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Effects of *Astragalus* Polysaccharides, *Achyranthes bidentata* Polysaccharides, and *Acantbepanax senticosus* Saponin on the Performance and Immunity in Weaned Pigs*

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ABSTRACT : Two trials were conducted to study the effects of two Chinese herbal polysaccharides, *Astragalus* polysaccharides (APS) and *Achyranthes bidentata* polysaccharides (ABPS), and one Chinese herbal saponin, *Acantbepanax senticosus* saponin (ASS), on the immunity and growth performance of weaned pigs. Experiment 1 was a 14-day growth assay, in which 32 weaned pigs were randomly allocated to one of four dietary treatments: i) 0.05% talcum powder control; ii) 0.05% APS; iii) 0.05% mixture of APS and ASS in a 1:1 ratio by weight; and iv) 0.05% mixture of APS, ASS, and ABPS in a ratio of 1:1:1 by weight. Blood samples were collected on day 14 to determine plasma parameters. Feed intake, body weight gain, and feed efficiency were also determined. Experiment 2 was a 21-day immunity assay, in which 16 weaned pigs were randomly allotted to one of two dietary treatments: i) 0.05% talcum powder control; and ii) 0.05% mixture of APS and ASS in a 1:1 ratio by weight. On day 21, pigs were challenged with lipopolysaccharide (LPS) and 3 h later blood samples were collected and analyzed for lymphocyte proliferation as well as interleukin 6 (IL-6), insulin-like growth factor 1 (IGF-1), growth hormone (GH), and cortisol levels. In Experiment 1, feeding Chinese herbal polysaccharides and saponin increased growth performance of the pigs. The effects of the mixture of APS and ASS were especially notable, as there was a significant improvement in growth performance compared with the control ($p<0.05$). The plasma concentration of immunoglobulin G (IgG), nitric oxide (NO), and nitric oxide synthase (NOS) were increased in all treatments groups, with the mixture of APS and ASS increasing the level of IgG and NOS significantly ($p<0.05$), compared with the control. There was no difference in the NO level between the control and treatment groups ($p>0.05$). In Experiment 2, Chinese herbal polysaccharides and saponin showed immunostimulating effects. The level of cortisol, GH, and IGF-I were significantly increased ($p>0.05$), and the level of IL-6 showed a significant decrease ($p<0.05$) in the APS and ASS treatment after the LPS challenge. The mixture of APS and ASS could stimulate the blood lymphocyte proliferation significantly whether the LPS was injected or not ($p<0.05$). These results show that Chinese herbal extracts can improve growth performance and stimulate immunity of weaned pigs. A mixture of APS and ASS, compared with APS alone, could be a new kind of immunostimulant for weaned pigs, which could result in greater positive effects on their growth performance and immunity. (**Key Words :** *Astragalus* Polysaccharides, *Achyranthes bidentata* Polysaccharides, *Acantbepanax senticosus* Saponin, Growth Performance, Immunity, Weaned Pigs)

INTRODUCTION

Some plant polysaccharides, as complex carbohydrates, were reported as having prebiotic effects (Cummings and Macfarlane, 2002). In China, natural medicinal products, especially polysaccharides, originating from fungi and herbs have been used as feed additives for farm animals for centuries and have shown antimicrobial activities, immune enhancement, and stress reduction (Xue and Meng, 1996; Chen et al., 2006). Polysaccharides derived from *Astragalus membranacea* (Huang Qi), *Lentinus edodes* (Shiitake), and *Tremella fuciformis* (White Jelly), have been used as immune enhancers (Guo et al., 2004).

Saponins found in many plant species are widely-distributed secondary plant metabolites (Figen, 2006). It has been shown that the soluble fraction and effective degradability starch for corn were increased *in situ* by a saponin-based surfactant (Hristov et al., 2007). Others have

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shown that soya-derived saponins increased the uptake of exogenous proteins, such as glycinin, by rabbit intestine cultured *in vitro*, and that lectin and saponins together stimulated this uptake to a greater degree than the sum of their individual effects (Alvarez and Torres-pinedo, 1982; Johnson et al., 1986). However, the combined effects of both polysaccharides and saponin on the performance and immunity of animals have not been well investigated.

