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Dietary Supplementation with *Acanthopanax senticosus* Extract Modulates Cellular and Humoral Immunity in Weaned Piglets

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ABSTRACT : This study was conducted to test the hypothesis that dietary supplementation with an herbal extract of *Acanthopanax senticosus* (AS) enhances the immune response in weaned piglets. Sixty piglets weaned at 21 days of age were randomly assigned to 3 treatment groups representing the addition of 0 or 1 g/kg of the AS extract or 0.2 g/kg of colistin (an antibiotic) to maize- and soybean meal-based diets (n = 20 per group). On days 7, 14 and 28 after initiation of the addition, total and differential counts of leucocytes, proliferating activity of peripheral lymphocytes, serum levels of immunoglobulins (Ig) and cytokines and the spleen index were determined. The AS extract decreased ($p<0.05$) the number of neutrophils on days 7 and 28 in comparison with the control group and reduced ($p<0.05$) serum interleukin-1β level on day 28 compared with the other 2 groups. Dietary supplementation with the AS extract increased ($p<0.05$) the lymphocyte/leukocyte ratio on day 28 compared with the control group and increased the proliferating activity of lymphocytes on days 14 and 28 compared with the other 2 groups. The AS extract increased ($p<0.05$) the serum content of IgG on day 7 and of IgG and IgM on day 28 compared with the other 2 groups, as well as increasing the serum content of tumor necrosis factor on day 7 and spleen index on days 7 and 28 compared with the control group. Collectively, these findings suggest that the AS extract as a dietary additive enhances the cellular and humoral immune responses of weaned piglets by modulating the production of immunocytes, cytokines and antibodies. (**Key Words :** Dietary Additive, *Acanthopanax Senticosus* Extract, Immunity, Weaned Piglets)

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 days of age to maximize the whole herd production (King et al., 1999; McGlone and Pond, 2003; Tang et al., 2005; Kong et al., 2007a). Because of the abrupt changes in feed composition and feeding conditions, early weaning interrupts the supply of immunologically important factors (e.g., glutamine and arginine) from sow's milk (Wu et al., 2004; Kong et al., 2007a,b), reduces feed intake and efficiency (Wu et al., 1996), impairs the production of antibodies, and compromises cellular immune functions (Touchette et al., 2002). Thus, early weaning increases the susceptibility of piglets to the gram-negative bacterial infection (e.g., *E. coli* infection) and disease incidence (e.g., diarrhea). Antibiotics have traditionally been suggested the main means for the prevention and treatment of diseases induced by the early-weaning stress. However, this therapy results in side effects, including reduced therapeutic effectiveness of antibiotics in treating a variety of bacterial infections in humans (Smith, 1999), which has prompted several countries to ban the use of dietary antibiotics for livestock. Recently, alternatives to antibiotics are being encouraged and are under active investigation. Imunomodulatory phytochemicals may offer

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alternatives to antimicrobial growth-promoters for early-weaned piglets.

Acanthopanax senticosus (AS), a tonic and sedative Chinese herb, is well known to be highly effective in treating various diseases. They include chronic bronchitis, hypertension, ischemia (Yi et al., 2001), allergies, rheumatism, diabetes (Park et al., 2000; Kang et al., 2001; Yi et al., 2002), stress-induced pathophysiologic changes (Nishibe et al., 1990; Fujikawa et al., 1996) and inflammation (Jung et al., 2003). The Chinese herbs are complex mixtures, consisting of up to hundreds or even thousands of different constituents, but only a few compounds are primarily responsible for the pharmaceutical and immunological effects (Huang et al., 2004). AS compounds include acanthosides, eleutherosides, triterpenic saponin, polysaccharide, flavone, chiisanoside, senticoside, sylrgin, organic acids, amino acids, vitamins and minerals (Yi et al., 2002; Yang et al., 2004). Saponin is an important class of natural products widely present in the plant kingdom, and most of them possess a variety of biological and pharmacological activities (Wina et al., 2005). Saponin may be responsible for the biological activities of AS (Han et al., 2003). Interestingly, the current literature indicates either a stimulatory (Schmolz et al., 2001) or an inhibitory (Yi et al., 2001) effect of AS on immune responses. Some evidence suggests that diterpenoids and phenolic substances are biologically active ingredients in *Acanthopanax* species (Nishibe et al., 1990).

