



Dietary Supplementation with *Acanthopanax senticosus* Extract Modulates Gut Microflora in Weaned Piglets

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ABSTRACT : This study was conducted to investigate the effects of *Acanthopanax senticosus* extract (ASE) as a dietary additive on gut microflora in weaned piglets. A total of sixty pigs were weaned at 21 d of age (BW = 5.64±0.23 kg) and allocated on the basis of BW and litter to three dietary treatments in a randomized complete block design. The dietary treatments were: control group (basal diet), antibiotics group (basal diet+0.02% colistin), and ASE group (basal diet+0.1% ASE). On d 7, 14 and 28 after consuming the experimental diets, five piglets per group were sacrificed and then the contents from the jejunum, ileum and cecum were collected to determine changes in the microbial community by using a polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) technique and estimating the contents of *Lactobacillus* and *E. coli* by *in vitro* culturing methods. The results showed that the ASE promoted the microflora diversity in the cecum. Enumeration of bacteria in the gut contents showed that the number of *Lactobacillus* increased ($p<0.05$), while that of *E. coli* decreased ($p<0.05$) when compared with the other 2 groups as the days of age progressed post-weaning. These findings suggested that the ASE, as a substitute for dietary antimicrobial products, could improve the development of the normal gut microflora and suppress bacterial pathogens, and effectively promote a healthy intestinal environment. (**Key Words :** *Acanthopanax senticosus* Extract, Dietary Additive, Weaned Piglet, Gut Microflora, PCR-DGGE)

INTRODUCTION

Natural weaning of piglets is a gradual process and occurs over several weeks or months. However, in modern

intensive pork production systems, piglets are weaned early, between 15 and 28 d postnatal, to maximize the whole herd production (King et al., 1999; McGlone and Pond, 2003; Deng et al., 2007; Yang et al., 2007a,b; Yao et al., 2008). Early-weanling pigs always suffer the challenges from many transitions, one of which is the transition from liquid to solid diets. The sudden changes interrupted the supply of immunological important factors from sow's milk (Wu et al., 2004; Wang et al., 2008), reduced feed intake and efficiency (Wu et al., 1996), increased nutrient malabsorption in small intestine, weaning diarrhea syndrome (Nabuurs, 1995; Frydendahl, 2002), and impaired the immune functions (Touchette et al., 2002; Hou et al., 2008). Therefore, early weaning increased the susceptibility of piglets to the gram-negative bacterial infection (e.g., *E. coli* infection) and disease incidence (e.g., diarrhea).

It is well established that the enhancement of weanling pig immunity is very important to prevent or attenuate the occurrence of disease derived from the continual exposure of weanling piglets to a wide variety of microorganisms (Blecha and Charley, 1990; Han et al., 2007). Antimicrobial have traditionally been the first priority for the prevention

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Table 1. Ingredient and chemical composition of the experimental diets (as-fed)

Item	Treatment		
	ASE ^a group	<i>Colistin</i> group	Control group
Ingredient composition (%)			
Corn	66.55	66.55	66.55
Soybean meal	24.00	24.00	24.00
Fish meal	6.00	6.00	6.00
Acidifier ^b	1.00	1.00	1.00
Corn starch	0.10	0.18	0.20
Calcium dihydrogen phosphate	0.60	0.60	0.60
Calcium carbonate	0.74	0.74	0.74
Vitamin premix ^c	0.04	0.04	0.04
Choline chloride (50%)	0.08	0.08	0.08
Mineral premix ^d	0.15	0.15	0.15
Salt	0.25	0.25	0.25
Flavor	0.05	0.05	0.05
L-lysine·HCl	0.25	0.25	0.25
DL-methionine	0.06	0.06	0.06
L-threonine	0.03	0.03	0.03
ASE	0.10	0	0
<i>Colistin</i>	0	0.02	0
Main nutrient composition			
Dry matter (%) ^e	90.04	89.92	90.18
Crude protein (%) ^e	20.32	19.55	20.26
Total calcium (%) ^e	1.05	0.72	0.87
Total phosphorus (%) ^e	0.57	0.54	0.53
Metabolic energy (MJ/kg) ^e	13.79	13.78	13.82

^a ASE = *Acanthopanax senticosus* extract.

