Effects of Dietary Synbiotics from Anaerobic Microflora on Growth Performance, Noxious Gas Emission and Fecal Pathogenic Bacteria Population in Weaning Pigs

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ABSTRACT : Synbiotics is the term used for a mixture of probiotics (live microbial feed additives that beneficially affects the host animal) and prebiotics (non-digestible food ingredients that beneficially affect the organism). This study investigated the effect of probiotics from anaerobic microflora with prebiotics on growth performance, nutrient digestibility, noxious gas emission and fecal microbial population in weaning pigs. 150 pigs with an initial BW of 6.80±0.32 kg (20 d of age) were randomly assigned to 5 dietary treatments as follows: i) US, basal diet+0.15% antibiotics (0.05% oxytetracycline 200 and 0.10% tiamulin 38 g), ii) BS, basal diet+0.2% synbiotics (probiotics from bacteria), iii) YS, basal diet+0.2% synbiotics (probiotics from yeast), iv) MS, basal diet+0.2% synbiotics (probiotics from mold), v) CS, basal diet+0.2% synbiotics (from compounds of bacteria, yeast and mold). The probiotics were contained in 10^9 cfu/ml, 10^5 cfu/ml and 10^3 cfu/ml of bacteria, yeast and molds, respectively. The same prebiotics (mannan oligosaccharide, lactose, sodium acetate and ammonium citrate) was used for all the synbiotics. Pigs were housed individually for a 16-day experimental period. Growth performance showed no significant difference between antibiotic treatments and synbiotics-added treatments. The BS treatment showed higher (p<0.05) dry matter (DM) and nitrogen digestibility while ether extract and crude fiber digestibility were not affected by the dietary treatment. Also, the BS treatment decreased (p<0.05) fecal ammonia and amine gas emissions. Hydrogen sulfide concentration was also decreased (p<0.05) in BS, YS and MS treatments compared to other treatments. Moreover, all the synbiotics-added treatments increased fecal acetic acid concentration while the CS treatment had lower propionic acid concentration than the US treatment (p<0.05) gas emissions but decreased in fecal propionate gas emissions. Total fecal bacteria and Escherichia coli populations did not differ significantly among the treatments, while the Shigella counts were decreased (p<0.05) in synbiotics-included treatment. Fecal bacteria population was higher in the YS treatment than other treatments (p<0.05). The BS treatment had higher yeast concentration than YS, MS and CS treatments, while US treatment had higher mold concentrations than MS treatment (p<0.05). Therefore, the results of the present study suggest that synbiotics are as effective as antibiotics on growth performance, nutrient digestibility and fecal microflora composition in weaning pigs. Additionally, synbiotics from anaerobic microflora can decrease fecal noxious gas emission and synbiotics can substitute for antibiotics in weaning pigs. (Key Words : Anaerobic Microflora, Digestibility, Probiotics, Synbiotics, Weaning Pigs)

INTRODUCTION

Supplemental antibiotics in animal feed improve growth performance and feed efficiency (Hay, 1977). However, supplemental antibiotics increase bacterial resistance and increase the risk of antibiotic residues in pork (Witte, 2000), making their use in swine production harmful to human health.

Recently, the most commonly used alternatives to antibiotics have been probiotics, prebiotics and synbiotics. Probiotics are defined as a live microbial feed additive that beneficially affects the host animal by improving the intestinal microbial balance (Fuller, 1989; Kelly, 1998; Ko and Yang, 2008a; Ko et al., 2008b). Mohan et al. (1996) reported that supplemental probiotics improved growth
performance and feed efficiency in animals. Prebiotics are defined as a non-digestible food ingredient that beneficially affects the organism by selectively stimulating growth and inhibiting harmful bacterial activity in the intestinal tract, thus improving health of the host (Gibson and Roberfroid, 1995). Prebiotics include oligosaccharide, dietary fiber, gluconic acid, and some other similar ingredients. Synbiotics is the mixture of probiotics and prebiotics.