Newly weaned piglets are highly susceptible to various stressors, resulting decreased growth and even death. The objective of this study was to investigate the possibility of using *Astragalus* polysaccharides (APS), *Achyranthes bidentata* polysaccharides (ABPS), and *Acantbepanax senticosus* saponin (ASS) as growth and immune stimulators for weaned pigs. Two strategies were used in our experiments. First, we determined the effects of APS, APS and ASS, APS, ASS and ABPS on performance and immunity markers in weaned pigs, and then the best mixture was used to investigate the effects on immunity in weaned pigs during lipopolysaccharides (LPS) stress.

MATERIALS AND METHODS

Animals and diets

Pigs (Large White) were obtained from a closed production system located in southeast of China (Hunan New Wellful Co., Ltd.). Pigs were weighed, allocated to their respective pens and provided *ad libitum* access to feed and water for the duration of the trial. The basal diets contained primarily corn (56.91%) and soybean (15.00%), and were prepared to meet or exceed NRC (1998) nutrient requirement (Table 1).

Table 1. Composition and nutrient content of basal diet[a]

Item	
Ingredients (%)	
Corn	56.91
Soybean meal	15.00
Fish meal	5.00
Dry whey powder	5.00
Soybean protein concentrated	4.73
Nuklospray K53[b]	3.00
Soybean oil	2.62
Glucose	2.00
Spray-dried plasma protein	2.00
Calcium hydrogen phosphate $CaHPO_4$	1.70
Limestone	0.26
Premix[c]	1.00
Acidifier	0.30
Lysine HCl	0.20
Threonine	0.12
DL- methionine	0.11
Total	99.95
Calculated analysis[d]	
Digestible Energy (MJ/kg)	14.65
Crude Protein (%)	19.50
Calcium (%)	0.80
Phosphorus (%)	0.78
Lysine (%)	1.50
Methionine (%)	0.42
Methionine+cystine (%)	0.83
NaCl (%)	0.68

[a] Expressed on air dry weight basis.
[b] Nuklospray, dairy based feed ingredient, Sloten, Netherlands.
[c] Premix provided for one kilogram of complete feed: 18,000 IU vitamin A, 4,000 IU vitamin D_3, 40 IU vitamin E, vitamin K_3 4 mg, vitamin B_1 6 mg, vitamin B_2 12 mg, vitamin B_6 6 mg, vitamin B_{12} 0.05 mg, biotin 0.2 mg, folic acid 2 mg, niacin 50 mg, calcium pantothenate 25 mg, Fe (as ferrous of sulfate) 100 mg, Cu (as copper sulfate) 150 mg, Mn (as manganese sulfate) 40 mg, Zn (as zinc sulfate) 100 mg, I 0.5 mg, Se (as sodium selenite) 0.3 mg.
[d] Calculated analysis based on analysis provided by companies furnishing product and standard feed tables (NRC, 1998).

Polysaccharides and saponins

APS and ASS were purchased from Sanjiang Biological Engineering Co., Ltd. (Xi'an, China). ABPS was purchased from Nantong Sihai Plant Extract Co., Ltd. (Jiangsu, China). The concentration of the heteroglycans in APS and ABPS, and saponins in ASS were 50%, 40%, and 1%, respectively. Of the five components, such as Eleutheroside-A, B, C, D, E, and F, in ASS, the products used in this experiment were B and E.

