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Effects of fermented whole-crop wheat and barley with or without supplementing inoculant (probiotics) on palatability, growth performance, nutrient digestibility, fecal microbiota and blood constituents in finishing pigs

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ABSTRACT

Two experiments were conducted to evaluate the effect of fermented whole crop cereal on palatability and performance in finishing pigs. In Exp. 1, a total of 20 finishing pigs (Landrace × Yorkshier) Duroc) were allotted to 4 dietary treatments to check the palatability of the dietary feed. Diet treatments were included a basal diet; FW = basal diets + 1% fermented wheat without inoculum, FWI = basal diets + 1% fermented wheat with inoculum, FB = basal diets + 1% fermented barley without inoculum, FBI = basal diets + 1% fermented barley with inoculum. Throughout the experimental period, pigs fed FWI and FBI diets had significantly higher feed palatability compared with FW, FB diets. In Exp. 2, a total of 20 finishing pigs were allotted to 4 dietary treatments (1 pigs/pen, 5 pigs/treatment). Dietary treatments were same as Exp. 1. In nutrient digestibility, pigs fed FWI had higher dry matter digestibility. The number of *Lactobacillus* in feces was significantly higher in FWI treatments inoculated with feed microorganisms. Our results indicated that dietary supplementation with fermented wheat and barley with supplementing inoculant had a beneficial effect in finishing pigs.

Key words: Fermented wheat and barley, Finishing pigs, Microbial inoculum, Nutrient digestibility, Palatability.

INTRODUCTION

Fermentation is an effective way to reduce dietary fibers, help in proliferation of lactic acid bacteria and produce VFA (volatile fatty acid; acetic acid, butyric acid, propionic acid), which have beneficial effect on nutrient digestibility and gut health. Jørgensen *et al.* (2010) reported that NSP was significantly reduced in fermented wheat and barley and that their dry matter and energy digestibility in the ileaum was higher than that of dry feed. Van Winsen *et al*., (2001) showed that the number of *lactobacillus* in the stomach and intestines significantly increased in pigs fed the fermented diets compared to dry diets. Furthermore, The added microorganisms in the feed can have a positive effect on growth rate, digestibility and microorganisms (Liu *et al*., 2018; Nguyen *et al*., 2018).

Therefore, the objective of the study was to determine the effect of fermented whole wheat and barley with or without supplementing inoculants (probiotics) on palatability, growth performance, nutrient digestibility, blood constituents and fecal microbiota in finishing pigs.

MATERIALS AND METHODS

The Animal Care and Use Committee of Chungbuk National University approved all the experimental protocols used in the current study.

Producing fermented whole crop wheat and barley with inoculum

Wheat and barley were harvested on the 40th and 35th day after heading, respectively and were ground in a hammer mill to pass through 5-mm screen. The inoculum was then supplemented and they were mixed in formula diets after fermentation and stabilization for a period of 60 days in gunny bag. When the fermentation was completed, the prepared wheat and barley silage was packed in vacuum. The inoculum supplemented as fermentation additives were *lactobacillus sp* 3-1, 5-1, 14-1, similar to *L. plantarum* and they were isolated from rice straw silage.

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Experiment 1: Palatability study

Experimental design, animals, housing and diets

A total of 20 cross-bred finishing pigs (Landrace Yorkshire) Duroc, having average body weight of 82.3 ± 2.6 kg were used in a 4-weeks experiment designed to evaluate the effects of fermented whole crop wheat and barley with inoculum on palatability. The 20 pigs were housed in an environmentally controlled room (24 to 26°C) having an area of 23.04 m^2. Pigs were free to choose four experimental diets from four feeders. The experimental diets were: FW = basal diet + 1% fermented wheat without inoculum, FWI = basal diet + 1% fermented wheat with inoculum, FB = basal diet + 1% fermented barley without inoculum, FBI = basal diet + 1% fermented barley with inoculum. The dietary treatments were formulated to meet or exceed the NRC (2012) estimates of nutrient requirements for finishing pigs and were isocalorific, isonitrogenous and had similar lysine levels (Table 1). On the 7^{th}, 14^{th} and 21^{st} days of the experiment, at 9:00 am, the experimental diets from each feeder were rotated to different feed treatments.

Experiment 2: Feeding study

Experimental design, animals, housing and diets

A total of 20 cross-bred finishing pigs (Landrace Yorkshire) Duroc, average body weight of 75.8 ± 0.4kg) were utilized in a 4-week experiment designed to assess the effects of fermented whole crop wheat and barley on growth performance, nutrient digestibility, serum characteristic, and fecal microbial shedding. Dietary treatments were same as in experiment 1. The pigs were placed in individually in metabolism cages (1.8 0.8 m^2) in an environmentally controlled room (24 to 26°C), equipped with slatted floors, stainless steel feeders, automatic water nipple drinkers.