^b Commercially obtained from Guangzhou Tianke Industry Co., Guangzhou, Guangdong, China.

^c Vitamin premix containing (per kg): 2,000,000 IU vitamin A, 400,000 IU vitamin D₃, 3,000 mg vitamin E, 300 mg vitamin K, 700 mg vitamin B₂, 200 mg vitamin B₆, 3 mg vitamin B₁₂, 8 mg biotin, 800 mg folic acid, 2,400 mg nicotinic acid.

^d Mineral premix containing (per kg): 165 mg Zn (ZnSO₄), 165 mg Fe (FeSO₄), 33 mg Mn (MnSO₄), 16.5 mg Cu (CuSO₄), 297 µg CaI₂ and 297 µg Se (Na₂SeO₃).

^e Analyzed.

and treatment of diseases induced by the weaning stress. Although antimicrobial agents have been used extensively on domestic animal as growth promoters for more than fifty years to improve animal health and production, the use of sub-therapeutic level of antimicrobial agents in the animal diets potentially impacted human health due to the veterinary drug residues and emergence of anti-antimicrobial strains of zoonotic microorganisms in food animals, which decreased the therapeutic effectiveness of antimicrobials of treating a variety of bacterial infections in humans (Peng and Bang, 2006; Huang et al., 2007). The ban of antibiotics has encouraged the research for alternative products. Therefore, novel antimicrobial alternatives with low residues and low resistance are nowadays being encouraged and under investigation in many countries. Among the new antimicrobial alternatives, herbal medicines and their extracts are some of the most

promising candidates. They are widely prescribed, marketed and used to improve general health of human for a long history in China (Bent et al., 2003). They not only are natural and low residual, but also possess the traits of obvious growth promotion and enhancement of cellular and humoral immunity in piglets (Kong et al., 2007a, b, c).

Many previous research showed that *acanthopanax senticosus* (AS) and its extract were promising and safe candidates in antimicrobial and anti-stress studies (Han et al., 2003; Lee et al., 2003; Wang et al., 2003; Yoon et al., 2004; Kong et al., 2007c). However, few concerned the effect of *acanthopanax senticosus* extract (ASE) on gut microflora in weaned piglets. The objectives of the present study were to investigate the effects of the ASE as a dietary additive on the gut microflora in weaned piglets in order to probe into the possibility of which in growth-promoting and diarrhea controlling mechanisms through the technique of denaturing gradient gel electrophoresis (DGGE) of polymerase chain reaction (PCR)-amplified 16S rRNA gene fragments and *in vitro* culturing methods.

MATERIALS AND METHODS

Preparation of ASE and its ingredients

ASE was prepared as previously described (Kong et al., 2007c). Briefly, 200 g dried and crushed roots and stems of AS were decocted in 1 L of boiling distilled water for 2 h. The decoction products were filtered, lyophilized and kept at 4°C. The yield of extraction was about 25% (w/w). Contents of total polysaccharides, flavone and organic acids in the ASE, as determined by vitriol-anthracene ketone (Zhou et al., 2005), rutin (Wang et al., 1996) and alkalimetric titration (Cai et al., 2000) methods were 2.94%, 0.19% and 1.04%, respectively.

Animals, experimental design and diets

Sixty crossed piglets (Landrace×Yorkshire×Duroc) with an average initial BW of 5.64±0.23 kg were used in the 28-d experiment. The pigs were weaned at 21 d of age and allocated on the basis of weight and litter of origin to three dietary treatments in a randomized complete block design (Tang et al., 2005; Fan et al., 2005). The dietary treatments were: control group (basal diet), antibiotics group (basal diet+0.02% *colistin*), and ASE group (basal diet+0.1% ASE). There were twenty pigs (ten barrows and ten gilts) for each treatment, with one pig per pen. Each 0.6×1.2 m pen was equipped with a single-hole feeder and a water nipple to allow *ad libitum* consumption of feed and water. Feed was added to the feeders thrice daily (0800, 1600 and 2400 h). The temperature was kept between 20 and 27°C, relative humidity was maintained from 40 to 70%, and lighting cycle was 12 h per day. This experiment was approved by Animal Care and Use Committee, the Chinese

Academy of Sciences (Li et al., 2008).

