



## The Effect of Dietary Fermented Soybean on the Growth Performance and Meat Quality of Pigs

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### Abstract

This study investigated the effects of fermented soybean (FS) on growth and meat quality in pigs. A total thirty-six pigs were divided into 2 groups (2 treatment×18 pigs each) and fed the experimental diets for 4 wk as follows: control (FS free); and T1 (FS 1%). The pigs in T1 had a higher feed efficiency compared with pigs fed control diets. pH was also significantly higher in the T1 group ( $p<0.05$ ). Water-holding capacity measured in T1 was slightly higher than that of the control. Cooking loss in T1 was significantly lower than controls ( $p<0.05$ ). CIE L\* and CIE b\* value were significantly higher in the control ( $p<0.05$ ), but CIE a\* value of T1 was higher than control ( $p<0.05$ ). The drip loss of T1 were significantly lower than controls ( $p<0.05$ ). Sensory characteristics of the treatment group showed higher than controls ( $p<0.05$ ). These results showed that FS could be served as a favorable feed additive and feedstuff for enhancing pork quality.

**Key words:** soybean, fermentation, pork quality, isoflavone

### Introduction

Interest in soybean as a food source for humans has increased recently all over the world. Soy and soy-based foods are rich sources of isoflavones (ISF), which have been under intensive investigation in recent years. It has been suggested that a diet rich in ISF is associated with a lowered risk for certain diseases, including breast and prostate cancers, osteoporosis and coronary heart disease (Barnes *et al.*, 1995; Messina *et al.*, 1994; Zheng *et al.*, 1999). In Asian countries where soy consumption is high, the occurrence of these diseases is much lower than in Western countries (Adlercreutz, 1990). ISF in soybeans are found in 4 chemical forms: aglycone, glycoside, acetylglycoside, and malonylglycoside; however, the glycoside forms are predominant, and variation in food processing techniques alter the relative content of acetylglycoside, malonylglycoside, and simple glycosides (Xu *et al.*, 2000). In soybean foods, ISF exists in both the

aglycone and glycoside forms and the fermentation process increases the aglycone forms in soy products. Generally, cooked soybeans, texturized vegetable protein, and soy-milk powder contain >95% of the total ISF as glycoside, whereas fermented soybean products such as tofu and tempeh contain 20-40%, respectively, of its ISF as aglycones (Wang and Murphy, 1994). Aglycones are formed by the hydrolysis of glycosides through  $\beta$ -glycosidase present in soybean. Fermentation, heat treatments, chemical and enzymatic hydrolysis changes composition of ISF profile (Cook, 1998; Ikeda *et al.*, 1995; Matsuura *et al.*, 1995; Pandjaitan *et al.*, 2000a; Pandjaitan *et al.*, 2000b; Xie *et al.*, 2003). Various estrogenic compounds when fed to or implanted into pigs have been shown to reduce fat and increase muscling (Bidner *et al.*, 1972; DeWilde and Lauwers, 1984; Plimpton and Teague, 1972).

Soybeans fermented with *Aspergillus* have been used widely in the orient as food for human consumption. These fermented soy foods are highly digestible and nutritious, contributing important nutrients including calcium and Vitamins A and B, as well as functional properties (Kim *et al.*, 1999; Lee, 1998). Hong *et al.* (2004) showed that the nutritional value of soybeans and soy-

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bean meal were improved after fermentation with *Aspergillus oryzae*, and suggested fermented soybean meal can be used as a specialized feed.

The objective of this study was to evaluate the utilization of fermented soybean on growth performance and meat quality of pigs.

## Materials and Methods

### Preparation of soybeans

Soybean varieties commonly grown in Yonchon, Korea, were obtained for use in this study. Soybeans were washed three times and soaked in distilled water at ambient temperature for 10 h. The soaked soybeans were drained and cooked in an autoclave at 121°C for 20 min. Cooked soybeans were cooled to room temperature and inoculated with *Bacillus subtilis* var. *natto* KCCM 11315 and *Aspergillus oryzae* KCCM 60241 mixed, and fermented in an incubator for 48 h. Fermented samples were dried in a hot-air oven at 80°C for 24 h. After drying, fermented soybean (FS) was stored at 4°C until used.

