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Achyranthes bidentata Polysaccharide Decreases Inflammatory Cytokine Secretion in Weaned Piglets after LPS Challenge

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Abstract: This study was conducted to investigate the effect of *Achyranthes Bidentata* Polysaccharide (ABP) on growth performance, inflammatory responses and hormones in weaned piglets after *Escherichia coli* Lipopolysaccharide (LPS) challenges. A total of 48 crossbred (Duroc x Large; White x Landrace) male pigs weaned at 28 days of age (8.45±0.14 kg) were randomly allotted to one of four treatments by initial BW in a 2×2 factorial design that included a dietary addition of ABP (0 or 500 mg kg^{-1}) and immunological challenge (with LPS or saline). On day 14 and 21, pigs were injected intraperitoneally with either 100 μg kg^{-1} BW of LPS or an equivalent amount of sterile saline. Blood samples were obtained 3 h after injection for analysis of Tumor Necrosis Factor-α (TNF-α), Prostaglandin E_2 (PGE_2), cortisol, Growth Hormone (GH) and Insulin-like Growth Factor (IGF)-I. The results showed that LPS challenge decreased Average Daily Feed Intake (ADFI) ($p<0.05$) from 14~21 and day 21~28. ABP increased ADG ($p<0.05$) and ADFI ($p<0.05$) from day 21~28. An interaction ($p<0.05$) between LPS challenge and diet was observed for the plasma concentration of TNF-α, PGE2, cortisol, and IGF-I after both LPS challenges such that among LPS-treated pigs, pigs fed the ABP diet were lower for TNF-α ($p<0.05$) and higher IGF-I ($p<0.05$) than those receiving the control diet. These results indicated that ABP alters the release of inflammatory cytokines that may lead to improved pig performance during an immunological stress.

Key words: *Achyranthes bidentata*, polysaccharide, lipopolysaccharide, inflammatory response, 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 (McGlone and Pond, 2003). 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), 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). The modern practice of feeding weaning pigs antibiotics as growth promoters has been established for a long time. 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 (WHO, 2002). Recently, alternatives to antibiotics are being encouraged and are under active investigation. Imunomodulatory phytochemicals may offer alternatives to antimicrobial growth-promoters for early-weaned piglets (Deng *et al*., 2007a, b; Kong *et al*., 2007a-c, 2009; Yin *et al*., 2008a, b).

Traditional Chinese Medicine (TCM) is a potential research target for dietary supplements as well as for new drug development (Lu *et al*., 2007). Ancient Chinese medical books indicated that Achyranthis Bidentatae Radix (the dried root of *Achyranthes bidentata* Blume, named Niu Xi, family Amaranthaceae) was used to nourish the liver and kidney, strengthen tendons and bones, eliminate blood stasis and stimulate menstrual discharge. *Achyranthis Bidentatae* (AB) is an important traditional Chinese medicine and is extensively used by TCM practitioners for the treatment of osteodynia of the lumbar region and knees, spasm and flaccidity of limbs (Committee of National Pharmacopoeia, 2005). Chemical investigations on AB disclosed that

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triterpenoids, phytoecdysones and polysaccharides existed in this herbal drug (Meng and Li, 2001). In modern researches, the medical effects of AB are inhibitory to osteoclast formation (Li *et al*., 2005), anti-inflammatory (Han *et al*., 2005), antivirus activity (Tian *et al*., 1995), immunomodulatory (Li and Li, 1997) and anti-tumor activity (Yu and Zhang, 1995; Xiang and Li, 1993). The previously studies have been shown that dietary supplementation with ABP to weaned piglets can enhance cellular and humoral immune responses (Chen *et al*., 2009), growth performance and health status (Chen *et al*., 2011). However, it is not clear about how ABP affects health status of weaned piglets under challenge situations.

On the basis of the foregoing, researchers hypothesized that dietary supplementation with *Achyranthis Bidentatae* Polysaccharides (ABP) decreases inflammatory cytokine secretion in weaned piglets after LPS challenge. This hypothesis was tested by determining the effect of ABP on growth performance, inflammatory responses and hormones in weaned piglets after *Escherichia coli* Lipopolysaccharide (LPS) challenges.