All diets were prepared according to NRC (1998) requirements. Vitamins and minerals were supplemented to meet or exceed NRC (1998) standards for pigs ranging in BW from 5 to 10 kg. Ingredient and nutrient composition for each diet was presented in Table 1.

Sample collection and preparation

On d 7, 14 and 28 after consumed the experimental diets, five piglets per treatment group were sacrificed under anesthesia with intraperitoneal sodium pentobarbital. Content samples were collected from the middle part of jejunum (24 cm in length), a 40-cm ileum segment beginning at a point 20 cm from the distal end, and the entire cecum (Yin et al., 2001). All gut samples were kept on ice and processed immediately after dissection. Parts of the content from the jejunum, ileum or cecum were collected and placed in separated sterile tube. Contents collection and bacterial sample preparation were performed as described previously (Apajalahti and Bedford, 1998). The remainder of fresh intestinal content samples was diluted with sterile phosphate-buffered saline (PBS, pH 7.3) for conventional culturing techniques.

Extraction and purification of DNA from gut contents

Extraction and purification of DNA from gut contents were carried out as previously described (Gong et al., 2002a; Gong et al., 2002b). All of the contents from the five pigs per group were combined as one sample, kept on ice, and processed further within 2 h. After three washes in saline containing 0.1% Tween 80 with vigorous hand shaking for 5 min per wash, bacterial cells were released from the contents, and then pelleted by centrifugation (27,000×g for 20 min) at 4°C. The total DNA from the contents was isolated and purified by QIAamp DNA Stool Kit (stool protocol; Qiagen, Hilden, Germany). Content in TE buffer was thawed on ice, centrifuged and resuspended in 60 µl of PBS. The samples were treated with buffer ALT, proteinase K, RNase A and buffer AL according to the manufacturer's instructions. The DNA was precipitated with ethanol, purified on a QIAamp spin column and eluted in 50 µl of AE buffer (10 mM Tris-HCl, 0.5 mM EDTA, pH 9.0).

PCR amplification

PCR-DGGE analysis of total bacteria was made as previously described (Nübel et al., 1996; Zetendal et al., 1998). Each DNA sample was standardized to 20 µg/ml and then amplified using primers specific for conserved sequences flanking the variable V6-V8 region of 16S rDNA. Part of 16S rRNA genes will then be amplified by PCR reaction using a pair of universal PCR primers to bacteria, followed by DGGE analysis. The DGGE profiles of

microflora from the piglets with different treatments will be compared and analysed. 16S rRNA genes were amplified by PCR from genomic DNA of content bacteria using universal bacterial primers HAD1-GC (5'-CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG GAC TCC TAC GGG AGG CAG CAG T-3') and HAD2 (5'-GTA TTA CCG CGG CTG CTG GCA C-3'). PCR reaction mixtures were the same as described previously (Walter et al., 2000). The amplification program was 93°C for 2 min and 30 cycles of 93°C for 30 s, 57°C for 30 s, and 72°C for 30 s, followed by 2 min at 72°C.

