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Effects of dietary soybean hulls and Lactobacillus reuteri on growth performance, nutrient digestibility and noxious gas mission from feces and slurry in finishing pigs

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A 6-week experiment was conducted to investigate the effects of supplementation of diets with soybean hulls (NSP) and Lactobacillus reuteri (L. reuteri) on growth performance, the apparent total tract digestibility (ATTD) of dry matter (DM) and nitrogen (N) and fecal and slurry noxious gas emission in finishing pigs. A total of 96 pigs with an average initial body weight (BW) of 80.70 ± 2.29 kg were assigned on the basis of weight to four treatment groups (6 replicates per treatment and 4 pigs per pen). In this study, dietary L. reuteri led to a higher ATTD of N than the negative groups ($P<0.05$). Pigs fed with soybean hull supplemental diet decreased ($P<0.05$) the fecal NH_3, H_2S and acetic acid emission compared with those without supplementation. The inclusion of L. reuteri significantly decreased ($P<0.05$) NH_3 emission from slurry as compared with those without supplementation. Moreover, a synergistic effect ($P<0.05$) between dietary soybean hulls and L. reuteri was observed on the emission of H_2S and R. SH emission from slurry in the current study. Taken together, both the supplementation of soybean hull or L. reuteri could decrease the noxious gas emission without any effect on the animals. Moreover, a synergistic effect between dietary soybean hulls and L. reuteri was observed on the H_2S and R. SH emission in finishing pigs.

Keywords: Soybean hulls/Lactobacillus reuteri/Noxious gas emission/Finishing pig

INTRODUCTION

It is well accepted that gas emissions from livestock production systems could result in various environmental problems and should be reduced. Schiffman, (1998) have suggested that pig manure contains several odor producing compounds, including NH_3, H_2S, R.SH and volatile organic compounds. Thus, many studies are being conducted to create new methods to minimize the environmental impacts of meat production because of the increasing pressure to sustain and improve the environment.
Generally, soybean hulls are regarded as an inexpensive by-product of soybean processing, which contains high levels of non-starch polysaccharides (NSP). Previous researches have demonstrated that NSP supplementation can reduce nitrogen excretion, noxious gas and volatile fatty acid (VFA) production from manure (Canh et al., 1997; Mroz et al., 2000). DeCam et al., (2001) shown that the addition of non-starch polysaccharides (NSP) has a positive impact on odor concentration in pig houses without hindering growth performance.
It is well suggested that noxious gas emission is highly related to utilization of nutrients and intestinal microbiota ecosystems (Ferket et al. (2002). Elina et al. (2003) have suggested that Lactobacillus reuteri are generally safe and are able to survive throughout the gastrointestinal tract. Han et al. (2001) also reported that supplementation with Lactobacilli is an effective way to reduce environmental pollutants from animal slurry because it improves feed efficiency and nutrients retention. Therefore, the present study was conducted to investigate the effects of supplementation with soybean hulls and L. reuteri on growth performance, apparent nutrient digestibility and fecal and slurry noxious gas emission in finishing pigs. 2.

MATERIALS AND METHODS

Experimental design, animals, housing and diets

This study was conducted using a 2 (control diet or soybean hulls diet) × 2 (L. reuteri, + or –) factorial experiments. A total of 96 crossbred ([Landrace×Yorkshire]×Duroc) pigs with an average initial BW of 80.70 ± 2.29 kg were used in a 6-week feeding trial to evaluate the effects of dietary soybean hulls and Lactobacillus reuteri supplementation on growth performance, nutrient digestibility and noxious gas emission in finishing pigs. At the beginning of the experiment, pigs were allotted to groups on the basis of their initial BW according to a completely randomized design. There were 6 replicate pens per treatment and 4 pigs per pen (2 gilts and 2 barrows). All diets were fed in meal form and formulated to meet or exceed the NRC (1998) recommendations for all nutrients (Table 1 and 2). Soybean hulls were used as a source of NSP in the diets and L. reuteri was added to the basal diet at the expense of corn. Each pen was equipped with a self-feeder and a nipple waterer to allow ad libitum access to feed and water throughout the experimental period.

Table 1. Diet composition (as fed basis)

Ingredient, %	Control diet	Soybean hulls diet
Corn	70.82	66.98
Soybean meal, dehulled	21.00	19.95
Soybean Hulls	-	5.00
Molasses	4.00	3.80
Tallow	2.00	1.90
Calcium carbonate	1.19	1.19
Difluorinated phosphate	0.46	0.46
NaCl	0.15	0.15
L-Lysine HCl	-	0.15
DL-Methionine	-	0.02
L-threonine	-	0.02
Choline chloride	0.03	0.03
Premix[1]	0.35	0.35

1Supplied per kg diet: 4,000 IU vitamin A, 800 IU vitamin D3, 171 IU vitamin E, 2 mg vitamin K, 4 mg vitamin B2, 1 mg vitamin B6, 16 g vitamin B12, 11 mg pantothenic acid, 20 mg niacin and 0.08 mg biotin; 15 mg Cu, 100 mg Fe, 100 mg Zn, 40 mg Mn, 0.3 mg I, 0.5 mg Co and 0.4 mg Se.

