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Evaluation of partial wheat grain replacement with fermented wheat on growth performance, blood characteristics and fecal noxious gas emission in finishing pigs

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ABSTRACT

The present study investigated the effects of partial wheat grain replacement with fermented wheat on growth performance, blood characteristics, fecal noxious gas emission and meat quality in finishing pigs. Finishing pigs 144 [(Yorkshire × Landrace) × Duroc] with an average body weight of 57.99 ± 1.92 kg were used. The dietary treatments were corn-soybean meal based diets with different inclusions of wheat grain and substitutions by fermented wheat, as follows: T1) 10% wheat grain and 0% fermented wheat; T2) 5% wheat grain + 5% fermented wheat; T3) 5% wheat grain and 0% fermented wheat; and T4) 2.5% wheat grain + 2.5% fermented wheat. Pigs fed T2 and T4 had increased body weight, ADG, and G/F ratio compared to those fed T1 and T3, respectively. Pigs fed T1 and T4 increased IgG concentration compared to those fed T3 at 12 week. T2 treatment increased glucose concentration compared to T1 treatment. Pigs fed T1 had increased BUN concentration compared to those fed T2 and T3 at 6th week. There was no significant difference on fecal noxious gas emission and meat quality among treatments. From the results, we suggest that partial wheat grain replacement with fermented wheat improved body weight, ADG, and G/F ratio in finishing pigs. Therefore, fermented wheat can be used as a raw cereal substitute, improving the productivity in finishing pig.

Key words: Average daily gain, Body weight, Finishing pigs, Glucose, Wheat

Wheat has slightly lower concentration of energy compared to corn but it has a greater concentration of amino acids than corn, making it more attractive for pig diets. Also, wheat contains variable amounts of non-starch polysaccharides (NSP) that can be used in the large intestine as a substrate for microbial fermentation (Knudsen *et al*. 1991). Feeding with fermented grains could improve the digestibility of protein and starch by increasing the activities of hydrolytic enzymes and gastrointestinal health, caused by decreased gastric pH, greater gastric lactic acid concentration, and decreased number of enteric pathogens (Chavan and Kadam 1989, Lindecrona *et al*. 2003). In previous reports, fermented wheat has improved feed efficiency and nutrient digestibility and decreased fecal noxious gas emission in pigs (Jorgensen *et al*. 2010, Cho *et al*. 2013). However, effect of fermented wheat on the growth performance was inconsistent depending on fermentation condition including a substrate for microbial fermentation (Chavan and Kadam 1989). It has been reported that fermented grains by *Aspergillus niger* (*A. niger*) that is one of the most common fungal species have improved digestibility of amino acids, decreased *Escherichia coli* infection, and shorter duration of diarrhea in pigs (Zhang *et al.* 2007, Cho *et al.* 2013). Thus, the objective of present study was to evaluate effects of partial wheat grain replacement with fermented wheat mediated *A. niger* on growth performance, blood characteristics, and fecal noxious gas emission, and meat quality in finishing pigs.

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MATERIALS AND METHODS

The Animal Care and Use Committee of Dankook University approved all experimental protocols used in the present study.

Preparation of fermented wheat: Dried raw wheat was soaked in distilled water (45%) for 1h. Hydrated wheat was then cooked in a steam tank at 60–70°C for 1h. Thereafter, cooked wheat was cooled to 25°C and mixed with inoculums of *Aspergillus niger GB-124* at an initial count of 10^3 or 10^4 cfu/g. After fermentation for 36 h, the final number of micro-organisms was guaranteed at a concentration of 10^9cfu/g; the fermented wheat was ground in a hammer mill and refrigerated (4°C) until it was mixed in the experimental diets.

