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# Dietary supplementation with *Acanthopanax senticosus* extract enhances gut health in weanling piglets

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ABSTRACT

The present study was conducted to investigate the effects of *Acanthopanax senticosus* extract (ASE) as a dietary additive on gut health in weanling piglets by examining diarrhea frequency, intestinal microbiota and morphology. A total of 96 Duroc× (Landrace × Yorkshire) piglets weaned at 21 days of age with an average initial body weight (BW) of 5.6 ± 0.4 kg were randomly assigned to 3 treatment groups with 4 duplicates of 8 piglets each. The piglets were fed basal diet to which had been added 0 or 1 g/kg of ASE, or 0.7 g/kg antibiotics, respectively. Fecal consistence was monitored twice daily and the frequency of diarrhea was calculated. On day 21 after the initiation of supplementation, 8 piglets were randomly selected from each treatment group (2 piglets per pen) and slaughtered. The jejunum, ileum, colon and cecum were then excised and fixed in 10% neutral formalin solution to determine villus height and crypt depth, after their contents were collected to determine microbiota. The results showed that dietary supplementation with ASE increased ($P<0.05$) the density of bacterial populations that co-migrated with *Lactobacillus amylovorus*, *Lactobacillus salivarius*, *Bacillus subtilis*, and *Clostridium lituseburens*, but decreased ($P<0.05$) those co-migrating with *Staphylococcus aureus*, *Salmonella typhimurium*, *Ruminococcus forques*, and *E. coli* O157:H7 in the PCR-DGGE profiling analysis when compared with the control group. The villus height of the duodenum, jejunum and ileum increased ($P<0.05$) by 14.8, 13.7 and 10.0%, while the crypt depth decreased ($P<0.05$) by 17.9, 9.1 and 12.1%, respectively, in response to dietary ASE supplementation. Additionally dietary supplementation with ASE or an antibiotic decreased ($P<0.05$) the frequency of diarrhea by 55.6 and 52.2%, respectively, compared with the control group. In conclusion, these findings suggest that dietary supplementation with ASE could regulate the microbiota composition and maintain a normal morphology of gut mucosa in weanling piglets, thereby decreasing diarrhea that resulted from weaning stress.



## 1. Introduction

In modern intensive swine-production systems, piglets are weaned between 14 to 28 days of age to maximize whole-herd production. Early weaning is commercially advantageous but is generally considered to be a stressful event and is often associated with a period of depressed feed intake and growth

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performance (Odle et al., 1996) as well as an increased incidence of diarrhea and disease (Frydendahl, 2002), overgrowth of pathogenic bacteria, villus atrophy and mortality. Weaning with widespread diarrhea could lead to considerable economic loss, and this could be counteracted by nutritional manipulation. Antibiotics have been traditionally used to prevent and treat enteric diseases (including diarrhea) induced by weaning stress. However, the continuous use and misuse of antibiotics have led to the emergence of drug- and antibiotic-residues in animal products (Monroe and Polk, 2000). Therefore, novel feed additives are needed to replace antibiotics. As potential alternatives to antimicrobial agents, traditional Chinese herbal medicines or their extracts may impair microbial growth in early-weaned piglets (Kong et al., 2007a, 2008).

*Acanthopanax senticosus* (AS), a tonic and sedative Chinese herb that contains glycoside, polyose, chromocor (Yi et al., 2002), organic acids and amino acids (AA) is well known to be highly effective in treating various diseases, including stress-induced pathophysiologic changes (Fujikawa et al., 1996) and inflammation (Yamazaki et al., 2007). Recent studies have shown that AS plays a role in enhancing immunity, antibiosis, antioxidation (Yang et al., 2004) and in reducing many kinds of stress (Gaffney et al., 2001) and fatigue (Dowling et al., 1996). We previously found that AS extract (ASE) as a dietary additive modified the cellular and humoral immune responses of weaned piglets by modulating the production of immunocytes, cytokines and antibodies (Kong et al., 2007b), and also enhanced digestion and the absorption of dietary AA in weaned piglets (Kong et al., accepted for publication). Although the various biological functions of AS have received extensive attention, there are few reports on the application of AS or ASE for preventing weaning stress in piglets, especially for preventing diarrhea and its underlying mechanisms.

The main function of the gastrointestinal system is to assimilate nutrients from the external environment into the animal's internal environment, where they are used for tissue growth and repair and for energy production (Xu et al., 1992). The lumen of the large intestine contains billions of microorganisms. These microorganisms use the food residues that are discharged from the small intestine and convert them into useful nutrients, such as short-chain fatty acids and vitamins, which can then be absorbed in the large intestine and be used by the animal (Hooper et al., 2002). Invasion of the gut by pathogens leads to epithelial cell damage. Thus, the gut may help to defend against this damage by increasing the rate of epithelial renewal (Gaskins, 1997) and affecting the villus/crypt architecture (Pluske et al., 1997). Therefore, we hypothesized that dietary supplementation with ASE may enhance gut health in weanling piglets. This hypothesis was tested by determining the frequency of diarrhea, intestinal microbiota and morphology in weanling piglets on day 21 after the initiation of dietary supplementation with ASE.