Experiment 1

A total of 32 barrows (initially 9.29±0.53 kg and 31±3 d of age) were used in a 14-day growth assay. Pigs were randomly assigned into one of four treatments i) A 0.05% talcum powder control; ii) 0.05% APS; iii) A 0.05% mixture of APS and ASS in a 1:1 ratio by weight; and iv) A 0.05% mixture of APS, ASS, and ABPS in a ratio of 1:1:1 by weight. Each treatment had four replicates and each replicate comprised two pigs. Feed consumption was measured and pigs were weighed on d 0, 7, and 14 to calculate ADG, ADFI, and gain: feed ratio (G/F). Blood samples were collected via the precaval vein into 10-ml heparinized vacuum tubes on d 14, and then centrifuged at 3,000 g for 10 min to collect plasma. Plasma was frozen at -20°C until analysis of immunoglobulin G (IgG), nitric oxide (NO), and total nitric oxide synthase (NOS) concentrations was performed. All blood samples were analyzed in duplicate.

Experiment 2

A total of 16 barrows (initially 6.51±0.19 kg and 23±3 d

of age) were used in a 21-day immunity assay. Pigs were randomly assigned into one of two treatments: i) A 0.05% talcum powder control; and ii) A 0.05% mixture of APS and ASS in a 1:1 ratio by weight. Each treatment had four replicates and each replicate two pigs. On d 21, pigs were challenged with lipopolysaccharide (LPS; *E. coli* serotype 055:B5; intraperitoneally injected at 100 mg/kg BW). Blood samples were collected 3 h after the LPS challenge into 10-ml heparinized vacuum tubes and centrifuged at 3,000 g for 10 min to separate plasma, which was stored at -20°C until analysis for interleukin 6 (IL-6), insulin-like growth factor 1 (IGF-1), growth hormone (GH), and cortisol concentrations. A second 10-ml whole-blood sample was taken from the same pig for the lymphocyte proliferation assay. All blood samples were analyzed in duplicate.

Chemical analysis

Plasma concentration of IgG was measured with an immunological turbidity kit (Bejing Sino-UK Institute of Biological Technology, China). Plasma concentration of NO and NOS was measured with the NO Detection Kit and Total Nitric Oxide Synthetase Detection Kit, respectively (Najing Jiancheng Bioengineering Institute, Nanjing, China). The assay sensitivity of the kit for IgG is 1.0 ng/ml.

Plasma concentration of GH, cortisol and IL-6 were measured with a RIA using a commercially available kit (Beijing Sino-UK Institute of Biological Technology, China). The assay sensitivity for GH, cortisol and IL-6 was 0.5 ng/ml, 1 ng/ml, and 50 pg/ml, respectively. Plasma concentration of IGF-1 was determined using a commercially available mouse IGF-1 double antibody radioimmunoassay assay kit (DSL-2900, Diagnostic Systems Laboratories, USA). Minimum detectability of this assay was 21 ng/ml.

Total blood lymphocyte proliferation assay

The total blood lymphocyte proliferation assay was performed as described previously (Flavey and Frankenburg, 1992). Briefly, 4-ml samples of blood were collected, 3 ml of Lymphocyte Separation Medium (Institute of Biomedical Engineering, Beijing, China) was added, the sample was then centrifuged at 2,500 g for 30 min, and lymphocytes were collected from the middle layer. The isolated cells were washed three times with RPMI 1640 cell-culture medium (GIBCO, Invitrogen Corporation, USA), and viable (trypan blue exclusion) cells were counted. All of the cells were cultured in RPMI 1640 cell-culture medium containing 10% fetal calf serum (FCS) (GIBCO BRL, Grand Island, NY, USA), penicillin (100 IU/ml) and streptomycin (100 IU/ml) (Amresco, Solon, OH, USA). The cells were added to 96-well plates, then Con A (Sigma, St. Louis, MO, USA) was added to each well at a final concentration of 16 μg/ml. The 96-well plates (Nunc, Roskilde, Denmark) were incubated for 72 h at 37°C in an atmosphere of air plus 5% CO_2; 6 h before harvesting, MTT (AMRESCO Inc., USA; 5 mg/ml), in a volume of 10 μl, was added to each well. Finally, a solution of 10% sodium dodecyl sulfate and 0.04 M hydrochloric acid (SDS-HCl) was added and cultured for an additional 2 h before optical density (OD) at 570 nm was examined.

