

THE APPARENT TOTAL TRACT DIGESTIBILITY, APPARENT ILEAL DIGESTIBILITY AND FECAL NOXIOUS GAS CONTENT OF GROWING PIGS FED PROBIOTICS SUPPLEMENTED DIETS

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The objective of this study was to determine the effects of probiotics (*Bacillus subtilis* endospores and *Clostridium butyricum* endospores complex) supplementation on the apparent ileal digestibility (AID), apparent total tract digestibility (ATTD) and fecal noxious gas content in growing pigs. Three barrows [(Landrace × Yorkshire) × Duroc] with an average initial BW of 26.48 ± 1.47 kg followed a 3 × 3 factorial arrangement of treatments (CON, P0.1, P0.2) with 3 levels of probiotics (0, 0.1, and 0.2%), respectively. In this study, the inclusion of probiotics increased (P<0.05) AID of dry matter (DM) and nitrogen (N) compared with CON group. Pigs fed P0.2 diet led to a higher (P<0.05) AID of energy than those fed CON diet. No difference was observed (P>0.05) on ATTD in the current study. Dietary probiotics decreased (P<0.05) the NH₃, propionic acid and butyric acid concentrations compared with CON diet. In conclusion, dietary added probiotics supplementation increased AID and reduced fecal noxious gas content in growing pigs.

Key words: growing pig, ileal digestibility, probiotics, noxious gas emission

During the last decades, it was well accepted that dietary probiotic could benefit the animal performance by producing antibacterial substances in the intestine (Hentges 1992); competition with harmful gut flora and stimulation of the immune system (Khajareern 1994). However, the efficacy of probiotic preparations in practice on digestibility is inconsistent because of the different diet composition, feeding strategy, strain differences, dose level, age of the pigs, as well as its interactions with environment

factors (Chesson 1994; Loh et al. 2008).

Generally, there were two phase in *Bacillus* species life cycle including vegetative cell and endospores (Sonenshein 2000). Previous studies had indicated that endospores are the most resistant cell type in the world, which are resistant to heat, radiation, desiccation, pH extremes and toxic chemicals (Setlow 1994; Nicholson et al. 2000). However, Ciffo et al. (1987) suggested that the acidic conditions in stomach may trigger the germination of the *Bacillus* spores. Ozawa et al. (1981) and Jadmus et al. (2001) also found that spores of *Bacillus subtilis* (*B. subtilis*) and *Bacillus cereu* (*B. cereu*) germinate rapidly and multiply to some extent in piglet stomach and intestine tract of pigs. Therefore, it is suitable to suggest that those kinds of endospores can germinate from the resistant form into the growing and dividing vegetative form after entering animal, and may evidence probiotic activity to the animal because of the rapidly bacilli multiply. Our previous study had suggested that the endospores increased the growth performance and nutrient digestibility in growing-finishing pig (Meng et al. 2010), but the mechanism by which the endospores affect the animal is unknown.

Thus, the objective of this study was to investigate the effect of a probiotics complex on the apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) and fecal noxious gas content in growing pigs.

MATERIALS AND METHODS

The experiment was performed at the Experimental Unit of the Dankook University, and the protocol of the current experiment was approved by the Animal

Care and Use Committee of Dankook University.

Source of probiotics

The probiotics preparation used in the current experiment is manufactured by a commercial company (Sporezyme; Woogene B&G Co., Ltd., Seoul, Korea). This product is composed of a mixture of spray-dried spore-forming *Bacillus subtilis* endospores and *Clostridium butyricum* endospores, which is guaranteed to contain at least 1.0×10^{10} viable spores/g of *Bacillus subtilis* endospores and 1.0×10^9 viable spores/g of *Clostridium butyricum* endospores.

Experimental animal, design and diets

A total of 9 growing barrows [(Landrace \times Yorkshire) \times Duroc] with an average initial BW of 26.48 ± 1.47 kg were used in this trial. Pigs were randomly allotted to a triplicate 3×3 factorial arrangement of treatments with 3 levels of probiotics (0, 0.1, and 0.2%) and 3 periods per square by their initial BW, and were surgically fitted with a T-cannula at the distal ileum by the direct method according to the procedures adapted from Stein et al. (1998). Following surgery, the barrows were housed individually in stainless steel metabolic cage (1.2×0.6 m) in a temperature controlled (28°C) room. Pigs were permitted 14 days of recovery and weighed prior to the initiation of the experiments according to the procedure described by Li et al. (1994), after which pigs were fed to 1 of the 3 experimental diets prepared. The ingredients and nutrient compositions of diets are provided in Table 1. All diets were formulated to meet or exceed the nutrient requirements recommended by NRC (1998). Chromic oxide (Cr_2O_3) was added (0.20%) in the diet as an indigestible marker to apparent digestibility determinations throughout the experiment.

