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EVALUATION OF *BACILLUS SUBTILIS* AND *LACTOBACILLUS ACIDOPHILUS* PROBIOTIC SUPPLEMENTATION ON REPRODUCTIVE PERFORMANCE AND NOXIOUS GAS EMISSION IN SOWS

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Abstract

The impacts of probiotics supplementation on reproduction performance and noxious gas emission in sows was evaluated in an experiment with a total of thirty sows (second-parity), from 4 weeks prior to farrowing, to day 21 of lactation. The gestation and lactation diets of sows were supplemented with probiotics containing *Bacillus subtilis* (1.2×10^7 cfu/g) and *Lactobacillus acidophilus* (1.15×10^6 cfu/g). Treatment included: basal diet (CON), basal diet + 0.1% probiotics (PB0.1), and basal diet + 0.2% probiotics (PB0.2). The supplementation of dietary probiotics significantly improved average daily feed intake during the lactation period (quadratic, P = 0.0429), sow backfat thickness during the weaning period (linear, P = 0.0385), and initial body weight of piglets (linear, P = 0.0054) as compared with CON, respectively. Furthermore, the supplementation of dietary probiotics reduced noxious gas emission as compared with CON (linear, P<0.05 for day 5 and day 10), respectively. In conclusion, dietary probiotics containing *B. subtilis* and *L. acidophilus* improved the growth performance of sows, resulted in increased weaning body weight of piglets, and induced an effective and significant reduction in fecal noxious gas emission in lactating sows, as compared with CON.

Key words: reproductive performance, noxious gas, piglet, probiotics, sow

Previous work from our laboratory (Wang et al., 2009; Yan and Kim, 2013) and others (Chen et al., 2005; Chen et al., 2006; Meng et al., 2010) have demonstrated the beneficial effect of pig feed supplementation with probiotics on piglet and pig performance. Probiotics tend to contain bacterial cultures capable of stimulating microflora, thereby modifying the intestinal microbial ecosystem, leading towards a favorable health status, improving feed efficiency and nutrient utilization. For example, supplementations with probiotics containing *Bacillus licheniformis* (*B. licheniformis*) and *Bacillus subtilis* (*B. subtilis*) in sows improved the diarrhea score, pre-weaning mortality, and weaning body weight of piglets (Alexopoulos et al., 2004), in addition

to increased growth performance and reduced noxious gas emission in growing pigs (Wang et al., 2009). Moreover, finishing pigs fed probiotics containing *B. subtilus*, *Bacillus coagulans*, and *Lactobacillus acidophilus* demonstrated improved growth performance and noxious gas emission (Chen et al., 2006). In addition, administration of *Bacillus cereus* var. *toyoi* has been shown to improve growth performance and promote beneficial, positive modification of intestinal microbial populations in weaning pigs (Jadamus et al., 2002; Papatsiros et al., 2011). However, contradictory results have arisen from some probiotic supplementation studies; in particular, a huge difference has existed amongst the species of probiotics used for supplementation, thereby influencing the degree of benefit (Bomba et al., 2002). For example, contradictory results have been obtained in probiotic supplementation feeding studies of growing-finishing pigs demonstrating that supplementation with *Lactobacillus* or *Bacillus* probiotics does not produce any observable effects on growth performance (Harper et al., 1983; Kornegay and Risley, 1996; Davis et al., 2008).

Therefore, the aim of the present study was to evaluate the efficacy of dietary supplementation of probiotics, specifically containing *B. subtilis* and *L. acidophilus*, on reproductive performance and noxious gas emission in sows.

Material and methods

Animals and housing

Thirty Landrace × Yorkshire multiparous sows (parity = 2) and their crossbred litters between the sows and Duroc were used in this study. The experiment lasted from 4 weeks prior to farrowing, to day 21 of lactation. Gestating sows were housed on a slat floor, in an environmentally regulated building. The ambient environments in the dry sow accommodation and the farrowing house were kept at a fairly constant temperature of 19–21°C, and 60% relative humidity. A nursery box equipped with an infrared spotlight and heating mat was provided to meet the requirements of piglets. Sows were individually fed, using specially installed troughs and nipple drinkers. All experiments in this study were carried out under the guidelines and approval of the Animal Care and Welfare Committee of Dankook University (South Korea).