Experimental design, animals, housing and diets: A total of 144 crossbred pigs [(Yorkshire × Landrace) × Duroc] with an average body weight (BW) of 57.99 ± 1.92 kg were used in a 12 week trial. The pigs were sorted into pen with six pigs per pen and six pens per treatment. The dietary treatments were corn-soybean meal based diets with different inclusions of wheat grain and substitutions by fermented wheat grain, as follows: T1, 10% wheat grain and 0% fermented wheat; T2, 5% wheat grain + 5% fermented wheat; T3, 5% wheat grain and 0% fermented wheat; and T4, 2.5% wheat grain + 2.5% fermented wheat. All diets were formulated to meet or exceed the NRC nutrition requirements (Table 1) (NRC 2012). Dietary calcium (Ca), phosphorus (P), and crude protein (CP) were analyzed according to the procedures described by the AOAC (AOAC 2012). Dietary Ca was assayed by atomic absorption spectrophotometry after wet ash procedures and P was determined by colorimetry. Energy was determined by using a Parr 6100 oxygen bomb calorimeter. Pigs were housed in an environmental controlled room with an average temperature of 22°C. Lights were supplied from 06.00 to 24.00 h. Each pen (1.8 m width × 1.8 m length) was equipped with a one-sided self-feeder and a nipple drinker to allow pigs *ad libitum* access to feed and water, respectively, throughout the experiment.

Table 1. Experimental based diet compositions (as fed-basis)

Ingredients,%	T1	T2	T3	T4
Corn	45.32	44.22	53.22	52.12
Soybean meal (48% CP)	35.00	35.00	35.00	35.00
Wheat	10.00	5.00	5.00	2.50
Fermented wheat		5.00		2.50
Fish meal (66% CP)	4.00	4.00	3.00	3.00
Animal fat	3.40	4.50	1.90	3.00
Dicalcium phosphate	1.00	1.00	0.10	0.10
Limestone	0.70	0.70	-	-
L-Lysine	0.26	0.26	-	-
DL-Methionine	0.02	0.02	-	-
Vitamin$_1$	0.20	0.20	0.08	0.08
Mineral$_2$	0.10	0.10	0.05	0.05
Limestone	-	-	1.15	1.15
Salt	-	-	0.50	0.50
Total	100.00	100.00	100.00	100.00
Calculated contents,%	0	0	0	0
Metabolizable energy, kcal/kg	3,396	3,446	3,254	3,304
Analyzed composition,%				
Crude protein	24.20	24.20	23.70	23.70
Lysine	1.60	1.60	1.36	1.36
Methionine	0.43	0.43	0.39	0.39
Calcium	0.82	0.82	0.73	0.73
Phosphorus	0.70	0.70	0.52	0.52

[1]Provided per kg of complete diet: vitamin A, 11,025 IU; vitamin D_3, 1,103 IU; vitamin E, 44 IU; vitamin K, 4.4 mg; riboflavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; pantothenic acid, 29 mg; choline, 166 mg; and vitamin B_{12}, 33 µg. [2]Provided per kg of complete diet: Cu, 12 mg; Zn, 85 mg; Mn, 8 mg; I, 0.28 mg; and Se, 0.15 mg.

Sampling and measurements: Individual pig body weight (BW) was recorded at the 0 (beginning), 6th and 12th week of the experimental period, and feed consumption was recorded on a per pen basis during the experiment to calculate average daily gain (ADG), average daily feed intake (ADFI), and G/F ratio.

At the beginning of the experiment, two pigs were randomly selected from each pen and 5 ml blood samples were collected via jugular venipuncture on 6th and 12th week. At the time of collection, blood samples were taken into K2 vacuum tubes. After collection, serum samples were centrifuged (2,000 '×' g) for 15 min at 4°C and serum was stored at –20°C for later analysis of IgG, glucose, BUN (blood urea nitrogen) by an automatic biochemistry.

Feces were collected by rectum massage on pen basis using a steel plate under the cage on 6th and 12th week to determine fecal ammonia (NH_3), hydrogen sulfide (H_2S), total mercaptans, and acetic acid emission. Amounts of 150 g fresh feces were stored in 2.6 L plastic boxes for replicates (Yan *et al.* 2012a, 2012b). The samples were fermented for 7 days at 28°C. During the fermentation period, a gas sampling pump was utilized for gas detection at 6th and 12th week. The adhesive plasters were punctured, and 100 ml of headspace air was sampled approximately 2 cm above the feces surface in 1min.