On the basis of the foregoing, we hypothesized that dietary supplementation with AS extract enhances the immune response in piglets weaned at 21 day of age. This hypothesis was tested by determining total and differential counts of leucocytes, lymphocyte proliferating activity, serum levels of immunoglobulins (Ig) and cytokines, and the spleen index on days 7, 14 and 28 after initiation of the treatment.

MATERIAL AND METHODS

Preparation of the AS extract

The extract of AS was prepared by decocting the dried herb in boiling distilled water (200 g/L) for 2 h. The decoction products were filtered, lyophilized and kept at 4°C. The yield of extraction was about 25% (w/w). The water-extract powder was dissolved in sterile saline (1 g/ml). Contents of total polysaccharides, flavone and organic acids in the AS extract, as determined by vitriol-anthracene ketone (Zhou et al., 2005), rutin (Wang et al., 1996) and alkalimetrictitration (Cai et al., 2000) methods were 2.94%, 0.19% and 1.04%, respectively. Concentrations (%) of amino acids in the extract, as analyzed using an HPLC method (Yin et al., 1993; Li et al., 2003) were: Phe 0.411;

Table 1. The dietary formulation and main nutrient levels

Dietary ingredients (g/kg)	1 g/kg AS extract	0.2 g/kg colistin	Control
Corn (CP 8.24%)	665.5	665.5	665.5
Soybean meal (CP 44.72%)	240.0	240.0	240.0
Fish meal (CP 65.05 %)	60.0	60.0	60.0
Corn starch	1.0	1.8	2.0
$Ca(H_2PO_4)_2$	6.0	6.0	6.0
$CaCO_3$	7.4	7.4	7.4
Vitamin premix[a]	0.4	0.4	0.4
Choline chloride (50%)	0.8	0.8	0.8
Trace element premix[b]	1.5	1.5	1.5
Salt	2.5	2.5	2.5
Flavor (Saccharin sodium)	0.5	0.5	0.5
Acidifier[c]	10.0	10.0	10.0
Lysine·HCl	2.5	2.5	2.5
Methionine	0.6	0.6	0.6
Threonine	0.3	0.3	0.3
Acanthopanax senticosus (AS) extract	1.0	0	0
Colistin	0	0.2	0
Main nutrient levels			
Metabolic energy (MJ/kg)	165.2	164.9	164.0
Crude protein	199.3	195.5	202.6
Calcium	8.7	8.7	8.7
Phosphorus	5.1	5.4	5.3

[a] Vitamin premix containing (per kg): 2,000,000 IU vitamin A, 400,000 IU vitamin D_3, 3,000 mg vitamin E, 300 mg vitamin K, 700 mg vitamin B_2, 200 mg vitamin B_6, 3 mg vitamin B_{12}, 8 mg biotin, 800 mg folic acid, 2,400 mg nicotinic acid.

[b] Trace element premix containing (per kg): 165 mg Zn ($ZnSO_4$), 165 mg Fe ($FeSO_4$), 33 mg Mn ($MnSO_4$), 16.5 mg Cu ($CuSO_4$), 297 μg CaI_2, and Se (Na_2SeO_3).

[c] Production of Guangzhou Tanke Industry Co. Ltd., composed of citric acid, calcium oxalate, and etc.

Table 2. Effects of *acanthopanax senticosus* (AS) extract on total and differential counts of leucocytes in weaned piglets