Experimental design

All sows were fed with complete feed specially formulated according to requirements at each stage of pregnancy or lactation (NRC, 1998). From day 86 to day 109 of pregnancy, a gestation diet was provided (Table 1). The amount of feed was set to meet the requirement of 2.5 kg/d during the gestation period. From day 110 of pregnancy to weaning (day 21 of lactation), sows were fed lactation diets (Table 1). Sows were allocated to one of three treatments according to their BW and two replicates per treatment and five pigs per pen. Sows in three dietary treatments were fed with diets that were supplemented as basal diet (CON), basal diet with probiotics mixture 0.1% (PB0.1), and basal diet with probiotics mixture 0.2% (PB0.2). The probiotics mixture included *B. subtilis* and *L. acidophilus*, at amounts of 1.2×10^7 cfu/g and 1.15×10^6 cfu/g, respectively.

Table 1. Composition of experimental diets (as-fed basis)

Ingredients	Gestation diet	Lactation diet[1]
Corn	571.0	511.2
Soybean meal (46% CP)	106.5	246.1
Wheat bran	120.0	40.0
Rapeseed meal	37.0	25.0
Rice bran	60.0	50.0
Tallow	35.9	60.5
Molasses	36.0	35.0
Dicalcium phosphate	15.2	16.4
Limestone	9.9	7.6
Salt	6.0	5.0
Lys (98%)	0.5	1.2
Vitamin premix[2]	1.0	1.0
Mineral premix[3]	1.0	1.0
Calculated compositions:		
metabolizable energy (MJ/kg)	31.9	34.4
crude protein	131.0	171.0
crude fat	68.9	91.0
Lys-Cl	6.5	10.0
calcium	8.7	8.5
phosphorus	7.6	7.3

[1]Supplemented with probiotics (*Bacillus subtilis* and *Lactobacillus acidophilus*).

[2]Provided per kilogram of complete diet: vitamin A – 10,000 IU; vitamin D_3 – 2,000 IU; vitamin E – 48 IU; vitamin K_3 – 1.5 mg; riboflavin – 6 mg; niacin – 40 mg; d-pantothenic acid – 17 mg; biotin – 0.2 mg; folic acid – 2 mg; choline – 166 mg; vitamin B_6 – 2 mg; and vitamin B_{12} – 28 mg.

[3]Provided per kilogram of complete diet: Fe (as $FeSO_4 \cdot 7H_2O$) – 90 mg; copper (as $CuSO_4 \cdot 5H_2O$) – 15 mg; zinc (as $ZnSO_4$) – 50 mg; Mn (as MnO_2) – 54 mg; I (as KI) – 0.99 mg; and Se (as $Na_2SeO_3 \cdot 5H_2O$) – 0.25 mg.

Sampling and measurements

Sows were weighed on day 110 of pregnancy, day of farrowing, and day of weaning. Piglets were weighed at birth and on day 21 of lactation, and the average daily feed intake (ADFI) was recorded. On day 110 of gestation, farrowing day, and day 21 of lactation, the backfat thickness of sows was determined, using an ultrasound instrument (Piglog 105, SFK Technology, Herlev, Denmark). The feces of sows were collected on day 0, 7, 14, and 21 of lactation, to measure the moisture content. At 8:00 every morning during lactation, the breeder observed the entire condition of feces and the incidence of diarrhea in piglets. The standard for fecal score was: 0, normal feces; 1, soft feces; 2, mild diarrhea; and 3, severe diarrhea. The rectum temperatures of sows were measured on day 0, 7, 14, and 21 of lactation.