### Experimental design and diets

All procedures were performed at the Department of Food Science and Biotechnology of Animal Resources, Konkuk University and met required departmental approvals. Purebred Yorkshire pigs were fed with one of the following two diets containing FS 0% and FS 1% for 4 weeks, respectively. The experimental diets were formulated to meet or exceed nutrient requirements of NRC (1998). FS was substituted in place of basal diet 1% levels (T1) on weight basis. The composition of the basal experimental diet is shown in Table 1. Thirty-six pigs were used in this experiment involving two separate trials, 18 pigs per trial. The pigs were allotted to two dietary treatments in each trial. Two pigs were assigned per pen (1.2 m<sup>2</sup>) in a totally slatted (concrete) environmentally regulated facility. Each pen, pigs had *ad libitum* access to feed and water via single-opening feeders and cup waterers. Individual pig body weight (BW) and feed disappearance were recorded weekly during each feeding phase to calculate average daily weight gain (ADG), average daily feed intake (ADFI), and gain:feed ratio on an as-fed basis.

### pH

The pH values of raw loins were measured in duplicate with a pH meter (340, Mettler-Toledo, Switzerland). Approximately 5 g of sample were cut into small pieces and homogenized with 20 mL of distilled water for 60 s

**Table 1. Composition of the experimental diet**

Ingredient (%)	Basal diet
Corn	68.76
Wheat bran	7.50
Soybean meal	17.30
Fat	4.00
Calcium carbonate	1.16
Salt	0.50
Dicalcium phosphate	0.28
Vit.+Min. mixture <sup>1)</sup>	0.24
Lys	0.15
Tylosin	0.05
Ethoxyquin	0.03
Phytase	0.03
Total	100
Calculated values	
TMEn <sup>2)</sup> (kcal/kg)	3,460
Crude protein (%)	15.23
Ca (%)	0.60
Total P (%)	0.43
Lysine (%)	0.86

<sup>1)</sup> Mineral mixture provided the following nutrients per kg of diet : vitamin A, 19,000 IU; vitamin D<sub>3</sub>, 4,000 IU; vitamin E, 90 IU; vitamin K<sub>3</sub>, 4.2 mg; vitamin B<sub>1</sub>, 3.0 mg; vitamin B<sub>2</sub>, 3.0 mg; vitamin B<sub>6</sub>, 4.5 mg; vitamin B<sub>12</sub>, 0.1 mg; niacin, 120 mg; pantothenic acid, 50 mg; folic acid, 2.5 mg; biotin, 0.08 mg; Fe, 75.0 mg; Co, 0.5 mg; I, 1 mg; Se, 0.2 mg.

<sup>2)</sup> Nitrogen corrected true metabolizable energy.

in an Ultra-Turrax (T25, Janke & Kunkel, Germany). The pH values were measured immediately after homogenization.

### Instrumental color

Objective color readings were determined in duplicate on a freshly cut loin surface adjacent to between the 7<sup>th</sup> and 9<sup>th</sup> ribs at 24 h postmortem. The samples were placed on a Styrofoam tray and over-wrapped with oxygen permeable film. After a 30 min bloom at 4°C, the color was determined using a Chromameter (CR 210, Minolta, Japan), which was set at CIE L\*, a\* and b\*, illuminant C. The instrument was standardized using white tile.

### Drip loss

Drip loss was measured as the weight loss during suspension of a standardized (5×4 cm) sample of about 50 g at 4°C. After 1 and 3 d storage time, the weight of the meat samples was measured.

### Cooking loss and shear force

The samples were cooked to an end-point temperature of 75°C on electric grills (Model CG20-1, Hobart, USA)

preheated to 180°C. The temperature of sample during cooking was monitored using an infrared thermometer (66, Fluke Corporation, USA). Each sample (7 mm thickness, about 15-20 g) was placed on the grill and cooked on the first side for 2 min, the second side for 2 min, and then turned every min until the endpoint temperature reached to 75°C. Cooked loins were cooled at room temperature for 30 min and then cooking loss was measured.

After cooling for 30 min, muscle sections of loins were compressed with a pate-type blade set attached to a Texture Analyzer (TA-XT2i, Stable Micro System Ltd., U.K.). Test speed was set at 2 mm/s. The average of the maximum force (Newton, SI unit of force) necessary to shear the samples was used for statistical analysis.

#### Water-holding capacity

Water-holding capacity (WHC) was measured by a modification of the procedure of Grau and Hamm (1953). Briefly, a 300 mg sample of muscle was placed in a filer-press device and compressed for 3 min. WHC was calculated from duplicate samples as a ratio of the meat film area to the total area.

