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Effects of *Achyranthes Bidentata* Polysaccharide on Growth Performance, Immunological, Adrenal, and Somatotropic Responses of Weaned Pigs Challenged with *Escherichia coli* Lipopolysaccharide

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ABSTRACT : A study was conducted with 48 weaned barrows (28±3 d, 8.45±0.14 kg) to determine the effect of *Achyranthes bidentata* polysaccharide (ABPS) supplementation on pig performance, immunological, adrenal and somatotropic responses following *Escherichia coli* lipopolysaccharide (LPS) challenge. The experiment was a 2×2 factorial design; the main factors included diet (supplementation with 0 or 500 mg/kg ABPS) and immunological challenge (LPS or saline). On d 14 and 21 of the trial, pigs were given an intraperitoneal injection with either 100 μg/kg 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), insulin-like growth factor (IGF)-I and immunoglobulin G (IgG). On d 2 after LPS challenge, peripheral blood lymphocyte proliferation (PBLP) was measured. LPS administration decreased average daily feed intake (ADFI) ($p<0.05$), had a tendency to decrease average daily gain (ADG) ($p<0.10$) during both the first and second challenge periods and increased ($p<0.05$) feed:gain ratio only during the first challenge period. ABPS tended to improve ADG ($p<0.10$) during the first challenge period, and improved ADG ($p<0.05$) and tended to improve ADFI ($p<0.10$) during the second challenge period. ABPS did not affect feed:gain ratio. An interaction ($p<0.05$) between LPS challenge and diet was observed for the plasma concentrations of TNF-α, PGE_2 and cortisol after both LPS challenges such that, among LPS-treated pigs, pigs fed the ABPS diet were lower for these indices than those receiving the control diet. In contrast, pigs fed the ABPS diet had higher IGF-I ($p<0.05$) compared with those fed the control diet. No effect of diet, LPS challenge or both on GH and IgG was observed after both LPS administrations. LPS challenge increased PBLP when these cells were incubated with 8 μg/ml of LPS during both the challenge periods, and did likewise when incubated with 8 μg/ml of concanavalin A only after the first challenge. ABPS had no effect on PBLP. These data demonstrate that ABPS alters the release of pro-inflammatory cytokines following an immunological challenge, which might enable pigs to achieve better performance. (**Key Words :** *Achyranthes Bidentata* Polysaccharide, Lipopolysaccharide, Inflammatory Response, Weaned Pigs)

INTRODUCTION

In swine production, the continual exposure of weanling piglets to a wide variety of microorganisms will lead to an immunological challenge, which can result in a series of physiological changes including temporary fever, depressed feed intake and activation of the immune system (Kelley et al., 1994). Consequently, the immunological challenge results in poor growth on animals and increases economic loss for pig producers. Johnson (1997) and Webel et al. (1997) propose that these changes are mostly ascribed to the release of pro-inflammatory cytokines, including tumor necrosis factor (TNF), interleukin (IL)-1 and IL-6. Overproduction of these pro-inflammatory cytokines has negative effects on growth and feed efficiency. Therefore, using immunomodulators to modulate the secretion of these cytokines is considered as a potential means to mitigate the negative effects induced by an immunological challenge (Lang et al., 1996).

Achyranthes bidentata polysaccharide (ABPS), a gray-white powder, which is isolated from the root of Chinese medicinal herb *Achyranthes bidentata* Blume, is composed of fructose and glucose residues and the molar ratio is 8:1 (Chen et al., 2005). ABPS is a graminans-type fructan that contains a β-D fructofuranosyl backbone having residues

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Table 1. Ingredient composition of the basal diet (as-fed basis)

Item	%
Ingredient	
Corn	57.47
Soybean meal (44% CP)	22.00
Wheat middling	6.00
Fish meal	6.00
Soy oil	1.20
Milk replacer	4.00
Limestone	0.65
Dicalcium phosphate	1.00
Salt	0.31
L-lysine·HCl (78.8% lysine)	0.32
Butylated hydroquinone	0.05
Vitamin and mineral premix[1]	1.00
Nutrient composition	
Digestible energy[2] (MJ/kg)	13.60
Crude protein[3]	20.00
Calcium[3]	0.80
Total phosphorus[3]	0.70
Total lysine[2]	1.35
Total methionine+cystine[2]	0.65

