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The effect of dietary addition of a polysaccharide from *Atractylodes macrophala* Koidz on growth performance, immunoglobulin concentration and IL-1β expression in weaned piglets

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SUMMARY

The present study was conducted to determine the effects of a polysaccharide of *Atractylodes macrophala* Koidz (PAM) as a dietary additive on growth performance, immunoglobulin concentration and IL-1β expression in weaned piglets. One hundred and twenty Landrace × Yorkshire piglets weaned at 28 days old (body weight 7·5 ± 0·07 kg) were assigned to five treatment groups (three pens/group, eight piglets/pen) fed maize/soybean-based diets supplemented with 0, 3, 6 or 9 g of PAM/kg diet or antibiotics (0·4 g flavomycin/kg + 0·13 g olaquindox/kg). The experimental period was 28 days. With increasing PAM supplementation levels, average daily gain was greater (quadratic, $P < 0·05$) and the ratio of amount fed to live weight (LW) gain (feed/gain) improved (quadratic, $P < 0·05$) during days 14–28 and overall, and diarrhoea incidence decreased (linear, $P < 0·05$) during days 14–28. Supplementation of PAM also increased (quadratic, $P < 0·05$) serum concentrations of interleukin (IL)-2 and IL-6 on day 14, and increased (quadratic, $P < 0·05$) IL-1β expression in jejunal mucosa and lymph nodes. Concentrations of PAM between 6 and 9 g/kg presented the strongest bioactivity compared to the control group or antibiotic-fed group. These findings indicate that PAM is effective in improving growth performance and cytokine response, which suggests that PAM can be used as a diet additive for weanling piglets.

INTRODUCTION

In recent years, it has been realized that dietary supplementation with antibiotics can result in side effects in treating a variety of bacterial infections in humans and is not the ultimate solution to disease control in livestock production (Adjiri-Awere & Van Lunen 2005). In order to overcome antibiotic resistance, scientists are searching for alternative yet effective dietary additives to prevent and treat emerging and re-emerging diseases.

The practice of early weaning can improve the sow's reproductive performance, but early weaned piglets are particularly sensitive to infection by pathogens that often lead to diarrhoea and even death.

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Many Chinese herbal ingredients have been reported to have immunity-enhancing effects in humans (Wang 1991) and animals (Kong *et al.* 2004, 2006, 2007*a*, *b*). Growing evidence shows that phytochemicals may have considerable potential use in animal production (Kong *et al.* 2007*c*, in press, 2009; Li *et al.* 2007). Kong *et al.* (2007*a*, *b*) reported that the Chinese herbal ultra-fine powder and *Acanthopanax senticosus* extract as a dietary additive enhances the cellular and humoral immune responses of weaned piglets by modulating the production of immunocytes, cytokines (including IL-1β, IL-2 and IL-6) and antibodies (including IgG and IgM). *Atractylodes macrophala* Koidz, as a herbal medicine, is traditionally used effectively to treat spleen and stomach diseases (including diarrhoea), and enhance immune function in humans. A polysaccharide complex is the major component of the aqueous extracts from *A. macrophala* Koidz. However, little is known about the effect of the complex (PAM) on the immune status and growth performance in livestock animals.

On the basis of the foregoing, it was hypothesized that PAM, as a dietary additive, may enhance the immune response and health, then increase the growth performance in weaned piglets (Kong *et al.* 2007*a*, *b*). This hypothesis was tested by analysing serum concentrations of IgG, IgM, IL-2 and IL-6, and expression level of IL-1β gene in gut-associated lymphoid tissues (GALTs), as well as growth performance and diarrhoea frequency in weaned piglets in order to develop immunomodulatory feed additives for livestock.

MATERIALS AND METHODS

Polysaccharide preparation

The PAM was isolated from fresh *A. macrophala* Koidz as previously described (Wen 2006). Briefly, sliced rhizomes of *A. macrophala* Koidz grown in Pingjiang county, Hunan Province of China, were extracted three times with boiling water. The supernatant was pooled after being centrifuged at 3000 g/min for 10 min and precipitated three times with ethanol (final concentration: 600 mg/g). The resultant polysaccharide extract was dialysed against several changes of water and then lyophilized by the multifunctional pulp thickener. All these procedures were performed using a TQ Multifunction Abstraction and Concentration Instrument (Liuyang, Hunan, China). The product is a coffee-coloured powder with a molecular weight of 10^4 Da. Content of total polysaccharides was 850 mg/g as determined by the vitriol-anthracene ketone method (Kong *et al.* 2004).

