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Research Article

The Effects of Fermented Soybean Meal on Growth Performance and Immune Characteristics in Weaned Piglets

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Abstract: This experiment was conducted to determine the effects of fermented soybean meal (FSBM) on growth performance and immune characteristics of piglets. In all, 60 crossbred piglets (Duroc × Landrace × Yorkshire) were allocated to 2 dietary treatments; 3 replications of 10 pigs were used for each treatment, and all piglets were weaned at 35 days and fed for 23 days. The results showed that average daily gain (ADG) improved (1.44% vs. 1.36%) in piglets fed the FSBM diet and feed gain ratio (FGR) decreased by 5.56% ($P < 0.05$) compared to the controls (soybean meal treatment). The level of serum IgG decreased 27.2% ($P < 0.01$) in piglets fed FSBM. Lymphocytes from whole blood had a lower proliferative response to concanavalin A (ConA) ($P < 0.01$) and lipopolysaccharide (LPS) ($P < 0.05$). Meanwhile, the splenocytes of piglets fed FSBM also showed lower proliferative response to ConA ($P < 0.05$) and LPS ($P < 0.01$). The results indicated that FSBM was beneficial to growth performance and decreased the immune response to soybean protein in piglets.

Key Words: Fermented soybean meal, growth performance, immune characteristics, piglets

Introduction

Soybean meal (SBM) is the most commonly used protein source in the animal feed industry (1). High protein content and widespread availability make soybean meal a good source of protein in animal diets (2); however, a variety of antinutritional factors (ANFs), such as trypsin inhibitor, lectins, and soybean globulins, have limited the application of soybean meal in animal feed, especially feed for young animals (3,4). The development of processing techniques that reduce the harmful and anti-nutritional properties of SBM would make possible a high-quality and inexpensive protein with valuable functional benefits available for young animals. Studies showed that lectins could usually be inactivated by thermal processing (5), and soybean globulins could be partly eliminated by ethanol treatment, but a reduction in growth can still be observed in piglets fed diets containing SBM (6). Hypersensitivity responses to soybean proteins glycinin and ß-conglycinin, and trypsin inhibitor may be one of the main causes of growth reduction in pigs (7) and calves (8).

As a result of developments in microbiology, the feed industry has found an effective and efficient method for the fermentation of soybeans and SBM. Kiers et al. (9) reported that fermented soybeans could be beneficial in the control of diarrhea in *Escherichia coli*-challenged weaned piglets (*Rhizopus microsporus* fermented) and that they significantly improved weight gain and feed intake (*Bacillus subtilis* fermented). However, data concerning fermented soybean meal (FSBM) as an excellent alternative for piglets are scare. Therefore, the objective of this study was to evaluate the effects of FSBM (*Aspergillus oryzae* fermented) on growth performance and immune characteristics in piglets.

Materials and Methods

Preparation of fermented soybean meal

The method used to ferment soybean meal was described in our previous study (10). Briefly, dried SBM was soaked with distilled water for 60 min. The hydrated SBM was then cooked in a steam tank at 60-70 °C for 1

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h. Then, the cooked SBM was cooled to room temperature for 1 h, inoculated with 0.3% *Aspergillus oryzae* 3.042 (± 10^4 counts/g of soybean meal, kindly provided by Microbial Institute of Zhejiang Province), mixed, and fermented in a bed-packed incubator for 48 h. The nutrient composition of FSBM is provided in Table 1. Fermentation reduced the level of trypsin inhibitor in FSBM compared to SBM (2.6 mg/g vs. 0.0 mg/g); trypsin inhibitor was measured as described by Kakade et al. (11).

Animals and experimental design

The study included 60 crossbred piglets (Duroc × Landrace × Yorkshire) that were identified by ear tags and housed in floored indoor pens. The piglets were allocated to 2 dietary treatments on the basis of weight, and ancestry was equalized across treatments by a randomized complete block design. For each treatment, 3 replications of 10 pigs per replicate were used. All piglets were weaned at 35 days and immediately began to feed on FSBM or SBM for 23 days. The control group was fed a corn-SBM diet, and in the treatment diet FSBM replaced SBM (Table 2). The basal diet was supplemented with minerals and vitamins to meet or exceed the requirements for piglets. All pigs were given ad libitum access to feed and water. Growth performance results as average daily gain (ADG), average daily feed intake (ADFI), and feed gain ratio (FRG) were collected on the 23rd day.