Blood samples of sows (1 sample per sow) were collected at 2 weeks prior to farrowing, and at weaning, via auricular vein, using a sterile syringe soaked with K_3EDTA solution, and then transferred into tubes for subsequent analysis (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA). Blood samples of piglets (3 pigs per litter) were collected at weaning, via jugular venipuncture using a sterile

needle into a 5-mL tube, with or without K_3EDTA, for subsequent analysis (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA). Serum aliquot was separated by centrifugation, and stored at 4°C, until analysis for IgG with an automatic biochemistry blood analyzer (HITACHI 747, Hitachi, Tokyo, Japan). The lymphocyte counts of the whole blood samples were determined, via an automatic blood analyzer (ADVIA 120, Bayer, Tarrytown, NY, USA).

The feces of sows were collected on day 21 of lactation, and then stored in 2.6 L plastic boxes, in duplicate. Each box had a small hole in the middle of one sidewall, which was sealed with adhesive plaster. The concentration of gas was determined on day 5 and 10. After a fermentation period of 10 days at room temperature (28°C), the plastic boxes were punctured, and the headspace air was sampled approximately 2.0 cm above the samples at a rate of 100 ml/min, using a Gastec detector (GV-100S; Gastec Corp., Kanagawa, Japan). Two samples from each sow were measured, and the average value was then calculated (Ao et al., 2011).

Statistical analysis

All data were analyzed using SAS 2003 (v. 9.1; SAS Institute Inc., Cary, NC, USA) using the Mixed procedure, with the following statistical model of

$$Y_{ijk} = \mu + t_i + r_k + e_{ijk}$$

where:

Y_{ijk} was an observation on the dependent variable *ij*,
μ was the overall population mean,
t_i was the fixed effect of probiotics supplementation,
r_k was the pen as a random effect,
e_{ijk} was the random error associated with the observation *ijk*.

A significant difference level of 0.05 was used to determine statistical significance, and a level of 0.10 was considered a trend. In addition, orthogonal comparisons were conducted, using polynomial regression, to measure the linear and quadratic effects of increasing the dietary concentration of probiotics.

Results

Growth performance

The highest amount of dietary probiotic supplementation increased the ADFI during lactation period (quadratic, P = 0.0429), and probiotic supplementation tended to decrease the sow backfat thickness during the gestation (linear, P = 0.0956) and weaning (linear, P = 0.0385) periods as compared with CON, respectively. However, there were no obvious differences in litters, sow BW, or piglet survival (Table 2). The body weights of piglets showed a significant difference amongst treatments at birth (linear, P = 0.0054) and tended to increase at weaning (linear, P = 0.0967) as compared with CON, respectively.

Table 2. Effect of probiotics supplementation on growth performance in lactating sows and piglets[1]

Item	CON	PB0.1	PB0.2	SEM[2]	P-value for contrast	
					linear	quadratic
Parity of sows	2	2	2			
Litter						
No. of pigs	11.7	12.0	12.0	0.23	0.6294	0.7853
Weaned pigs	11.6	11.5	11.8	0.24	0.6635	0.7048
Sow body weight (kg)						
gestation	239.5	241.1	234.2	3.49	0.8804	0.5870
farrowing	212.8	215.6	212.2	3.63	0.9491	0.7036
weaning	204.5	214.5	206.5	5.12	0.9990	0.3816
loss	35.0	26.6	27.6	3.03	0.2321	0.8016
Average daily feed intake (kg)						
gestation	2.38	2.40	2.37	0.06	0.9275	0.8648
lactation	5.31	5.75	6.23	0.22	0.4449	0.0429
Sow backfat thickness (mm)						
gestation	20.1	17.1	16.9	0.81	0.0956	0.3854
farrowing	19.6	16.5	16.4	0.84	0.1127	0.3747
weaning	13.9	11.7	10.9	0.60	0.0385	0.5323
loss	6.2	5.4	5.7	0.41	0.4905	0.3180
Days to estrus	4.1	4.0	4.0			
Piglet survival (%)	98.6	96.0	98.7			
Initial weight (kg)	1.26	1.26	1.32	0.04	0.0054	0.1039
Weaning weight (kg)	6.29	6.60	6.86	0.14	0.0967	0.9402
Average daily gain (g/d)	240.2	254.0	254.6	6.20	0.3818	0.7074

[1]Abbreviations: CON – basal diet; PB0.1 – basal diet + 0.1% probiotics; PB0.2 – basal diet + 0.2% probiotics.
[2]Standard error of means.