#### Visual and sensory evaluations

Visual and sensory evaluations were assessed to 20 samples (10 pigs per treatment) obtained from farm and transported to Konkuk University. For these tests, the pork loin cuts were chilled at 4°C. Samples were cut from 7th to 9th ribs and used for visual and sensory evaluations. Panelists were screened and selected following procedures of directional difference test (paired comparison test). Initially, 10 panelists began the screening procedure. 2 pieces (7 mm thickness, each 25-30 g/person) of raw loins were presented on the same plate to the panelist to evaluate the visual characteristics. 20 samples were evaluated by each panelist (2 treatments, 10 samples per treatment). The pieces were scored for color, fat/meat structure, and overall preference using a 10-point scale (10= extremely desirable and 1= extremely undesirable). After visual evaluation, the samples for sensory test were cooked as previously described. Gas heaters placed under hot plates were used for cooking the pork samples, after pre-heating a hot plate for 3 min, the slices were cooked and served in front of panelists. The samples were provided in a coded manner and presented in a random manner. The hot plate was changed between samples, and, crackers and water were provided to cleanse the plate of the panelists.

A 10-point category scale was used for rating the dif-

ferent characteristics of the treatments. The cooked loin slices were assessed for visual appearance, color, flavor, tenderness, juiciness, and overall appeal. Visual appearance, color, flavor, and overall appeal were scored as follows; 10=extremely acceptable and 1=extremely unacceptable. Tenderness and juiciness were scored as follows; 10=extremely tender, extremely juicy; 1=extremely tough, dry.

#### Statistical analysis

All statistical analyses were performed using the GLM procedures of SAS software (SAS Institute, 2001). A software program using Duncan's multiple range test to compare treatment means was applied. A  $p < 0.05$  was considered statistically significant. One replicate was considered as the experimental unit for each performance parameter. The experimental unit was on pig for the other parameters. All data were expressed as mean $\pm$ SD.

## Results and Discussion

#### Growth performance

Our results showed that T1 animals had higher feed:gain ratio (FGR) than controls (Fig. 1). Average daily gain (ADG) and average daily feed intake (ADFI) in pigs fed FS were lower than controls. Overall, the effects of soy isoflavones on weight gain, feed intake, and feed efficiency appeared somewhat variable. Winters and Banz (1997) reported supplemental isoflavones increased weight gain in female rats with no change in feed efficiency, however, weight gain and feed efficiency decreased in male rats fed a similar diet. Cook (1998) reported that supplemental soy ISF (0 or 1,585 mg/kg) increased ADG in gilts from 6 to 30 kg BW. However, in a second study, Cook (1998) reported that dietary genistein (one form of ISF; 0, 200, 400, 600, or 800 mg/kg) did not affect ADG in barrows from 5 to 28 kg BW. A decrease in ADG and ADFI was seen in Sprague-Dawley rats fed a C-SPC diet supplemented with 0, 431, 862, or 1,724 mg/kg soy ISF (Cook, 1998).

Our results showed that FS significantly improved the growth performance of pigs. Similar growth-promoting effects were also found by Kim *et al.* (2005) who reported that pigs fed a diet with soybean meal fermented with *A. oryzae* had a greater ( $p < 0.05$ ) ADG than control. Zamora and Veum (1979) showed that whole soybeans fermented with *A. oryzae* increased growth performance of growing pigs. Previous studies using broiler chickens also produced a similar improvement in growth perfor-

