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Evaluation of *Lactobacillus* and *Bacillus*-based probiotics as alternatives to antibiotics in enteric microbial challenged weaned piglets

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The present study investigates the efficacies of two probiotic products as alternative to antibiotics on growth performance, nutrient digestibility, immunity, and fecal microbiota in piglets challenged with *Salmonella enterica* serovar Typhimurium KCTC 2515 and *Escherichia coli* KCTC 2571. Ninety-six 28-day-old piglets were randomly allotted to four dietary treatments consisting of four replicate pens with six piglets each. The dietary treatments were: negative control (NC), positive control (PC, 0.002% apramycin), 0.5% *Lactobacillus* probiotic (P1), and 0.04% *Bacillus* probiotic (P2). Average daily gain (ADG) and average daily feed intake (ADFI) were improved by treatment with PC and P1, whereas, feed conversion ratio (FCR) was improved by treatment with P2 compared to NC ($P < 0.05$). Digestibility of dry matter, crude protein, and crude fat increased upon treatment with PC, P1, and P2 compared to NC. All dietary treatments showed significant reduction of fecal *Salmonella* and *E. coli* counts with an increase of *Lactobacillus* and *Bacillus* spp counts compared to NC ($P < 0.05$). The serum IgG level was elevated by P2 treatment compared to others ($P < 0.05$). Overall, both *Lactobacillus* and *Bacillus* probiotics had beneficial effects on weaned piglets under challenged condition and therefore, can be used as potential alternatives to antibiotics.

Key words: *Lactobacillus*, *Bacillus*, fecal microbiota, immunoglobulin, challenged piglet.

INTRODUCTION

One of the most important aspects of veterinary research is to improve the quality and delivery of safe livestock products (meat, milk and egg) for human consumption. Swine meat, widely consumed worldwide, can be a source of food borne pathogens such as *Salmonella* and *Escherichia coli* (Korsak et al., 2003). Therefore, it becomes critical to the producers to identify the best methods to mitigate *Salmonella* and *E. coli* infection from pig meats. In the past, antibiotics were included at sub-therapeutic levels, acting as growth promoters (Antibiotics Growth Promoters, AGPs) and reducing the pathogen load (Dibner and Richards, 2005). However, there is a recent consumer rising trend in having AGPs removed from animal agriculture due to health and environmental issues, together with the increase of bacterial strain resistant against many human antibiotics. These concerns have resulted in the severe restriction or total elimination of antibiotics as growth promoters (EC, 2003) in many countries. Langlois et al. (1988) demonstrated that complete removal of antibiotics from animal production diminished resistance of lactose-fermenting fecal coliform bacteria. However, that has put tremendous pressure on the livestock industry to identify viable therapeutic alternatives against food borne pathogens, such as probiotics, which have used successfully in livestock feeds (Alexopoulos et al., 2004; Chen et al., 2005, 2006).

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Table 1. Ingredients and nutrient levels of basal diet.

Item	Value
Ingredients (%,as-fed basis)	
Yellow corn	45.15
Wheat	23.00
Wheat bran	4.00
Soybean meal	18.00
Limestone	0.98
Calcium phosphate	1.10
Salt	0.25
Vitamin premix[A]	0.55
Animal fat	2.50
Molasses	4.30
L-Lysine	0.17
Chemical composition (as fed basis)[B]	
ME (kcal/kg)	3265
Crude protein (%)	18.0
Ca (%)	0.70
Available phosphorus (%)	0.55
Lysine (%)	0.95
Methionine (%)	0.30

[A]Contains the following nutrients per kg of diet: vitamin (V) A 6000 IU; VD3 800 IU; VE 20 IU; VK3 2 mg; VB1 2 mg; VB2 4 mg; VB6 2 mg; VB12 1 mg; pantothenic acid 11 mg; niacin 10 mg; biotin 0.02 mg; Cu 21 mg; Fe 100 mg; Zn 60 mg; Mn 90 mg; I 1.0 mg; Co 0.3 mg; Se 0.3 mg. [B]Calculated values.