Animals, housing and treatment

One hundred and twenty Landrace × Yorkshire piglets, weaned at 28 days of age (body weight (BW) $7{\cdot}5 \pm 0{\cdot}07$ kg), were balanced for initial BW and ancestry across five treatment groups and fed maize/soybean-based diets formulated on National Research Council (NRC 1998) requirements supplemented with 0 (Control), 3, 6 or 9 g PAM/kg diet, or 0·53 g antibiotics/kg (0·4 g flavomycin/kg + 0·13 g olaquindox/kg as a positive control) (Table 1). Chemical analysis of the experimental diets was conducted as described by Kong *et al.* (2007*c*). There were three pens of piglets (four castrates and four gilts) per treatment group. The piglets were housed in a nursery facility with hard plastic and slatted flooring, and had free access to diets and drinking water. The temperature and relative humidity of the room were maintained at 28 ± 2 °C and 65–75%, respectively (Kong *et al.* in press). The experimental period was 28 days.

The study was carried out in accordance with the Chinese guidelines for animal welfare and experimental protocol approved by the Animal Care Committee, Institute of Subtropical Agriculture, the Chinese Academy of Sciences, Changsha, China (Yin *et al.* 2008*a*).

Growth performance

BWs and feed intakes were measured at the beginning of the trial and twice weekly thereafter. On the basis of these data, average daily gain (ADG), average daily feed intake (ADFI) and feed:gain (F:G) ratio were calculated for the period of days 0–14, 15–28 and 0–28, according to the method of Kong *et al.* (2007*c*).

Diarrhoea frequency

To evaluate the frequency of diarrhoea, the number of pigs per pen that suffered was recorded daily throughout the study. Faecal consistency was monitored twice daily and quantified using a scale ranging from 0 to 3, with 0 = normally shaped faeces, 1 = shapeless (loose) faeces, 2 = thick, liquid (soft) faeces, and 3 = thin, liquid faeces (watery diarrhoea). When the score was higher than 1, the piglet was considered to have diarrhoea. The frequency of diarrhoea per pen was determined as described by Vente-Spreeuwenberga *et al.* (2004).

$$\text{Diarrhoea frequency} = \frac{\text{pigs per pen suffering diarrhoea}}{\text{8 experimental pigs per pen} \times \text{2 days}}$$

The frequency of diarrhoea per treatment group is shown as the mean calculated using the data from three pens per treatment group.

Blood sampling and analysing

On days 14 and 28 after introducing the dietary herbal supplementation, jugular venous blood samples

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Table 1. *Ingredients and nutrient levels of the experimental diets (g/kg, as fed basis)*

Ingredients	Control	Antibiotics*	3 g/kg PAM†	6 g/kg PAM	9 g/kg PAM
Maize	605·3	604·8	602·3	599·3	596·3
Soybean meal	238·5	238·5	238·5	238·5	238·5
Fish meal	50·0	50·0	50·0	50·0	50·0
Plasma globulin meal	20·0	20·0	20·0	20·0	20·0
Whey powder (pure)	40·0	40·0	40·0	40·0	40·0
Soybean oil	6·2	6·2	6·2	6·2	6·2
Premix‡	40·0	40·0	40·0	40·0	40·0
PAM	0	0	3·0	6·0	9·0
Antibiotics	0	0·53	0	0	0
Composition§					
DE (MJ/kg)	16·8	16·7	16·8	16·7	16·7
CP	212·3	212·2	212·3	212·2	212·1
Ca	8·0	7·9	8·0	7·9	7·8
P	7·9	7·9	7·9	7·8	7·8
Lysine	12·4	12·3	12·4	12·3	12·3
Methionine	3·6	3·6	3·5	3·5	3·4
Threonine	8·6	8·6	8·5	8·5	8·4