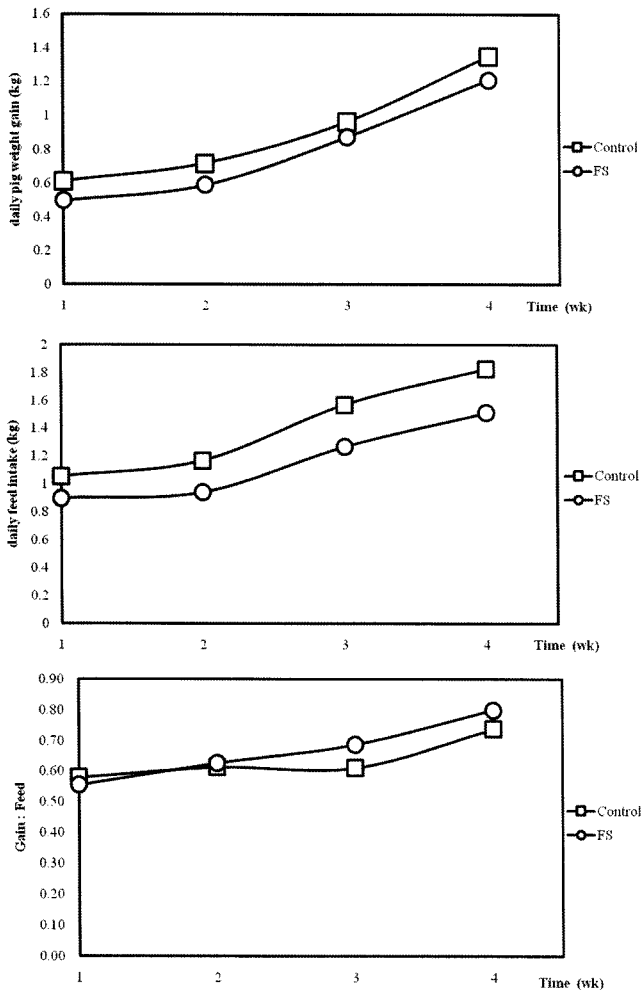


Fig. 1. Effects of FS on growth performance of pigs offered feed *ad libitum*.

mance. These growth-promoting effects may be due mostly to the increased nutrition value of fermented soybean (Feng *et al.*, 2007) because fermentation may improve digestibility in legumes (Odunfa, 1986; Sarkar and Tamang, 1995). Early observations by Van Veen and Schaefer (1950) showed that digestibility of whole soybeans were improved by fermentation. These data showed an increase in nutrient utilization in soybeans, particularly protein and energy, caused by fermentation. The improvement of protein and energy utilization may be due mostly to the elimination of trypsin inhibitor and the degradation of large-size (>60 kDa) protein in soybean meal after fermentation. Hong *et al.* (2004) found that fermentation with *A. oryzae*, significantly reduced trypsin inhibitor contents and the size of soybean peptide. The improvement of activities of intestinal enzymes in piglets fed with FS may suggest that lectins were degraded by *A. oryzae*. Lectins have been known to disrupt microvillar membrane integrity and to induce *Escherichia coli* overgrowth

in rats and piglet intestine (Kelly *et al.*, 1990). The mechanism by which FS affects the activities of intestinal enzymes needs further research. We also observed that the off-flavor of FS affected ADFI, ADG and overall appeal of feed. The more researches that about decrease the off-flavor of FS and improve the efficiency of feed should be need.

### Meat quality

Meat color has a significant effect on consumers' palatability, and is an important indicator determining meat quality. In meat color, CIE L\* that indicates lightness (whiteness) was higher in controls compared to T1 ( $p < 0.05$ ). In the value of CIE a\* that indicates redness, T1 samples were higher than controls ( $p < 0.05$ ). In the value of CIE b\* that indicates yellowness, controls were higher than T1 ( $p < 0.05$ ). The rate of discoloration of meat is believed to be related to the effectiveness of oxidation processes and enzymic reducing systems in controlling metmyoglobin levels in meat (Faustman and Cassens, 1989). The CIE L\* value of chicken meat color was significantly increased by supplemental 40 and 80 mg ISF/kg (Jiang *et al.*, 2007). Payne *et al.* (2001a) demonstrated that the CIE a\* and CIE b\* color scores were decreased linearly as ISF increased. Supplemental daidzein in the maternal diet during late gestation did not affect meat color of the progeny (Rehfeldt *et al.*, 2007). These results suggested that different mechanisms or factors possibly influence oxidative damage to lipids and proteins (Sen *et al.*, 1997).