Sampling and measurements

Pigs were fed twice daily (0800 and 2000, equal portion at each meal). The daily feed allowance was $0.05 \times \text{BW}^{0.9}$, as proposed by Armstrong and Mitchell (1955). The feed intake was increased 150 g each subsequent period to account for BW increase over the

Table 1. Composition of basal diet (as-fed basis)

Items	%
Corn	54.87
Soybean meal	31.77
Rapeseed meal	1.60
Rice bran	1.50
Tallow	4.35
Molasses	3.10
Dicalcium phosphate	1.18
Limestone	0.79
Salt	0.20
L-Lys HCL, 78%	0.34
Vitamin premix ¹	0.20
Trace mineral premix ²	0.10
Calculated composition	
DE, kcal/kg	3400
CP, %	17.00
Lys, %	0.98
Ca, %	0.75
Total P, %	0.62

¹ Provided per kg of complete diet: 6500 IU vitamin A, 950 IU vitamin D₃, 27 IU vitamin E, 2.0 mg vitamin K₃, 3.6 mg vitamin B₂, 1.3 mg vitamin B₆, 15 mg pantothenic acid, 26.0 mg niacin, and 0.03 mg biotin.

² Provided per kg of complete diet: 50 mg Mn (as manganese oxide), 70 mg Zn (as zinc oxide), 54 mg Cu (as copper sulfate), 0.5 mg I (as calcium iodate), 0.5 mg Co (as $\text{Co}_2\text{O}_3 \cdot 7\text{H}_2\text{O}$), and 0.25 mg Se (as $\text{Na}_2\text{SeO}_3 \cdot 5\text{H}_2\text{O}$).

duration of the experiment. Water was provided for *ad libitum*.

Each period lasted 7 days, and animals were deprived of feed overnight before the following period diet was offered. The initial 5 days was an adaptation period to the diet, which were stored at -20°C until analysis. Ileal digesta were collected for a 12 h periods between the morning and evening feeding (0800 and 2000) at day 7 and 8. A plastic bag (225 mL) was attached to the cannula barrel using a cable tie and digesta flowing into the bag were collected. Bags were removed whenever they were filled with digesta, or at least once every 30 min, and stored at -20°C . Feces were collected a minimum of 2 times per day at 0800 and

1600, using plastic bags attached to the skin around the anus (Van Kleef et al., 1994). Collected digesta and feces were pooled by pig and frozen at -20°C . Before analyses, feces and digesta were thawed, homogenized, subsampled, and freeze-dried. Feed, feces and digesta were analyzed for dry matter (DM) and nitrogen (N) content (AOAC, 2000), and gross energy content was measured using the Adiabatic Bomb Calorimeter (Model 1241, Parr Instrument Co., USA). The chromium concentration was determined via UV absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan) and the apparent digestibility were calculated via indirect methods described by Williams et al. (1962).

Duplicate pH values for each collected feces sample were measured using a pH meter (WTW pH 340-A, WTH Measurement Systems Inc.). $\text{NH}_3\text{-N}$ concentration was determined according to the methods of Chaney and Marbach (1962). The VFA measured in this experiment included acetic acid, propionic acid and butyric acid. For analysis of VFA, 2 g of fecal samples were added to 8 ml of distilled water. With the addition of HCl (2 drops), samples were centrifuged ($17400 \times \text{g}$) for 10 min and VFA were analyzed by gas chromatography (Hewlett Packard 6890 Plus, USA).

Statistical analyses

The 3×3 Latin square design was used with the analysis of variance procedure (PROC ANOVA) of SAS (SAS Inst. 1996) to perform analysis of variance for balanced data. Variability in the data was expressed as the pooled SE, and a $P < 0.05$ was considered to be statistically significant and $P < 0.10$ was considered as a tendency.

RESULTS

The apparent ileal and apparent total tract digestibility

No effect was observed on ATTD of DM, N and energy at the end of the experiment (Table 2).

Fecal noxious gas content

Pigs fed CON diet showed higher ($P < 0.05$) NH_3 , propionic acid and butyric acid

concentrations than those fed P0.1 and P0.2 diets (Table 4), while acetic acid was not affected by dietary treatments.

Table 2. Effects of probiotics supplementation on apparent ileal digestibility in growing pigs

Items, %	CON ¹	P0.1 ¹	P0.2 ¹	SE ²
DM	70.74 ^b	76.97 ^a	77.45 ^a	2.73
N	73.08 ^b	78.32 ^a	79.00 ^a	2.56
Energy	74.05 ^b	76.56 ^{ab}	78.71 ^a	2.34

¹Abbreviated CON, basal diet; P0.1, CON + 0.1% probiotics; P0.2, CON + 0.2% probiotics.

²Pooled standard error.

^{a,b}Means in the same row with different superscripts differ ($P < 0.05$).

Table 3. Effects of probiotics supplementation on apparent total tract digestibility in growing pigs

Items, %	CON ¹	P0.1 ¹	P0.2 ¹	SE ²
DM	85.67	85.49	86.08	3.98
N	85.35	84.78	84.92	2.38
Energy	86.55	85.43	85.68	2.09

¹Abbreviated CON, basal diet; P0.1, CON + 0.1% probiotics; P0.2, CON + 0.2% probiotics.