	1 g/kg AS extract	0.2 g/kg colistin	Control
Day 7 after the addition			
Leucocyte number (10^9/L)	16.00±6.73	15.45±2.08	14.55±3.50
Lymphocyte number (10^9/L)	8.30±2.78	7.18±1.32	9.58±2.43
Neutrophil number (10^9/L)	0.60^{b}±0.28	0.78^{ab}±0.13	0.93^{a}±0.13
Monocyte number (10^9/L)	4.20±2.67	5.25±1.34	4.48±1.25
N/(L+M)-ratio (%)	5.31±0.58	4.96±0.60	5.02±0.35
Day 14 after the addition			
Leucocyte number (10^9/L)	16.94±8.11	17.03±4.78	21.38±3.73
Lymphocyte number (10^9/L)	9.08±5.08	12.32±5.26	12.38±3.26
Neutrophil number (10^9/L)	1.20±0.35	1.05±0.17	0.96±0.19
Monocyte number (10^9/L)	4.68±1.00	6.14±2.39	7.95±2.85
N/(L+M)-ratio (%)	5.58±1.46	5.30±0.57	6.19±0.98
Day 28 after the addition			
Leucocyte number (10^9/L)	14.00±6.09	15.94±2.93	17.35±2.19
Lymphocyte number (10^9/L)	7.70±1.85	10.64±3.13	9.53±1.57
Neutrophil number (10^9/L)	0.54^{b}±0.19	0.58^{ab}±0.10	0.80^{a}±0.14
Monocyte number (10^9/L)	3.20^{b}±0.48	3.60^{b}±0.80	9.08^{a}±3.32
N/(L+M)-ratio (%)	3.83^{b}±0.65	4.51^{ab}±0.45	5.05^{a}±0.41

[a-b] Means±SEM with different superscript letters within a row are different ($p<0.05$).

Leu 0.232; Ile 0.067; Val 0.077; Ala 0.214; Gly 0.187; Asp 0.286; Glu 0.471; Cys 0.245; His 0.041; Lys 0.095; Arg 0.378; Thr 0.130; Ser 0.247 and Met 0.028. On the basis of results from our previous study, the decocted AS extract was serially diluted with RPMI-1640 (GIBCO) into 6 concentrations: 125.0, 62.5, 31.3, 15.6, 7.8 and 0 mg/ml before use. The diluted preparations were filtered through a 0.22 μm filter and the resultant solution was stored at 4°C until use for determining its direct effect on *in vitro* lymphocyte proliferating activity.

Animal, housing and treatment

Sixty 21-day-old Duroc×Landrace×Yorkshire piglets with an average body weight (BW) of 5.64 kg were weaned and then randomly assigned on the basis of litter, BW and sex to 3 treatment groups (male:female = 1:1) representing supplementation with 0 or 1 g/kg of the AS extract, or 0.2 g/kg of colistin to a maize- and soybean meal-based diet formulated based on National research Council (NRC, 1998) requirements (Table 1). Chemical analysis of the experimental diets was conducted as described by Yin et al. (1993) and Huang et al.(2005). There were 20 piglets per treatment group. Colistin (an antibiotic) was used as a positive control. The piglets were housed individually in an environmentally-controlled nursery facility with hard plastic and slatted flooring, and had free access to diets and drinking water (Yin et al., 2001a,b,c; ; Li et al., 2007a; Li et al., 2007b; Yang et al., 2007). The experiment was carried out in accordance with the Chinese guidelines for animal welfare and experimental protocol (Yin et al.,2002; Fan et al., 2005; Tang et al., 2005; Deng et al.,2007a; Huang et al., 2007).

Experimental design and analyses

Sampling and preparations : On days 7, 14 and 28 after initiation of the addition, five piglets were sampled randomly from each group. Blood sample (15 ml per piglet) was collected by venepuncture from the jugular vein between 8:00 and 10:00 a.m. after the piglet was weighed. One milliliter of the sample was drawn in K_2EDTA-coated tubes for determination of total and differential counts of leucocytes; five milliliters were transferred immediately into aseptic capped tubes with sodium heparin for analysis of the AS extract effects on lymphocyte proliferation; the remainder was drawn in plastic uncoated normal tubes, kept at room temperature and processed for sera within 2 h. The sera were obtained by centrifugation at 3,000 r/min and 4°C for 20 min and stored at -20°C until analysis of IgG, IgM, interleukin (IL)-1β, IL-2 and tumor necrosis factor (TNF). On day 21, five piglets were sampled randomly from the control group for analysis of direct effects of the AS extract on *in vitro* lymphocyte proliferating activity.

Determination of total and differential counts of leucocytes : Total and differential numbers of leucocytes were determined using an Auto- hemocytometer (BT-2100, Dyn, USA) within 2 h after sampling according to the manufacturer's instructions. The N/(L+M)-ratio was calculated as the number of neutrophils divided by the number of lymphocytes+monocytes, and used as an indicator of stress (Gross and Siegel, 1983).