Many studies have evaluated the effects of different synbiotic preparations (Haghighi et al., 2005; Metzler et al., 2005). However, variable results were obtained due to the different composition of synbiotics. Several factors, such as bacterial species, dosage level, storage condition, diet composition, feeding strategy and interactions with drugs, significantly influenced the efficiency of synbiotics (Fuller, 1989; Kelly, 1998). Supplemental Aspergillus oryzae, one kind of prebiotic, increased growth performance and nitrogen retention in pigs, while supplemental Fermacto 500® from Aspergillus oryzae culture increased the digestibility of protein and fat in pigs (Grimes et al., 1997). The prebiotics used were mannan oligosaccharides, fructo-oligosaccharides, transgalacto-oligosaccharides, non-digestible oligosaccharides and others. Probiotics from aerobic microflora, such as Lactobacillus sp. (Taylor et al., 2002) and Saccharomyces cerevisiae (Williams et al., 1991), have been used normally in the livestock production industry. Therefore, prebiotics, probiotics and synbiotics may be useful for the substitution of antibiotics to stimulate growth performance in pigs. We used probiotics from anaerobic microflora with several prebiotics in this study. We investigated also whether probiotics from anaerobic microflora with several prebiotics could substitute for antibiotics by examining effects on growth performance and nutrient digestibility. Additionally, we investigated whether synbiotics from anaerobic microflora with several prebiotics could improve noxious gas emission and the fecal bacteria population in weanling pigs.

**MATERIALS AND METHODS**

**Animals and diets**

A total of 150 weanling pigs ((Yorkshire×Landrace) ×Duroc) with average BW of 6.80±0.32 kg were randomly assigned to 5 dietary treatments based on sex, BW and litter (3 replications per treatment with ten pigs per replication). The pigs were housed individually and given pre-feeding for 3 days and thereafter had free access to water and experimental feed for 13 days (aged 23 to 36 days). The care of these animals was in accordance with the Guide for the Care and Use of Laboratory Animals (Cheonan Yonam College Animal Care Committee).

<table>
<thead>
<tr>
<th>Table 1. Composition of experimental diet as % air dry matter (as-fed basis)</th>
<th>Ingredient (%)</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expanded pellet corn</td>
<td>25.07</td>
<td></td>
</tr>
<tr>
<td>Whey</td>
<td>23.85</td>
<td></td>
</tr>
<tr>
<td>Wheat</td>
<td>12.00</td>
<td></td>
</tr>
<tr>
<td>Dehulled soybean meal</td>
<td>12.00</td>
<td></td>
</tr>
<tr>
<td>Full fat soybean meal</td>
<td>7.00</td>
<td></td>
</tr>
<tr>
<td>White fish meal</td>
<td>5.58</td>
<td></td>
</tr>
<tr>
<td>Soy oil</td>
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<tr>
<td>Pop gold®</td>
<td>3.50</td>
<td></td>
</tr>
<tr>
<td>Brewer’s yeast</td>
<td>1.50</td>
<td></td>
</tr>
<tr>
<td>White sugar</td>
<td>3.00</td>
<td></td>
</tr>
<tr>
<td>Limestone</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
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</tr>
<tr>
<td>Sodium chloride</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>Zinc oxide</td>
<td>0.30</td>
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</tr>
<tr>
<td>Vitamin premix²</td>
<td>0.30</td>
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</tr>
<tr>
<td>Mineral premix³</td>
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</tr>
<tr>
<td>Lysine 78%</td>
<td>0.32</td>
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<tr>
<td>Methionine 99%</td>
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<td></td>
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<tr>
<td>Oxytetracycline 200⁴</td>
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<td></td>
</tr>
<tr>
<td>Tiamulin 38 g⁵</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>Antioxidant</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Flavor and sweetener</td>
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<tr>
<td>Organic acid</td>
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<tr>
<td>Choline chloride</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>Chemical composition</td>
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</tr>
<tr>
<td>Crude protein (%)⁶</td>
<td>21.0</td>
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</tr>
<tr>
<td>Ether extract (%)⁶</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td>Crude fiber (%)⁶</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Crude ash (%)⁶</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>DE (Mcal/kg)⁷</td>
<td>3.6</td>
<td></td>
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<tr>
<td>Calcium (%)⁸</td>
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<td></td>
</tr>
<tr>
<td>Phosphorus (%)⁶</td>
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<td></td>
</tr>
<tr>
<td>Lysine (%)⁷</td>
<td>1.50</td>
<td></td>
</tr>
</tbody>
</table>