MATERIALS AND METHODS

Preparation of *Achyranthes bidental* polysaccharide: The dried and sliced rhizome of Radix AB (from Henan Province, China) was decocted three times with distilled water (200 g L^{-1}), 1.5 h per time. All of the decoctions were pooled and condensed under hypopiesis condition into 1 g herb per mL decoction. The condensed solution was purified as follows: removed protein and pigment by Sevag's Method (Xue, 1985) and active carbon adsorption, dialyzed against several changes of distilled water for 24 h, extracted by one-step precipitation with dehydrated ethanol whose final content was 80% in the decoction (Kong *et al*., 2004a, b), precipitates isolated by centrifuged were lyophilized and then dissolved into distilled water (200 g L^{-1}) the solution was dropwised with 30% H_2O_2 at 50°C and pH 8-9 until the color not changing and water bathed at 50°C for 2 h, dialyzed against distilled water for 48 h and condensed under hypopiesis condition, extracted by one-step precipitation with dehydrated ethanol whose final content was 75% and the precipitates were dissolved into distilled water, the solution was applied to a DEAE-Cellulose (2.6×100 cm) column and G-50 sephadex column in turn (Zhang, 1999). The polysaccharide was a white power after lyophilized and consisted of D-mannose and D-glucose in a molar ratio 8:1 by HPLC analysis (Shanghai Institute of Chemical Physics, Chinese Academy of Sciences, China). Carbohydrate content of the final product was 95% measured by Vitriol-anthrone (Xue, 1985) taking anhydrous glucose as standard control, protein and nuclear acid contamination in the extract was negligible (absorbance at 280 and 260 nm wavelengths close to zero). The relative molecular mass of the polysaccharide was about 1300-1400 as determined by the Gel Filtration Method. The final extract was used as feed additive in this study.

Animal, housing and experimental design: A total of 48, three-hybreded (Duroc x Large; White x Landrace) piglets weaned at 28 days of age were acquired from Hunan Zhenghong Pig Farm and allocated into four treatment groups on the basis of body weight and litter of origin in a complete randomized design (Yao *et al*., 2008). A soybean meal basal diet (Table 1) was formulated to meet the nutrient requirements of growing pigs as suggested by NRC (1998). Two diets were tested with LPS or saline challenges including the basal control diet and one treatment diets in which ABP were added to basal control diet with 500 mg kg^{-1} (Liu *et al*., 2009). There were four pens of piglets for each treatment with 3 piglets per pen. Each pen was equipped with a feeder and a water nipple to allow *ad libitum* consumption of feed and water (Deng *et al*., 2007a, b). 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 40-70% and lighting cycle was 12 h per

Table 1: Dietary ingredients and nutrient levels in basal diet (air-dry basis; %)

Composition	Values
Dietary ingredients	
Corn	66.90
Wheat bran	4.00
Decorticated soybean meal	24.00
Fermented soybean meal	2.00
$CaHPO_4$	1.20
$CaCO_3$	0.80
Choline chloride (50%)	0.10
NaCl	0.35
Lysine•HCl	0.24
Methionine	0.06
Premix*	0.35
Nutrient levels**	
Digestible energy (MJ Kg^{-1})	13.50
Crude protein	18.60
Lysine•HCl	1.14
Methionine	0.32
Methionine+Cystine	0.63
Calcium	0.68
Total phosphorus	0.59
Available phosphorus	0.35

*Premix provided for per kg diet: 80 mg Fe ($FeSO_4$), 80 mg Zn ($ZnSO_4$), 5 mg Cu ($CuSO_4$), 3 mg Mn ($MnSO_4$), 0.25 mg Se (Na_2SeO_3), 0.14 mg I (CaI_2); 2250 IU vitamin A, 220 IU vitamin D_3, 16 IU vitamin E, 0.5 mg vitamin K_3, 2 mg vitamin B_1, 4.5 mg vitamin B_2, 7 mg vitamin B_6, 0.03 mg vitamin B_{12}, 0.2 mg biotin, 0.3 mg folic acid, 30 mg nicotinic acid, 25 mg pantothenic acid and 20 mg vitamin C; **Data of crude protein, calcium and total phosphorus were measured values and others were calculated values day (Yin *et al*., 2000). This experiment was approved by Animal Care and Use Committee, the Chinese Academy of Sciences (Yin *et al*., 2010).