The probiotic preparation used in current experiment is manufactured by Genebiotech Co., Ltd. (Republic of Korea) under the name of Lactocare®. It is guaranteed to contain at least 1.5×106 CFU/g of Lactobacillus reuteri GB-LC 1 in a liquid form.

Table 2. Nutrient composition of experimental diets (as fed basis)

Items	Control diet	Soybean hulls diet
Calculated composition		
DE, kcal/kg	3,490	3,420
CP, %	16.00	15.80
CF, %	2.38	3.97
EE, %	2.98	2.94
Ash, %	2.58	2.69
Starch, %	45.40	43.10
Sugar, %	4.95	4.78
Sulfur, %	0.19	0.18
NSP[1], %	16.50	19.40
Lys, %	0.78	0.78
Met, %	0.26	0.26
Cys, %	0.29	0.28
Met+Cys, %	0.55	0.54
Trp, %	0.16	0.16
Thr, %	0.60	0.57
Tyr, g/kg	0.57	0.56
Analysis composition, %		
DM	82.50	82.90
CP	16.80	16.20
Ca	0.71	0.70
P	0.60	0.59
ADF	5.31	6.11
NDF	7.40	11.82

[1]Non-starch polysaccharides (NSP) were determined as DM − Ash − (Crude protein + Crude fat + Starch + Sugar).

Sampling and measurements

Body weight and feed consumption were measured at the end of the experiment to monitor the average daily gain (ADG), average daily feed intake (ADFI) and gain/ feed (G/F) ratio. Chromium oxide (Cr_2O_3) was added to the diet at 0.20% as an indigestible marker at the beginning of the 6th week to calculate the apparent total tract digestibility for dry matter (DM) and nitrogen (N). Fecal grab samples were then collected randomly from at least two pigs in each pen (1 gilt and 1 barrow) after the pigs were fed diet containing the indicator for 4 d. All feed and fecal samples from one pen were then pooled and mixed, after which the fecal samples were dried for 72 hours at 70 C, and were finely ground to be able to pass through a 1-mm sieve screen and then frozen and stored in a refrigerator at -20 C until analysis. All the fed and fecal samples were then analyzed for DM and N according to the AOAC (1995). Chromium levels were determined via by using UV absorption spectrophotometry (Shimadzu, UV-1201, Japan) and the apparent digestibility of DM and N were calculated using indirect-ratio methods.

Fresh feces and urine samples were collected from two pigs in each pen on the last 2 days of 5th week of the experiment and then mixed well for each respective pen. Analysis Analyses was were then conducted using fresh fecal samples (300 g) and feces +urine samples (150 g + 150 ml) obtained from each pen that were stored in 2.6-L sealed plastic boxes. Each box had a small hole in the middle of one side wall that was sealed by adhesive plaster and all samples were collected and analyzed in duplicates. After being sealed in the boxes, samples were allowed to ferment for a period of 30 days at room temperature (21 °C) following the method described by Otto et al. (2003). After the fermentation period, the gases produced were evaluated using a Gastec (model GV-100) gas sampling pump (Gastec Corp., Gastec detector tube No. 3L and 3La for NH_3; No. 4LL and 4LK for H_2S; No. 81 and 81L for acetic acid; No. 70 and 70L for R.SH, Gastec Corp, detector tube, Japan). Prior to measurement, the slurry samples were manually shaken for approximately 30 s to disrupt any crust formation on the surface of the slurry sample and to homogenize them. The adhesive plaster was then punctured and 100 mL of the headspace air was sampled approximately 2.0 cm above the feces or slurry surface. Following air sampling, each box was re-sealed with adhesive plaster. The headspace measurement was repeated 24 and 48 h after the initial measurement and the concentration of gases were determined using the average of the 3 head space measurements.

Statistical analysis

The experimental data were analyzed by a 2 × 2 factorial using the GLM Procedure described by the Statistics Analysis Systems Institute (1996). The model evaluated the main effect of soybean hulls, L. reuteri, and soybean hulls × L. reuteri, with the individual pen serving as the unit. The variability of all of the data was expressed as the standard error (SE) and a $P<0.05$ was considered to be statistically significant.

RESULTS

Growth performance

No significant differences in the ADG, ADFI and G/F of pigs were observed among treatment groups in this study (Table 3).