Sample Collected

At the end of the feeding trial, 12 pigs (6 piglets from each treatment) were selected and sacrificed, dehaired, and eviscerated. Blood and spleen samples were taken to determine T and B lymphocyte proliferation to soybean proteins. Serum was collected and snap-frozen in liquid nitrogen for analyzing IgA, IgM, and IgG. The level of immunoglobulin was analyzed according to immunoturbidimetry assay (10).

Table 1. Nutrient composition of SBM and FSBM.

Diet (%)	SBM	FSBM
Crude protein (%)[a]	43.54	49.41
Dry matter (%)[a]	88.17	91.19
Calcium (%)[a]	0.54	0.53
Phosphorus (%)[a]	0.80	0.82
Trypsin inhibitor (mg/g)	2.63	0.00

[a] On a dry matter basis.

Table 2. Ingredient and nutrient composition of the experiment basal diet (g kg^{-1} as fed).

Ingredient	SBM	FSBM
Maize	564.5	604.5
SBM	285	0
FSBM	0	245
Fishmeal	45	45
Wheat middling	10	10
Calcium hydroxide	15	15
Limestone	8	8
Salt	2.5	2.5
Whey powder	50	50
Soybean oil	10	10
Premix[1]	10	10
Analyzed value (% as feed)		
Crude protein	211	210
Lysine	15	15
Ash	71	70

[1]Supplied the following in mg/kg of diet: Fe, 15; Cu, 5; Zn, 140. Vitamin A, 10,000 IU; VE, 60 mg; VD_3, 1500 IU; VK, 0.5 mg; VB_1, 5 mg; VB_2, 20 mg; Niacin, 20 mg; dl-a-tocopherol acetate, 78 IU; D-pantothenic acid, 20 mg; Biotin, 0.4 mg.

Proliferation assay of splenocyte and peripheral lymphocyte

Mitogen-induced splenocyte and peripheral lymphocyte proliferation were examined according to Kong et al. (12) and Zhou et al. (13). Spleen samples were gently smashed by pressing with the flat surface of a syringe plunger against a stainless steel sieve (200 mesh). Splenocytes were washed twice and then resuspended in RPMI 1640 (Gibco, NY, USA) supplemented with benzyl-penicillin (100 IU/ml), streptomycin (100 IU/ml), and 10% fetal bovine serum. Blood samples with sodium heparin were diluted with an equal volume of Hanks' solution and carefully layered on the surface of the lymphocyte separation medium. The

lymphocytes' bands were collected and washed twice with RPMI 1640, without fetal bovine serum. The splenocytes and peripheral lymphocytes were incubated in 96-well tissue culture plates, 100 µl/well, with either 100 µl of ConA (25 µg/ml, Sigma, USA), LPS (100 µg/ml, Sigma, USA), or complete medium (controls) added. After 44 h of incubation at 37 °C in a 5% CO_2 humid incubator, 20 µl of MTT (5 mg/ml, Amresco, Solon, USA) was added to each well and incubated for another 4 h. Then, 100 µl of DMSO was added to each well and shaken for 10 min to dissolve the precipitation completely. The absorbance was measured at 570 nm using an EIA reader (Model BIO-RAD-550, USA). The results are reported as a stimulation index (SI). SI is calculated as the absorbance of mitogen-stimulated cells divided by the absorbance of non-stimulated control (media only) cells.

Statistical analysis

One-way ANOVA was performed using the GLM procedure in SAS software (14). A significance level of $P < 0.05$ was used.

Results

The results showed that piglets fed the FSBM diet had higher ADG ($P < 0.05$) and FGR ($P < 0.05$) than piglets fed raw soybean protein (Table 3). ADG and FGR in piglets fed FSBM increased by 8.33% and decreased by 5.56%, respectively, compared to piglets fed SBM.

Piglets fed FSBM had lower ($P < 0.01$) levels of serum IgG than piglets fed SBM (Table 4). There was no difference in the level of serum IgA and IgM between the piglets fed FSBM and SBM.

Table 3. Effects of FSBM on growth performance of piglets offered feed ad libitum.