Probiotics refer to a group of non-pathogenic organisms that, when ingested in sufficient number, have beneficial effects on the health of the host (Reid et al., 2003). There are three main categories of organisms that are commonly referred to as probiotics: Lactic acid bacteria (LAB), spore-forming *Bacillus* spp., and yeast. *Lactobacilli* are non-pathogenic, Gram-positive bacteria as well as natural inhabitants of the porcine gastro-intestinal tract. Previous studies have discovered that native gut microbes can successfully prevent infection by *Salmonella* spp. (Nurmi and Rantala, 1973) and reduce shedding of pathogenic *E. coli* (Watkins et al., 1982).

However, their concentration decreases dramatically immediately after weaning (Huis in't Veld and Havenaar, 1993) which allows the proliferation of pathogenic bacteria. On the other hand, some *Bacillus* spp., with soil as their natural habitat, are used as probiotics, either alone or in combination (Hong et al., 2005). They cannot colonize in the gastrointestinal tract; but stimulate the growth of *Lactobacilli* through production of catalase and subtilisin (Hosoi et al., 2000). Several studies reported improvements in growth performance (Huang et al., 2004; Alexopoulos et al., 2004), nutrient digestibility (Shon et al., 2005; Chen et al., 2006), humoral and cell-mediated immune responses (Fernandes and Shahani, 1990; European food safety authority, 2010), and the microbial ecosystem (Huang et al., 2004; Baker et al., 2013) upon dietary supplementation with *Lactobacillus* or *Bacillus*-based probiotics, although inconsistencies in result have also been reported (Cromwell, 2001). The discrepancies observed can be attributed to different strains, dose levels, diet compositions, feeding strategies, age of animals, etc (Chesson, 1994). This indicates the need of specific studies to elucidate the efficacies of the several probiotic preparations.

This study was done to investigate the efficacy of *Lactobacillus*- and *Bacillus*-based probiotic preparations as an alternative to typical AGPs and assess their effects on growth performance, nutrient digestibility, immunity, and microbial ecology of weaned piglets challenged with *Salmonella enterica* serovar Typhimurium KCTC 2515 and *Escherichia coli* KCTC 2571.

MATERIALS AND METHODS

Experimental studies with piglets were approved by the Animal Care and Use Committee of Sunchon National University, Suncheon, Republic of Korea.

Source of probiotics

The *Lactobacillus*-based probiotic preparation, Avilac, used in the current experiment was manufactured by Daesung Microbiologica Labs Co., Ltd. (Seoul, Korea) and containing at least 10^{10} cfu of *Lactobacillus reuteri avibro*/kg of diet. The *Bacillus*-based probiotic, Bioplus 2B, was manufactured by Easy Bio System Inc. (Seoul, Korea) and containing *Bacillus subtilis* and *Bacillus licheniformis* both at 3.2×10^9 cfu/kg of diet.

Probiotic supplementation diet

A total of 96 three-line crossbred [(Landrace × Yorkshire) × Duroc] weaned piglets (28-days-old, average body weight of 8 kg) were housed for a period of 28 days. A completely randomized design was used with four treatments and four replicates (pens of 6 piglets with an equal sex ratio of three male and three female) per treatments, where piglets were allotted by body weight. Four dietary groups were formed; each consisted of 24 piglets (four replicates of six pigs per pen). The dietary groups included: basal diet without any supplement (NC; negative control), basal diet added with 0.002% apramycin (PC; positive control), basal diet added with 0.5% *Lactobacillus*-based probiotic (P1), and basal diet added with 0.04% *Bacilli*-based probiotic (P2). The dose levels of antibiotic and probiotics used in this experiment were determined in accordance with previous research (Ahmed et al., 2013; Harper et al., 1983; Gracia et al., 2004).

The antibiotic and probiotic products were mixed on a weight:weight ratio basis by replacing an equal amount of basal diet. The basal diet used in this experiment was in pellet form and was formulated to provide the nutrient requirements recommended by the NRC (1998). The ingredients and composition of the experimental diet are presented in Table 1. All pigs were housed in an environmentally controlled isolation trailer with a slatted plastic floor in 12 adjacent pens. Each pen was equipped with a one-sided

self-feeder and a nipple drinker to allow *ad libitum* access to feed and water throughout the experimental period. The target room temperature and humidity were 25°C and 60%, respectively. Individual pig body weights were measured at the beginning (day 1), middle (day 14), and end (day 28) of the experiment, and ADG was calculated. Feed consumption was recorded on a pen basis every other week, and the ADFI and FCR were calculated.