Sample collection and preparation: Individual piglet Body Weight (BW) was assessed on day 0, 14 and 21 (weaning) and 7 days after weaning to calculate the Average Daily Gain (ADG), Average Daily Feed Intake (ADFI) and Feed/Gain (F/G) ratio (Yin *et al*., 2001). On days 14 and 21 after consumed the experimental diets, all piglets from each treatment group were injected intraperitoneally with a dosage of 100 μg kg^{-1} BW of LPS (Sigma, USA) or saline. Blood samples (15 mL per piglet) were collected 3 h after challenge by venipuncture of the jugular vein between 8 and 9 a.m. before feeding. Sera were obtained by centrifiguation at 3000 g for 10 min and stored at -20°C until analysis for cytokines and hormones (Tan *et al*., 2009).

Analysis of serum concentrations of cytokines and hormones: Serum TNF-α concentrations were determined using ELISA kits (R and D System, USA). PGE_2 concentrations were determined using ^{125}I RIA kits (Suzhou University, China). The concentrations of cortisol, GH and IGF-I were determined using ^{125}I RIA kits (Beijing Sino-UK Institute of Biological Technology, China (Tang *et al*., 2005).

Statistical analysis: Data were expressed as mean±SEM. Results were statistically analyzed the GLM procedure of the Statistical Analysis System (SAS Institute, Cary, NC). A $p<0.05$ was taken to indicate statistical significance.

RESULTS AND DISCUSSION

Effects of ABP on growth performance in weaned piglets after LPS challenges: As shown in Table 2, LPS challenge decreased Average Daily Feed Intake (ADFI) ($p<0.05$) from 14~21 and day 21~28. ABP increased ADG ($p<0.05$) and ADFI ($p<0.05$) from day 21~28. APS had no effect on the feed conversion rate ($p>0.05$). An interaction ($p<0.05$) between LPS challenge and diet was not observed in growth performance.

Effects of ABP on serum contents of cytokines and hormones in weaned piglets after LPS challenges: Effects of ABP on serum contents of cytokine and hormones in the weaned piglets were listed in Table 3. An

Table 2: Effect of Achyranthes Bidentata Polysaccharide (ABP) and on performance of weaned pigs after Lipopolysaccharide (LPS) challenges

	-LPS		+LPS		
Items	ABPS (0 mg kg^{-1})	ABPS (500 mg kg^{-1})	ABPS (0 mg kg^{-1})	ABPS (500 mg kg^{-1})	SEM
ADG (g)					
0~14 day	210.000	210.000	202.000	206.000	23.000
14~21 day[a]	594.000	631.000	447.000	510.000	42.000
21~28 day[b]	610.000	698.000	566.000	613.000	35.000
Overall[a]	382.000	402.000	339.000	369.000	22.000
ADFI (g)					
0~14 day	330.000	353.000	348.000	336.000	18.000
14~21 day[a]	779.000	788.000	663.000	729.000	52.000
21~28 day[a]	995.000	1056.000	922.000	972.000	44.000
Overall[a]	582.000	610.000	551.000	568.000	25.000
F/G					
0~14 day	1.571	1.691	1.723	1.631	0.095
14~21 day[a]	1.311	1.249	1.483	1.429	0.118
21~28 day	1.631	1.513	1.629	1.586	0.092
Overall	1.523	1.517	1.625	1.539	0.046

[a]LPS effect ($p<0.05$); [b]Diet effect ($p<0.05$)

Table 3: Effect of *Achyranthes Bidentata* Polysaccharide (ABP) on plasma Tumor Necrosis Factor-α (TNF-α), Prostaglandin E_2 (PGE_2), cortisol, Insulin-like Growth Factor (IGF)-I and Growth Hormone (GH) levels in weaned pigs after Lipopolysaccharide (LPS) challenges