Table 3. Effect of supplemental soybean hulls and Lactobacillus reuteri on growth performance in finishing pigs[1]

Items	Diet				
NSP	CON[2]		NSP[2]		SE[3]
L. reuteri	-	+	-	+	
ADG, g	925	913	911	974	26
ADFI, g	2,869	2,771	2,777	2,802	35
gain/feed	0.322	0.330	0.328	0.348	0.01

[1]Ninety six pigs with an initial body weight of 80.70 ± 2.29 kg.

[2]Abbreviation: CON, the control diet; NSP, CON + 5% soybean hulls; -, without Lactobacillus reuteri 0.2% supplementation; +, with Lactobacillus reuteri 0.2% supplementation.

[3]Pooled standard error.

Apparent total tract digestibility (ATTD) of dry matter (DM) and nitrogen (N)

There were no significant differences in DM digestibility observed among the treatments (Table 4). However, N digestibility was significantly greater when pigs fed diets with a L. reuteri ($P<0.05$) than that in control group.

Table 4. Effect of supplemental soybean hulls and Lactobacillus reuteri on apparent nutrient digestibility in finishing pigs[1]

Items	Diet				
NSP	CON[2]		NSP[2]		SE[3]
L. reuteri	-	+	-	+	
DM, %	72.75	73.76	72.36	73.04	0.82
N, %[4]	71.42	76.89	71.51	73.44	1.16

[1]Ninety six pigs with an initial body weight of 80.70 ± 2.29 kg.

[2]Abbreviation: CON, the control diet; NSP, CON + 5% soybean hulls; -, without Lactobacillus reuteri 0.2% supplementation; +, with Lactobacillus reuteri 0.2% supplementation.

[3]Pooled standard error.

[4]L. reuteri effect, $P<0.05$.

Fecal and slurry noxious gas emission

In this study, pig fed the soybean hull supplemental diet decreased ($P<0.05$) the fecal NH_3, H_2S and acetic acid emission as compared with those without supplementation. the inclusion of L. reuteri significantly decreased ($P<0.05$) slurry NH_3 emission compared with those without supplementation. Moreover, a synergistic effect ($P<0.05$) between dietary soybean hulls and L. reuteri was observed on the slurry H_2S and R. SH emission in the current study.

Table 5. Effect of supplemental soybean hulls and Lactobacillus reuteri on noxious gas emission and pH in finishing pigs[1]

Items, mg/kg	Diet				
NSP	CON[2]		NSP[2]		SE[3]
L. reuteri	-	+	-	+	
NH_3					
Feces[6]	31.5	27.5	26.5	25.0	1.0
Slurry[7]	110.0	57.5	90.0	50.0	12.3
H_2S					
Feces[6]	29.0	15.0	65.0	50.0	13.0
Slurry[8]	6.3	3.0	8.0	1.0	1.0
R.SH					
Feces	27.5	25.0	25.0	22.5	7.0
Slurry[8]	17.5	7.0	13.0	5.0	1.8
Acetic acid					
Feces[6]	17.6	18.5	20.4	19.6	0.8
Slurry	21.1	20.8	27.5	26.6	1.1

[1]Ninety six pigs with an initial body weight of 80.70 ± 2.29 kg.

[2]Abbreviation: CON, the control diet; NSP, CON + 5% soybean hulls; -, without Lactobacillus reuteri 0.2% supplementation; +, with Lactobacillus reuteri 0.2% supplementation.

[3]Pooled standard error.

[4]Feces (300 g) were kept in a sealed box and fermented for 30 d, a Gastec detector was then used to measure noxious gas emission.

[5]Feces (150 g) + urine (150 mL) were kept in a sealed box and fermented for 30 days, a Gastec detector was then used to measure noxious gas emission.

[6]Soybean hulls effect, $P<0.05$.

[7]L. reuteri effect, $P<0.05$.

[8]Soybean hulls × L. reuteri interaction, $P<0.05$.

DISCUSSION

Growth performance and apparent total tract digestibility of DM and N

Previously, it is well suggested that dietary NSP could decrease the gastrointestinal emptying rate in pigs because of its higher viscous material (Guérin et al., 2001). Dilger et al. (2004) demonstrated that gastrointestinal pH may be increased by the buffering capacity of NSP, and reduce the gastric secretion in response to the alkaline environment. Therefore, it is reasonable to hypothesize that dietary soybean hulls (NSP) could decrease the growth performance and nutrient digestibility in the current study. However, the inclusion of soybean hulls did not affect the growth performance and nutrient digestibility compared with the no-supplemental soybean hulls diets, which is in agreement with Radar matrix (1996) and Van Oeckel (1998), who suggested that pig fed soybean hulls supplemental diet (5 or 15%) did not affect the growth performance in growing and finishing pigs, respectively. It is well accepted that fermentation of NSP and dietary fiber takes place in the cecum and colon of monogastric animals and can produce increased amount of VFA, which may be responsible for up to 30% of the maintenance energy needs of growing pigs (Varel and Yen, 1997). They also reported that this amount is higher in finishing pigs due to the greater maturity of cecal function. Therefore, we hypothesized the higher NSP level (soybean hulls) in the diet might results in energy production in the hindgut, which would compensate the lower DE levels in the soybean hulls diet. Furthermore, the CP levels in the current study (16.2% and 16.8%) were higher than the NRC requirement (13.2%). This excess protein may be another reason for the absence of any negative effects in pigs that were provided with the diet containing lower nutrient levels.

