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Effects of Fermented non-conventional Feed on Organ Indices and Immune-Related Gene Expression in Fattening Bamei Pigs

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Abstract

【Objective】 To investigate the effects of substituting fermented non-conventional feed for the basal diet in fattening Bamei pigs.【Methods】Seventy-two Bamei pigs during the fatling period were randomly divided into two groups, each containing six replicates with six pigs per replicate. The control group (CK) was fed a corn-soybean meal basal diet, while the experimental group (FK) received the basal diet supplemented with 10% fermented non-conventional feed (dry matter basis) as an equal replacement. After 60 days of feeding, twelve pigs from each group were slaughtered for sample collection and performance evaluation. 【Results】 The results demonstrated that fermented non-conventional feed significantly reduced the cardiac organ index ($P < 0.05$) and increased the renal organ index ($P < 0.05$). Regarding immune-related gene expression, the feed markedly elevated the relative mRNA expression levels of IL-1, IL-2, TNF, IgA, and IgM in both lymphoid and hepatic tissues ($P < 0.01$). 【Conclusion】 In summary, replacing 10% of the basal diet with non-conventional fermented feed improves the health status and immune function of fattening Bamei pigs. Thus, substituting conventional feed with fermented non-conventional feed is not only feasible but also offers an economically efficient nutritional strategy for local pig breeds farming.

Keywords: Microbial-enzyme synergy; Non-conventional feed; Bamei pigs; Organ index; Immunity.

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1. Introduction

Organ indices serve as critical indicators for evaluating animal nutritional metabolism and physiological health. The developmental status of organs such as the heart, liver, and spleen directly influences energy allocation efficiency and disease resistance in pigs[1]. Studies have shown that hepatic index is positively correlated with protein synthesis capacity, while splenic index serves as a key parameter for assessing immune potential in swine populations [2,3]. Additionally, the expression levels of immunoglobulins and cytokines are vital molecular markers for evaluating immune response capacity in pigs. Specifically, IgA plays a pivotal role in mucosal immunity, and IL-2 levels are closely associated with lymphocyte proliferative activity [3-6].

Fermented non-conventional Feed, through microbial processing, exhibits significant reductions in antinutritional factors and increased concentrations of bioactive small molecules. These changes may regulate organ development by improving nutrient absorption efficiency [7]. However, research on the effects of Fermented non-conventional Feed on immune function in indigenous pig breeds remains limited. Therefore, this study investigated Bamei pigs during the fattening phase, measuring organ indices (heart, liver, spleen, lungs, and kidneys) and analyzing the relative expression levels of immune-related genes (IL-1, IL-2, TNF, and three immunoglobulins) in lymphoid and hepatic tissues. The objectives were to clarify the impact of Fermented non-conventional Feed on organ development and immunity in Bamei pigs, provide data-driven insights for optimizing dietary formulations for indigenous breeds, and offer practical guidance for reducing farming costs and enhancing herd health.

2. Materials and Methods

2.1 Animal care

All experimental methods and protocols were approved by the Research Ethics Committee of the Academy of Animal Science and Veterinary, Qinghai University (Ethics Approval Number: 2024-QHMKY-002).

2.2. Experimental materials

The rapeseed meal, rapeseed straw, potato residue, and basal diet for fattening pigs used in this trial were provided by the Qinghai Huzhu Bamei Pig Original Breeding & Multiplication Farm. The basal diet was formulated according to the nutritional standards of the NRC (2012) for swine.

The compound probiotic bacterial solution was provided by the Animal Nutrition and Feed Science Laboratory, Academy of Animal Science and Veterinary, Qinghai University. It comprised the following strains: Lactobacillus rhamnosus YLW00 (CCTCC NO: M 2018759),

Pediococcus acidilactici YLW002 (CCTCC NO: M 2018760), Lactobacillus casei YLW003 (CCTCC NO: M 2018761), Saccharomyces cerevisiae JZ9 (CCTCC NO: M 2020844), Pichia pastoris JZ10 (CCTCC NO: M 2020843), Kluyveromyces marxianus MN29 (CCTCC NO: M 2020845). The viable bacterial count was ≥ 10^8 CFU/mL, with a uniform strain ratio of 1:1:1:1:1:1.

The Jiaolijia compound enzyme preparation was purchased from Boyd (Beijing) Biotechnology Co., Ltd. Its main components included: Xylanase ≥ 1000 U/g, β-Glucanase ≥ 200 U/g, β-Mannanase ≥ 500 U/g, Cellulase ≥ 500 U/g.