At the end of the experiment, all pigs were transferred to the slaughter house and treated with conventional procedures. Carcasses were chilled at 2°C for 24 h and a piece of the right loin was taken through a perpendicular cut between the 10th and 11th ribs. Before the meat quality evaluation, meat samples were thawed at ambient temperature. The color measurements of lightness (L*), redness (a*), and yellowness (b*) values were determined with a Minolta CR410 chromameter. Sensory evaluation (color, marbling, and firmness scores) was conducted according to the National Pork Producers Council standards (NPPC 1991). At the same time, duplicate pH values of each sample were directly measured with a pH meter. The water-holding capacity (WHC) was measured according to a previous report (Kauffman *et al.* 1986). Briefly, 0.3 g sample was pressed at 3,000 psi for 3 min on a 125 mm diameter filter paper. The areas of the pressed sample and expressed moisture were delineated and then determined with a digitizing area-line sensor. The ratio of water:meat area was calculated as a measure of WHC (a smaller ratio indicates higher WHC). The loin muscle area (LMA) was measured by tracing the loin muscle surface at the 10th rib, which also used the above-mentioned digitizing area-line sensor. Drip loss was measured using a ~2 g meat sample according to a previous report described as the 'plastic bag method' (Honikel 1998).

Statistical analysis: All data were subjected to statistical analyses as a randomized complete block design using the GLM procedure of the SAS software (SAS Institute 1996), with the pen as the experimental unit. Orthogonal contrasts used to separate treatment means were: 1) T1 vs T2, 2) T1 vs T3 and 3) T3 vs T4. Variability in the data was expressed as the pooled standard error (SE). P values<0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

Growth performance: Effect of dietary supplementation of wheat on growth performance is shown in Table 2. Pigs fed T2 had increased body weight compared to those fed T1 at 6 and 12 wk. Also, pigs fed T4 had increased body weight compared to those fed T3 at 6 and 12 wk. On phase I, pigs fed T2 and T4 had increased ADG and G/F ratio compared to those fed T1 and T3, respectively. On phase II, pigs fed T2 had increased ADG compared to those fed T1. On overall, pigs fed T2 and T4 had increased ADG and G/F ratio compared to those fed T1 and T3, respectively. These results indicated that feeding with 10% wheat replaced by 5% fermented wheat increased growth performance except for ADFI compared to feeding with non-fermented wheat in finishing pigs.

In the present study, growth performance was improved by dietary supplementation of fermented wheat. However, effect of dietary supplementation of fermented wheat on growth performance has inconsistent results. In disagreement with the present report, piglets fed liquid diets with fermented wheat had un-improved ADG, ADFI, and G/F ratio (Canibe *et al.* 2007). Also, different dose of wheat inclusion did not significantly affect live weight gain (LWG) or feed conversion ratio (FCR) (Ball and Magowan 2012). However, in another previous report, the ADG was improved when pigs were fed with fermented wheat grain containing 45%, as compared with those fed non-fermented wheat grain (Cho *et al.* 2013). In addition, the G/F was improved when pigs were fed liquid feed containing 45% fermented wheat, as compared with those fed non-fermented liquid feed (Scholten *et al.* 2002). The exact reason how fermented diets improved growth performance of pigs is not yet clear. However, the facts that fermented diets reduce the gastric pH and the number of coliform bacteria in the gastrointestinal tract compared with non-fermented diets can explain that fermented diets improve growth performance of pigs.

Blood characteristics: Effect of dietary supplementation of wheat on blood characteristics is indicated in Table 3.

Table 2. The effects of dietary supplementation of fermented wheat on growth performance in finishing pigs

	T1	T2	T3	T4	SEM	T1 vs T2	T1 vs T3	T3 vs T4
Body weight, kg								
Initial	52.34	51.85	51.89	51.35	0.77	0.66	0.84	0.63
6 wk	80.92	83.36	79.22	82.31	1.13	0.04	0.45	0.01
12 wk	117.63	122.88	114.75	119.06	1.13	<0.01	0.19	0.01
Phase 1 (0 to 6 wk)								
Average daily gain, g	680.63	750.24	650.79	737.22	14.02	<0.01	0.11	<0.01
Average daily feed intake, g	2005.91	2009.76	1941.19	1953.85	43.10	0.95	0.85	0.84
G/F	0.34	0.37	0.34	0.38	0.01	<0.01	0.06	<0.01
Phase 2 (6 to12 wk)								
Average daily gain, g	874.11	940.89	845.87	874.92	19.81	0.03	0.51	0.31
Average daily feed intake, g	2515.93	2616.49	2696.85	2682.62	41.69	0.10	0.97	0.81
G/F	0.35	0.36	0.31	0.33	0.01	0.28	0.56	0.29
Overall (0 to 12 wk)								
Average daily gain, g	777.37	845.57	748.33	806.07	9.13	<0.01	0.09	<0.01
Average daily feed intake, g	2260.92	2313.13	2319.02	2318.23	34.21	0.29	0.89	0.99
G/F	0.34	0.37	0.32	0.35	0.01	<0.01	0.20	<0.01