Statistical analysis

Data were subjected to analysis of variance using the GLM procedure in the SAS software package (SAS Inst. Inc., Cary, NC, USA). The significance level for all tests was set at $p<0.05$.

RESULTS

Experiment 1

Overall, pigs gained 377±28 g/d during the test period (Table 2), which resulted in pigs averaging 14.242±0.487 kg BW across all treatments by the end of the experiment. Compared with the control, the mixture of APS and ASS treatment increased both the ADFI and ADG significantly ($p<0.05$). The mixture of APS and ASS decreased the ratio of feed to gain, but there was no difference among treatments for the entire 14 d trial. The Chinese herbal extracts increased the concentrations of IgG, NO, and NOS in the plasma, but the mixture of APS and ASS increased the level of IgG and NOS significantly ($p<0.05$), compared with the control groups. There was no difference in NO level between the control group and the treatment groups ($p>0.05$) (Table 3).

Experiment 2

As shown in Table 4, the mixture of APS and ASS could stimulate the blood lymphocyte proliferation significantly whether the LPS jnjected or not ($p<0.05$). Before the injection of LPS, the mixture of ASS and APS increased the concentrations of GH and IGF-I, and decreased the concentrations of cortisol and IL-6, but there was no difference between the two groups ($p>0.05$). Blood lymphocyte proliferation, and cortisol, GH and IGF-1 levels were increased ($p>0.05$), while the level of IL-6 was decreased significantly ($p<0.05$) by the APS-ASS mixture after the LPS challenge (Table 4).

DISCUSSION

In China, natural medicinal products, especially polysaccharides, have been used as feed additives for farm animals for centuries and have been shown to have immunity-enhancing properties (Xue and Meng, 1996; Chen et al., 2006). APS is a large-molecular weight

Table 2. The effects of Chinese herbal polysaccharides and saponin on performance of weaned pigs (Exp. 1)

Item	Control	APS	Mixture of APS and ASS (1:1)	Mixture of APS, ASS, and ABPS (1:1:1)	SEM
			Mixture A	Mixture B	
ADFI (kg/d)					
0-7 d	511	539	570	534	19
7-14 d	551	551	618	573	27
0-14 d	531^{b}	545^{ab}	594^{a}	553^{ab}	17
ADG (kg/d)					
0-7 d	358^{b}	416^{ab}	444^{a}	352^{b}	23
7-14 d	353	297	392	404	39
0-14 d	356^{b}	360^{ab}	418^{a}	374^{ab}	16
Feed/gain					
0-7 d	1.43^{a}	1.30^{b}	1.28^{b}	1.52^{a}	0.05
7-14 d	1.56^{b}	1.85^{a}	1.58^{b}	1.42^{b}	0.08
0-14 d	1.49	1.51	1.42	1.48	0.06

A total of 32 piglets were used, 2 pigs/pen and four replicate pens/per treatment. Values are presented as means; SEM means standard error of the mean. Means in a row with different letters differ significantly ($p<0.05$).

Table 3. The effects of Chinese herbal polysaccharides and saponin on the immune index of the plasma of weaned piglets (Exp. 1)

Item	Control	APS	Mixture of APS and ASS (1:1)	Mixture of APS, ASS, and ABPS (1:1:1)	SEM
IgG (g/L)	6.72^{b}	7.24^{ab}	8.04^{a}	7.52^{ab}	0.22
NO (μmol/L)	50.32	55.77	57.79	55.04	7
NOS (U/ml)	28.78^{b}	30.10^{b}	35.14^{a}	33.26^{ab}	2

A total of 32 piglets were used, 2 pigs/pen and four replicate pens/per treatment. Values are presented as means; SEM means standard error of the mean. Means in a row with different letters differ significantly ($p<0.05$).