²Pooled standard error.

Table 4. Effects of probiotics supplementation on fecal NH_3 and VFA concentrations in growing pigs

Items	CON ¹	P0.1 ¹	P0.2 ¹	SE ²
NH_3 , mg/kg	235.30 ^a	219.24 ^b	212.07 ^b	3.18
Volatile fatty acids, % ³				
Propionic acid	18.65 ^a	16.09 ^b	15.81 ^b	1.05
Butyric acid	17.05 ^a	13.49 ^b	14.08 ^b	1.67
Acetic acid	17.15	18.31	17.01	1.17

¹Abbreviated CON, basal diet; P0.1, CON + 0.1% probiotics; P0.2, CON + 0.2% probiotics.

²Pooled standard error.

³Percentage of total VFA.

^{a,b}Means in the same row with different superscripts differ ($P < 0.05$).

DISCUSSION

In the current study, a marginal beneficial effect was observed on AID with dietary

probiotics, which to some extent indicated that the endospores benefited the digestibility in the anterior small intestine. Generally, Nicholson et al. (2000) have suggested that endospores are generated when the living, vegetative cells of these genera are exposed to harsh environmental conditions such as heat, radiation, desiccation and toxic chemicals (Nicholson et al. 2000), they also suggested that spores are resistant to heat, radiation, desiccation, pH extremes, and toxic chemicals (Setlow 1994; Nicholson et al. 2000). However, acidic conditions in the stomach may trigger germination of the bacillus spores (Ciffo et al. 1987). Ozawa et al. (1981) and Jadmus et al. (2001) also found that spores of *B. subtilis* and *B. cereu* germinate rapidly and multiply to some extent in piglet stomach and intestine tract of pigs. Therefore, those kinds of endospores can germinate from the resistant form into the growing vegetative form after entering animal, and evidence probiotic activity to the animal because of the rapidly bacilli multiply. Supportably, our previous study also documented that endospores supplementation could improve the growth performance and nutrient digestibility in pigs. Jonsson and Conway (1992) have demonstrated that addition of *Bacillus* spp. led to an improved growth performance and health status of pigs. Our previous study also reported an increased ADG in growing pigs fed diets supplemented with complex probiotics (*Lactobacillus acidophilus*, *Saccharomyces cerevisiae*, and *B. subtilis*) at the level of 0.2% (Chen et al. 2006). Therefore, we hypothesized the supplemental probiotics was germinated prior to the intestinal and benefited the nutrient utilization in the anterior small intestine in the current study. However, the ATTD of DM and N were not affected by endospores supplementation. It is well accepted that fecal digestibility represents nutrient disappearance through absorption, endogenous losses and bacterial assimilation of nutrients in the small and large intestine, whereas the AID represents the net disappearance of nutrients from the digestive tract prior to the distal ileum. Therefore, we hypothesized the microbial fermentation and bacterial assimilation of

nutrient in the large intestine may have led to the similar ATTD in the current study. Probiotics could reduce the environmental pollutants from animal manure by improving feed efficiency and nutrient retention (Han et al. 2001). Our previous studies have found *Bacillus*-based probiotics reduced the fecal NH₃-N in finishing pig in spite of the absence of an improvement in apparent N utilization (Wang et al. 2009; Chen et al. 2006). Similar in this study, dietary probiotics reduced fecal noxious gas content without affecting the ATTD. It is evident from the current study that the inclusion of probiotics led to a greater ileal digestibility compared with CON group, indicating that probiotics supplementation increased the nutrient digestibility in the anterior small intestine and subsequently decreased the residues nutrients passed to the large intestine. Previous study has suggested that intestinal fermentation mainly occurs in the distal ileum and in the hindgut (Decuyper and Van der Heyde 1972). Ferket et al. (2002) suggested that fecal odor and NH₃ emission are related to the intestinal microbiota ecosystem and microbial fermentation in the large intestine. Therefore, the reason for the decreased fecal noxious gas content in the current study should be the decreased residue nutrients passed to the large intestine. Moreover, it is well accepted that VFA is the main metabolite product of the microbial fermentation in the large intestine. Nagamine et al. (1998) have found VFA concentration was lower in the feces of pigs fed a diet supplemented with dietary microbes complex compared with pigs given the non-supplemented diets. Our previous study also reported that butyric acid content was decreased when finishing pigs were fed *Bacillus*-based probiotic diets at 0.1 and 0.2% level (Chen et al. 2006). Therefore, we suggested that the reason for the decreased fecal noxious gas content may not only to be the increased digestibility but also the beneficial effect of the probiotics on the intestinal microflora in the large intestine. Collectively, dietary supplementation of probiotics can improve AID and reduce fecal NH₃-N and VFA concentrations in growing pigs.

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