* Flavomycin + olaquindox, 4 + 1·3.
† Polysaccharides of *Atractylodes macrophala*.
‡ The premix provides following per kg of diets: VD_3 386 IU; VA 3086 IU; VE 15·4 IU; VK_3 2·3 mg; VB_2 3·9 mg; D-calcium pantothenate 15·4 mg; nicotinic acid 23 mg; choline 500 mg; VB_{12} 0·016 mg; Cu (Gly-Cu, 210 mg/g) 17 mg; Fe (Gly-Fe 14 %) 133 mg; Zn (Met-Zn 17·5 %) 133 mg; Mn (Gly-Mn 22 %) 33·3 mg; I ($Ca(IO_3)_2$) 0·83 mg; choline chloride (50 %) 1000 mg; antimildew/acidifying agent (propanoic acid) 2·5 g; antioxidant (ethyoxyquin) 200 mg; edulcorant (crystallose) 400 mg; flavour 600 mg; salt 1·3 g; lysine·HCl 2·7 g; methionine 660 mg; threonine 440 mg.
§ Analysed values, except DE which was calculated.

(10 ml per piglet) were withdrawn randomly from two piglets (one castrate and one gilt, the chosen pigs for collecting blood samples were different on days 14 and 28) per pen by venipuncture into plastic uncoated tubes between 08·00 and 10·00 h. Serum samples were obtained by centrifugation at 3000 g for 10 min and stored at −20 °C until analysis. The serum concentrations of IgG, IgM, IL-2 and IL-6 were determined using radio-immunodiffusion kits (Jiancheng Bioengineering Ltd, Nanjing, Jiangsu, P. R. China), respectively, according to the manufacturer's instructions.

Sampling and analysing of jejunal mucosa and mesentery lymph node

After the collection of blood samples on day 28, the two piglets (one castrate and one gilt) per pen were slaughtered under general anaesthesia (administered via intravenous injection of 4 % sodium pentobarbital solution at 40 mg/kg BW) and jugular puncture (Kong *et al*. 2007*b*) and then immediately eviscerated. Approximately 1 g of both jejunal mucosa and mesentery lymph node were collected respectively, then immediately frozen in liquid nitrogen and stored at −80 °C until the extraction of total RNA.

The expression levels of IL-1β mRNA in jejunal mucosa and mesentery lymph node were determined by reverse transcription–polymerase chain reaction (RT–PCR). Total RNA was isolated using the TRIZOL Reagent (Invitrogen). The quantity of the RNA was checked by measuring the optical density at 260 and 280 nm. Total RNA (10 mg) was reverse-transcribed to cDNA using the RevertAid First Strand synthesis kit (Fermentas). The cDNA samples were amplified using PCR reagents (Taq polymerase and dNTP Mix, MBI Fermentas, USA). Porcine glyceraldehydes-3-phosphate dehydrogenase (GAPDH) was used as a housekeeping gene to normalize expression of the IL-1β gene (Huang *et al*. 2007). The primers used were as follows: porcine IL-1β (forward, 5′-GGCTA ACTACGGTGA CAACA ATAAT G-3′; reverse, 5′-CAGATTCTTT CCCTT GATCC CTAA-3′) and porcine GAPDH (forward, 5′-GA-AGG TCGGA GTGAA CGGAT T-3′; reverse, 5′-GCCTT CTCCA TGGTC GTGA-3′). Each 25 μl of the PCR reaction mixture contained: 12·3 μl of sterile and de-ionized H_2O, 2·5 μl of 10 × PCR buffer, 2·5 μl of dNTP mix (2 mmol/l), 1·25 μl each of forward and reverse IL-1β primers (10 μmol/l), 0·8 μl each of forward and reverse GAPDH primers (10 μmol/l), 0·1 μl of TaqDNA polymase (5 units/μl), 1·5 μl of $MgCl_2$ (25 mmol/l), and 2 μl of 125 pg to 1·25 μg cDNA. The amplification conditions were as follows: 95 °C, 2 min; 95 °C, 15 s; 60·6 °C, 90 s and 72 °C, 45 s for 30 cycles; 72 °C, 10 min. The PCR product of IL-1β was

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Table 2. *Effect of dietary supplementation with polysaccharides of* A. macrophala (*PAM*) *on growth performance in weaned piglets* ($n=3$)