	-LPS		+LPS			p-values		
Items	ABPS (0 mg kg^{-1})	ABPS (500 mg kg^{-1})	ABPS (0 mg kg^{-1})	ABPS (500 mg kg^{-1})	SEM	Diet	LPS	Interaction
Day 14								
TNF-α (pg mL^{-1})	329.00	303.00	2998.00	2114.00	288.00	0.031	<0.001	0.040
E_2 PGE_2 (pg mL^{-1})	671.25	693.66	1019.22	844.91	55.86	0.062	<0.001	0.017
Cortisol (ng mL^{-1})	50.98	71.02	221.15	168.90	11.17	0.045	<0.001	<0.001
GH (ng mL^{-1})	4.30	4.43	5.22	4.62	0.66	0.736	0.258	0.465
IGF-I (ng mL^{-1})	169.00	171.00	108.00	151.00	16.00	0.042	0.001	0.075
Day 21								
TNF-α (pg mL^{-1})	332.00	353.00	2470.00	1769.00	225.00	0.039	<0.001	0.028
E_2 PGE_2 (pg mL^{-1})	553.56	580.20	751.33	613.92	45.75	0.096	0.001	0.015
Cortisol (ng mL^{-1})	66.73	81.25	165.77	129.34	8.11	0.059	<0.001	<0.001
GH (ng mL^{-1})	5.65	4.98	4.90	5.42	0.60	0.809	0.711	0.148
IGF-I (ng mL^{-1})	146.00	179.00	128.00	138.00	10.00	0.004	<0.001	0.078

interaction ($p<0.05$) between LPS challenge and diet was observed for the plasma concentration of TNF-α and PGE2. ABP tended to decrease the plasma concentration of TNF-α and PGE2 in LPs-challenged piglets. ABP had no effect on TNF-α and PGE2 concentrations in saline-challenged piglets ($p>0.05$). On day 14~21, LPS increased cortisol concentrations and decreased IGF-1 concentrations ($p<0.05$). ABP decreased ($p<0.05$) cortisol concentrations in LPS-challenged piglets but not in saline-treated piglets ($p>0.05$). LPS and ABP had no effect on GH concentrations ($p>0.05$).

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 (Van 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 3-4 weeks when the piglets are usually weaned on commercial farms (Van Beers-Schreurs *et al*., 1992). 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 Jr., 2000). In this regard, components of innate immunity may serve as predictors of the overall immunity as well as pig health. For this reason, researchers determined the effect of ABP on growth performance, inflammatory responses and hormones in weaned piglets after *Escherichia coli* Lipopolysaccharide (LPS) challenges.

There has been growing interest in recent years in the use of Chinese herbal medicines or its ingredients as new, alternative growth promoters for domestic animals and poultry (Deng *et al*., 2007a, b; Guo *et al*., 2004; Ma and Chan, 2004; Kong *et al*., 2004a, b, 2006, 2007a; Yin *et al*., 2006; Chen *et al*., 2009, 2011). This study is the first time to determine the effect of ABP on growth performance, inflammatory responses and hormones in weaned piglets after *Escherichia coli* Lipopolysaccharide (LPS) challenges. The results showed that dietary supplementation with the ABP increased dietary supplementation of the ABP significantly affect the growth performance which is agreed with the previously study (Chen *et al*., 2011). Moreover, supplementation of ABP increased plasma concentrations of hormones, antibodies and alkaline phosphatase ($p<0.05$) and IL-1β mRNA abundance in liver, jejunal mucosa and lymph nodes (Chen *et al*., 2011). These results suggested that supplementation of ABP can increase immune responses without challenges (Chen *et al*., 2011). However, in this study researchers found that supplementation of ABP can decrease serum contents of cytokines and hormones in weaned piglets after LPS challenges suggesting that the reasons leading to improved growth performance are different with or without challenges.

Serum cortisol concentration is an indicator of stress status in pigs (Shen *et al*., 2012). In this study, results indicate that supplementation of ABP reduces stress associated with LPS-challenges.

CONCLUSION

It can be concluded that dietary supplementations of ABP alters the release of inflammatory cytokines and regulates the immune resposnes that may lead to improved pig performance during an immunological stress.

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