Fecal moisture content of sows and diarrhea incidence of piglets

The fecal moisture content of sows was unaffected by dietary probiotics supplementation amongst treatments during the lactation period as compared with CON (Table 3). Similarly, no obvious effect on piglet diarrhea incidence was detected amongst all dietary treatments as compared with CON (Table 4).

Table 3. Effect of probiotics supplementation on fecal moisture in lactating sows[1]

Item (°C)	CON	PB0.1	PB0.2	SEM[2]	P-value for contrast	
					linear	quadratic
day 0	44.10	39.51	42.13	1.28	0.5308	0.2050
day 7	30.43	30.08	31.42	0.62	0.5373	0.5467
day 14	30.60	29.79	29.34	0.57	0.3880	0.8847
day 21	30.63	30.06	29.28	0.31	0.1778	0.8739

[1]Abbreviations: CON – basal diet; PB0.1 – basal diet + 0.1% probiotics; PB0.2 – basal diet + 0.2% probiotics.

[2]Standard error of means.

Table 4. Effect of probiotics supplementation on diarrhea in piglets[1]

Item (%)	CON	PB0.1	PB0.2	SEM[2]	P-value for contrast	
					linear	quadratic
Number of piglets	0.17	0.25	0.18	0.03	0.9751	0.2809
Fecal score[3]	0.20	0.21	0.15	0.04	0.5113	0.7554

[1]Abbreviations: CON – basal diet; PB0.1 – basal diet + 0.1% probiotics; PB0.2 – basal diet + 0.2% probiotics.
[2]Standard error of means.
[3]Fecal score: 0 – normal; 1 – soft feces; 2 – mild diarrhea; 3 – severe diarrhea.

Rectum temperature and blood profile

The rectum temperatures of sows at day 0, 7, 14, and 21 were unaffected by probiotics supplementation during lactation period, as compared with CON (Table 5); similarly, no significant difference was observed in any of the blood profile criteria examined (Table 6). However, diets with probiotics supplementation significantly increased lymphocyte numbers and amount of IgG in piglets as compared with CON.

Table 5. Effect of probiotics supplementation on rectal temperature in lactating sows[1]

Item (°C)	CON	PB0.1	PB0.2	SEM2	P-value for contrast	
					linear	quadratic
day 0	39.1	39.1	39.0	0.07	0.6974	0.9484
day 7	39.7	39.7	39.8	0.11	0.7475	0.9684
day 14	39.1	39.1	39.1	0.06	0.6760	0.8136
day 21	38.8	38.8	38.7	0.10	0.7100	0.9477

[1]Abbreviations: CON – basal diet; PB0.1 – basal diet + 0.1% probiotics; PB0.2 – basal diet + 0.2% probiotics.
[2]Standard error of means.

Table 6. Effect of probiotics supplementation on blood profiles in lactating sows and piglets[1]

Item (%)[3]	CON	PB0.1	PB0.2	SEM[2]	P-value for contrast	
					linear	quadratic
Sows						
Lymphocyte (%)						
initial	43.8	44.6	44.2	1.58	0.9272	0.8707
final	47.1	50.1	48.1	1.32	0.3903	0.6373
IgG (mg/dL)						
initial	304	302	303	7.76	0.9871	0.9552
final	326	340	332	10.51	0.8282	0.6469
Piglets at weaning						
Lymphocyte (%)	49.2	55.6	54.7	1.45	0.1153	0.2373
IgG (mg/dL)	193	224	212	7.44	0.2832	0.1767

[1]Abbreviations: CON – basal diet; PB0.1 – basal diet + 0.1% probiotics; PB0.2 – basal diet + 0.2% probiotics.
[2]Standard error of means.
[3]Initial – 2 weeks prior to farrowing; final – weaning.