[1] Provided the following amounts of vitamins and trace minerals per kilogram of complete diet: retinol acetate, 2,700 μg; cholecalciferol, 62.5 μg; dl-α-tocopheryl acetate, 20 mg; menadione, 3 mg; vitamin B_{12}, 18 μg; riboflavin, 4 mg; niacin, 40 mg; pantothenic acid, 15 mg; choline chloride, 400 mg; folic acid, 700 μg; thiamin,1.5 mg; pyridoxine, 3 mg; biotin, 100 μg; Zn, 80 mg ($ZnSO_4 \cdot 7H_2O$); Mn, 20 mg ($MnSO_4 \cdot 5H_2O$); Fe, 83 mg ($FeSO_4 \cdot H_2O$); Cu, 25 mg ($CuSO_4 \cdot 5H_2O$); I, 0.48 mg (KI); Se, 0.36 mg ($Na_2SeO_3 \cdot 5H_2O$).
[2] Calculated. [3] Analyzed.

linked (2→1)- and (2→6)- with branches and an α-D-glucopyranose residue on the nonreducing end of the fructan chain (Jin et al., 2007). Compared with other polysaccharides, ABPS has a smaller molecular mass. In humans and rodents, it has been used as an immune modulator (Li and Li, 1997; Shao et al., 2002; Jin et al., 2007). Because of its traits of natural origin, as well as diverse pharmacological effects, producing no drug residues and low side effects, ABPS is an attractive alternative to antibiotics. Within our knowledge, no study has been conducted to observe the effect which ABPS exerts its activities in pigs. Therefore, this study was conducted to evaluate the effects of dietary ABPS supplementation on performance, as well as the immunological, adrenal, and somatotropic response in weaned pigs challenged with *Escherichia coli* lipopolysaccharide (LPS).

MATERIALS AND METHODS

Experimental design

Our experimental protocol was approved by the Animal Care and Use Committee of Hubei Province. A total of 48 crossbred (Duroc×Large White×Landrace) male pigs weaned at 28±3 d of age (8.45±0.14 kg) were randomly allotted to one of four treatments by initial BW in a 2×2 factorial arrangement that included a dietary addition of ABPS (0 or 500 mg/kg) and LPS challenge (with or without).

Pigs were housed in 1.20×1.10 m pens with six replicate pens per treatment with two pigs per pen. Each pen was equipped with plastic slotted floor, and a feeder and a nipple waterer to allow pigs *ad libitum* access to feed and water. The temperature in the room was controlled at 25-27°C by air condition. The basal diet (Table 1) was formulated to meet or exceed NRC (1998) requirements for all nutrients. ABPS, which contained polysaccharide no less than 90% and had a molecular weight of 2,680, was purchased from Zhejiang Jingxin Pharmaceutical Company (Zhejiang, China). Pigs were weighed and feed disappearance was measured on days 0, 14, 21 and 28 throughout the 28-day trial.

Immunological challenge model

On d 14 and 21 of the trial, the challenged group was injected intraperitoneally with LPS (*Escherichia coli* Serotype O55:B5; Sigma Chemical Co., St. Louis, MO, USA) at 100 μg/kg BW and the unchallenged group was administrated an equivalent amount of 0.9% (wt/vol) NaCl solution. The LPS was dissolved in sterile 0.9% NaCl solution such that 0.2 ml of solution/kg of BW would achieve the desired dosage.

Blood sample collections and assays

On d 14 and 21, 3 h after the LPS or saline administration, blood samples (all pigs) were respectively collected into heparinized vacuum tubes (Becton Dickinson Vacutainer System; Franklin Lake, NJ) and centrifuged (3,500×g for 10 min) to separate plasma, and then stored at -80°C until analysis.

Plasma tumor necrosis factor-α (TNF-α) was analyzed using a commercially available ELISA kits (R&D Systems Inc., Minneapolis, MN). Minimum detectable concentration was 3.7 pg/ml with an intra- and inter-assay CV were 4.9% and 8.9%. The average recovery of porcine TNF-α in porcine serum is 95%.

Plasma prostaglandin E_2 (PGE_2) was analyzed using a commercially available ^{125}I RIA kit (College of Medical Science of Suzhou University, Jiansu, China), which we have also previously validated in pigs (Liu et al., 2003). Minimum detectability of PGE_2 was 6.25 pg/ml with an intra-assay CV less than 10%.

Plasma cortisol was analyzed using a Coat-a-CountTM assay kit (Diagnostic Products, Los Angeles, CA). Minimum detectable dose of cortisol was 2 ng/ml. Intra- and inter-assay CV were 4.6% and 9.0%, respectively.