Table 3. The effects of dietary supplementation of fermented wheat on blood profiles in finishing pigs

	T1	T2	T3	T4	SEM	T1 vs T2	T1 vs T3	T3 vs T4
IgG, mg/dL								
0 wk	659.60	801.40	716.60	670.20	86.57	0.26	0.59	0.71
6 wk	971.00	814.20	993.00	871.60	68.05	0.12	0.06	0.23
12 wk	1011.80	1029.80	761.00	987.60	54.55	0.82	0.04	0.01
Glucose, mg/dL								
0 wk	87.20	85.80	89.20	95.60	6.61	0.88	0.71	0.50
6 wk	75.20	89.00	66.60	76.80	2.31	<0.01	<0.01	0.01
12 wk	88.20	81.00	68.40	78.00	3.25	0.14	0.72	0.05
Blood urea nitrogen, mg/dL								
0 wk	11.46	12.26	12.68	11.12	0.92	0.55	0.68	0.25
6 wk	16.40	12.92	12.82	11.64	0.65	<0.01	<0.01	0.21
12 wk	18.42	15.92	12.36	12.08	1.03	0.11	0.20	0.85

Pigs fed T1 and T4 had increased IgG concentration compared to those fed T3 at 12 wk. On glucose concentration, T2 treatment increased glucose concentration compared to T1 treatment and pig fed T1 and T4 had increased glucose concentration compared to those fed T3 at 6 wk. On BUN concentration, pigs fed T1 had increased BUN concentration compared to those fed T2 and T3 at 6 wk.

In the present study, pigs fed fermented wheat had higher concentration of the blood parameters than those fed non-fermented wheat. Fermented grain diets reportedly alter blood profiles such as BUN. Plasma urea nitrogen was decreased in pigs fed 5% fermented potato pulp in growing pigs (Li *et al.* 2011). In addition, feeding of 10 or 15% fermented soy protein to nursery pigs improved BUN and total protein concentrations in blood (Cho *et al.* 2007). Fermentation of grains in feedstuffs increased glucose and cholesterol concentration by increasing availability of sugars in the small intestine and absorption of absorbed short-chain fatty acids (Yen *et al.* 1991). Plasma glucose and cholesterol concentrations in the blood increased in pigs fed fiber-fermenting bacteria, as compared with pigs fed no bacterium (Ziemer *et al.* 2012). De Roda s*et al.* (1996) showed that growing-finishing pigs fed a *Lactobacillus acidophilus* had decreased serum cholesterol concentrations, as compared with no probiotics. In addition, inclusion of a *Bacillus lichenformis* and *Bacillus subtilis* product in feed did not alter cholesterol concentrations in blood at 1 d postpartum; but by 15 d postpartum, pigs fed the probiotic treatment had greater cholesterol concentration than pigs fed no probiotics (Alexopoulos *et al.* 2004). The exact reason how fermented diets influenced blood profiles including glucose of pigs is not yet clear. However, the facts that fermented diets increased energy in diet composition compared with non-fermented diets can explain that fermented diets increased glucose concentration in blood of pigs.