polysaccharide that has been intensively studied in human medicine and utilized in animal production, such as broilers (Chen et al., 2003). *Achyranthes* (ACH) is also suggested to have the potential to be used as a feed additive to improve broilers' immunity (Chen et al., 2003). Because the molecular weight of ACH was much smaller than that of APS, it was more easily absorbed by the intestinal enterocytes and utilized by the immune system (Tian and Feng, 1994). Guo et al. (2008) reported that ABPS altered the release of pro-inflammatory cytokines following an immunological challenge, which could help pigs achieve better performance. It was in agreement with our experiments that ABPS, combined with APS and ASS, increased pig performance, but there was no difference between the mixture of APS and ASS group and the mixture of APS, ASS, and ABPS group. It seems that ABPS do not enhance the effective of APS and ASS.

Saponins found in many plant species are widely-distributed secondary plant metabolites (Figen, 2006). Previous studies have shown that saponins could improve the digestibility and absorptivity in some animals, especially for ruminants (Mader and Brumm, 1987). The combined effects of both polysaccharides and saponin on the performance and immunity of animals, however, have

Table 4. Effects of a mixture of APS and ASS on immune index of the plasma after a LPS challenge in weaned pigs (Exp. 2)

Item	-LPS			+LPS		
	Control	Mixture of APS and ASS (1:1)	SEM	Control	Mixture of APS and ASS (1:1)	SEM
Blood lymphocyte proliferation	0.46^{a}	0.502^{b}	0.02	0.51^{a}	0.56^{b}	0.015
Cortisol (ng/ml)	168.81	159.86	12	269.34	288.44	12
IL-6 (pg/ml)	207.44	200.51	14	236.50^{a}	178.60^{b}	18
Growth factor (ng/ml)	4.96	4.74	0.25	5.59	5.93	0.22
IGF-1 (ng/ml)	242.623	267.853	17	193.073	209.350	17

A total of 16 piglets were used, 2 pigs/pen and four replicate pens/per treatment. Values are presented as means; SEM means standard error of the mean. Means in a row with different letters differ significantly ($p<0.05$).
Lipopolysaccharide was intraperitoneally injected on day 21, blood was collected at 0 and 3 h after LPS injection.

not been well investigated. Therefore, we hypothesized that the mixture of polysaccharides and saponins would have better effects on pig performance. Our findings suggested that both the mixture of APS and ASS, and the mixture of APS, ASS, and ABPS increase the pig performance, but there was no difference between them.

NO is a highly reactive molecule with innate immune functions, and as a multifunctional mediator in immune defenses, it has been reported to have protective functions against a number of viruses, many intracellular bacteria and parasites, and has been implicated in the control of cancers (Karupiah et al., 1993; Xie et al., 1996; Bogdan, 1997; Fang, 1997; MacMicking et al., 1997). NO can be produced by the nitric oxide synthase (NOS), which is a family and include neuronal NOS (nNOS), immunologic NOS (iNOS), and endothelial NOS (eNOS). nNOS and eNOS are both constitutively expressed enzymes regulated by the availability of intracellular Ca (Wallace et al., 1998). But the expression of iNOS is triggerred by inflammatory cytokines. The action of NO occurs through activity of the inducible isoform of NOS (iNOS). The relatively large concentrations of NO produced by iNOS (compared with eNOS or nNOS) are potentially damaging to tissue (Lyons, 1995). Previous study have suggested that iNOS activity could be increased intestinal inflammation for the animals (Rachmilewitz, et al., 1995; Ribbons, et al., 1995). In the current study, the concentration of the NOS in the plasma was the total one, and it was higher in the mixture of APS and ASS (1:1) than the control, but for the plasma concentration of the NO, there was no difference between them. It maybe that the the mixture of APS and ASS, or the mixture of APS, ASS, and ABPS could situmulate the expression of eNOS and nNOS, but not the iNOS, and suggested that both the mixture could stimulate the absoption of Ca^{2+}.