Oral challenge

The *S. enterica* serovar Typhimurium KCTC 2515 and *E. coli* KCTC 2571 used in the study are parts of the Korean Collection for Type Cultures (KCTC) and the stocks were purchased from the Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea. The bacterial cultures were prepared by growth in LB (Luria-Bertani) Broth (Difco, Detroit, MI, USA) for 24 h at 37°C by using 1% inoculum volume from the stocks. Tenfold dilution of the bacterial cultures were made prior to challenge and plated in agar media to enumerate the cell concentration per ml. All piglets were orally challenged with 5 ml of culture fluid containing 5.9×10^8 cfu/ml of *S. enterica* serovar Typhimurium KCTC 2515 and 2.3×10^8 cfu/ml of *E. coli* KCTC 2571 at the back of the oral cavity using a micropipette tip. Bacterial solution was slowly dribbled into piglet's throat in order to trigger the swallowing reflex and minimize the passage of inoculants into the lungs. The goal was to use the challenge as a model of post-weaning lag phase, which is manifested by an imbalanced microbiota in the intestine, resulting in poor growth performance and immunity. The piglets were housed in an environmentally controlled isolation trailer to prevent possible cross-contamination.

Sampling and measurement

A digestibility trial was conducted using chromium oxide (0.20%) as an indigestible marker (Fenton and Fenton, 1979). All piglets were fed diets mixed with chromium oxide (Cr_2O_3) on day 21, and fecal grab samples were collected from all pigs on day 28 and stored immediately in sealed plastic bags at -20°C until analysis. For chemical analysis, the fecal grab samples were dried in a force-air drying oven at 70°C for 72 h and then finely ground to pass through a 1 mm screen. Analyses of feed and fecal samples were done in accordance with the methods established by the AOAC (2000). The chromium concentration was measured with an atomic absorption spectrophotometer (Model AA-6200; Shimadzu Corp., Kyoto, Japan) in a cuvette blanked with distilled water at 440 nm. Standard curves were prepared by using a stock solution of pure Cr_2O_3 (100 mg/100 ml), diluted to several working standards of 5, 10, or 20 mg/100 ml and carrying them through each method. The optical density was plotted against milligrams of Cr_2O_3. The digestibility was then calculated using the following formula:

$$\text{Digestibility (\%)} = [1-\{(N_f \times C_d) / (N_d \times C_f)\}] \times 100$$, in which

N_f = Nutrient concentration in feces (%DM)
N_d = Nutrient concentration in diet (%DM)
C_f = Chromium concentration in feces (%DM)
C_d = Chromium concentration in diet (%DM)

For microbial analysis, two piglets (one male and one female) were identified from each pen by a double ear-tag on day 1 of the trial. Fresh fecal samples were collected on day 7, 14, 21 and 28 of the experiment, directly from the rectum of these piglets in sterile polyethylene bags, via manual stimulation of the internal and external anal sphincters, in order to avoid any additional contamination of the samples. The samples were than serially diluted 10-fold in sterile saline (0.9%). Microbial assay of fecal samples was carried out by culture techniques. The microbial groups analyzed were *S. typhimurium* [Salmonella-Shigella (SS) agar], *E. coli* [MacConkey (MAC) agar], *Lactobacillus* spp. [de Man, Rogosa and Sharpe (MRS) agar], and *Bacillus* spp. [Mannitol Egg Yolk Polymyxin (MYP) agar]. The microbial plates were inoculated with three dilutions each in duplicate. The agar plates were then incubated anaerobically at 37°C for 24 h, after which microbial colonies were immediately counted. Microflora enumerations were expressed as $\log^{10}$ cfu/mL.