Plasma growth hormone (GH) was measured using a commercially available porcine GH ^{125}I RIA kit (Linco Research, Inc., St. Charles, MO). The minimum detection

Table 2. Effect of feeding *achyranthes bidentata* polysaccharide (ABPS) and lipopolysaccharide (LPS) challenge on performance of weaned pigs (0 to 28 d) [a]

Item	-LPS		+LPS		SEM
	0 mg of ABPS/kg	500 mg of ABPS/kg	0 mg of ABPS/kg	500 mg of ABPS/kg	
Average daily gain (g)					
0 to 14 d	212	212	203	207	23
14 to 21 d [bc]	592	632	447	511	42
21 to 28 d [de]	611	699	567	614	35
Overall [b]	381	401	339	369	22
Average daily feed intake (g)					
0 to 14 d	330	353	349	335	18
14 to 21 d [b]	779	788	664	729	52
21 to 28 d [bc]	995	1,056	924	971	44
Overall [b]	582	610	550	568	25
Feed:gain					
0 to 14 d	1.557	1.666	1.719	1.619	0.095
14 to 21 d [b]	1.316	1.247	1.486	1.426	0.118
21 to 28 d	1.628	1.579	1.631	1.582	0.092
Overall [d]	1.527	1.522	1.622	1.539	0.046

[a] Lipopolysaccharide was injected on d 14 and 21. Values are means (n = 6) for six pens per treatment with two pigs per pen.
[b] LPS effect ($p<0.05$). [c] Diet effect ($p<0.10$). [d] LPS effect ($p<0.10$). [e] Diet effect ($p<0.05$).

limit was 1 ng/ml, with an intra-assay CV of 4.0%.

Plasma insulin-like growth factor-I (IGF-I) was determined by using a commercially available porcine IGF-1 IRMA kit (Diagnostic Systems Laboratories, Inc.). The minimum detection limit was 2 ng/ml, with an intra-assay CV of 3.9%.

IgG concentration was determined using a radial immunodiffusion test kit according to the methods of Hicks et al. (1998) as a representative humoral immune response. Five microliters of standard solutions and diluted plasma samples were pipetted to a separately identified well of the test plates. The plate was securely covered and placed in a 37°C, humidified incubator for 48 to 72 h. After incubation, plates were removed and placed over a source of illumination to clearly see precipitin rings. The external diameters of the rings were measured to the nearest 0.1 mm using the scale provided. A reference curve was plotted using the diameters measured from standard solutions. From the reference curve, the IgG concentration of each diluted test sample was calculated by multiplying the concentration read from the curve by the dilution factor to obtain the actual concentration.

All blood related measurements were analyzed in duplicate.

Lymphocyte proliferation

Lymphocyte proliferation was measured using a colorimetric assay, with 3-(4,5-dimethlthiazol-2-yl)-2,5-diphenyltetrazolium bromide (M-2128, Sigma Chemical Inc., St. Louis, USA) in cultures of purified peripheral blood mononuclear cells according to the method of Liu et al. (2003) and Kong et al. (2007). Briefly, mononuclear cells were collected by gradient centrifugation from the peripheral blood obtained d 2 after the first and the second LPS injection, at 3,000×g for 30 min. The cells were washed three times, and then suspended in RPMI-1640 complete culture medium supplemented with 10% (vol/vol) heat-inactivated fetal calf serum, 100 U/ml of penicillin, 100 μg/ml of streptomycin and 25 mM N-(2-hydroxyethyl)-piperazine-N'-2-ethane-sulfonic acid. Cell activity was detected by trypan blue dye exclusion, and the cell density was counted to adjust to 2×10^6 cells/ml culture medium. Then the cellular suspension was added to 96-well microtiter plates with a total culture volume of 200 μl.

Lymphocyte mitogen concanavalin A (ConA; Type IV, C-2010, Sigma Chemical Inc., St. Louis, USA) or LPS was added at a final concentration of 8 μg/ml culture medium, and then the plates were incubated at 37°C in a 5% CO_2 incubator. After 66 h of incubation, 10 μl MTT solution (5 mg MTT/ml in 1/15 M phosphate-buffered saline, pH 7.6) was added to each well and the plates were incubated at 37°C for another 6 h. At the end of incubation, 100 μl of a 10% sodium dodecyl sulfate in 0.04 M HCl solution was added to lyse the cells and solubilize the MTT crystals. Plates were read at 570 nm using an automated microplate reader (Bio-Rad, Model 550, Hercules, CA). Lymphocyte proliferation was expressed as a stimulation index, which was calculated as the absorbance of wells incubated with ConA or LPS divided by the absorbance of wells incubated without ConA or LPS.