2.3 Preparation of non-conventional fermented feed

Potato residue, rapeseed meal, and rapeseed straw were mixed at a ratio of 6:3:1 (dry matter basis) and thoroughly homogenized. Subsequently, the mixture was inoculated with 5% (w/w) complex probiotic inoculum and 1 g/kg complex enzyme preparation. Distilled water was added to adjust the moisture content to 50%. After complete mixing, the substrate was transferred into fermentation bags, sealed, and anaerobically fermented at room temperature (25 ± 2°C) for 10 days before being stored for subsequent use.

2.4 Experimental design, animals, and diets

A total of 72 female Bamei pigs in the fattening period (6 months of age, initial body weight 73.40 ± 3.38 kg) were selected and randomly assigned to a control group (CK) and an experimental group (FK) using a single-factor completely randomized design, with 6 replicates per group and 6 pigs per replicate. The CK group was fed a basal diet, while the FK group received a modified diet in which 10% of the basal diet (dry matter basis) was replaced with an equal mass of fermented non-conventional feed, freshly mixed with the basal diet before each feeding. The trial comprised a 7-day pre-trial period and a 53-day formal trial period, totaling 60 days. The composition and nutritional levels of the basal diet are presented in Table 1. The experiment was conducted at the Huzhu Bamei Pig Original Breeding and Multiplication Farm in Qinghai Province. Prior to the trial, all pigs were dewormed, and the pens were thoroughly disinfected. During the trial, pigs had ad libitum access to water and were fed a fixed ration twice daily at 07:50 and 17:25 hours. Residual feed in each replicate was recorded 30 minutes after feeding (08:35 and 17:55 hours), followed by cleaning of troughs and pens.

Table 1. The nutrient compositions of the basal diet (air-dry basis).

Ingredients	Contents(%)	Nutritional Indicators 2	
corn	65.00	Digestible energy (MJ/kg)	13.85
soybean meal	20.00	Crude protein (%)	15.85

sunflower meal	5.00	Lys(%)	0.773
wheat bran	3.00	Met(%)	0.279
soybean oil	3.00	Calcium(%)	0.728
Premix 1	4.00	Total phosphorus(%)	0.478
Total	100.00		

Note: 1 Premix provided per kilogram of diet: Vitamin A 12,000 IU, Vitamin D 3,200 IU, Vitamin E 5.5 mg, Vitamin K 2 mg, Vitamin B_1 2 mg, Vitamin B_2 5 mg, Vitamin B_6 4 mg, Vitamin B_{12} 25 μg, Niacin 20 mg, D-calcium pantothenate 13 mg, Folic acid 1.3 mg, Choline chloride 1.1 g. Ferrous sulfate 100 mg, Copper sulfate 6 mg, Zinc sulfate 110 mg, Manganese sulfate 8 mg, Sodium selenite 0.25 mg, Potassium iodide 0.25 mg.2 Nutrient analysis:Digestible energy (DE) was calculated according to China Feed Composition and Nutritional Value Tables (34th Edition, 2023); all other values are measured data.

2.5 Sample Collection

At the conclusion of the feeding trial, Bamei pigs were fasted for 12 hours and humanely euthanized via electrical stunning followed by exsanguination through jugular vein bleeding. Hearts, livers, spleens, lungs, and kidneys were collected from 12 randomly selected pigs per group. Organ surfaces were blotted with clean absorbent paper to remove residual blood before weighing. Liver and lymphoid tissues were immediately placed in sterile Eppendorf (EP) tubes, flash-frozen in liquid nitrogen, and stored at -80°C for subsequent analyses.

2.6 Organ Index

The organ index was determined according to the methodologies described by GHALAMKARI et al.[8] and GUO et al.[9]. The calculation formula is as follows:

Organ Index = (Organ Weight / Body Weight) × 100%

2.7 Primer Design and Synthesis

Total RNA was extracted from lymphoid and liver tissues using the RNAiso Plus Kit (TAKARA) according to the manufacturer's instructions. RNA concentration was measured based on absorbance at 260 nm. RNA integrity was assessed via agarose gel electrophoresis, and RNA quality was confirmed by verifying A 260-280 absorbance ratios between 1.8 and 2.0. Total RNA was reverse-transcribed using the PrimeScript™ RT Reagent Kit (TAKARA). Real-time PCR was performed on an ABI 7500 Fast Real-Time PCR System with the TB Green™ Premix Ex Taq™ II Kit (TAKARA) following the manufacturer's protocol. Primer sequences are listed in Table 2.