DGGE analysis

Parallel DGGE was performed essentially as described previously (Muyzer and Smalla, 1998; Simpson et al., 1999) using a Bio-Rad D-Code System™ (Bio-Rad, CA, USA). PCR fragments were separated using 8% polyacrylamide gels with 1.0×TAE buffer (20 mM Tris-acetate, pH 7.4, 10 mmol/L sodium acetate, 0.5 mmol/L Na₂EDTA) with 35-60% linear gradients of denaturant (100% denaturant corresponds to 7 mol/L urea and 40% deionized formamide). The polyacrylamide was diluted from a nondeionized 40% acrylamide/bis stock solution 37.5: 1 (Bio-Rad, CA, USA). Gradients were formed using a Bio-Rad Gradient Former™ Model 385 and gels were polymerized onto a gel support film (FMC, ME, USA). PCR samples were applied to gels in aliquots of 5 µl per lane. Electrophoresis was performed at 60°C for 16 h at 100 V. Additionally, bacterial reference ladders representing known bacterial strains were loaded to allow standardization of band migration and gel curvature among different gels (Simpson, 2000). The reference ladder comprised the following species, listed in order from the top of the gel to the bottom: *Staphylococcus aureus*, *Lactobacillus salivarius*, *Ruminococcus forques*, *Bacillus subtilis*, *E. coli* O157:H7, *Clostridium perfringens*, *Salmonella typhimurium*, and *Clostridium lituseburens*. After electrophoresis, the gels were silver-stained and scanned using a GS-710 calibrated imaging densitometer (Bio-Rad). Each individual amplicon was then visualized as a distinct band representing at least one bacterial species on the gel.

DGGE image analysis

DGGE images were analyzed using Quantity One v. 4.5.2 software (Bio-Rad, CA, USA). The software was configured to automatically detect bands on gels. Automatic band detection criteria were identical on all lanes for each gel. When gel imperfections and features were automatically detected as bands by the software, these false bands were manually removed and not included in subsequent numerical analyses. Anomalous staining

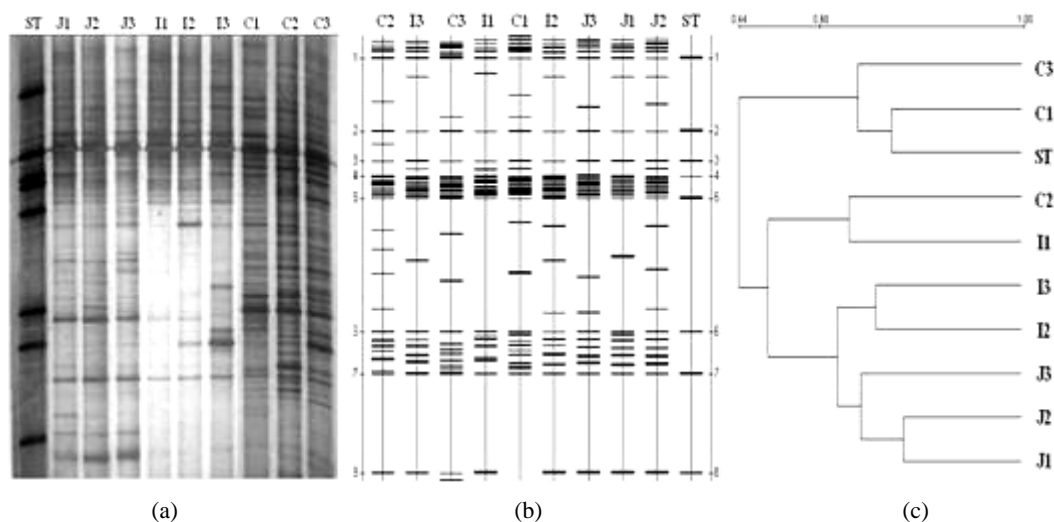


Figure 1. DGGE profiles, sketch map and similarity indices illustrating the bacterial diversity of jejunal (J), ileal (I) and cecal (C) in pigs on d 7 after consumed the experimental diets. 1, 2 and 3 represented *acanthopanax senticosus* extract group, *colistin* group and control group, respectively. a, b and c represented DGGE profiles, sketch map of DGGE profiles and similarity index of DGGE profiles, respectively. ST represented the standard strains, which listed in order from the top of the gel to the bottom were *Staphylococcus aureus*, *Lactobacillus salivarius*, *Ruminococcus forques*, *Bacillus subtilis*, *E. coli* O157: H7, *Clostridium perfringens*, *Salmonella typhimurium* and *Clostridium lituseburens*, respectively.

residues (spotting and peppering) were removed from digital images of gels as necessary. DGGE profiles were also compared using Sorenson's index, a pairwise similarity coefficient C_s , which was determined by: $C_s = (2j/(a+b)) \times 100$, where a is the number of DGGE bands in lane 1, b is the number of DGGE bands in lane 2, and j is the number of common DGGE bands (Gillan et al., 1998; Simpson et al., 2000; Grandlic et al., 2006). Dendrograms were constructed using the unweighted pair group method with arithmetic mean (Ward's).