Statistical analysis

All data were analyzed by ANOVA using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC) appropriate

Table 3. Effect of feeding *achyranthes bidentata* polysaccharide (ABPS) and lipopolysaccharide (LPS) challenge on the proliferation of lymphocytes isolated from peripheral blood in weaned pigs after both lipopolysaccharide injection [a, b]

Item [c]	-LPS		+LPS		SEM
	0 mg of ABPS/kg	500 mg of ABPS/kg	0 mg of ABPS/kg	500 mg of ABPS/kg	
Following the first LPS injection					
LPS (8 μg/ml [c])	1.008	1.011	1.045	1.099	0.031
ConA (8 μg/ml [c])	1.024	1.038	1.166	1.121	0.050
Following the second LPS injection					
LPS (8 μg/ml [c])	1.113	1.157	1.398	1.355	0.112
ConA (8 μg/ml)	1.148	1.102	1.072	1.074	0.050

[a] Lipopolysaccharide was injected on d 14 and 21. Values are means (n = 12) for all pigs per treatment. Values are expressed as stimulation index, which is calculated as: absorbance of wells incubated with concanavalin A (ConA) or LPS divided by the absorbance of wells incubated without ConA or LPS.
[b] Blood was obtained 2 d after the first and the second LPS administration. [c] LPS effect ($p<0.05$).

for a factorial arrangement of treatments in a randomized complete block design. The statistical model consisted of the effects of challenge (saline or LPS), diet (ABPS or no ABPS) and their interactions. Pen was used as experimental unit for the performance data, whereas individual pig data were used as the experimental unit for blood analysis and measurement of lymphocyte proliferation. A level of $p<0.05$ was used as the criterion for statistical significance, whereas $p<0.10$ was taken to indicate a statistical trend.

RESULTS

Growth performance

There was no LPS challenge×diet interaction for average daily gain (ADG), average daily feed intake (ADFI) and feed/gain observed throughout the 28-d trial (Table 2). Prior to LPS challenge (from d 0 to 14), dietary treatment did not affect growth performance. During the first challenge period (from d 14 to 21), pigs fed ABPS tended to have a 10.0% higher ADG ($p<0.10$) than the control pigs, but ADFI and feed:gain were not affected. Correspondingly, LPS challenge reduced ADG ($p<0.05$) by 21.7% and ADFI ($p<0.05$) by 11.1%, and increased feed:gain ($p<0.05$) by 13.6% compared to the saline-injected pigs regardless of dietary treatment. During the second challenge period (from d 21 to 28), supplementation of ABPS increased ADG ($p<0.05$) by 8.9% and tended to increase ADFI ($p<0.10$) by 5.6%; whereas LPS challenge had a tendency to decrease ADG ($p<0.10$) by 7.7% and decreased ADFI ($p<0.05$) by 7.6%. In the whole performance trial (from d 0 to 28), there was no significant affect on ADG, ADFI and feed:gain observed by addition of ABPS, but LPS challenge reduced ADG ($p<0.05$) by 9.5% and ADFI ($p = 0.05$) by 6.2%, and had a tendency to increase feed:gain ($p<0.10$) by 3.7% compared with the saline-treated pigs.

Lymphocyte proliferative and humoral responses

The results of lymphocyte proliferation are shown in Table 3. There was no LPS challenge×diet interaction observed for lymphocyte proliferation when incubated with either LPS or ConA. Dietary treatment had no effect on lymphocyte proliferation. LPS challenge increased ($p<0.05$) lymphocyte proliferation when incubated with 8 μg/ml LPS after the first and second LPS challenges, and did likewise with 8 μg/ml ConA only after the first LPS challenge.

The concentration of serum IgG was measured to represent the humoral immune response, and the results are presented in Table 4. There was neither LPS challenge nor diet effect on the concentration of serum IgG after the first and the second LPS challenge.