The pH of meat is closely related to change in meat qualities such as water holding capacity (WHC) and tenderness. The pork samples in our study showed pH ranging between 5.48-5.62 (Table 2). T1 samples showed increased pH compared to controls ( $p < 0.05$ ). This result may be attributed to a decreased lactic acid production in muscles postmortem (Lee *et al.*, 1979; Raj *et al.*, 1990, 1992). The inability of muscle cells to rid themselves of metabolic by-products such as lactic acid caused a decrease in pH (Judge *et al.*, 1989). This decrease in pH can affect WHC (Ferket and Foegeding, 1994; Pearson, 1994). WHC is known to affect meat color, texture, the hardness in fresh meat, and tenderness and juiciness of cooked meat. In general, WHC is higher when pH or marbling scores are high. T1 samples showed slightly increased WHC values compared with controls, but this increase was not statistically significant (Table 2). The cooking loss is the measurement of drip loss in meat caused by physical force (heat). It is one of methods that

can be used to assess the WHC of meat. T1 samples had significantly lower drip loss compared to controls ( $p < 0.05$ ). The drip loss increased steadily as time postmortem increased, after 1 d and 3 d postmortem storage, yet drip loss in T1 samples was significantly lower than controls ( $p < 0.05$ ). We also measured the shear force of meat in order to assess tenderness objectively. Shear force is a force applied when meat is cut in the direction perpendicular to the muscular fiber of the meat. The shear force of pork loins depends on the structure of the meat tissue layer. In the present experiment, we minimized variation (deviation) among the samples by measuring shear force using a plate-type blade set. In the experiment (Table 2), the shear force was similar among T1 samples and controls ( $p > 0.05$ ).

**Table 2. Dietary effects of fermented soybean on pork quality**

Items	Control	FS <sup>1)</sup>
pH	5.48±0.04 <sup>b2)</sup>	5.62±0.07 <sup>a</sup>
CIE color score		
L*	54.88±2.60 <sup>a</sup>	48.97±1.34 <sup>b</sup>
a*	13.97±0.36 <sup>b</sup>	14.39±0.45 <sup>a</sup>
b*	3.37±0.69 <sup>a</sup>	2.02±0.56 <sup>b</sup>
Drip loss (%)		
1 d	6.27±1.25 <sup>a</sup>	4.38±1.25 <sup>b</sup>
3 d	9.50±1.03 <sup>a</sup>	6.85±1.66 <sup>b</sup>
Cooking loss (%)	31.55±2.09 <sup>a</sup>	28.29±2.03 <sup>b</sup>
Water holding capacity (%)	33.28±7.28	38.36±4.86
Shear force (kg)	5.84±1.14	5.99±0.63

<sup>1)</sup> FS: fermented soybean.

<sup>2)</sup> Mean±SD.

<sup>a, b</sup> Mean±SD values in a same row with no common superscripts are significantly different ( $p < 0.05$ ).

**Table 3. Dietary effects of fermented soybean on sensory characteristics of pork**

Items	Control	FS <sup>1)</sup>
Visual evaluations		
Color	6.90±0.70 <sup>b2)</sup>	7.83±0.27 <sup>a</sup>
Fat/meat structure	7.58±0.21 <sup>b</sup>	7.78±0.32 <sup>a</sup>
Overall preference	5.83±0.61 <sup>b</sup>	7.07±0.50 <sup>a</sup>
Sensory evaluations (after cooking)		
Color	7.25±0.14 <sup>b</sup>	8.22±0.20 <sup>a</sup>
Visual appearance	7.70±0.05 <sup>b</sup>	7.90±0.09 <sup>a</sup>
Flavor	8.83±0.15	8.19±0.32
Tenderness	6.08±0.23 <sup>b</sup>	7.14±0.40 <sup>a</sup>
Juiciness	6.73±0.25 <sup>b</sup>	7.02±0.43 <sup>a</sup>
Overall appeal	7.07±0.23 <sup>b</sup>	8.36±0.19 <sup>a</sup>

<sup>1)</sup> FS: fermented soybean.

<sup>2)</sup> Mean±SD.

<sup>a, b</sup> Mean±SD values in a same row with no common superscripts are significantly different ( $p < 0.05$ ).

Sensory evaluations were made through visual evaluations (color, fat/meat structure, overall preference) before cooking pork loins and sensory evaluations (color, visual preference, flavor, tenderness, juiciness, and overall appeal) after cooking. These results are presented in Table 3. In visual evaluation, the color, fat/meat structure and overall preference of T1 samples were evaluated higher than controls ( $p < 0.05$ ). In sensory evaluation, visual preference and flavor were not significantly different among T1 samples and controls. Color, tenderness, juiciness and overall preference of T1 were significantly higher than controls.

Overall, our results indicate that FS added to in pig feed can improve both the feed efficiency and pork quality. Also we presented evidence that suggests that FS could be used for lowering cholesterol levels in meats.

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