Dietary additive intervention on the growth of *E. coli* and *Lactobacillus*

Surface plate counting technique will be used for enumeration of viable *lactobacilli* and *E. coli* by pour-plating on agar plate. A 0.5 g aliquot of each content from the jejunum, ileum and cecum will be aseptically serially diluted in 0.1% peptone water and plated on appropriate agar media, respectively, using three plates for each dilution. *Lactobacilli* was estimated by counting viable bacteria based on counting of colonies growing on lactic acid bacteria agar (Parentel et al., 2001), incubated anaerobic at 37°C for 48 h. *E. coli* was enumerated by pour-plating on Eosin-Methylene blue agar medium, incubated at 37°C for 24 h. Colony forming units on plates containing between 30 and 300 colonies were counted. Data were expressed by colony forming units per gram content (CFU/g).

Chemical analysis

Dry matter, crude protein, total calcium and total

phosphorus contents of diets were analyzed according to AOAC (2003) procedures. Gross energy in feed, urines and feces were measured with an adiabatic bomb calorimeter (modelWZR-1, Changsha Bente Instrument Co., China).

Statistical analysis

Data were analyzed using one-way ANOVA (SAS Inst. Inc., Cary, NC, 2002). The Duncan's multiple comparison tests was used to determine differences among means of treatment groups. A probability value <0.05 was taken to indicate statistical significance.

RESULTS

Bacterial communities in contents of jejunal, ileal and cecal of pigs fed the experimental diets

DGGE analysis targeted to the predominant bacterial population showed considerable intra-individual diversity as well as uniqueness of gut contents' microbiota under different dietary additive intervention. The DGGE profile, sketch map and similarity indices of bacterial communities from contents of jejunal, ileal and cecal in pigs fed the experimental diets on d 7, 14 and 28 were presented in Figure 1, 2 and 3, respectively. The results indicated that Jejunal and ileal microflora DGGE bands of the ASE group were less than the other 2 groups, and cecal microflora DGGE bands were more than that of the control group (27 vs. 26), but less than that of the *colistin* group (27 vs. 30). The jejunal similarity between the ASE and *colistin* groups was high (C_s value >76.3%), which could be grouped into