Jin et al. (1997) suggested that continuous feeding of direct-fed microbes to livestock could maintain the beneficial intestinal microflora by inducing competitive exclusion of pathogenic bacteria, and by exerting antagonistic activity towards pathogenic bacteria. Our previous study also suggested that dietary Lactobacillus brevis improved the growth performance of growing-finishing pigs (Chen et al., 2006 a). Thus, it is reasonable to expect beneficial results could be observed in response to supplementation of probiotics such as Lactobacillus species. However, no effect was detected for the growth performance in this study, which is in agreement with Nousiainen and Setala (1993), who found that the inclusion of Lactobacillus did not affect the growth performance of growing-finishing pigs. The reason for the absence is likely to be the more developed digestive system, improved immunity and increased resistance to intestinal disorders as pig become older (80 kg) (Nousiainen and Setala, 1993). Interestingly, the inclusion of Lactobacillus reuteri significantly increased the N digestibility in the current study, which is in agreement with Shon et al. (2005), who suggested that 0.2% Lactobacillus reuteri did not affect the DM digestibility, but did increase N digestibility during the finishing phase. Previously, Wenk (2000) have suggested that Lactobacillus spp. could stimulate and stabilize the digestion processes by enhancing the population of beneficial micro-organisms and improving microbial enzyme activity. Therefore, the increased gut health and microbial enzyme activity may be responsible for the higher N digestion in this study.

Noxious gas emission

In this study, pig fed the soybean hulls led to a significant depression on the noxious gas emission compared with the no-supplemental diet. It is well suggested that the addition of soybean hulls led to a 0.6% decrease in the level of crude protein and its metabolites, which are precursors for odor production in the gut of animals and in slurry stores (Mackie et al., 1998). Kiene and Hines, (1995) have suggested that variations in the concentrations of sulfuric odorous compounds (H2S and R.SH.) occur primarily as a result of differences in the sulfur-containing protein of diets and the metabolism of sulfur-containing amino acids (methionine, cystine and cysteine). Wang et al. (2008) also suggested that higher NSP diet could enhance microbial activities within the gut of the animal, and might be responsible for the lower excreta pH and higher slurry VFA concentrations. Our study suggested that soybean hull supplemented diet reduced crude protein concentration compared with the none-supplemented diets. Therefore, it is suitable to hypothesize that the decreased protein levels and the improved intestinal environment may be responsible for the depression effect on the odorous compounds in the current study. Moreover, Ji and Kim (2002) found that addition of complex probiotics (Lactobacillus acidophilus, Bacillus spp. and Aspergillus oryzae) could decrease the NH3 production of pigs by increasing the nutrient digestibility. Our previous study also suggested that bacillus-based probiotic reduced fecal NH3-N excretion by improving the intestine environment and nutrient digestibility in finishing pigs (Chen et al., 2006b). Similarly, in this study, the inclusion of dietary probiotic significantly decreased the N digestibility. Therefore, the decreased NH3 emission from slurry in the current study may have occurred as a result of increased digestibility of nitrogen.

Moreover, a significant interaction between soyabean hulls and L. reuteri was observed on the H2S and R.SH emission in the current study. Kiene and Hines, (1995) have suggested that variations in the concentrations of sulfuric odorous compounds (H2S and R.SH.) occur primarily as a result of differences in the sulfur composition of diets and the metabolism of sulfur-containing amino acids (methionine, cystine and cysteine). It is well accepted that the inclusion of NSP could benefit the populations of Bifidobacteria and Lactobacilli in the large intestine (O'Connell et al., 2005). Our previous study also suggested that dietary probiotic could increase the health of the gastro-intestine tract in the animals (Chen et al., 2006 a,b). Therefore, the reason for the synergistic effect between L. reuteri and soybean hulls is likely to be due to their beneficial effect on the intestinal bacteria ecosystem and subsequently its protein digestibility. However, the exact underline mechanism is still unknown. Thus, further research need be conducted to investigate the mechanism responsible for the above effects between soybean hulls and dietary L. reuteri supplementation.

In conclusion, both the supplementation of soybean hull and L. reuteri could decrease the noxious gas emission without adverse effect on the animals. Moreover, a synergistic effect between dietary soybean hulls and L. reuteri was observed on the H2S and R. SH emission in finishing pigs.

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