Noxious gas emission

The effects of dietary probiotics supplementation on the emission of excreta nox-

ious gas emission are shown in Table 7. Overall, probiotics supplementation reduced ammonia (linear, P = 0.0009 for day 5; linear, P = 0.0093 for day 10), hydrogen sulfide (linear, P = 0.0714 for day 5; linear, P = 0.0121 for day 10), and total mercaptans (linear, P = 0.0216 for day 5; linear, P = 0.0019 for day 10) emissions as compared with CON, respectively.

Table 7. Effect of probiotics supplementation on fecal gas emission in lactating sows[1]

Item (ppm)	CON	PB0.1	PB0.2	SEM[2]	P-value for contrast	
					linear	quadratic
Ammonia						
day 5	20.00	17.43	13.63	0.98	0.0009	0.7485
day 10	24.00	22.29	17.76	1.18	0.0093	0.4860
Hydrogen sulfide						
day 5	4.43	4.49	3.78	0.18	0.0714	0.2150
day 10	4.66	4.45	4.26	0.07	0.0121	0.8326
Total mercaptan						
day 5	0.53	0.50	0.46	0.02	0.0216	0.7011
day 10	0.57	0.54	0.48	0.02	0.0019	0.5755

[1]Abbreviations: CON – basal diet; PB0.1 – basal diet + 0.1% probiotics; PB0.2 – basal diet + 0.2% probiotics.
[2]Standard error of means.

Discussion

One of the mechanisms in which probiotics act is to improve intestinal health, leading to better general health and productivity amongst animals (Cho et al., 2011). With regards to growth performance, *B. subtilis* and *L. acidophilus* supplementation significantly increased the ADFI of sows during lactation (quadratic, P = 0.0429) and initial weight of piglet (linear, P = 0.0054) as compared with CON, respectively, in our study. The results corroborated with those of Alexopoulos et al. (2004) who demonstrated that 0.04% probiotic (*B. licheniformis* and *B. subtilis*) supplementation on commercial farms improved sow ADFI and significantly reduced weight loss during lactation. Interestingly, Jørgensen and Hansen (2006) reported that dietary probiotics supplementation can also influence reproduction performance in pigs by increasing litter size and piglet weight at weaning, and reducing the pre-weaning mortality and piglet diarrhea score. One possible reason to explain the small size and piglet weight outcome is most likely the higher concentration of serum cholesterol and total lipids in probiotics-treated sows in mid-lactation due to probiotic induced improvements to nutrient utilization. With regards to the reduction of the incidence of higher diarrhea score and pre-weaning mortality, this could be an indirect positive effect of probiotics arising from the sow into piglets (Alexopoulos et al., 2004). Demeckov et al. (2002, 2003) suggested that piglets contacting probiotic-containing feces from sows fed probiotics supplemented diets might help beneficial strains colonize the gut of piglets. However, in our current study, litter size, mortality, and piglet diarrhea score were not affected by dietary probiotics supplementation (*B. subtilis* 1.2×10^7 cfu/g

and *L. acidophilus* 1.15×10^6 cfu/g), which may have been due to the differences in bacterial concentrations and bacterial species in the probiotics used as compared to other previous studies (Demeckov et al., 2002, 2003; Alexopoulos et al., 2004; Jørgensen and Hansen, 2006).