Plasma TNF-α, PGE_2, cortisol, GH, and IGF-I

As indicated by Table 5, after the first and second LPS challenges, an interaction between ABPS and LPS was observed for all plasma indices with the exception of GH. Plasma TNF-α ($p<0.05$), PGE_2 ($p<0.10$) and cortisol ($p<0.10$) responses to the LPS challenge were lower in pigs receiving the ABPS diet than the LPS-treated pigs fed the control diet, whereas there was no difference for these

Table 4. Effect of feeding *achyranthes bidentata* polysaccharide (ABPS) and lipopolysaccharide (LPS) challenge on serum immunoglobulin G (IgG) level after both lipopolysaccharide challenges in weaned pigs[a, b]

Item	-LPS		+LPS		SEM
	0 mg of ABPS/kg	500 mg of ABPS/kg	0 mg of ABPS/kg	500 mg of ABPS/kg	
IgG (g/L)					
d 14	2.29	2.13	2.13	2.30	0.08
d 21	2.22	2.26	2.34	2.35	0.11

[a] Lipopolysaccharide was injected on d 14 and 21. Values are means (n = 12) for all pigs per treatment.
[b] Blood was obtained 3 h after the first and the second LPS administration.

Table 5. Effect of feeding *achyranthes bidentata* polysaccharide (ABPS) and lipopolysaccharide (LPS) challenge 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 after both lipopolysaccharide challenges in weaned pigs[a, b]

Item	-LPS		+LPS		SEM	p-value		
	0 mg of ABPS/kg	500 mg of ABPS/kg	0 mg of ABPS/kg	500 mg of ABPS/kg		Diet	LPS	Interaction
d 14								
TNF-α (pg/ml)	328	304	2,998	2,113	288	0.031	<0.001	0.040
PGE_2 (pg/ml)	671	694	1018	845	56	0.062	<0.001	0.017
Cortisol (ng/ml)	51	71	221	169	11	0.045	<0.001	<0.001
GH (ng/ml)	4.24	4.43	5.12	4.62	0.66	0.736	0.258	0.465
IGF-I (ng/ml)	168	171	108	152	16	0.042	0.001	0.075
d 21								
TNF-α (pg/ml)	331	353	2,468	1,767	225	0.039	<0.001	0.028
PGE_2 (pg/ml)	554	580	751	615	46	0.096	0.001	0.015
Cortisol (ng/ml)	67	81	166	129	8	0.059	<0.001	<0.001
GH (ng/ml)	5.68	4.96	4.90	5.42	0.60	0.809	0.711	0.149
IGF-I (ng/ml)	144	177	129	137	10	0.004	<0.001	0.079

[a] Lipopolysaccharide was injected on d 14 and 21. Values are means (n = 12) for all pigs per treatment.
[b] Blood was obtained 3 h after the first and the second LPS administration.

indices response in the saline-injected pigs. In contrast, plasma IGF-I in pigs fed the ABPS diet was higher ($p<0.05$) than in those fed the control diet among LPS-injected pigs, whereas there was no difference among saline-injected pigs. No effects of diet, LPS challenge or both on plasma GH concentration were observed following both the first and the second LPS challenge.

DISCUSSION

In the present study, we used the well-recognized model for inducing sickness in pigs by injecting LPS (Johnson and von Borell, 1994; Lien et al., 2005) which is a component of the outer membrane of gram-negative bacteria, to determine the effect of ABPS supplementation on immunological challenge. Until now, few researches were conducted to evaluate the effect of ABPS on growth performance of livestock. In our study, before LPS challenge, dietary treatment had no effect on pig performance. This finding is similar to Chen (2002) who reported that there was no effect on broiler performance when ABPS added to 200 mg/kg. However, pigs fed 500 mg/kg ABPS had higher weight gain during both LPS challenge periods and higher feed intake during the first LPS challenge period compared with those fed the control diet, which indicates the importance of ABPS supplementation under stress, infection and diseases. The LPS challenge significantly decreased pig weight gain and feed intake, and this finding is consistent with some previous studies in pigs (Lee et al., 2000b; Liu et al., 2003; Mao et al., 2005).

In the current study, the circulating concentrations of the inflammatory indices TNF-α and PGE_2 were increased after LPS injection, which is consistent with Lee et al. (2000a) and Liu et al. (2008a, b). It is well known that, during an immunological challenge, one of the first responses of an animal is to release pro-inflammatory cytokines such as IL-1β and TNF-α from macrophages (Spurlock, 1997), which will induce an increase in the secretion of PGE_2 in muscle (Hellerstein et al., 1989) and an activation of the immune system. This response directs nutrients away from tissue growth to support immune function (Spurlock, 1997), and finally decreases the efficiency of nutrient utilization for growth. The pigs fed ABPS had lower concentrations of TNF-α and PGE_2 than did pigs fed the control diet among the LPS-challenged pigs, which indicates an anti-inflammatory role of ABPS. Until now, no other research was conducted to evaluate the anti-inflammatory role of ABPS. The exact mechanism(s) by which ABPS exerts its anti-inflammatory role on the animals is unclear. However, there are three possible pathways for anti-inflammatory effects of ABPS.