For immunoglobulins quantification, blood samples were collected directly from the jugular vein on day 28 using a 22-gauge sterile needle in a 10 ml syringe and then transferred to a BD Vacutainer (Becton Dickinson, Franklin Lakes, NJ) without anticoagulant. The blood was then quickly transferred to a centrifuge tube and centrifuged for 15 min at 3,000 rpm (1610 × g) in a cold chamber (4°C). The sera were carefully removed to plastic vials and stored at -20°C until immunoglobulin analysis was performed. The concentrations of serum IgG, IgM, and IgA were assayed using Pig IgG (Cat. No. E100-104), IgM (Cat. No. E100-100), and IgA (Cat. No. E100-102) ELISA Quantitation Kits (BETHYL Laboratories Inc., USA), respectively, whereas TNF-α was assayed using a Porcine TNF-α Quantikine ELISA Kit (Cat. No. PTA00) according to manufacturer's instructions. Each experiment was run in duplicate, and the results represent the means of three experiments. The absorbance of each well was measured using a microplate reader (Thermo Lab Systems, Finland) at 450 nm (Correction wavelength, 570 nm). The results were expressed as mg/ml of serum.

Statistical analysis

All data were subjected to analysis of variance (ANOVA) appropriate for a completely randomized design by using the general linear model procedures (GLM) of the SAS Institute Inc. (SAS, 2003). The pen was used as the experimental unit to analyze growth performance and nutrient digestibility, whereas an individual piglet was used as the experimental unit for analysis of serum immunoglobulins and fecal microbiota. Statistically significant effects were further analyzed, and means were compared using Duncan's multiple range tests. Probability values of $P < 0.05$ were considered as statistically significant, whereas $P < 0.10$ was considered a tendency.

RESULTS

Growth performances

The effects of probiotics on growth performance of weaned piglets are shown in Table 2. During phase 1 (days 0 to 14), ADG of piglets treated with P1 was greater ($P = 0.0004$) compared to NC, PC, or P2. On the other hand, during phase 2 (days 14 to 28) and the overall experimental period (days 0 to 28), ADG of piglets treated with PC and P1 were greater ($P < 0.0001$) compared to P2 or NC treatment. Moreover, ADG induced by P2 treatment was non-significantly higher compared to NC treatment during all phases.

During phase 1, phase 2, and the overall experimental period, ADFIs of the P1 and PC-supplemented groups were higher ($P < 0.0001$) compared to NC or P2 treatment, with P2 showing a non-significantly lower effect than NC. During phase 2 and the overall experimental

Table 2. Effects of *Lactobacillus*- and *Bacillus*-based probiotics on growth performance of challenged piglets.

Parameter[A]	Treatment[B]				SEM[C]	P-value
	NC	PC	P1	P2		
Initial BW (kg/piglet)	8.01	8.44	7.97	8.17	0.33	0.75
ADG (g/piglet)						
Phase 1 (day 0 to 14)	296^{c}	383^{b}	481^{a}	309^{c}	22.88	0.0004
Phase 2 (day 14 to 28)	191^{c}	362^{a}	298^{ab}	255^{bc}	21.51	0.001
Overall (day 0 to 28)	243^{b}	373^{a}	390^{a}	282^{b}	13.36	<.0001
ADFI (g/piglet)						
Phase 1(day 0 to 14)	451^{c}	492^{b}	670^{a}	440^{c}	8.90	<.0001
Phase 2 (day 14 to 28)	517^{c}	848^{a}	719^{b}	511^{c}	12.87	<.0001
Overall (day 0 to 28)	484^{b}	670^{a}	695^{a}	475^{b}	8.01	<.0001
FCR (feed/gain)						
Phase 1(day 0 to 14)	1.59	1.36	1.41	1.43	0.13	0.70
Phase 2 (day 14 to 28)	2.78^{a}	2.38^{ab}	2.42^{ab}	2.07^{b}	0.17	0.12
Overall (day 0 to 28)	2.00^{a}	1.81^{ab}	1.79^{ab}	1.69^{b}	0.08	0.08

^{a,b,c} Means in a row with no common superscripts differ significantly ($p < 0.05$) or tend to differ ($p < 0.10$). [A]BW, Body weight; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio. [B]NC: negative control; basal diet, PC: positive control; basal diet + 0.002% Apramycin, P1: basal diet + 0.5% *Lactobacillus*-based probiotics, P2: basal diet + 0.04% *Bacillus*-based probiotics. [C]Standard error of the means.