Previous studies have shown that many Chinese herbal medicinal ingredients affected humoral and cellular immunity in chickens, mice, and rabbits (Liang et al., 1998; Tang et al., 1998; Chu et al., 2004). Several Chinese herbal medicinal ingredients, *Astragalus* polysaccharide (APS), *Isatis* root polysaccharide (IRPS), *Propolis* polysaccharide (PPS), and *Epimedium* flavone (EF), were found to have strong immune-enhancing effects (Liang et al., 1998; Kong et al., 2004). Qiu et al. (2007) reported that a high dosage of ARPS and CYPS, as well as APS and IRPS resulted in higher Con A-stimulated lymphocyte proliferation compared with control values.

Lymphocytes are important immune-response cells that play a critical role in immune functions, and an optical density value is an indicator of lymphocyte proliferation. In this experiment, both with and without a LPS stress, the mixture APS and ASS stimulated lymphocyte proliferation significantly. As there was no treatment groups consisting of only APS or ASS, this stimulatory effect could result from either APS or ASS, or both.

An increase in plasma concentrations of cortisol or corticosterone is usually regarded as indicator of stressful conditions where a greater concentration indicates more stressful conditions (Ernest, 1994). In our experiments, after the LPS challenge the concentration of cortisol had increased, indicating that pigs were undergoing a stress response in both control and treatment groups. Though the level of plasma cortisol in the treatment groups was higher than that of the control, there was no significant difference between them.

IL-6 is thought to be a proinflammatory cytokine involved in chronic inflammatory responses. Several cytokines, including TNF-a and IL-1, can stimulate the synthesis of IL-6 (Okuda et al., 1995; Stephan et al., 1997). Some traditional Chinese medicine, such as Bu-Zhong-Yi-Qi-Tang, could significantly inhibit IL-6 production in chronic fatigue syndrome (CFS) patients (Shin et al., 2003). It is also a cytokine involved in the interaction between the immune system and the pituitary-adrenocortical axis (HPA) (Ernst et al., 1994). Previous studies have demonstrated that IL-6 stimulated the HPA in humans (Woloski et al., 1985; Salas et al., 1990; Navarra et al., 1991; Tominaga et al., 1991; Harbuz et al., 1992). These results, however, did not agree with our findings, although a possible reason for this may be that shorter experimental time in our experiment could result in an insensitive IL-6 response to the plasma cortisol. Compare to the control group, the mixture of APS and ASS could decrease the concentration of IL-6 in plasma significantly, suggested that the mixture of APS and ASS could inhibited the IL-6 secretion, which could relieve the stress from LPS in weaned pigs.

Soto et al. (1998) also concluded that lowered IGF-1 and GH release was an important causative factor of decreased growth during an inflammatory challenge. Fan et al. (1994) reported that anorexia induced by a LPS inflammatory challenge was associated with the decreased release of IGF-1. Hasselgren (1993) suggested that decreases in IGF-1 were indicative of the repartitioning of nutrients away from normal growth to the activation of immune system after a LPS challenge. Consistent with this previous study, our results show that the circulating concentration of IGF-1 was decreased after LPS challenge. Compared with the control a mixture of APS and ASS increased IGF-1 secretion, suggesting they might have positive effects on the growth in weaned pigs after a LPS challenge.

Gianotti et al. (1998) concluded that low IGF-1 was not always concomitant with a corresponding decreased concentration of GH. In the current study, the IGF-1 decrease induced by an immune challenge did not follow the changes in GH levels, which increased for both the

control and treatment groups. This may be partly attributed to a change in the release of cortisol that could enhance GH gene expression by pituitary cells as demonstrated in a previous *in vitro* study (Giustina and Veldhuis, 1998).

In summary, this research suggested that a mixture of APS and ASS, compared with the control or APS alone, have a positive effects on growth performance and immunity, but there was no difference between the mixture of APS and ASS, and the mixture of APS, ASS and ABPS, it seemed that ABPS did not enhance the effective of APS and ASS on the performance for the pigs.

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