Analysis of lymphocyte proliferation : Blood peripheral lymphocytes were prepared from control, AS-supplemented and colistin-supplemented piglets, using Percoll gradient as described by Kong et al. (2004). The cells were diluted to 5×10^6/ml with RPMI 1640 media supplemented with

Table 3. Effects of *acanthopanax senticosus* (AS) extract on lymphocyte proliferating activity of weaned piglets *in vivo*

	1 g/kg AS extract	0.2 g/kg colistin	Control
Day 7 after the addition			
None ConA	0.56[a]±0.03	0.50[b]±0.02	0.50[b]±0.03
+ConA	0.62±0.03	0.62±0.01	0.62±0.01
Day 14 after the addition			
None ConA	0.60[a]±0.03	0.54[b]±0.03	0.55[b]±0.04
+ConA	0.74[a]±0.02	0.64[c]±0.01	0.70[b]±0.02
Day 28 after the addition			
None ConA	0.63[a]±0.04	0.58[b]±0.03	0.56[b]±0.02
+ConA	0.80[a]±0.03	0.71[b]±0.03	0.71[b]±0.04

[a-c] Means±SEM with different superscript letters within a row are different ($p<0.05$).

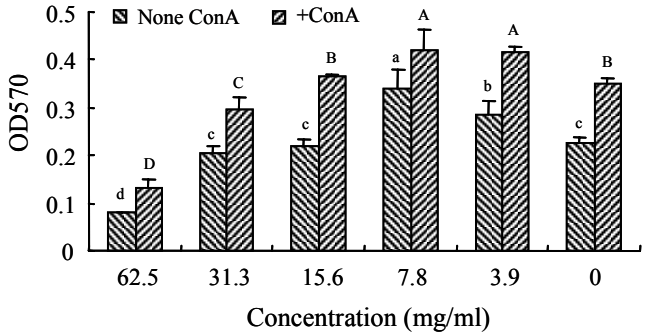

Figure 1. Direct effects of *acanthopanax senticosus* (AS) extract on *in vitro* lymphocyte proliferating activity of weaned piglets. At each concentration of the AS extract, lymphocyte proliferating activity was higher ($p<0.05$) in the presence of ConA than in its absence. [a-d] Means±SEM with different letters are different ($p<0.05$) for lymphocytes cultured in the absence of ConA. [A-D] Means±SEM with different letters are different ($p<0.05$) for lymphocytes cultured in the presence of ConA.

benzylpenicillin 100 IU/L, streptomycin 100 IU/L and 10% fetal bovine serum (growth media). For analysis of proliferation, 90 μl of cell suspension and 10 μl of Concanavalin (ConA, 80 μg/ml; a specific T-cell mitogen) were added to a well in a 96-well tissue culture plate. The assays were performed in quadruplet. After a 44 h incubation at 37°C in a 5% CO_2 incubator, 20 μl 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 5 mg/ml, Amresco Inc.) were added into each well, followed by a 4-h incubation and then addition of 100 μl of Dimethyl Sulfoxide. The solutions were mixed for 5 min to completely dissolve the precipitation. Light absorbance was measured at 570 nm with MQX200 Enzyme-linked Immunosorbent Assay Reader (Bio-Tek instruments, Inc., USA).

Effect of different AS concentrations on lymphocyte proliferation : Peripheral blood lymphocytes were prepared from unsupplemented piglets as previously described (Kong et al., 2004). Cell proliferation was determined as described above, except that adding another 100 μl of the culture medium contained 0, 3.9, 7.8, 15.6, 31.3 or 62.5 mg/ml AS extract into each well.

Analysis of serum immunoglobulins and cytokines : Serum IgG and IgM were analyzed using CX-4 Auto-blood biochemical Analyzer (Beckman Inc., America) according to the reagent kit manufacturer's instructions (immunoturbidimetry; Beijing Leadman Biochemistry Technology Co. Ltd., Beijing, China). Serum IL-1β, IL-2 and TNF were determined using radioimmunoassay kits (Beijing Chemclin Biotech Co., Ltd.; Deng et al., 2007b; Huang et al., 2007; Li et al., 2007).