Table 3. Effects of *Lactobacillus*- and *Bacillus*-based probiotics on nutrient digestibility of challenged piglets.

Nutrient digestibility (%)	Treatment[A]				SEM[B]	P-values
	NC	PC	P1	P2		
Dry Matter	72.6^{c}	85.8^{a}	77.1^{b}	76.2^{b}	0.88	<.0001
Crude Protein	65.2^{c}	77.1^{a}	71.7^{b}	69.9^{b}	1.53	0.001
Crude Fat	66.3^{b}	78.4^{a}	76.9^{a}	78.6^{a}	1.18	<0.001
Crude Fiber	68.1^{b}	82.3^{a}	73.1^{b}	73.0^{b}	1.80	0.009
Crude Ash	45.3^{b}	66.7^{a}	53.4^{b}	47.7^{b}	2.47	0.002

^{a,b,c} Means in a row with no common superscripts differ significantly ($P < 0.05$). [A]NC: Negative control; basal diet, PC: Positive control; basal diet + 0.002% Apramycin, P1: basal diet + 0.5% *Lactobacillus*-based probiotics, P2: basal diet + 0.04% *Bacillus*-based probiotics. [B]Standard error of the means.

period, FCR of piglets treated with P2 was lower ($P < 0.10$) compared with other treatments, with PC and P1 showing intermediate effects.

Apparent nutrient digestibility

Apparent digestibility of dry matter (DM) and crude protein (CP) were greater ($P < 0.01$) in piglets treated with PC, P1, and P2 compared to NC, with P1 and P2 showing intermediate effects (Table 3). Digestibility of crude fat (EE) was elevated ($P < 0.01$) upon treatment with PC, P1, and P2 in relation to NC. Apparent digestibility of crude fiber (CF) and crude ash (CA) increased upon PC treatment ($P < 0.01$) compared to treatment with P1, P2, or NC.

Fecal microflora population

The results of the study on fecal microflora concentration are shown in Table 4. On day 7, piglets treated with P1

Table 4. Effects of *Lactobacillus*- and *Bacillus*-based probiotics on fecal microbial concentrations in challenged piglets (log_{10} cfu/ml).

Day post infection	Treatment[A]				SEM[B]	P-value
	NC	PC	P1	P2		
S. typhimurium						
D 7	4.09[a]	3.54[ab]	2.96[b]	4.04[a]	0.25	0.07
D 14	4.00[a]	1.92[b]	1.49[b]	3.34[a]	0.29	0.007
D 21	4.26[a]	0.77[c]	2.73[b]	3.03[b]	0.21	<.0001
D 28	4.17[a]	0.33[c]	1.85[b]	0.33[c]	0.25	<.0001
E. coli						
D 7	4.73	4.68	4.88	4.95	0.14	0.54
D 14	5.42	5.44	5.40	5.14	0.22	0.75
D 21	5.62[a]	4.60[b]	5.71[a]	5.07[b]	0.15	0.004
D 28	6.19[a]	4.97[b]	5.38[b]	5.48[b]	0.17	0.008
Lactobacillus spp.						
D 7	7.10[a]	6.38[b]	7.42[a]	7.28[a]	0.11	0.0005
D 14	7.24[ab]	7.03[ab]	7.40[a]	6.73[b]	0.15	0.91
D 21	7.19	6.91	7.20	7.21	0.18	0.70
D 28	6.71[b]	7.08[a]	7.26[a]	7.08[a]	0.10	0.03
Bacillus spp.						
D 7	5.91	6.01	6.16	6.05	0.20	0.87
D 14	6.33	6.53	6.06	6.48	0.23	0.52
D 21	6.51	6.40	6.49	6.36	0.14	0.86
D 28	6.35[b]	7.13[a]	6.93[a]	7.34[a]	0.11	0.002

[a,b,c] Means in a row with no common superscripts differ significantly ($P < 0.05$). [A]NC: Negative control; basal diet, PC: Positive control; basal diet + 0.002% Apramycin, P1: basal diet + 0.5% *Lactobacillus*-based probiotics, P2: basal diet + 0.04% [B]Standard error of the means.