Items	Control	Antibiotics*	3 g/kg PAM	6 g/kg PAM	9 g/kg PAM	*P* (linear)	*P* (quadratic)
Body weight (BW)							
Day 0	7·5±0·01	7·5±0·28	7·5±0·12	7·5±0·21	7·5±0·14	0·975	0·993
Day 14	11·1±0·21	10·9±0·28	11·2±0·25	11·2±0·37	11·2±0·14	0·778	0·941
Day 28	14·9±0·22	15·5±0·31	15·7±0·50	16·5±0·14	16·0±0·21	0·016	0·029
Days 0–14 after the supplementation							
ADFI (g)	370±4	371±34	393±7	388±9	375±20	0·648	0·277
ADG (g)	259±15	257±48	264±10	272±26	259±10	0·830	0·890
F:G	1·4±0·08	1·5±0·14	1·5±0·05	1·5±0·10	1·4±0·02	0·990	0·884
Diarrhoea incidence (proportions)	0·11±0·036	0·12±0·008	0·13±0·005	0·08±0·044	0·13±0·008	0·991	0·972
Days 14–28 after the supplementation							
ADFI (g)	656±14	622±64	669±53	687±10	696±6	0·254	0·534
ADG (g)	275±7	302±13	327±31	374±20	352±18	0·011	0·030
F:G	2·4±0·01	2·2±0·06	2·1±0·03	1·9±0·07	2·0±0·12	0·004	0·003
Diarrhoea incidence (proportions)	0·04±0·012	0·01±0·003	0·02±0·012	0·02±0·007	0·01±0·003	0·044	0·144
Overall							
ADFI (g)	513±5	495±45	535±27	542±5	522±18	0·522	0·426
ADG (g)	267±7	286±14	295±14	323±6	306±6	0·008	0·014
F:G	1·9±0·05	1·9±0·05	1·8±0·02	1·7±0·03	1·7±0·09	0·008	0·033
Diarrhoea incidence (proportions)	0·08±0·020	0·06±0·005	0·08±0·007	0·05±0·025	0·07±0·004	0·549	0·834

* Flavomycin + olaquindox, 4 + 1·3.

508 bp. PCR products (10 μl) and 2 μl of loading dye were mixed. Subsequently, PCR products were electrophoresed on 15 g/l agarose gel containing ethidium bromide (0·5 μg/ml) for 1 h at 100 V. A low DNA mass ladder (MBI Fermentas) was utilized as a molecular weight marker. DNA bands were visualized and densitometric analysis was performed using a UV transilluminator (UVP Biolmaging System, Upland, CA, USA).

Statistical analysis

All data were subjected to analysis of variance (ANOVA) appropriate for randomized complete block design by using the GLM procedure of SAS (SAS Institute, Cary, NC). Orthogonal polynomial contrasts were used to determine linear and quadratic effects of increasing PAM supplementation levels on all measurements. $P<0·05$ was taken to indicate statistical significance.

RESULTS

Effect of PAM on growth performance

Changes of growth performance in weaned piglets after the supplementation are listed in Table 2. There was no difference in BW on days 0 and 14, ADFI, ADG or F:G during days 0–14, and ADFI during days 14–28 and overall among five treatments. Increasing PAM supplementation level resulted in increased BW on day 28 (quadratic, $P<0·05$), and increased ADG during days 14–28 (quadratic, $P<0·05$) and overall (quadratic, $P<0·05$), and decreased F:G during days 14–28 (quadratic, $P<0·01$) and overall (quadratic, $P<0·05$). Supplementation of 6 g PAM/kg increased BW on day 28, and improved ADG and feed conversion ratio during days 14–28 and overall compared with the control and antibiotics groups ($P<0·05$). Overall, 9 g PAM/kg also improved F:G in comparison with piglets fed the control or antibiotics diet.

With increasing PAM supplementation level, diarrhoea incidence was decreased (linear, $P<0·05$) during days 14–28. The antibiotics and 9 g PAM/kg groups had the lowest diarrhoea incidence among the five groups during days 14–28 ($P<0·05$). PAM or antibiotics had no effect on diarrhoea incidence during days 0–14 and overall.

Effects of PAM on serum concentrations of IgG, IgM, IL-2 and IL-6

Changes in serum concentrations of IL-2, IL-6, IgG and IgM in weaned piglets following supplementation are listed in Table 3. There was no difference in serum IgG and IgM on day 14, and serum IL-2, IL-6, IgG and IgM on day 28 among the five groups. With increasing PAM supplementation level, serum concentrations of IL-2 (quadratic, $P<0·01$) and IL-6 (quadratic, $P<0·05$) were increased on day 14. On

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Table 3. *Effect of dietary supplementation with polysaccharides of* A. macrophala (*PAM*) *on serum concentrations of IL-2, IL-6, IgG and IgM in weaned piglets* ($n=6$)