Sampling and measurements

Pigs were weighed at the beginning and the end of the experiment period, feed intake was recorded on pen basis during the experiment to calculate average daily gain (ADG), average daily feed intake (ADFI) and gain to feed ratio (G: F). Chromium oxide (Cr_2O_3) was added to the diet at 0.20% as an indigestible marker to calculate the digestibility of dry matter (DM), crude protein (CP) for 7 days prior to fecal collection at the 4^{th} week. The fecal samples were pooled within pen and dried in a forced air-drying oven at 70°C for 72 h and finely ground to pass through a 1mm screen. The procedure utilized for the determination of DM and CP digestibility was conducted in accordance with the methods established by the AOAC (2000). On last day of experiment, 5-ml blood sample was collected by jugular vein puncture from pigs in each pen. Blood samples were collected into vacuum tubes containing no additives to obtain serum. After centrifugation (3000 ×g for 15 min at 4°C), blood urea nitrogen (BUN), creatinine, total cholesterol, triglyceride and glucose were determined by the automatic biochemistry analyzer (HITACHI 747, Tokyo, Japan). Fecal samples were collected directly via massaging the rectum of pigs in each pen at the end of the experiments. Samples from each pen were pooled and placed on ice for transportation to the laboratory, 1 g of fresh sample from each pen was then diluted with 9 ml of 1% peptone broth (Becton, Dickinson and Co.) and homogenized. Viable counts of bacteria in the fecal samples were then conducted by plating serial 10-fold dilutions (in 1% peptone solution) onto MacConkey agar plates (Difco Laboratories, Detroit, MI, USA) and lactobacilli medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany) to isolate the *E. coli* and *Lactobacillus*, respectively.

Statistical analysis

All data were subjected to the statistical analysis as a randomized complete block design using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC) and Two-way ANOVA was used to examine the difference between the dietary ingredients and inoculum and the interaction effect.

Table 1: Basal diet composition for Experiment 1 and Experiment. 2 (as-fed basis).

Items	FW	FB
Ingredients, %		
Corn	56.71	56.71
Soybean meal (44%)	32.45	32.45
Fermented whole crop wheat	1.00	-
Fermented whole crop barley	-	1.00
Wheat bran	5.00	5.00
Soybean oil	2.00	2.00
Dicalcium phosphate	1.47	1.47
Limestone	0.63	0.63
Salt	0.25	0.25
DL-Methionine	0.08	0.08
L-Lysine-HCI	0.08	0.08
L-Threonine	0.03	0.03
Vitamin premix[a]	0.2	0.2
Mineral premix[b]	0.1	0.1
Calculated nutrients		
ME kcal/kg	3286	3282
Crude protein, %	19.99	19.99
Lysine, %	1.164	1.164
Methionine, %	0.388	0.388
Calcium, %	0.68	0.68
Total P, %	0.7	0.71

FW = 1% fermented whole crop wheat diets, FB = 1% fermented whole crop barley diets, ME = metabolizable energy.

[a]Provided per kilogram of diet: 4,500 mg of vitamin A, 93.75 mg of vitamin D3, 37.5 mg of vitamin E, 2.55 mg of vitamin K3, 3 mg of thiamin, 7.5 mg of riboflavin, 4.5 mg of vitamin B6, 24 µg of vitamin B12, 51 mg of niacin, 1.5 mg of folic acid, 0.2 mg of biotin and 13.5 mg of pantothenic acid.

[b]Provided per kilogram of diet: 37.5 mg of Zn, 37.5 mg of Mn, 37.5 mg of Fe, 3.75 mg of Cu, 0.83 mg of I, 62.5 mg of S and 0.23 mg of Se.

Differences among treatment means were determined using the duncan's multiple range test with a P < 0.05 indicating a significance.

RESULTS AND DISCUSSION

Experiment 1

Palatabilit

Palatability of pigs fed experimental diets is presented in Table 2. FB group had lowest (p < 0.05) ADFI compared to other treatments. ADFI was higher (p < 0.05) in the inoculum group than in the non - inoculum group. ADFI was higher (p < 0.05) in fermented wheat than in fermented barley. An interactive effect between inoculum and ingredient were observed on ADFI. Using fermenting agent during storage could be a useful way to improve nutrient content and animal performance, it could help to make good quality silage and microbial inoculation is environmentally friendly and safe to handle (Yitbarek and Tamir, 2014). When the fermentation was complete, the number of lactic acid bacteria was found to have increased as compared with other organic acids (Hackl *et al*. 2010; Hristov and MaAllister 2002). In the state of natural fermentation, acetic acid and biogenic amine, which negatively affect palatability and intake, were further increased (Brooks *et al.,* 2003; Brooks, 2008). Brooks (2008) demonstrated that using inoculants can result in consistent fermentation and high lactic acid concentrations. In previous study, concentration of lactic acid was significantly higher in the silage supplemented with inoculum compared with silage supplemented without inoculum this result is in agreement with Hristov and MaAllister, (2002).

