hydrolyzes neutral fat into FFA for use as an energy source, and FFA are also associated with oxidative stress, making serum FFA content an important indicator for determining whether animals are experiencing oxidative stress. CREA is a metabolic product of muscle in animals, almost entirely excreted in urine, and serves as one method for assessing kidney function [15]. Serum T3 and T4 contents are reference indicators for normal thyroid function [16]. This experiment demonstrated that replacement of fish meal with fermented silkworm pupa and soybean meal had no significant effect on these indicators, suggesting that such replacement does not affect the corresponding functional levels in piglets.

### 3.3 Effects of Replacement of Fish Meal by Fermented Silkworm Pupa and Soybean Meal on Immune Function and Organ Indexes of Weaned Piglets

Serum IgG and IgM in mammals have antibacterial, antiviral, and antitoxic effects and constitute important components of humoral immunity. The thymus and spleen are vital immune organs in livestock; the former is the central organ of cellular immunity and the primary site for T cell differentiation and development, while the latter is an important peripheral immune organ involved in systemic cellular and humoral immune responses. Studies have shown that adding microbial fermented feed to weaned piglet diets can significantly increase serum IgG and IgM contents and enhance humoral immune function [17], but few studies have reported on the effects of replacing fish meal with fermented silkworm pupa and soybean meal on immune function in weaned piglets. This experiment primarily investigated the effects of such replacement on immune organ indexes and serum IgG and IgM contents. The results showed no significant differences among groups in immune organ indexes or serum IgG and IgM contents, but serum IgG and IgM contents showed a clear upward trend with increasing replacement levels. This is mainly because the three beneficial

bacterial strains inoculated in our fermented silkworm pupa and soybean meal have immunoenhancing effects, as the bacteria themselves or their metabolites can stimulate the intestinal immune system, activate gut-associated lymphoid tissue to enhance IgA antibody secretion, and induce T and B lymphocytes and macrophages to produce cytokines, thereby activating the systemic immune system through lymphocyte recirculation and enhancing immune function [18-19]. Additionally, various bioactive peptides and chitosan generated through silkworm pupa fermentation have obvious immunoenhancing effects [7,20].

Visceral organs are the foundation for physiological functions in animals, and organ indexes reflect whether organ development is normal, metabolism is vigorous, and functions are complete, while also indirectly indicating growth performance and health status. This experiment demonstrated no significant differences in organ indexes between experimental and control groups, and no abnormal development or enlargement of these organs was observed during dissection, indicating that replacing fish meal with fermented silkworm pupa and soybean meal in weaned piglet diets had no adverse effects on organ development or health.

## Conclusion

Under the conditions of this experiment, fermented silkworm pupa and soybean meal can replace 25% of fish meal in weaned piglet diets without affecting piglet growth performance.

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