Items	Control	Antibiotics*	3 g/kg PAM	6 g/kg PAM	9 g/kg PAM	*P* (linear)	*P* (quadratic)
Day 14 after the supplementation							
IL-2 (ng/ml)	2·3±0·03	3·1±0·62	3·5±0·15	3·4±0·22	3·5±0·35	0·004	0·003
IL-6 (pg/ml)	269±32	283±24	356±17	363±28	383±21	0·006	0·023
IgG (mg/dl)	270±15	282±23	282±9	332±26	296±9	0·152	0·259
IgM (mg/dl)	45±2·4	48±2·9	49±3·2	54±3·0	49±2·3	0·121	0·216
Day 28 after the supplementation							
IL-2 (ng/ml)	2·7±0·30	3·0±0·12	3·0±0·34	3·5±0·05	3·2±0·20	0·112	0·237
IL-6 (pg/ml)	290±22	303±45	310±32	340±24	326±17	0·245	0·492
IgG (mg/dl)	303±15	359±31	338±45	381±8	360±18	0·117	0·267
IgM (mg/dl)	47±0·7	50±4·7	49±3·7	56±2·2	52±4·2	0·097	0·264

* Flavomycin + olaquindox, 4 + 1·3.

Table 4. *Effect of dietary supplementation with polysaccharides of* A. macrophala (*PAM*) *on IL-1*β *gene expression level of jejunum mucous and mesentery lymphatic nodes in weaned piglets* ($n=6$)

Items	Control	Antibiotics*	3 g/kg PAM	6 g/kg PAM	9 g/kg PAM	*P* (linear)	*P* (quadratic)
IL-1β/GAPDH in jejunal mucosa	0·34±0·035	0·61±0·029	0·60±0·037	0·66±0·036	0·75±0·031	<0·001	<0·001
IL-1β/GAPDH in lymph nodes	0·50±0·095	0·58±0·015	0·56±0·022	0·664±0·042	0·67±0·025	0·010	0·044

* Flavomycin + olaquindox, 4 + 1·3.

day 14, serum concentrations of IL-2 did not differ from the antibiotics group. Serum concentrations of IL-6 were greater ($P<0·05$) in piglets fed the 9 g PAM/kg diet compared with piglets fed the antibiotics diet.

*Effect of PAM on expression level of IL-1*β *mRNA in jejunal mucosa and mesentery lymph nodes*

The expression levels of IL-1β mRNA in jejunal mucosa and mesentery lymph nodes are shown in Table 4. Increasing PAM supplementation level increased IL-1β expression in jejunal mucosa (quadratic, $P<0·001$) and lymph nodes (quadratic, $P<0·05$). Referring to the jejunal mucosa, IL-1β expression in piglets supplemented 9 g PAM/kg were greater ($P<0·05$) than those in the control and antibiotics, as did jejunal mucosa in piglets supplemented with 6 g PAM/kg than that in control group. Expression levels of IL-1β gene from mesentery lymph nodes in piglets supplemented 6 or 9 g PAM/kg were greater ($P<0·05$) than that in the control group. There was no difference among antibiotics and PAM groups.

DISCUSSION

Growth of animals is an outcome of complex metabolic transformations, including glucose and amino acid utilization, intracellular protein turnover and fat deposition as well as their regulation by hormones and other factors (Jobgen *et al.* 2006). In the current study, dietary supplementation of PAM improved ADG and feed efficiency in weaned pigs. The current results agree with previous research which showed that PAM supplementation improved the growth performance of ducks (Wen 2006). These results suggest that PAM may be effective as a novel dietary additive in weaned piglets.

Immediately after weaning, piglets normally consume little feed (Wu *et al.* 1996) and exhibit tremendous stress, as indicated by high circulating levels of cortisol (Wu *et al.* 2000). This catabolic state can contribute to impaired immunity and compromised growth (Li *et al.* 2007). A lack of antibiotics or PAM in the diet probably results in an increased amount of pathogenic micro-organism (e.g. *Escherichia coli*) in the piglet intestine, therefore leading to intestinal dysfunction and diarrhoea. In the current study, dietary supplementation with PAM

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decreased the diarrhoea frequency during days 14–28. These findings suggest that PAM could inhibit gut pathogens in piglets. Similarly, some research has shown that the Chinese herbal ultra-fine powder (He *et al.* 2008) and *A. senticosus* extract (Yin *et al.* 2008*b*; Fang *et al.* 2009) could promote the development of the normal gut microbiota, suppress bacterial pathogens and lead to a healthy intestinal environment.