## 2. Materials and methods

### 2.1. Preparation of ASE

ASE was prepared by decocting the dried herb in boiling distilled water (200 g/L) for 2 h. The AS decoction was filtered, lyophilized and kept at 4 °C. The yield of extraction was about 25% (w/w). The water-extracted powder was dissolved in sterile saline (1 g/ml). The contents (g/kg) of total polysaccharides, flavone and organic acids in the ASE were 29.4, 1.9 and 10.4, as determined by the vitriol-anthracene ketone, rutin (Kong et al., 2004) and alkalimetric titration (Cai et al., 2000) methods, respectively. The contents (g/kg) of AA in the extract, as analyzed by high-pressure liquid chromatography (HPLC, Hitachi L-8800 Auto-Analyzer, Tokyo, Japan) method were: Phe 4.11; Leu 2.32; Ile 0.67; Val 0.77; Ala 2.14; Gly 1.87; Asp 2.86; Glu 4.71; Cys 2.45; His 0.41; Lys 0.95; Arg 3.78; Thr 1.30; Ser 2.47 and Met 0.28.

### 2.2. Animal housing and treatment

A total of 96 Duroc×(Landrace×Yorkshire) piglets weaned at 21 days of age with an average initial body weight (BW) of 5.6 ± 0.4 kg were randomly assigned into one of 3 groups based on litter, BW and sex. There were 4 pens per treatment group, with 8 piglets (4 barrows and 4 gilts) per pen. The 3 treatment groups received a maize–soybean-based diet [based on National Research Council (NRC, 1998) requirements] that had been supxplemented with 0 or 1 g/kg of ASE, or 0.7 g/kg antibiotics (10% bacitracin zinc 400 mg + 15% carbadox 300 mg) (Table 1). The basal diet did not contain antibiotics or growth-promoting levels of Cu and Zn. The piglets were housed in an environmentally-controlled nursery facility (temperature, 20–27 °C; relative humidity, 60–70%; lighting cycle, 12 h/d) with a hard-plastic completely slatted flooring, and had free access to food and drinking water. The study was carried out in accordance with the Chinese guidelines for animal welfare and experimental protocols.

**Table 1**
Dietary ingredients and main nutrient levels (g/kg, as-fed basis).

| Dietary ingredients | Control | *Acanthopanax senticosus* extract | Antibiotics |
|---|---|---|---|
| Corn (CP 7.84%) | 572.0 | 571.1 | 571.3 |
| Soybean meal (CP 43%) | 180.0 | 180.0 | 180.0 |
| Fermn soybean meal (CP 51.5%) | 68.0 | 68.0 | 68.0 |
| Whey powder | 50.0 | 50.0 | 50.0 |
| Soybean oil | 30.0 | 30.0 | 30.0 |
| Fish meal (CP 63.5%) | 60.0 | 60.0 | 60.0 |
| Zeolit meal | 9.0 | 9.0 | 9.0 |
| *Acanthopanax senticosus* extract | 0 | 1.0 | 0 |
| Antibiotics | 0 | 0 | 0.7 |
| Calcium phosphate dibasic | 12.0 | 12.0 | 12.0 |
| $CaCO_3$ | 6.0 | 6.0 | 6.0 |
| Premix [a] | 12.5 | 12.5 | 12.5 |
| Main nutrient levels | | | |
| Digestible energy (MJ/kg) | 14.30 | 14.30 | 14.30 |
| Crude protein | 199.6 | 199.4 | 198.6 |
| Calcium | 7.8 | 7.8 | 7.8 |
| Available phosphorus | 5.8 | 5.8 | 5.8 |
| Lysine | 13.6 | 13.6 | 13.6 |
| Methionine | 4.3 | 4.3 | 4.3 |
| Threonine | 9.0 | 9.0 | 9.0 |

[a] The premix provides the following for kilogram of diets: $VD_3$ 386 IU; VA 3086 IU; VE 15.4 IU; $VK_3$ 2.3 mg; $VB_2$ 3.9 mg; D-calcium pantothenate 15.4 mg; nicotinic acid 23 mg; choline 500 mg; $VB_{12}$ 0.016 mg; Cu (Gly-Cu, 21%) 17 mg; Fe (Gly-Fe 14%) 133 mg; Zn (Met-Zn 17.5%) 133 mg; Mn (Gly-Mn 22%) 33.3 mg; I ($Ca(IO_3)_2$) 0.83 mg; choline chloride (50%) 1000 mg; antimildew/acidifying agent (propanoic acid) 2.5 g; antioxidant (ethyoxyquin) 200 mg; edulcorant (crystallose) 400 mg; flavor 600 mg; salt 1.3 g; lysine·HCl 2.7 g; methionine 660 mg; threonine 440 mg. All of the feedstuffs were provide by Hunan Guang'an Biology Technology Co. Ltd., Changsha, Hunan, China.