First of all, ABPS may inhibit pro-inflammatory cytokine synthesis through the synthesis of anti-inflammatory cytokines. Anti-inflammatory cytokines such as IL-10, by suppressing the activity of the signal transduction of nuclear transcription factor κB which is a major transcription factor of pro-inflammatory cytokines (Schottelius et al., 1999) inhibit the synthesis of pro-inflammatory cytokine to maintain the balance between the pro- and anti-inflammatory mediators (Hoqaboam et al., 1998). Secondly, ABPS may increase the synthesis of IL-1 receptor antagonist. IL-1 receptor antagonist is a competitive inhibitor of interleukin-1 that binds to type I interleukin-1 receptors (Poutsiaka et al., 1993). Regretfully, we did not measure the anti-inflammatory cytokines and interleukin receptor antagonists in the current study. Finally, ABPS may inhibit pro-inflammatory cytokines synthesis by

decreasing the production of arachidonic acid metabolites such as prostaglandins (PGE_2 in the current study). Prostaglandins play a major role in the inflammatory and immune responses and are capable to decrease cytokines production (Calder, 1997).

In our study, that ABPS supplementation significantly suppressed the increase of pro-inflammatory cytokine TNF-α and PGE_2 release, which indicates less activation of the immune system, and consequently more nutrients to support tissue growth. This may partially explain why pigs fed 500 mg/kg ABPS had higher gain and feed intake compared with the control.

Besides depressed gain and feed intake, a reduction in plasma IGF-I was observed in pigs administrated with LPS. The study of Hasselgren (1993) suggested that the decrease in IGF-I was indicative of the repartitioning of nutrients away from normal growth to the immune response after LPS challenge. Soto et al. (1998) also proposed that the decrease in release of IGF-I was an important causative factor of decreased growth during an immunological challenge. Therefore, that the significantly mitigated reduction of plasma IGF-I due to addition of ABPS observed in our study may be an indication that ABPS supplementation alleviates the alterations in somatotropic axis after an LPS challenge, which might have been associated with the improved performance in pigs supplemented with ABPS compared with control pigs.

In the present study, in those LPS-challenged pigs, feeding ABPS partially attenuated the reduction of plasma IGF-I, concurrent with lower plasma TNF-α levels compared with the control. Therefore, the effect of ABPS on alleviating plasma IGF-I reduction seems to be closely associated with the decrease of pro-inflammatory cytokine release, such as TNF-α. McCarthy et al. (1995) proposed that anorexia induced by pro-inflammatory cytokines after LPS challenge was associated with the suppressed release of IGF-I. Accordingly, in our study, those LPS-challenged pigs fed with ABPS mitigated the decrease in feed intake, correspondingly attenuated the reduction of IGF-I. This may also explain why pigs fed ABPS had a higher feed intake compared with control pigs.

Pro-inflammatory cytokines are not only primarily associated with immune function, but also have the potential to change many aspects of neuroendocrine function, including the hypothalamic-pituitary-adrenal axis (HPAA) (Mandrup-Poulsen et al., 1995). Pro-inflammatory cytokines have been shown to stimulate neurons in the hypothalamus to release corticotropin-releasing hormone (Perlstein et al., 1993), which stimulated the adrenal cortex to produce cortisol (George and Chrousos, 1995). Concurred with aforesaid viewpoint, in the current study, we observed the increase in TNF-α induced by LPS challenge was concomitant with a rapid elevation of plasma cortisol, which is in agreement with previous study of Liu et al. (2003). However, feeding ABPS significantly decreased the level of plasma pro-inflammatory cytokine TNF-α and cortisol induced by LPS challenge. Therefore, ABPS may decrease the production of cortisol by suppressing pro-inflammatory cytokine TNF-α release.

In our study, ABPS supplementation decreased the release of pro-inflammatory cytokine TNF-α and PGE_2 in LPS-treated pigs, but did not reduce the production of TNF-α and PGE_2 in saline-injected pigs, indicating that ABPS mitigated the excessive activation of immune system in animals subjected to immunological challenge, but did not affect the immune function of normal animals. Furthermore, we did not observe an effect of ABPS on the concentration of IgG and lymphocyte proliferation in the current study. So ABPS did not decrease the immune function of normal animals. Therefore, it is rational to say that it is not bad at least to animals to fed ABPS when pigs are challenged by infectious diseases. Further evaluation of effects of ABPS on growth and inflammatory response in pigs challenged by infectious diseases is warranted before it is used in commercial practice.