Determination of spleen index : After blood samples were collected, piglets were sacrificed by intravenous injection of 4% sodium pentobarbital solution (40 mg/kg BW). The spleen was sampled and its weight was determined. The spleen index was calculated as the spleen weight divided by the BW of the same piglet and used as an indicator of immune function.

Statistical analysis

Data were expressed as mean±SEM. Results were statistically analyzed using ANOVA and the GLM procedure of the Statistical Analysis System (SAS Institute, Cary, NC). Duncan's multiple range test was used to compare differences among the three treatment groups. A *P*-value of less than 0.05 was taken to indicate statistical significance.

RESULTS

Effects of AS extract on total and differential counts of leucocytes

Total and differential counts of blood leukocytes in piglets are summarized in Table 2. Dietary supplementation with the AS extract decreased ($p<0.05$) the neutrophils number on days 7 and 28 after the initiation of the addition, when compared with the control group. On days 7 and 14, the numbers of total leukocytes did not differ ($p>0.05$) between the control and colistin-supplemented groups. On day 28, the colistin treatment decreased ($p<0.05$) the number of monocytes and had no effect ($p>0.05$) on the numbers of lymphocytes, neutrophils, or total leukocytes in comparison with the control group.

Table 4. Effects of *acanthopanax senticosus* (AS) extract on contents of serum cytokines in weaned piglets

	1 g/kg AS extract	0.2 g/kg Colistin	Control
Day 7 after the addition			
Interleukin-1β (ng/ml)	0.11±0.04	0.10±0.01	0.08±0.02
Interleukin-2 (ng/ml)	1.96^{ab}±0.19	2.63^{a}±0.62	1.78^{b}±0.49
Tumor necrosis factor (ng/ml)	0.62^{a}±0.08	0.53^{a}±0.09	0.35^{b}±0.14
Day 14 after the addition			
Interleukin-1β (ng/ml)	0.21±0.07	0.24±0.03	0.23±0.02
Interleukin-2 (ng/ml)	4.26±1.39	4.98±0.65	4.46±0.55
Tumor necrosis factor (ng/ml)	0.51^{ab}±0.17	0.49^{b}±0.06	0.69^{a}±0.04
Day 28 after the addition			
Interleukin-1β (ng/ml)	0.11^{c}±0.04	0.20^{b}±0.02	0.26^{a}±0.03
Interleukin-2 (ng/ml)	4.52±1.31	3.61±0.76	3.24±0.86
Tumor necrosis factor (ng/ml)	0.48±0.05	0.52±0.11	0.47±0.06

$^{a-c}$ Means±SEM with different superscript letters within a row are different ($p<0.05$).

Table 5. Effects of *acanthopanax senticosus* (AS) extract on contents of serum immunoglobulin (Ig) and spleen index in weaned piglets

	1 g/kg AS extract	0.2 g/kg colistin	Control
Day 7 after the addition			
IgG (mg/dl)	468.42±25.5^{a}	401.00±56.79^{b}	381.84±50.09^{b}
IgM (mg/dl)	16.72±4.84	9.19±2.45	19.35±5.20
Spleen index	0.22±0.03^{a}	0.14±0.02^{b}	0.17±0.04^{b}
Day 14 after the addition			
IgG (mg/dl)	295.03±27.00	316.38±12.53	325.15±27.50
IgM (mg/dl)	18.22±0.38^{ab}	13.70±5.16^{b}	18.83±2.58^{a}
Spleen index	0.21±0.02	0.23±0.03	0.19±0.03
Day 28 after the addition			
IgG (mg/dl)	342.73±42.93^{a}	275.10±17.66^{b}	288.79±24.51^{b}
IgM (mg/dl)	26.15±4.99^{a}	18.15±3.35^{b}	21.15±7.35^{b}
Spleen index	0.25±0.05^{a}	0.24±0.02^{a}	0.17±0.02^{b}

$^{a-b}$ Means±SEM with different letters are different ($p<0.05$) among three treatment groups within each age group.

Effects of dietary supplementation with the AS extract on lymphocyte proliferation

Table 3 summarizes data on proliferation of lymphocytes from the control, AS- supplemented and colistin-supplemented pigs. On days 7, 14 and 28 after the initiation of the addition, lymphocyte proliferation in the absence of ConA was higher ($p<0.05$) in piglets fed the AS extract-supplemented diet in comparison with the other 2 groups of pigs. Similar results were obtained for lymphocyte proliferation in the presence of ConA on days 14 and 28.