showed lower ($P < 0.10$) fecal counts of *Salmonella* in relation to P2, PC, or NC. On day 14, fecal *Salmonella* counts were significantly reduced ($P < 0.01$) by PC and P1 treatments in relation to P2 or NC. On days 21 and 28, piglets treated with PC, P1, and P2 all showed lower ($P < 0.0001$) fecal *Salmonella* counts compared to NC treatment. Dietary supplementation of antibiotic (PC) or probiotic products (P1 and P2) had no effects on fecal counts of *E. coli* on days 7 and 14. On day 21, treatment with PC and P2 showed reduced ($P < 0.01$) *E. coli* counts compared to P1 or NC treatment. However, on day 28, piglets treated with PC, P1, and P2 all showed lower ($P < 0.01$) fecal *E. coli* counts in relation to NC treatment.

On days 7 and 14, PC treatment had reduced ($P < 0.01$) fecal counts of *Lactobacillus* spp. in relation to P1, P2, or NC ($P < 0.10$). Whereas the dietary treatments showed no effects on fecal *Lactobacillus* spp. counts on day 21. Fecal *Lactobacillus* spp. counts increased at day 28 ($P < 0.05$) following treatment with P1, P2, and PC compared to NC. There were no effects of dietary supplementation of antibiotics (PC) and probiotic products (P1 and P2) on fecal *Bacillus* spp. counts in piglets on days 7, 14 and 21. However, on day 28, piglets treated with PC, P1, and P2 all showed higher ($P < 0.01$) fecal *Bacillus* spp. counts in relation to NC.

Serum levels of IgG, IgM, IgA and TNF-α

Table 5 shows the effects of dietary treatments on serum immunoglobulin and TNF-α concentrations in challenged piglets. The serum IgG concentration in piglets treated with P2 was greater ($P < 0.01$) compared to those treated with NC, PC, or P1. However, the concentrations of IgM and IgA were unaffected by the dietary treatments ($P > 0.05$). Antibiotics and both probiotic groups showed significantly lower concentrations of serum TNF-α in relation to control ($P < 0.0001$).

DISCUSSION

A well balanced gut microbiota is able to positively affect

Table 5. Effects of *Lactobacillus*- and *Bacillus*-based probiotics on serum immunoglobulins and TNF-α concentration of challenged piglets.

Parameter	Treatment[A]				SEM[B]	P-values
	NC	PC	P1	P2		
IgG (mg/mL)	409[b]	366[b]	417[b]	527[a]	17.5	0.001
IgM (mg/mL)	30.2	29.9	30.8	30.2	0.47	0.66
IgA (mg/mL)	6.33	5.80	6.10	6.50	0.42	0.69
TNF-α (pg/mL)	133[a]	98.7[b]	101[b]	99.7[b]	1.14	<.0001

[a,b]Means in a row with no common superscripts differ significantly (P < 0.05). [A]NC: Negative control; basal diet, PC: Positive control; basal diet + 0.002% Apramycin, P1: basal diet + 0.5% *Lactobacillus*-based probiotics, P2: basal diet + 0.04% *Bacillus*-based probiotics. [B]Standard error of the means.

the integrity of the intestinal barrier against pathogen colonization through its protective and metabolic function and can stimulate the immune system in an anti-inflammmatory manner (Gaggia et al., 2010). However, physiological or psychological stresses such as weaning lead to dysfunction of the intestinal barrier function by negatively altering gut microbial composition (Gareau et al., 2009). Probiotics are mainly used to reinforce or re-establish the gut microbial balance, especially when hosts are confronted with challenges or stress (Vanbelle, 2001), generally associated with poor growth rate and immunity. Probiotic bacteria such as *Lactobacilli* and *Bacilli* have been shown to improve growth performance of pigs by maintaining the intestinal microbial balance (Alexopoulos et al., 2004; Shon et al., 2005). In the current study, we examined the efficacy of *Lactobacillus*- or *Bacillus*-based probiotics for improving the growth performance, nutrient digestibility, microbial ecosystem, and immune response of weaned piglets challenged with the enteric pathogens *Salmonella enterica* serovar Typhimurium and *E. coli*. The antibiotic apramycin was used as a positive control with the objective of evaluating the efficacies of the probiotic products as alternatives to AGPS.