Experiment 2

Growth performance

In growth performance, there were no significant difference (P > 0.05) in final body weight, average daily gain, average daily feed intake and gain to feed ratio among all treatments (Table 3). Feeding of acidified feeds is a good way to maintain pig intestinal health and biological performance without the use of antibiotics (Brooks, 2008).The fermented diets fed with lactic acid bacteria were able to reduce the amount of -glucan, which is an part of dietary fiber, the growth rate of broilers fed fermented wheat and barley was increased (Skrede *et al.,* 2001; Skrede *et al*. 2003). Broilers fed fermented feeds have a higher growth rate than those fed dry feeds (Chiang *et al.,* 2010; Yasar *et al.,* 2016).

Nutrient digestibility

The DM digestibility of the treatments inoculated with probiotics were significantly higher (P < 0.05) than those notinoculated with probiotics (Table 4). Fermented whole crop wheat showed significantly higher (P < 0.05) digestibility

Table 2: Effect of fermented whole-crop wheat and barley with or without supplementing inoculant (probiotics) on palatability in finishing pigs.

Items, kg/pen	FWI	FW	FBI	FB	SE[2]	*P* - value		
						Inoculum	Ingredient	Inoculum × Ingredient
Total ADFI	3.16[a]	3.06[a]	3.13[a]	2.81[b]	0.03	<.0001	<.0001	0.007
Total FI	88.6	85.6	87.6	78.6	0.1			

FWI = 1% fermented whole crop wheat diets supplemented with microbial inoculum, FW = 1% fermented whole crop wheat diets supplemented without microbial inoculum, FBI = 1% fermented whole crop barley diets supplemented with microbial inoculum, FB = 1% fermented whole crop barley diets supplemented without microbial inoculum.

[1]Data are the means of 4 replicates.

[a,b]Means bearing differant superscript within row differ significantly (p < .05).

[2]Standard error.

Table 3: Effect of fermented whole-crop wheat and barley with or without supplementing inoculant (probiotics) on growth performance in finishing pigs.

Parameters	FWI	FW	FBI	FB	SE[2]	*P* - value		
						Inoculum	Ingredient	Inoculum × Ingredient
Initial BW, kg	76.0	76.2	75.6	75.4				
Final BW, kg	104.5	108.4	107.4	104.5	2.02	0.431	0.331	0.214
ADG	1.02	1.15	1.14	1.04	0.21	0.115	0.213	0.445
ADFI	2.43	2.49	2.40	2.47	0.11	0.311	0.441	0.391
G:F	0.42	0.46	0.47	0.42	0.05	0.245	0.221	0.204

FWI = 1% fermented whole crop wheat diets supplemented with microbial inoculum, FW = 1% fermented whole crop wheat diets supplemented without microbial inoculum, FBI = 1% fermented whole crop barley diets supplemented with microbial inoculum, FB = 1% fermented whole crop barley diets supplemented without microbial inoculum.

[1]Date are the means of 4 replicates.

BW = body weight, ADG = average daily gain, ADFI = average daily feed intake.

[2]Standard error.

of DM and CP than fermented whole crop barley. Boisen *et al*. (1996) reported that bacterial inoculants could increase DM digestibility as well as animal performance. Wheat and barley silage inoculated with bacteria were higher in crude protein digestibility than dry wheat and barley and crude protein digestibility of wheat was higher than barley (Hackl *et al.*, 2010). Lyberg *et al.* (2006) reported that fermentation of barley and wheat based feeds increased the ileal digestibility of the organic matter, nitrogen, phosphorus and calcium. Fermented wheat showed higher ileal digestibility of fermented barley than that of fermented barley in growing pigs (Jørgensen *et al.,* 2010). Our result implicated the probiotics inoculated at the time of fermentation seem to have improved digestibility by improving intestinal environment of finishing pigs.

Blood constituents

The pigs fed diets supplemented with probiotics tended to have a higher creatinine concentration than the non-treated group (Table 5). Eggum (1970) showed that BUN content increased with increasing protein content in feed, Whang and Easter (2000) argues that the BUN value has a negative

Table 4: Effects of fermented whole-crop wheat and barley with or without supplementing inoculant (probiotics) on nutrient digestibility in finishing pigs.