Probiotics act to improve intestinal health, which is directly related to nutrient utilization, leading to better general health and productivity amongst animals. Scharek et al. (2005) and Böhmer et al. (2006) have reported slightly lower body temperatures of sows receiving probiotics, which is a positive factor, since this effect can be ascribed to an enhancement of the immune system. However, several studies using probiotics containing *Bifidobacteria* revealed that these Gram-positive lactic acid producing bacteria showed no immune stimulating effect, either on the mucosal or systemic immune response in rodents (Perdigon et al., 2003; Scharek et al., 2000). The rectum temperature of pigs directly reflects whole body temperature (Lucas et al., 2000). When we measured rectum temperatures of sows put onto probiotic supplementation in our study, no differences were observed as compared to sows fed on a regular non-probiotic supplemented diet. Our results indicate that *L. acidophilus*, also Gram-positive lactic acid producing bacteria, and *B. subtilis*, Gram-positive non-lactic acid producing bacteria, do not appear to have any influence on the immune system of pigs. These contradictory results among studies may be due to the different species of bacteria used as probiotics (Lessard et al., 2009; Cho et al., 2011).

With a good growth rate, farmers move to focus their production on attaining carcass quality and finally meat quality. Carcass quality focuses principally on higher percentage lean meat and reduced thickness of backfat. Reduced thickness of backfat gives a better conformity of the animal, allowing higher selling price per kg of live weight. Jasek et al. (1992) provided diets containing *B. subtilis* and *B. licheniformis* to growing pigs, and observed improvement of slaughter traits, such as a reduction *inter alia* of backfat thickness by 3.6%. Additionally, Grela et al. (2001) supplemented mannan-oligosaccharide to feed for growing pigs, and reported an increase in the ham weight of fatteners. Our current study results corroborate with the aforementioned studies, showing tendencies of reduced sow backfat thickness, during gestation (linear, $P = 0.0956$) and weaning period (linear, $P = 0.0385$) with probiotic supplementation as compared with CON, without significant reduction in sow body weight. Alternately, Shen et al. (2011) reported that probiotic supplementation containing *Saccharomyces cerevisiae* demonstrated no effect on sow backfat thickness, whereas Cui et al. (2013) reported that probiotic supplementation containing *B. subtilis* showed a 16.77% higher backfat thickness as compared with CON. These contradictory results may be due to differences in bacteria species used and genotypes of pigs (Rekiel et al., 2005).

Lastly, regarding fecal noxious gas emission, Ferket et al. (2002) have suggested that fecal odor and ammonia emission are directly related to nutrient utilization and the intestinal microbial ecosystem. Consequently, dietary probiotic supplementation has been theorized to beneficially influence the intestinal microbial ecosystem, inducing a shift in the intestinal microflora, resulting in enhanced nitrogen absorption, and thereby indirectly reducing excreta noxious gas emission. Dietary probiotic sup-

plementation in our study resulted in a significant reduction in excreta ammonia, hydrogen sulfide, and total mercaptans gas emissions as compared with CON, respectively. Our results corroborate with studies by Chen et al. (2006) and Wang et al. (2009). Chen et al. (2006) reported that finishing pigs fed with *Bacillus*-based probiotics for 6 weeks resulted in a significant reduction in fecal ammonia and hydrogen sulfide emission. In addition, Wang et al. (2009) confirmed that probiotic supplementation with *B. subtilis* and *B. licheniformis* in growing pigs, significantly decreases slurry noxious ammonia emission.

In conclusion, dietary probiotic supplementation containing *B. subtilis* and *L. acidophilus* improved the ADFI and backfat thickness of sows, and resulted in the increased initial BW of piglets. Moreover, probiotic supplementation induced an effective and significant reduction in fecal noxious gas emissions of ammonia, hydrogen sulfide, and total mercaptans, in response to increasing probiotic concentration, as compared with CON, respectively. However, it is important to note that the efficacy of a probiotic is primarily determined by the efficacy of the selected bacterial strain and the physiology of the pig. Additionally, it is well known that not all probiotics work with pigs because of the complexity of the intestine and variation between individual animals. Therefore, further studies need to be carried out, in order to confirm which probiotics can be positively utilized in a beneficial manner and note their characteristics in pigs.

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