1. Enzyme-treated 50% soybean protein imported from Taiwan.
2. Supplied per kilogram of diet: 4,800 IU vitamin A, 960 IU vitamin D₃, 20 IU vitamin E, 2.4 mg vitamin K₃, 4.6 mg vitamin B₉, 1.2 mg vitamin B₁₂, 13 mg pantothenic acid, 23.5 mg niacin and 0.02 mg biotin.
3. Supplied per kilogram of diet: 12.5 mg manganese, 179 mg zinc, 5.0 mg copper, 0.5 mg iodine and 0.4 mg selenium.
4, ⁵ 0.15% antibiotics composed of 0.05% oxytetracycline 200 and 0.10% tiamulin 38 g used for antibiotics diet and replaced with 0.20% synbiotics in the other diets.
6. Analytical values.
7. Based on composition values from Rural Deveropment Administration (2007).

Weanling pigs (Table 1). The 5 dietary treatments were: i) US, basal diet+0.15% antibiotics (0.05% oxytetracycline and 0.1% tiamulin) ii) BS, basal diet+0.2% synbiotics (probiotics from bacteria), iii) YS, basal diet+0.2% synbiotics (probiotics from yeast), iv) MS, basal diet+0.2% synbiotics (probiotics from mold), v) CS, basal diet+0.2% synbiotics (probiotics from compounds of bacteria, yeast and mold). The probiotics were contained in 10⁶ cfu/ml, 10⁵ cfu/ml and 10³ tfu/ml of bacteria, yeast and molds,
Oxide (Cr₂O₃) as a non-digestible marker to calculate the apparent nutrient digestibility. The ADG, ADFI and gain/feed on d 7, the disappearance were recorded weekly and then used to determine the ADG, ADFI and gain/feed. On d 7, the experimental diets were supplemented with 0.2% chromium oxide (Cr₂O₃) as a non-digestible marker to calculate the apparent nutrient digestibility. At the end of the experiment, fresh fecal samples were obtained from each pig. For nutrient digestibility, feed and fecal samples were dried at 60°C in an air-forced oven for 3 days and then were ground finely to pass through a 0.1 mm screen. After grinding, the samples were stored at -20°C until the analyses were performed.

All of the feed and fecal samples were analyzed for DM, nitrogen, ether extract and crude fiber following the procedures outlined by the AOAC (AOAC, 1995). Chromium was analyzed via UV absorption spectrophotometry (Shimadzu, UV-1201, Kyoto, Japan) following the method described by Williams et al. (1962).

Fresh fecal sample from each pen was separated into several parts in order to analyse noxious gas contents and counts of fecal bacteria. Fecal noxious gas emission was determined using Gastec (SKC Gulf Coast, G-100, TX, USA) according to the method described by Cho et al. (2008). The fecal VFA concentration was determined by the method of Erwin et al. (1961). The VFA concentration in the supernatant liquid was determined using a gas chromatograph (VARIAN, CP-3800, CA, USA).

Microbial analysis
For analysis of fecal pathogenic bacteria, 10 g of fresh fecal sample was diluted with 9 ml distilled water and then homogenized for 1 min, and thereafter, the homogenized sample was diluted to 10⁻¹¹ times. The total bacteria and Shigella sp. counts were determined using plate count agar (Cat. No. 247940, Difco, USA) and Salmonella-Shigella agar (Cat. No. 274500, Difco), respectively, after incubation in an anaerobic chamber at 37°C for 48 h. The Escherichia coli and Salmonella sp. counts were determined using MacConkey agar (Cat. No. 212123, Difco) and Salmonella-Shigella agar, respectively, after incubation in an anaerobic chamber at 37°C for 24 h. The colony count on each plate was measured using a colony counter (Suntex-570, Sung Kwang, Korea). Colony forming units (cfu) were defined as being distinct colonies measuring at least 1 mm in diameter.