Fecal noxious gas emission: Effect of dietary supplementation of wheat on fecal oxious gas emission is indicated in Table 4. The present study indicated that fermented wheat had no affect on noxious gas emission. In disagreement with the present study, a number of reports

Table 4. The effects of dietary supplementation of fermented wheat on fecal noxious gas emission in finishing pigs

	T1	T2	T3	T4	SEM	T1 vs T2	T1 vs T3	T3 vs T4
6 wk								
Ammonia	16.90	16.43	14.90	15.53	0.51	0.54	0.41	0.96
Total mercaptans	25.13	23.03	22.80	23.07	0.67	0.06	0.79	0.87
Hydrogen sulfide	14.27	15.10	13.47	13.80	0.69	0.42	0.74	0.97
Acetic acid	19.10	18.63	17.97	18.20	0.53	0.55	0.76	0.93
12 wk								
Ammonia	16.80	16.47	14.60	14.33	0.52	0.66	0.73	0.82
Total mercaptans	23.60	24.07	22.20	22.53	1.09	0.77	0.83	0.98
Hydrogen sulfide	14.37	13.57	13.67	13.27	0.77	0.48	0.72	0.91
Acetic acid	18.87	18.47	18.00	16.90	0.49	0.58	0.15	0.87

Table 5. The effects of dietary supplementation of fermented wheat on meat quality in finishing pigs

	T1	T2	T3	T4	SEM	T1 vs T2	T1 vs T3	T3 vs T4
pH	5.69	5.68	5.65	5.70	0.03	0.86	0.46	0.23
Longissimus muscle area (cm^2)	48.15	49.67	49.52	49.70	0.86	0.23	0.34	0.88
Water holding capacity (%)	62.41	62.93	62.80	65.54	1.76	0.89	0.37	0.29
Meat color								
L (lightness)	59.68	60.02	61.56	60.83	0.91	0.80	0.83	0.58
a (redness)	18.00	19.00	19.21	18.25	0.42	0.17	0.97	0.12
b (yellowness)	10.33	9.67	10.80	10.14	0.47	0.34	0.18	0.34
Cooking loss (%)	29.09	27.90	29.30	28.72	0.85	0.34	0.31	0.63
Sensory evaluation								
Color	1.63	1.73	1.67	1.75	0.06	0.20	0.11	0.31
Marbling	1.58	1.69	1.54	1.69	0.85	0.20	0.19	0.08
Firmness	1.54	1.69	1.63	1.67	0.10	0.14	0.35	0.11
Drip loss (%)								
D1	7.21	5.17	8.86	5.41	1.12	0.21	0.09	0.06
D3	10.88	11.05	14.64	9.33	1.88	0.95	0.19	0.06
D5	13.24	14.94	17.21	13.25	1.67	0.48	0.51	0.11
D7	16.06	17.98	19.55	16.64	1.42	0.35	0.73	0.16

discussed how fermented diets alter fecal noxious gas emission (Awati *et al.* 2006, Cho *et al.* 2013). In previous report, fermented diets decreased fecal NH_3 content, as compared to non-fermented diets in weanling pigs (Awati *et al.* 2006). In addition, fermented corn diets decreased fecal H_2S and acetic acid contents, as compared to non-fermented corn diets in growing pigs (Cho *et al.* 2013). They suggested that the decreased fecal noxious gas emission by fermented diets may be due to a shift in microbial population and a shift in fermentation acids produced by microbes (Scholten *et al.* 2002, Cho *et al.* 2013).

Meat quality: No significant differences were observed on pH, LMA, WHC, meat color, sensory evaluation, and drip loss among all treatments ($P>0.05$). Meat quality is an important criterion for meat processors and consumers (Wood *et al.* 2004, Raes *et al.* 2004). In the present study, meat quality was not influenced by dietary supplementation of fermented wheat. However, according to a number of previous reports, fermented diets influence meat quality in pigs. The supplementation of fermented garlic powder at the level of 2 and 4g/kg in finishing pig diet increased marbling and firmness scores, as compared to non-fermented garlic powder (Yan *et al.* 2011). In addition, fermented apple diet supplementation increased WHC and pH and improved marbling of fresh meat and tenderness, juiciness, flavor and overall acceptability of cooked meat, as compared to non-fermented apple diets in the finishing pigs (Lee *et al.* 2009). The result that pigs fed fermented wheat showed a higher marbling score, as compared to non-fermented wheat diet may be due to stimulation of feed intake by dietary fermented wheat addition. However, the exact mechanism remains unknown and further research is necessary to investigate how fermented wheat affects meat quality.

In conclusion, results indicated that partial wheat grain replacement with fermented wheat improved body weight, ADG, and G/F ratio in finishing pigs. Therefore, fermented wheat can be used as raw cereal substitutes, improving the productivity in finishing pigs.

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