Items%	FWI	FW	FBI	FB	SE[2]	*P* - value		
						Inoculum	Ingredient	Inoculum × Ingredient
Dry matter	81.64[a]	80.83[c]	81.10[b]	79.94[d]	0.08	<.001	<.001	<.001
Crude protein	74.67	77.71	76.71	75.07	0.05	0.041	0.249	0.06

FWI = 1% fermented whole crop wheat diets supplemented with microbial inoculum, FW = 1% fermented whole crop wheat diets supplemented without microbial inoculum, FBI = 1% fermented whole crop barley diets supplemented with microbial inoculum, FB = 1% fermented whole crop barley diets supplemented without microbial inoculum.

[1]Date are the means of 4 replicates.

[a,b,c,d]Means bearing differant superscripts within row differ significantly (p < .05).

[2]Standard error.

Table 5: Effect of fermented whole-crop wheat and barley with or without supplementing inoculant (probiotics) on blood profiles in finishing pigs.

Parameters mg/dl	FWI	FW	FBI	FB	SE[2]	*P* - value		
						Inoculum	ngredient	Inoculum × Ingredient
BUN[3]	12	19	12	9	1.94	0.119	0.412	0.214
Creatinine	1.46	1.36	1.44	1.25	0.41	0.081	0.101	0.209
Cholesterol	101	91	93	97	4.09	0.111	0.243	0.405
Triglyceride	37	31	34	35	3.15	0.109	0.304	0.541
Glucose	85	98	79	78	1.92	0.449	0.801	0.641

FWI = 1% fermented whole crop wheat diets supplemented with microbial inoculum, FW = 1% fermented whole crop wheat diets supplemented without microbial inoculum, FBI = 1% fermented whole crop barley diets supplemented with microbial inoculum, FB = 1% fermented whole crop barley diets supplemented without microbial inoculum.

[1]Date are the means of 4 replicates.

[2]Standard error.

[3]Blood urea nitrogen.

Table 6: Effect of fermented whole-crop wheat and barley with or without supplementing inoculant (probiotics) on fecal microbiota in finishing pigs.

Items% log10/g	FWI	FW	FBI	FB	SE[2]	*P* - value		
						Inoculum	Ingredient	Inoculum × Ingredient
E.coli	6.1[a]	5.7[b]	5.4[c]	6.2[a]	0.5	0.389	0.824	0.041
Lactobacillus	7.1[a]	6.5[c]	6.7[bc]	6.9[b]	0.6	0.001	0.603	0.001

FWI = 1% fermented whole crop wheat diets supplemented with microbial inoculum, FW = 1% fermented whole crop wheat diets supplemented without microbial inoculum, FBI = 1% fermented whole crop barley diets supplemented with microbial inoculum, FB = 1% fermented whole crop barley diets supplemented without microbial inoculum.

[1]Date are the means of 4 replicates.

[a,b,c]Means bearing differant superscripts within row differ significantly (p < .05).

[2]Standard error.

correlation with average daily lean gain and feed efficiency in growing-finishing pigs. BUN and serum creatinine concentrations can be used to assess kidney function, and the normal concentrations of BUN and serum creatinine are about 8-24mg/dl and 0.8-2.3 mg/dl, respectively (Zimmerman *et al.,* 2012). The creatinine concentration tended to increase in the fermented group, consistent with the report in Li *et al. (*2011), which showed a decrease in creatinine in the 5% fermented potato pulp group in piglets.

Fecal microbiota

Inoculum treatment led to an increase in *Lactobacillus* count compared to the non-treated group (Table 6). The number of *Lactobacillus* was highest in FWI treatments. Fermented wheat and barley did not affect the number of *E. coli*, *Lactobacillus* ($P > 0.05$). High lactic acid concentration inhibits *Salmonella* in the feed and hence eliminates it at the start of the food chain (Brooks *et al.,* 2003). Bacterial strains used as inoculum for production are highly lactic acid producing and should be active against intestinal pathogens (van Winsen *et al.,* 2001). Use of *B. subtilis* MA139 as a starter strain co-inoculated with *S. cerevisae* and *Lactbacillus fermentum* successfully controlled the growth of Enterobacteriaceae (Ying *et al.,* 2009). Adding 2.0 g of formic acid to the fermented feed may prevent the proliferation of undesired strains such as Enterobacteriaceae (Canibe *et al.,* 2007).

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CONCLUSION

In conclusion,this study showed that fermented whole crop wheat and barley diets supplemented with microbial inoculum had no effect on growth performance but had a beneficial effect on the number of *Lactobacillus* in the feces and decreased the creatinine concentration in blood.

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