Ten grams of feces sample was also diluted by 90 ml distilled water, following injection of oxide-free carbon dioxide gas and then homogenized for 2 min. The homogenized sample was sterilized at 121°C for 15 min, and then the sterilized sample was diluted 10⁻¹¹ times using anaerobic diluent solution by the method of Bryant and Burkey (1953). The anaerobic diluted solution, pre-reduced medium, and anaerobic microbial populations were measured using the anaerobic incubator, as described by Holdman et al. (1977). Anaerobic bacterial population was determined using Modified Dehority’s Artificial Medium (MDAM) agar by the method of Dehority (1965). The MDAM agar was added to a 2% Bacto agar at Dehority’s Artificial Medium agar. The anaerobic yeast count was determined using Potato Dextrose Agar Medium (PDA) agar (Cat. No. 6278625, Difco). One ml of rumen fluid (diluted 10⁻³ to 10⁻⁶) was combined with 9 ml of PDA agar including 1 ml of antibiotic (2×10⁴ IU/ml benzylpenicillin G with 2 mg/ml of streptomycin sulfate) to guard against bacterial population. The total yeast population was counted by the same method used for the total bacterial count. The anaerobic mold population was determined using Modified Lowe’s agar Medium according to the method of Lowe et al. (1985). Modified Lowe’s agar Medium was added 2% Bacto agar at Lowe’s agar Medium. One 1 ml of fecal sample (diluted 10⁻³ to 10⁻⁶) was added to 9 ml of Modified Lowe’s agar with antibiotic (2×10⁴ IU/ml benzylpenicillin G with 2 mg/ml of streptomycin sulfate) and was then incubated in an anaerobic condition at 38.5°C for 5 days.

Statistical analyses
The data were analyzed using the General Linear Model (GLM) procedures of SAS (1996), and significant differences among the means were determined using Duncan’s Multiple Range Test method (Duncan, 1955), with p<0.05 indicating significance.

RESULTS

Growth performance and nutrient digestibility
Effects of synbiotics supplementation on growth performance and nutrient digestibility are presented in Table 2. There was no significant difference in ADG, ADFI and gain/feed between the synbiotics and antibiotics treatments. However, the DM and nitrogen digestibility in the BS group was significantly higher (p<0.05) than in the CS, MS and CS treatments. In addition, the ether extract and crude fiber digestibility were not affected by dietary treatments.

Fecal gas emission
Ammonia gas emission was significantly lower (p<0.05) in the BS and YS treatments than in the US treatment (Table 3). Ammonia gas emission was also significantly decreased (p<0.05) in the BS group compared to other treatments, but did not differ among the US, YS,
Hydrogen sulfide gas emission was significantly lower (p<0.05) in the BS, YS and MS groups than in the US and CS treatments, but did not differ among the BS, YS and MS groups.

Fecal acetate concentration was higher (p<0.05) in the MS and CS treatments than in the US group, and fecal acetate concentration in the BS and YS groups did not differ significantly compared to the US, MS and CS treatments.

Fecal propionate concentration was lower (p<0.05) in the CS group than in the US group, and its concentration in the BS, YS and MS groups did not differ compared to the US and CS groups. Fecal valerate concentration was lower (p<0.05) in the YS treatment than in the BS and MS treatment. The butyrate concentration was not affected by the dietary treatments.

Fecal microflora population

Effects of synbiotics supplementation on fecal bacteria counts are shown in Table 4. Symbiotic type did not affect the counts of total pathogenic bacteria. The fecal Escherichia coli and Salmonella counts were also not affected by dietary treatments. The US treatment had higher (p<0.05) Shigella count than other treatments. In addition, MS and CS treatments presented lower (p<0.05) Shigella count than BS and YS treatments. Salmonella was not detected in any of the experimental treatments.