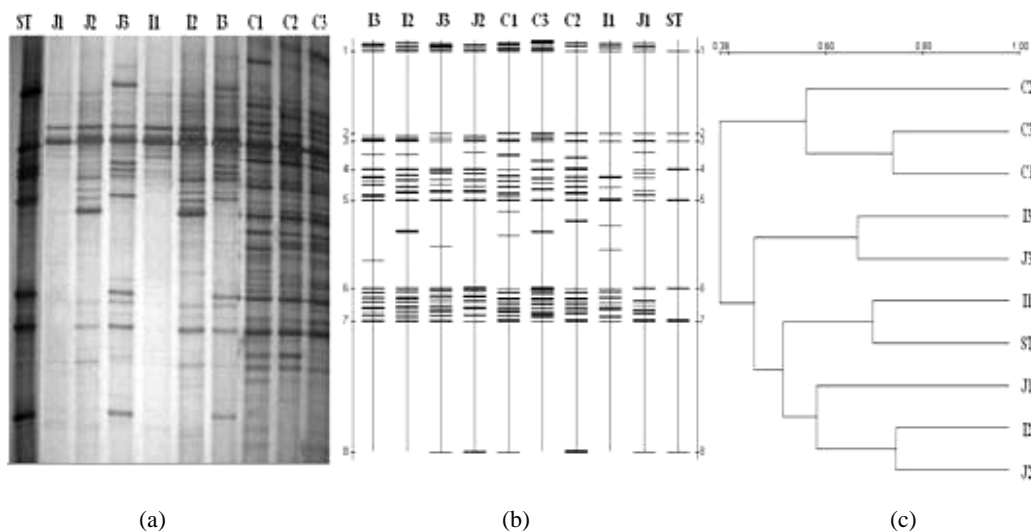


Figure 2. DGGE profiles, sketch map and similarity indices illustrating the bacterial diversity of jejunal (J), ileal (I) and cecal (C) in pigs on d 14 after consumed the experimental diets. 1, 2 and 3 represented *acanthopanax senticosus* extract group, *colistin* group and control group, respectively. a, b and c represented DGGE profiles, sketch map of DGGE profiles and similarity index of DGGE profiles, respectively. ST represented the standard strains, which listed in order from the top of the gel to the bottom were *Staphylococcus aureus*, *Lactobacillus salivarius*, *Ruminococcus forques*, *Bacillus subtilis*, *E. coli* O157: H7, *Clostridium perfringens*, *Salmonella typhimurium* and *Clostridium lituseburens*, respectively.

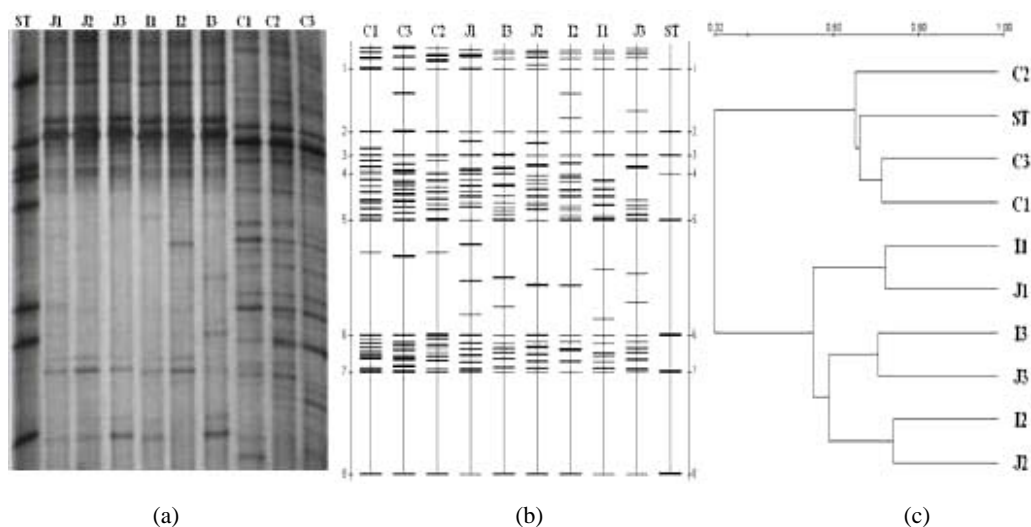


Figure 3. DGGE profiles, sketch map and similarity indices illustrating the bacterial diversity of jejunal (J), ileal (I) and cecal (C) in pigs on d 28 after consumed the experimental diets. 1, 2 and 3 represented *acanthopanax senticosus* extract group, *colistin* group and control group, respectively. a, b and c represented DGGE profiles, sketch map of DGGE profiles and similarity index of DGGE profiles, respectively. ST represented the standard strains, which listed in order from the top of the gel to the bottom were *Staphylococcus aureus*, *Lactobacillus salivarius*, *Ruminococcus forques*, *Bacillus subtilis*, *E. coli* O157: H7, *Clostridium perfringens*, *Salmonella typhimurium* and *Clostridium lituseburens*, respectively.

to one cluster. The ileal similarities among the 3 groups were not very high (C_s value $>57.3\%$) on d 7 (Figure 1). Jejunal and ileal microflora DGGE bands of the ASE group were less than the other 2 groups, and cecal microflora DGGE bands were more than those of *colistin* (26 vs. 31) and control groups (26 vs. 23). The similarity of jejunal microflora among the 3 groups were also high (C_s value $>$