Table 2. Primer sequences

Gene Name	Primer Sequence （5'→3'）	Length, bp	Annealing Temperature, ℃
IL-1	F：GCCAACGTGCAGTCTATGGAGTG	91	60.9
	R：GGTGGAGAGCCTTCAGCATGTG	91	61.2
IL-2	F：TTTACATGCCCAAGCAGGCTACAG	120	59.9
	R: TAGCACTCCCTCCAGAGCTTTGAG	120	60.8
TNF	F：CAACGGCGTGAAGCTGAAAGAC	135	59.6
	R: ATGCGGCTGATGGTGTGAGTG	135	60.9
IgA	F：GCCACCATCACCAAACCCAAAG	149	59.7
	R: AGCCATCGCACCAGCACATC	149	61.5
IgG	F：AGAGGGCAATTACCGCACCAC	125	60.7
	R: GCATCACCGCACACTGGAATATG	125	59.0
IgM	F：ACTGTCTCTGTGGTGTGCTTGATC	144	59.2
	R: GCTGAGGCTGTAGGTGCTGTC	144	61.2
β-actin	F：GATCTGGCACCACACCTTCTACAAC	107	60.0
	R: TCATCTTCTCACGGTTGGCTTTGG	107	59.7

Note: IL-1, Interleukin-1; IL-2, Interleukin-2; TNF, Tumor Necrosis Factor; IgA, Immunoglobulin A; IgG Immunoglobulin G; IgM Immunoglobulin M ; β-actin, internal reference.

2.8 Statistical Analysis

Experimental data were initially organized using Microsoft Excel 2019 and analyzed statistically with SPSS 27.0 software. Differences between groups were compared using independent samples t-tests. Results are expressed as mean ± standard deviation (SD). A threshold of $P > 0.05$ was considered not statistically significant, $P < 0.05$ as statistically significant, and $P < 0.01$ as highly statistically significant.

3. Results and Analysis

3.1 Effects of Fermented non-conventional Feed on Organ Indices of Bamei Pigs During the Fattening Period

As shown in Table 3, compared with the CK group, the FK group showed no significant effects on the liver, spleen, or lung organ indices of Bamei pigs during the fattening period ($P > 0.05$). However, it significantly reduced the heart organ index by 15.54% ($P < 0.05$) and increased the kidney organ index by 18.46% ($P < 0.05$).

Table 3. Effects of Fermented non-conventional Feed on Organ Indices of Bamei Pigs During the Fattening Period

Item	Group		P-value
	CK	FK	
Heart organ index (%)	6.35±0.96a	5.39±0.67b	0.010
Liver organ index (%)	16.63±1.64	16.33±2.54	0.741
Spleen organ index (%)	2.32±0.65	2.22±0.41	0.644
Lung organ index (%)	13.48±2.76	12.57±1.99	0.362
Kidney organ index (%)	2.98±0.45a	3.51±0.56b	0.018

3.2 Effects of Fermented non-conventional Feed on Relative Expression Levels of Immune Genes in Bamei Pigs During the Fattening Period

The results are shown in Figure 1. As seen in Figure 1A, compared with the CK group, the relative expression levels of IL-1, IL-2, TNF, IgA, and IgM in the lymph nodes of Bamei pigs in the FK group were extremely significantly upregulated ($P < 0.01$). From Figure 1B, compared with the CK group, the relative expression levels of IL-1, IL-2, TNF, and IgM in the liver of Bamei pigs in the FK group also showed extremely significant upregulation ($P < 0.01$).