	SBM	FSBM	SEM
Initial weight (kg)	8.63	8.62	0.15
Final weight (kg)	17.01	17.67	0.26
Average daily feed intake (kg)	0.53	0.53	0.01
Average daily gain (kg)	0.36[a]	0. 39[b]	0.01
Feed gain ratio	1.44[b]	1.36[a]	0.02

Values are presented as means: n = 3 for ADG, ADFI, and FC per treatment. [a,b]Means within the same row with different superscripts differ significantly ($P < 0.05$).
SEM: Standard error of the mean.

Table 4. Effects of FSBM on immunoglobulins in piglets.

	SBM	FSBM	SEM
IgG (g/l)	0.56[c]	0.44[a]	0.03
IgA (g/l)	0.71	0.72	0.09
IgM (g/l)	0.10	0.12	0.02

Values are the mean for 6 pigs. [c, a]Means within rows with different superscript letters differ significantly ($P < 0.01$).
SEM: Standard error of the mean.

Table 5. Effect of FSBM on splenocyte and peripheral lymphocyte proliferation (SI*) in pigs.

	SBM	FSBM	SEM
Whole blood			
CONA[4] -induced	0.91[c]	0.33[a]	0.04
LPS[4] -induced	0.86[c]	0.45[a]	0.04
Splenocyte lymphocyte			
ConA[4]-induced	2.53[b]	2.27[a]	0.07
LPS[4] -induced	2.56[c]	2.03[a]	0.1

Values are the mean for 6 pigs. [a,b]Means within rows with different superscript letters differ significantly ($P < 0.05$). [a,c]Means within rows with different superscript letters differ significantly ($P < 0.01$).
[4]ConA: Concanavalin A ; LPS: Lipopolysaccharide.
* SI = OD 570 nm of experimental value/OD 570 nm of control value.

The results of lymphocyte proliferation are shown in Table 5. FSBM significantly decreased ($P < 0.01$) both ConA- and LPS-induced peripheral lymphocyte proliferation when compared to SBM. Meanwhile, lymphocytes from the spleens of pigs fed FSBM also showed a lower proliferation response to ConA ($P < 0.05$) and LPS ($P < 0.01$).

Discussion

The present results showed that FSBM significantly improved the growth performance of piglets. Similar growth-promoting effects were reported by Kim et al. (15). They found that pigs fed a diet of 5% FSBM (fermented with *A. oryzae*) had greater ($P < 0.05$) ADG and FGR than the control group. Zamora and Veum (16) showed that whole soybeans fermented with *A. oryzae* improved the growth performance of pigs. Our previous experiment with broilers also found similar improvements in growth performance. These growth-promoting effects may be primarily due to the improvement in the

nutritional value of FSBM and the elimination of ANFs after fermentation (trypsin inhibitor 2.63 vs. 0.00 mg/g).

Consistent with previous reports (4,8,17), piglets fed SBM had a high level of IgG antibodies against dietary soybean protein. Stimulation of the systemic immune system by undenatured glycinin and ß-conglycinin absorbed from digesta in the gut lumen could account for these circulating antibodies. In the present experiment, the decrease in serum IgG concentration suggested that fermentation could denature antigenic materials in SBM. Visessanguan et al. (18) indicated that the almost complete breakdown of 3 subunits from ß-conglycinin and both polypeptides from glycinin were observed after fermentation of SBM. Our previous study also (19) indicated that fermentation could degrade soybean antigenic protein (such as glycinin and ß-conglycinin).

Lymphocyte proliferation is an important phase in the immune response of the animal body. The level of lymphocyte proliferation, therefore, is a significant method for determining cell immunity and clinical immune function (20). T and B lymphocytes have receptors to identify antigen and mitogen, and lymphocytes can be stimulated by a specific antigen and then have a proliferative response. In the present experiment, peripheral lymphocytes and splenocytes had a lower proliferative response to ConA and LPS in the pigs fed FSBM. Li et al. (4) reported that intraepithelial lymphocytes showed higher proliferative responses to mitogens in piglets fed a diet containing SBM. The present results confirmed this point. The decrease of proliferative responses observed in our study might be associated with the degradation of soybean antigenic materials (11S glycinin and 7S ß-conglycinin). Hong et al. (21) showed that FSBM (*Aspergillus oryzae* fermented) did not contain large peptides (> 60 kD) and significantly increased the number of small peptides (< 20 kD).

In conclusion, FSBM improved the growth performance of piglets, decreased serum IgG, and lowered whole blood and spleen lymphocyte proliferative response to ConA and LPS. Further work is required to better define the mechanisms of effect of the nutrients in FSBM on immune function in piglets.

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