In the present study, application of *Lactobacillus*-based probiotics and antibiotics resulted in improvement of ADG and ADFI, whereas *Bacillus*-based probiotics resulted in improvement of FCR. These beneficial effects of antibiotic and probiotic supplementation on growth performance are consistent with the results of Cromwell (2001), Shon et al. (2005), and Wang et al. (2011). *Lactobacilli* bacteria are natural inhabitants of the gastrointestinal tracts of piglets. Their metabolites, including lactic acid, digestive enzymes etc., stimulate gastrointestinal peristalsis and promote apparent nutrient digestibility, which improves the appetites of piglets and maintains microbial equilibrium in the intestine (Wang et al., 2011). However, it was previously shown that *Lactobacilli* counts decline dramatically immediately after weaning (Huis in't Veld and Havenaar, 1993), resulting in microflora imbalance, digestive disturbance and poor performance of piglets. Therefore dietary supplementation of *Lactobacillus*-based probiotic could be beneficial (Djouzi et al., 1997). In the present study, increased ADG and ADFI in piglets fed *Lactobacillus*-based probiotics could be attributed to improved digestibility of nutrients and microbial ecology in the intestine. Supplementation of *Bacillus*-based probiotic had no significant effect on ADG and ADFI of weaned piglets which is partially consistent with previous studies (Kritas and Morrison, 2004; Min et al., 2004). However, these results have not always been consistent. For example, Gracia et al. (2004) reported improved ADG and ADFI during prestarter and overall prestarter-finishing period by dietary 0.04% Bioplus 2B. However, Robert and Gabriel (2006) reported that addition of 0.04% Bioplus 2B at different periods had different effects on the ADG of young pigs. In our study, lower improvement in ADG in *Bacillus*-probiotic treatments may be due to the short treatment period (Wang et al., 2009). The numerical reduction of ADFI in the *Bacillus*-based probiotic-treated group can be considered as a contributing factor in the increased feed to gain ratio. The reduced feed intake and improved FCR with no effect on weight gain indicates that the *Bacillus* probiotics indeed exerted some beneficial effects in the piglets. *Bacillus* can produce some useful enzymes such as amylase, protease (Ohno et al., 1995), that improves the apparent digestibility of complex carbohydrates and proteins, thus increasing the FCR (Anjum et al., 2005).

Supplementation of probiotic products and apramycin improved the apparent digestibility of DM, CP and EE. In agreement with the findings of the present study, Meng et al. (2010), and Shim et al. (2010) reported greater apparent digestibility of DM and CP in pigs and broilers supplemented with probiotics complex. Min et al. (2004) reported the positive effects of 0.04% Bioplus 2B in the DM and N digestibilities of nursery pigs. Conversely, Shon et al. (2005) and Wang et al. (2009) reported no improvement in nutrient digestibility of growing pigs by dietary *Lactobacillus* or *Bacillus*-based probiotic supplementation, respectively. Improvement of apparent digestibility of nutrients by probiotics and antibioitcs could be attributed to increased nutrient availability for absorption via suppression of growth and metabolic activities of

harmful gut microbiota along with simultaneous alteration of the intestinal morphology (Shim et al., 2010). *Bacillus* and *Lactobacillus* are also known to increase the rate of glucose transport, intestinal villous height, and crypt depth ratio (Breves et al., 2000; Rao and Wang, 2010), which may have contributed to improved nutrient uptake in pigs. Moreover, probiotic products may compete with other intestinal microorganisms for nutrients or result in production of antibacterial substances (Hentges, 1992) if continuously administered to the animals, which would explain the results regarding nutrient digestibility.