Effects of addition of the AS extract to culture medium on lymphocyte proliferation

At each concentration of AS extract, lymphocyte proliferating activity was higher ($p<0.05$) in the presence of ConA than in its absence (Figure 1). Regardless of addition of ConA to culture medium, lymphocyte proliferating activity was the highest in the presence of 3.9 and 7.8 mg/ml AS extract and was the lowest in the presence of 62.5 mg/ml AS extract, whereas intermediate values were observed in the presence of 0 and 15.6 mg/ml AS extract (Figure 1). Lymphocyte proliferating activity decreased ($p<0.05$) progressively with increasing the concentration of the AS extract from 7.8 to 62.5 mg/ml.

Effects of dietary supplementation with the AS extract on serum concentrations of immunoglobulins

Effects of the AS extract on serum contents of IgG and IgM in weaned piglets are illustrated in Figure 1. On days 7 and 28 after initiation of the addition, serum IgG contents were higher ($p<0.05$) in piglets fed the AS extract-supplemented diet when compared with the other 2 groups of pigs. The AS extract also increased ($p<0.05$) serum concentration of IgM on day 28 compared with the other 2 groups.

Effects of dietary supplementation with the AS extract on serum concentrations of cytokines

Dietary supplementation with the AS extract for 7 days increased ($p<0.05$) serum concentrations of TNF compared with the control group (Table 4). On day 14, serum levels of IL-1β and IL-2 did not differ ($p>0.05$) among the 3 treatment groups. Four weeks after initiation of the treatment, serum IL-1β content was lower ($p<0.05$) in the AS extract-supplemented piglets compared with the other 2

groups (Table 4).

Effects of AS extract on spleen index

Dietary supplementation with the AS extract increased ($p<0.05$) the spleen index on day 7 after the initiation of the addition when compared with the other 2 groups (Table 5). On day 28, the spleen index in the extract- or colistin-supplemented piglets was higher ($p<0.05$) than that in the control group (Table 5).

DISCUSSION

The abrupt change in feed composition and feeding conditions at weaning of piglet causes a dramatic change in the small-intestinal structure and digestive function, which often results in nutrient malabsorption in the small intestine (Beers-Schreurs et al., 1992; Nabuurs, 1995). The small-intestinal damage affects the metabolic and nutritional states of the neonates. Moreover, an increase in plasma cortisol occurs at the time of weaning (Wu et al., 2000; Herskin and Jensen, 2002) and may affect immune functions (Kusnecov and Rossi-George, 2002). The immune system, specially the acquired immunity, plays an important role in protecting piglets against pathogenic infection. However, the acquired immunity is underdeveloped at the age of 3 to 4 weeks when the piglets are usually weaned on commercial farms (Van et al., 1996). Thus, exposure of pigs to various pathogens results in reduced productivity (Balaji et al., 2000; Greiner et al., 2000). A severe problem is the diagnosis and management of subclinical infections, especially when the pathogens are unknown. Therefore, it is imperative that appropriate measures be taken to enhance the host's ability to resist a wide variety of pathogens (Mallard et al., 1998). The innate immune system is potentially useful in this regard, as it is non-specific and it recognises a large number of different pathogens with a restricted set of receptors (Medzhitov and Janeway, 2000). In this regard, components of innate immunity may serve as predictors of the overall immunity as well as pig health. For this reason, we determined the concentrations of blood leukocytes (including neutrophils, monocytes and lymphocytes), antibodies, and cytokines in controls and AS extract-supplemented piglets.

The neutrophil is an important component of the innate immunity involved in antibacterial defence (Burg et al., 2001). An increase in the number and activity of neutrophils can be indicative of an underlying infection (Dunkley et al., 1995) or stress (McGlone et al., 1983; Kelley et al., 1994). Gross et al. (1983) also reported that the neutrophil to lymphocyte+monocytes ratio could be used as an indicator of stress (Gross and Siegel, 1983). The determination of total and differential counts of leucocytes in weaned piglets indicated that the AS extract decreased the number of neutrophils on days 7 and 28 and the N/(L+M) ratio on day 28 compared with the control group. This result suggests that the AS extract enhances the resistance of piglets to bacterial infection or weaning stress. The AS's effect may involve both a peripheral action on tissue metabolism and a direct action on the brain, because oral administration of the AS affected the levels of dopamine and noradrenaline in the whole brain of rats 30 min after administration (Fujikawa et al., 2002). Changes in the brain levels of dopamine and noradrenaline may be an underlying mechanism for the AS extract to enhance the resistance of piglets to the weaning stress.