67.3%), the same as ileal (C_s value $>65.6\%$) and cecal (C_s value $>56.9\%$) on d 14 (Figure 2). Jejunal microflora DGGE bands of the ASE group were gradually increased, and ileal microflora DGGE bands were still less than those of *colistin* (22 vs. 32) and control groups (22 vs. 25), but cecal microflora DGGE bands were more than those of *colistin* (30 vs. 24) and control groups (30 vs. 27),

Table 2. Culture counts of in intestinal contents of weaned-piglets (LgCFU/g)

	Bacteria species	Intestinal section	ASE ¹ group	Colistin group	Control group	Pooled SEM ²
D 7	<i>E. coli</i>	Jejunum	5.22 ^a	4.24 ^b	5.37 ^a	0.067
		Ileum	5.21 ^b	4.88 ^c	6.12 ^a	0.076
		Cecum	5.87 ^a	4.52 ^b	6.02 ^a	0.188
	<i>Lactobacilli</i>	Jejunum	6.49 ^a	5.86 ^c	6.08 ^b	0.063
		Ileum	6.98 ^a	6.58 ^b	6.81 ^a	0.049
		Cecum	6.52 ^a	6.45 ^a	6.25 ^b	0.045
D 14	<i>E. coli</i>	Jejunum	5.34 ^c	5.73 ^b	5.97 ^a	0.081
		Ileum	5.79 ^c	6.17 ^b	6.43 ^a	0.233
		Cecum	5.78 ^b	6.17 ^a	6.11 ^a	0.045
	<i>Lactobacilli</i>	Jejunum	7.29 ^a	7.00 ^b	6.83 ^b	0.210
		Ileum	7.86	7.57	7.52	0.049
		Cecum	7.66 ^a	7.70 ^a	7.55 ^b	0.045
D 28	<i>E. coli</i>	Jejunum	5.67 ^b	5.82 ^a	5.92 ^a	0.072
		Ileum	5.55 ^c	6.08 ^b	6.27 ^a	0.045
		Cecum	5.80 ^b	5.68 ^c	6.10 ^a	0.072
	<i>Lactobacilli</i>	Jejunum	6.88	6.82	6.85	0.036
		Ileum	7.26 ^a	6.78 ^b	6.80 ^b	0.045
		Cecum	7.29 ^a	7.34 ^b	7.34 ^b	0.027

^{a, b, c} Values within a row with different letters differ ($p < 0.05$), $n = 5$.

¹ ASE = *Acanthopanax senticosus* extract. ² SEM = Standard error of the mean.

respectively. The similarities of the 3 intestinal sections' microflora among the 3 groups were also high (Cs value >56%) on d 28.

The influence of dietary additive intervention on the growth of *E. coli* and *Lactobacillus* in vitro

The effects of dietary additive intervention on the growth of *E. coli* and *Lactobacillus* were presented in Table 2. Results showed that the number of *E. coli* decreased, while of *lactobacillus* increased in the ASE group relative to other 2 groups on d 14 and 28 after initiation of the experiment. To be detailed, on d 7, counts of *E. coli* in ileal contents of the ASE group were lower ($p < 0.05$) than that of the control group, while higher ($p < 0.05$) than that of the colistin group. Counts of *lactobacillus* in jejunum and cecum of the ASE group were higher ($p < 0.05$) compared with the control group, and of *lactobacillus* in all of the 3 intestinal section's contents were increased ($p < 0.05$) relative to the colistin group. On d 14, counts of *E. coli* in all 3 intestinal contents of the ASE group were lower ($p < 0.05$) than those of the other 2 groups, the same as those on d 28. Counts of *lactobacillus* increased ($p < 0.05$) in jejunum contents of the ASE group when compared with the other 2 groups. On d 28, Counts of *lactobacillus* in ileum and cecum of ASE group were also ($p < 0.05$) higher than those of the other 2 groups.