Anaerobic bacteria population was greater (p<0.05) in the US treatment than in the other treatments but did not differ among the US, BS, MS and CS groups.

Fecal volatile fatty acid concentrations (μmol/N)

Acetate 73.98b 79.83ab 81.90ab 83.38ª 82.51ª 4.99
Propionate 48.03ª 45.93ab 43.85ab 41.95ab 41.13b 3.93
Butyrate 19.68 23.10 22.38 25.13 21.90 4.67
Valerate 7.13ª 7.95ª 6.38ª 7.80ª 7.33ª 0.76

1 Mean of 30 pigs individually housed in pens.
2 i) US = basal diet+0.15% antibiotics, ii) BS = basal diet+0.2% synbiotics (probiotics from bacteria), iii) YS = basal diet+0.2% synbiotics (probiotics from yeast), iv) MS = basal diet+0.2% synbiotics (probiotics from mold), v) CS = basal diet+0.2% synbiotics (from compounds of bacteria, yeast and mold). Same prebiotics (mannan oligosaccharide, lactose, sodium acetate and ammonium citrate) was used for all the synbiotics.
3 Standard error of the mean.

a, b, c Values in the same row with different superscripts differ at p<0.05.
DISCUSSION

Either probiotics or prebiotics have been reported to have beneficial effects on pigs. Kim et al. (2001) suggested that supplementation of probiotics improved ADG and feed efficiency in finishing pigs. Moreover, Pollman et al. (1980) reported that dietary administration of probiotics had no influence on growth performance of growing pigs while a positive effect was obtained in nursery pigs. They suggested that the positive effects of probiotics tended to be higher in early-weaning pigs than in growing pigs. For the symbiotics, Smith and Jones (1963) reported that supplemental symbiotics changed intestinal bacteria colonies, increased the production of lactate and antibody and decreased harmful bacteria growth in animals. Collington et al. (1988) also reported increased enzyme activity and enzymatic reaction due to symbiotics administration. Min et al. (1992) suggested that supplemental symbiotics improved growth performance and feed efficiency in weaning pigs. In addition, supplemental symbiotics from Lactobacillus sp. decreased diarrhea, as well as increased performance and feed efficiency in initial weaning pigs (Pollman, 1986).

Antibiotics have been used in swine diets since the 1950s to improve productivity, prevent disease, provide medical treatment and promote growth performance (Hays, 1977). Supplemental antibiotics improve growth performance and feed efficiency by decreasing enteropathogenic bacteria (Kim and Kim, 1992). In the current study, the growth performance in symbiotics treatments was similar to antibiotics treatment, which indicated that symbiotics performed a positive effect on pigs and such effect was comparable with antibiotics (Witte, 2000).

Generally, pigs show evidence of decreased feed intake and growth at weaning. The dietary factors (such as digestibility, structure, composition, taste and flavor) are largely different from those of sow milk (Le-Dividich and Herpin, 1994). Therefore, the digestive tract of weanling pigs must make adaptations for acidic control, enzyme secretion, motility and absorption (Hansen et al., 1993). Under this situation, the digestive enzymes change rapidly between 2 and 8 weeks of age in pigs.

Many researchers have shown that supplemental probiotics increase protein availability and decrease nitrogen excretion (Han et al., 1984; Noh et al., 1995). Results from the present experiment indicated that supplemental symbiotics increased DM and CP digestibility in early-weaning pigs. Symbiotics are also considered to decrease harmful bacteria counts and aid the adhesion of beneficial bacteria through the decrease of intestinal pH (Underdahl et al., 1982), as well as increasing feed palatability and nutrient digestibility (Jurgens et al., 1997) through the increased production of beneficial enzymes (Collington et al., 1988). In some other reports, supplemental probiotics increased crude ash or P digestibility in the intestinal tract, which may also indicate that these substances have a positive influence on nutrient digestibility.