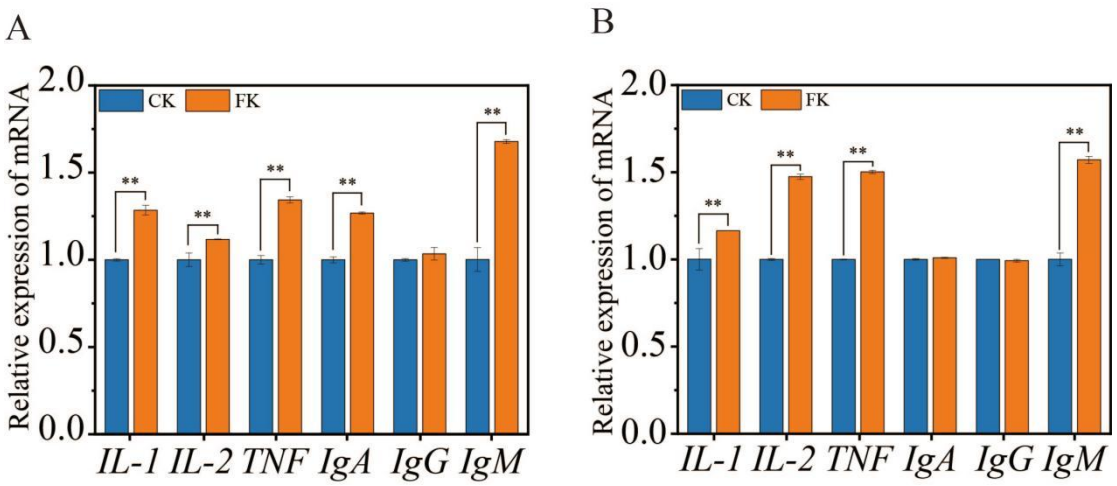

Figure 1. Effects of Unconventional Fermented Feed on Relative Expression Levels of Immune Genes in Bamei Pigs During the Fattening Period

Note: (A) Lymph nodes; (B) Liver. * indicates a significant difference at ($P < 0.05$); ** indicates an extremely significant difference at ($P < 0.01$).

4. Conclusions and Discussions

Organ indices can reflect, to some extent, the growth, nutritional status, and health of animals[1]. JIANG H J et al.[10] found that dietary supplementation with fermented cassava residue significantly increased the heart index. LIU X N et al.[11] reported that feeding diets containing 5% or 10% whole-plant silage maize numerically increased organ indices, but no significant differences were observed between groups. LIU B H et al.[12] demonstrated that replacing soybean meal with fermented rapeseed meal in low-protein diets had no significant effect on organ indices in finishing pigs. In this trial, the unconventional fermented feed showed no significant impact on the indices of the liver, spleen, or lung but significantly reduced the heart index and increased the kidney index. These findings partially align with the trends reported by LIU X N et al. and LIU B H et al., but contrast with the results of J H J et al. The observed discrepancies may arise from differences in fermentation substrates (e.g., potato residue and rapeseed meal versus cassava residue and rapeseed meal, which vary in nutritional composition and fermentation products), microbial composition (e.g., Lactobacillus rhamnosusYLW00 may influence cardiovascular function via nitric oxide synthesis, while other strains lack similar effects), and breed-specific responses (Bamei pigs may metabolically respond to fermented feed differently compared to conventional finishing pigs)[13,14]. These findings suggest that the effects of unconventional fermented feed on organ indices may depend on substrate specificity, microbial strain activity, and animal breed, warranting further research to clarify the underlying mechanisms.

The probiotics and their metabolites in fermented non-conventional feed can be recognized by the body as independent antigens, stimulating the intestinal mucosa, epithelial cells, and associated immune organs, thereby promoting the proliferation of T lymphocytes and B lymphocytes and enhancing immune function[15,16]. MIZUMACHI et al.[17] demonstrated that fermented liquid feed significantly increased serum IgM and IgG levels in weaned piglets compared to non-fermented liquid feed, thereby improving systemic immunity. WANG et al.[18] found that probiotic supplementation in feed elevated serum IL-2 content in weaned piglets. In this trial, the relative expression levels of IL-1, IL-2, TNF, IgA, and IgM genes in the lymph nodes and liver of Bamei pigs in the FK group during the fattening period were all extremely significantly upregulated. The expression trends of IL-1, IL-2, IgA, and IgM aligned with previous studies, while the marked increase in TNF expression diverged from prior findings. This discrepancy may arise from the following mechanisms: (1) Immunomodulatory components in the FK group`s feed, such as short-chain fatty acids, might activate the B cell receptor (BCR) signaling pathway to promote IgA and IgM secretion; (2) The feed may enhance arginine availability, activating the mTOR pathway to drive T cell proliferation and IL-2 synthesis; (3) Elevated TNF expression could be linked to NF-κB pathway activation induced by feed components like lipopolysaccharides[19,20]. These findings suggest that fermented non-conventional feed may regulate immunoglobulin and cytokine

This study demonstrates that supplementing the basal diet of Bamei pigs during the fattening period with 10% fermented non-conventional feed yields the following effects: (1) Significantly increases the renal organ index and relative expression levels of IL-1, IL-2, TNF, and IgM genes in the lymph nodes and liver; (2) Significantly reduces the cardiac organ index. These results confirm the dietary feasibility of replacing 10% of the basal diet with fermented non-conventional feed. Furthermore, the strategy is proven to optimize organ development, activate synergistic effects between cellular and humoral immune responses, and enhance immune function in pigs.

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