DISCUSSION

After birth, the piglets' gastrointestinal tract microflora developed rapidly and characteristic microbial populations include *E. coli*, *lactobacillus*, *streptococcus*, *clostridium*, *bifidobacterium* and so on (Simpson et al., 1999). After

weaning, the piglet's gut is colonized by a diverse and unstable community, which may induce to diarrhea in poor environmental condition. The pig gastrointestinal tract microflora impacts growth efficiency and disease susceptibility through some mechanisms, which may be increase of *lactobacillus* and *bifidobacterium*, and decrease of *E. coli* in the intestine or apparent selection for *lactobacillus*, commensals known to competitively exclude potentially pathogenic species from colonizing the intestine (Zhu et al., 2000).

The bacterial diversity and main flora of gastrointestinal tract under different diets have been extensively studied with traditional cultivation methods, but the information from these studies is limited because a majority of naturally occurring bacterial population cannot be cultured and isolated (Suau et al., 1999; Li et al., 2006). The use of PCR-DGGE technique could greatly help microbial ecologists in surveying the differences or changes of complex microbial communities within environmental area (Muyzer et al., 1998, Deng et al., 2007), and then this technique was successfully applied to molecular analysis of the animal and human gastrointestinal tract bacterial community, which is not only highly complex but also difficult to characterize due to its abundant and varied populations (Zcetendal et al., 1998; Deng et al., 2007). Not only can the differences be assayed between the animals on different diets, but also among different gut compartments and specific locations within these gut compartments. The ability to detect these differences will allow investigations to more closely monitor the effects of treatments or regimens on the gut microflora (Simpson et al., 1999; Zhu et al., 2000). However, DGGE profiles and analysis of similarity in bands present difficultly the quantitative data. Therefore, the gut

microflora and their development of viable *Lactobacillus* and *E. coli* influenced by the ASE in the diets were studied by PCR-DGGE technique and culturing methods, respectively.

AS is a typical Chinese herb. Its roots are an important Chinese folk medicine for the treatment of a variety of human diseases, such as isochemic heart diseases, hypertension, rheumatism, allergies and diabetes and tumor, etc. AS is also known to be effective for reducing many kinds of stress or fatigue. In the present study, dietary ASE gradually reduced the microbial population of *E. coli*, and also gradually increased the amounts of *Lactobacillus* in jejunum, ileum and cecum contents compared with those in antibiotic-free treatment as the experiment proceeded. However, dietary *colistin* not only inhibited the proliferation of *E. coli*, but also that of *Lactobacillus* in jejunum and ileum contents except in cecum contents on d 7 and 14. The DGGE profiles and similarity indices of piglet's gut microorganism showed that the ASE had obvious impacts on gut microflora during the period of supplementation, especially reduced the diversity of pathogenic bacterium, and the impacts were enhanced with the proceeding of supplementation. A possible explanation for the anti-pathogenic bacterium function of ASE could be attributed to its biomedical active molecules, such as polysaccharides, flavone and organic acids. Previous researches showed that compounds isolated from AS had various biological activities and could profoundly modulate the body function. For example, polysaccharide isolated from AS activated B cells and macrophages by interacting with TLRs and leading to the subsequent activation of mitogen-activated protein kinases and NF- κ B (Hana et al., 2003), and possessed good inhibition against irradiation-induced injury of rat (Li and Zhou, 2007; Yin et al., 2008). Yi et al. (2002) reported that extracts from AS stem inhibited mast cell-mediated anaphylaxis *in vivo* and *in vitro* murine model. Our previous study has demonstrated that ASE profoundly enhanced the cellular and humoral immune responses of weaned piglets by modulating the production of immunocytes, cytokines and antibodies (Kong et al., 2007).

In summary, our study suggested that the ASE, as a promising alternative of antimicrobial, could effectively promote a healthy intestinal environment postweaning and then reduce the incidence of diarrhea in the weaned piglets. Our findings also support the concept that antimicrobial substitutes might ideally promote the growth of beneficial commensal bacteria, while suppressing those that are deleterious.

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