IMPLICATIONS

The results of current study indicate that supplementation with *achyranthes bidentata* polysaccharide to pigs diet is able to relax the immunological and adrenal response to an *Escherichaia coli* lipopolysaccharide challenge by mediating the release of pro-inflammatory cytokines, and make pigs achieve better growth performance finally. However, source of *achyranthes bidentata* polysaccharide obtained from different methods or producers may vary in their structure, chemical composition, or both, and further evoke different effects on pigs. Accordingly, further investigation is warranted to focus on not only the performance and immune effect of *achyranthes bidentata* polysaccharide, but also the method producing the polysaccharide when it is added to animal diets.

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REFERENCES

Calder, P. C. 1997. n-3 Polyunsaturated fatty acids and immune cell function. Adv. Enzyme Reg. 37:197-237.

Chen, H. L. 2002. Studies on the extraction, immunomodulating activities of Chinese Herbal polysaccharides and approach to the mechanism. Ph.D. Dissertation, Chinese Academy of Agriculture Science, Beijing, China.

Chen, X, M., Y. J. Xu and G.. Y. Tian. 2005. Physical-chemical properties and structure elucidation of ABPS isolated from the root of achyranthes bidentata. Acta Pharm Sinica 40:32-35 (Chinese).

George, P. and M. D. Chrousos. 1995. The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. N. Engl. J. Med. 332:1351-1363.

Hasselgren, P. O. 1993. Protein metabolism in sepsis. R. G. Landes Co., Austin, TX.

Hellerstein, M. K., S. N. Meydani, M. Meydani, K. Wu and C. A. Dinarello. 1989. Interleukin-1 induced anorexia in the rat. Influence of prostaglandins. J. Clin. Invest. 84:228-235.

Hicks, T. A., J. J. McGlone, C. S. Whisnant, H. G. Kattesh and R. L. Norman. 1998. Behavioral, endocrine, immune, and performance measures for pigs exposed to acute stress. J. Anim. Sci. 76:474-483.

Hoqaboam, C. M., M. L. Steinhauser, H. Schock, N. Lukacs, H. Schock, N. Lukacs, R. M. Strieter, T. Standiford and S. L. Kunkel. 1998. Therapeutic effects of nitric oxide inhibition during experimental fecal peritonitis: Role of interleukin-10 and monocyte chemoattractant protein 1. Infect. Immun. 66: 650-655.

Jin, L. Q., Z. J. Zheng, Y. Peng, W. X. Li, X. M. Chen and J. X. Lu. 2007. Opposite effects on tumor growth depending on dose of *achyranthes bidentata* polysaccharides in C57BL/6 mice. Int. Immunopharmacol. 7:568-577.

Johnson, R. W. 1997. Inhibition of growth by pro-inflammatory cytokines: An integrated view. J. Anim. Sci. 75:1244-1255.

Johnson, R. W. and E. von Borell. 1994. Lipopolysaccharide-induced sickness behavior in pigs is inhibited by pretreatment with indomethacin. J. Anim. Sci. 72:309-314.

Kelley, K. W., R. W. Johnson and R. Dantzer. 1994. Immunology discovers physiology. Vet. Immunol. Immunopathol. 43:157-165.

Kong, X. F., Y. L. Yin, G. Y. Wu, H. J. Liu, F. G. Yin, T. J. Li, R. L. Huang, Z. Ruan, H. Xiong, Z. Y. Deng, M. Y. Xie, Y. P. Liao and S. W. Kim. 2007. Dietary supplementation with acanthopanax senticosus extract modulates cellular and humoral immunity in weaned piglets. Asian-Aust. J. Anim. Sci. 20:1453-1461.

Lang, C. H., J. Fan, R. Cooney and T. C. Vary. 1996. Interleukin-1 receptor antagonist attenuates sepsis-induced alterations in the IGF system and protein synthesis. Am. J. Physiol. 270:E430-E437.

Lee, D. N., T. F. Shen, H. T. Yen, C. F. Weng and B. J. Chen. 2000a. Effects of chromium supplementation and lipopolysaccharide injection on the immune responses of weanling pigs. Asian-Aust. J. Anim. Sci. 13:1414-1421.