Application of either antibiotic or probiotic treatments resulted in reduced numbers of fecal *Salmonella* and *E. coli* as well as increased *Lactobacillus* spp. and *Bacillus* spp. counts compared to control. It has been reported that probiotic bacteria maintain normal gut microflora in two ways: competitive exclusion or antagonism. Once established in the gut, probiotic bacteria may produce compounds with bactericidal or bacteristatic properties (bacteriocins) such as organic acids, hydrogen peroxide, lactoferrin, etc (Jin et al., 1997). These substances are thought to inhibit the growth of pathogenic bacteria by reducing the pH in the gut (FEFANA EU Feed Additives and Premixtures Association, 2005). Researchers found that *L. reuteri* can secrete sufficient amounts of a broad spectrum antibiotic substance, reuterin, resulting in the desired anti-microbial effects (Talarico et al., 1988). *Lactobacilli* themselves can also colonize the gut mucosa to form a biological barrier to pathogenic microbes. Huang et al. (2004) reported that *Lactobacilli* isolated from weaned pigs are able to reduce gut *E. coli* and increase gut *Lactobacilli* counts. Reduction of fecal shedding of *Salmonella* was also observed upon dietary supplementation of a mixture of probiotics (mainly *Lactobacilli*) in weaned pigs (Casey et al., 2007).

Although *Bacillus* spp. is not a principal member of the normal intestinal flora and could not colonize the intestine for long periods, it consumes oxygen rapidly and reduces pH, which favors *Lactobacilli* and inhibits *E. coli* and *Salmonella* (Wu et al., 2011). On the other hand, the digestive enzymes secreted by *Bacillus* spp. have limited effects on improving production performance in animals, although the various nutrients yielded by these enzymes may contribute to population changes in the fecal microflora to some extent. The increased number of *Lactobacillus* and *Bacillus* counts in the antibiotic-treated group during the last week of the experiment can be attributed to the development of resistance against antibiotics (Sarra et al., 1982).

The capacity of probiotics to modulate the immune system is one of the more recent developments in the livestock field. In the present study, immune responses were evaluated by determining levels of serum immunoglobulins (IgG, IgM, and IgA) and the cytokine TNF-α. Our results showed that serum IgG values significantly increased in the *Bacillus*-based probiotics-treated group. Our results are consistent with a report by Pătrăscanu et al. (2011), who observed increased IgG and IgM levels in pregnant sows supplemented with *Bacillus*-based probiotics Bioplus 2B®.

Chen et al. (2005) also observed increased IgG levels in growing pigs upon supplementation of a probiotics complex (*Lactobacillus acidophilus*, 1.0×10^7 CFU/g; *Saccharomyces cerevisae*, 4.3×10^6 CFU/g; *Bacillus subtilis* 2.0×10^6 CFU/g). Some researchers have reported that *Bacilli* and *Lactobacilli* bacteria alone or in combination can enhance humoral and cell-mediated immune responses (European Food Safety Authority, 2010) as well as further promote anti-bacterial and anti-viral activities. The protective effects of feeding immune-enhancing probiotics can reduce the severity of *E. coli* infection, and this reduction may be associated with enhanced humoral and cellular immune responses (Shu and Gill, 2002). Probiotics also enhance the systematic antibody response to soluble antigens in the serum and participate in the development of immunity (Christensen et al., 2002).

The poorest level of immunoglobulins in the antibiotics-treated group may be due to the immunosuppressive action of aminoglycoside, which has been reported to reduce the production of antibodies after enteric challenge (Roura et al., 1992). We found reduced serum TNF-α concentrations in both the antibiotics - and probiotics-supplemented groups. Our result are consistent with Isolauri et al. (2001), who reported that probiotics mediate the suppression of lymphocyte proliferation and cytokine production by T cells, thereby down regulating the expression of proinflammatory cytokines such as tumor necrosis factor-α (Stewart et al., 1996).

Conclusion

In total, the findings of the present study indicate that dietary supplementation of *Lactobacillus*-based probiotics positively affected body weight gain and feed intake, whereas feed conversion ratio was improved by *Bacillus*-based probiotics. Both probiotics positively altered the microbial environment. Moreover, *Bacillus*-based probiotics increased serum IgG production. Considering these results, we suggest further feeding trials in order to better understand the effects of such additives as antibiotic alternatives as well as to elucidate their mechanisms of action underlying immunity enhancement in weaned piglets.

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