Several innate traits, particularly levels of circulating monocytes are indicative of growth performance in Large White pigs (Clapperton et al., 2005). Monocytes act as effectors, phagocytosing pathogens and destroying them by production of free radicals and also by producing cytokines as signaling molecules (Underhill and Ozinsky, 2002). In addition, monocytes produce inflammatory chemokines and cytokines, which increase the proportion of circulating neutrophils and also activate and attract neutrophils to the site of infection. In the present study, the AS extract increased the ratio of monocytes to leukocytes in weaned piglets, suggesting a role for the extract in increasing immune functions and reducing inflammation. The underlying mechanisms are likely multifactorial and need to be elucidated in future studies.

Lymphocytes are important circulating immune cells that play a critical role in maintaining immune functions and regulating inflammation (Sean et al., 2005). Remarkably, the AS extract increased the ratio of lymphocytes to leukocytes in weaned piglets. Lymphocyte proliferation, a critical event of the immune response in the host, was markedly increased in piglets in response to the dietary supplementation with the AS extract. Interestingly, there was a synergistic effect between the extract and ConA. Notably, the AS extract exhibited a concentration-dependent effect on lymphocyte proliferation, in that reduced values were observed at either a low or a high dosage. These findings clearly demonstrate that an appropriate concentration of the AS extract is critical for eliciting a beneficial effect on lymphocyte proliferation and function.

The serum immunoglobulin titer is an indicator of humoral immunity. Our results of higher concentrations of serum IgG and IgM in weaned piglets supplemented with the AS extract indicate its efficacy in enhancing their humoral immunity. The spleen is the important organ for antibody production. The data on the spleen index suggest that the AS extract increased splenocyte proliferation in weaned piglets, thereby sustaining an increase in with the production of immunoglobulins. Our result is in agreement

with the recent report that AS stimulates the proliferation and immunoglobulin production of B cells (Han et al., 2003).

Acute infection can lead to the release of cytokines such as IL-1 and TNF-αthat act to cause anorexia and muscle protein breakdown, thereby reducing productivity (Johnson, 1998). IL-1β, secreted from macrophages, is an inflammatory cytokine, which plays an important role in activating T cells and rejecting tumor cells (Tanigawa et al., 2000). The pro-inflammatory cytokine TNF-α is one of the first inflammatory mediators to appear in response to infection, after which it induces a number of different biological effects in various cells, initiating and regulating the immune response (Bemelmans et al., 1996). A reduced secretion of TNF-α was observed in severely malnourished children, compared to the cytokine secretions in the same patients after nutritional rehabilitation. Thus, a low serum level of TNF-αmay contribute to an increased susceptibility to gram-negative bacterial infections commonly seen in malnourished children (Doherty et al., 1994). An evident observation from our study is that dietary supplementation with the AS extract decreased ($p<0.05$) serum IL-1β level on day 28 and increased ($p<0.05$) serum TNF concentration on day 7 after initiation of the addition in piglets. Collectively, these results indicated an increase in cellular and humoral immunities in the AS extract-supplemented weaned piglets, conferring an important protective role in the non-specific defense against infections (Bogdan Petrunov et al., 2006).

CONCLUSION

Early weaning is a major stress factor that impairs intestinal metabolism and function in swine production and is associated with compromised immunity. Dietary supplementation with low-cost immunomodulatory phytochemicals may enhance immune responses in weaned piglets. The AS extract as dietary additive enhances the cellular and humoral immune responses of weaned piglets by modulating the production of immunocytes, cytokines and antibodies. Because the large numbers of components in the Chinese herbs make their screening and analysis extremely challenging, our findings help identify the water-soluble extract of the AS as a natural green dietary additive for promoting the immune response and healthy growth in weaned piglets.

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