Lee, D. N., C. F. Weng, H. T. Yen, T. F. Shen and B. J. Chen. 2000b. Effects of chromium supplementation and lipopolysaccharide injection on physiological responses of weanling pigs. Asian-Aust. J. Anim. Sci. 13:528-534.

Li, Z. K. and D. D. Li. 1997. The immunomodulatory effect of *achyranthes bidentata* polysaccharides. Acta Pharm Sinica 32: 881-887 (Chinese).

Lien, T. F., K. H. Yang and K. J. Lin. 2005. Effects of chromium propionate supplementation on growth performance, serum traits and immune response in weaned pigs. Asian-Aust. J. Anim. Sci. 18:403-408.

Liu, Y. L., J. J. Huang, Y. Q. Hou, H. L. Zhu, S. J. Zhao, B. Y. Ding, Y. L. Yin, G. F. Yi, J. X. Shi and W. Fan. 2008a. Dietary arginine supplementation alleviates intestinal mucosal disruption induced by *Escherichia coli* lipopolysaccharide in weaned pigs. Br. J. Nutr. doi:10.1017/S0007114508911612.

Liu, Y. L., D. F. Li, L. M. Gong, Z. Y. Feng, G. F. Yi, A. M. Gaines and J. A. Carroll. 2003. Effects of fish oil supplementation on performance as well as immunological, adrenal and somatotropic responses of weaned pigs after *Escherichia coli* lipopolysaccharide challenge. J. Anim. Sci. 81:2758-2765.

Liu, Y. L., J. Lu, J. X. Shi, Y. Q. Hou, H. L. Zhu, S. J. Zhao, H. M. Liu, B. Y. Ding, Y. L. Yin and G. F. Yi. 2008b. Increased expression of the peroxisome proliferator-activated receptor γ in the immune system of weaned pigs after *Escherichia coli* lipopolysaccharide challenge. Vet. Immunol. Immunopathol. 10.1016/j.vetimm.2008.02.014.

Mandrup-Poulsen, T., J. Nerup, J. I. Reimers, F. Pociot, H. U. Anderson, A. Karlsen, U. Bjerre and R. Bergholdt. 1995. Cytokines and the endocrine system. I. The immunoendocrine network. Eur. J. Endocrinol. 133:660-671.

Mao, X. F., X. S. Piao, C. H. Lai, D. F. Li, J. J. Xing and B. L. Shi. 2005. Effects of β-glucan obtained from the Chinese herb *Astragalus membranaceus* and lipopolysaccharide challenge on performance, immunological, adrenal, and somatotropic responses of weanling pigs. J. Anim. Sci. 83:2775-2782.

McCarthy, H. D., S. Dryden and G. Williams. 1995. Interleukin-1β-induced anorexia and pyrexia in rat: Relationship to hypothalamic neuropeptide Y. Am. J. Physiol. 269:E852-E857.

Perlstein, R. S., M. H. Whitnall, J. S. Abrams, E. H. Mougey and R. Neta. 1993. Synergistic roles of interleukin-6, interleukin-1, and tumor necrosis factor in the adrenocorticotropin response to bacterial lipopolysaccharide *in vivo*. Endocrinol. 132:946-952.

Poutsiaka, D. D., M. Mengozzi, B. Sinha and C. A. Dinarello. 1993. Cross-linking of the beta-glucan receptor on human monocytes results in interleukin-1 receptor antagonist but not interleukin-1 production. Blood 82:3695-3700.

Schottelius, A. J. G., M. W. Mayo, R. B. Sartor and A. S. Baldwin, Jr. 1999. Interleukin-10 signaling blocks inhibitor of kappa B kinase activity and nuclear factor kappa B DNA binding. J. Biol. Chem. 274:31868-31874.

Shao, S. J., L. Mai and Y. Chen. 2002. The effect of *achyranthes bidentata* polysaccharide on mice red blood cell immunological function. Chinese Remedies and Clinics, 5: 281-282 (Chinese).

Soto, L., A. I. Martin, S. Millan, E. Vara and A. López-Calderón. 1998. Effects of endotoxin lipopolysaccharide administration on the somatotropic axis. J. Endocrinol. 159:239-246.

Spurlock, M. E. 1997. Regulation of metabolism and growth during immune challenge: An overview of cytokine function. J. Anim. Sci. 75:1773-1783.

Webel, D. M., B. N. Finck, D. H. Baker and R. W. Johnson. 1997. Time course of increased plasma cytokines, cortisol, and urea nitrogen in pigs following intraperitoneal injection of lipopolysaccharide. J. Anim. Sci. 75:1514-1520.