In the past, the effects of supplemental probiotics were mainly directed toward the improvement of swine production. Recently, the effects of supplemental probiotics have been used to address environmental concerns because noxious gas emissions and odors decrease swine production, increase diseases, and result in problems with civil petitions (Ra et al., 2004) reported that supplemental symbiotics with ficus-indica var. saboten could reduce ammonia and sulfide gas emissions of finishing pigs. Santoso et al. (1999) reported that supplemental Bacillus subtilis improved broiler production and decreased ammonia gas emissions due to reduced fecal

**Table 4.** The effects of dietary symbiotics from anaerobic microflora on fecal pathogenic counts and fecal microbial population in weanling pigs

<table>
<thead>
<tr>
<th>Items</th>
<th>Treatments</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecal pathogenic counts (cfu)</td>
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</tr>
<tr>
<td>Total bacteria (×10⁶)</td>
<td>61.33</td>
<td>50.33</td>
</tr>
<tr>
<td>Escherichia coli, (×10³)</td>
<td>6.00</td>
<td>12.00</td>
</tr>
<tr>
<td>Salmonella (×10⁴)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Shigella (×10⁴)</td>
<td>22.00</td>
<td>14.67</td>
</tr>
<tr>
<td>Fecal microbial population</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteria (cfu, ×10⁵)</td>
<td>0.33</td>
<td>24.00</td>
</tr>
<tr>
<td>Yeast (cfu, ×10⁶)</td>
<td>40.00</td>
<td>23.33</td>
</tr>
<tr>
<td>Mold (tfu, ×10⁵)</td>
<td>23.00</td>
<td>18.67</td>
</tr>
</tbody>
</table>

1 Mean of 30 pigs individually housed in pens.
2 i) US = basal diet+0.15% antibiotics, ii) BS = basal diet+0.2% symbiotics (probiotics from bacterial), iii) YS = basal diet+0.2% symbiotics (probiotics from yeast), iv) MS = basal diet+0.2% symbiotics (probiotics from mold), v) CS = basal diet+0.2% symbiotics (from compounds of bacteria, yeast and mold). Same prebiotics (mannan oligosaccharide, lactose, sodium acetate and ammonium citrate) was used for all the symbiotics.
3 Standard error of the mean.
4 Colony forming units.
5 Thallus forming units.
6 Not detected.

a-c Values in the same row with different superscripts differ at p<0.05.
nitrogen excretion. Supplemental synbiotics also can decrease the emission of noxious gases such as ammonia, sulfides, amines, indoles and phenol (Hill et al., 1970). Visek (1978) reported that supplemental synbiotics reduced noxious gas emissions by decreasing harmful intestinal bacteria populations caused by increased urease secretion. The present experiment showed that ammonia gas emission was decreased in the BS group compared to the US group and that sulfide gas emission also decreased in the BS, YS and MS groups compared to the US group. Therefore, we considered that supplemental probiotics from anaerobic microflora with prebiotics can decrease odor in the swine industry.

The main effects of probiotics include decreasing enteropathogenic bacteria by changing the intestinal bacteria colony (Hill et al., 1970) and protecting bacteria colony production in the digestive intestinal wall (Muralidhara et al., 1977). Schierack et al. (2004) also reported that supplemental probiotics from Enterococcus faecium decreased Escherichia coli in growing pigs by more than 50%. Huang et al. (2004) reported that supplemental probiotics decreased counts of Escherichia coli and aerobic bacteria, and increased counts of Lactobacillus sp. and anaerobic bacteria in the gastrointestinal tract.

The present experiment showed that supplementation of synbiotics resulted in similar fecal pathogenic counts compared to treatment with antibiotics. In addition, the Shigella counts were much less than on the antibiotics-added treatment.

In conclusion, supplemental probiotics from anaerobic microflora with prebiotics did not affect performance. However, supplemental probiotics from anaerobic microflora with prebiotics increased DM and CP digestibility as well as decreasing noxious gas emission and enteropathogenic bacteria in early-weaning pigs. Supplemental synbiotics can be expected to improve swine production by improving the feeding environment of early-weaning pigs.

ACKNOWLEDGMENT

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REFERENCES