Until now, little research has been conducted to investigate the effect of dietary PAM supplementation on humoral immunity in weaned piglets. IgG and IgM, the major serum immunoglobulins, are key components of the humoral immunity in all mammals (Li *et al.* 2007). In the present study, dietary supplementation of PAM had no effect on the concentration of IgG and IgM. The current data is in contrast to Wen (2006), who reported that 2 g PAM/kg increased serum IgG concentration of Sprague–Dawley rats and increased serum IgG and IgA concentrations of weaned pigs. The reason for the discrepancy might be associated with the amount of PAM supplemented to the experimental diets and the varieties of experimental animals.

Cytokines are important molecules mediating antibody production and thus the immune response in the host. IL-2 and IL-6 are involved in immune regulation and host defence (Li *et al.* 2007). IL-2 regulates the differentiation and activation of T cells, B cells, natural and lymphocyte-activated killer cells, monocytes and macrophages that are involved in cellular and humoral immune responses (Wang 1991; Liu & Liu 1992). IL-6 plays a critical role in B-cell differentiation (Liu & Liu 1992). Compelling evidence shows that many types of bioactive polysaccharides can increase the production of cytokines (including IL-2 and IL-6), which play important roles in immune responses (Liu & Liu 1992). In the current study, PAM supplementation increased serum concentrations of IL-2 and IL-6 on day 14. A dose of 6 or 9 g PAM/kg kept the highest serum concentrations of IL-2 and IL-6. The current findings are in agreement with Wen (2006), who reported that 2 g PAM/kg improved serum IL-2 concentration of weaned pigs. In addition, Wen (2006) found that PAM administration increased serum IL-2 and IL-6 concentrations of Cherry Valley ducks. Thus, the current results suggest that PAM provokes a cytokine response in weaned piglets.

The gut is a major immune organ in mammals. Specifically, the T and B lymphocytes proliferate and mature in the GALT, mounting a successful immune response to antigens. Many studies have shown that the GALT is composed of immune cells and lymph nodes, conferring both non-specific and specific immune functions (Poussier & Julius 1994). IL-1β is produced by monocytes, macrophages and dendritic cells, which are present in the intestinal mucosa of weaned piglets (Deng *et al.* 2007). IL-1β probably plays a key role in activation, proliferation and differentiation of T-cells, as well as improving both specific and non-specific immune responses in weaned piglets. The current study showed for the first time that dietary supplementation with PAM enhanced mRNA levels for IL-1β gene in jejunal mucosa and mesentery lymph nodes, which may provide a molecular mechanism for the beneficial effect of PAM on increasing gut defence capacity. PAM can exert its effect not only on the circulating immunocytes but also on the gut-associated lymphatic immune system. This will aid in protecting the piglet small intestine from infections by food- and air-borne pathogens (He *et al.* 2008; Yin *et al.* 2008*a*).

In conclusion, dietary supplementation with PAM increased ADG and feed efficiency, while decreasing the diarrhoea frequency in weanling piglets. Dietary supplementing PAM also increased serum concentrations of cytokines (IL-2 and IL-6) and expression level of IL-1β mRNA in GALT. The current findings indicate PAM is effective in improving growth performance and cytokine response, which suggests that PAM can be used as a diet additive for weanling piglets.

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REFERENCES

Adjiri-Awere, A. & Van Lunen, T. A. (2005). Subtherapeutic use of antibiotics in pork production: risks and alternatives. *Canadian Journal of Animal Science* **85**, 117–130.

Deng, Z. Y., Zhang, J. W., Wu, G. Y., Yin, Y. L., Ruan, Z., Li, T. J., Chu, W. Y., Kong, X. F., Zhang, Y. M., Fan, Y. W., Liu, R. & Huang, R. L. (2007). Dietary supplementation with polysaccharides from *Semen cassiae* enhances immunoglobulin production and interleukin gene expression in early-weaned piglets. *Journal of Science of Food and Agriculture* **87**, 1868–1873.

Fang, J., Yan, F. Y., Kong, X. F., Ruan, Z., Liu, Z. Q., Huang, R. L., Li, T. J., Geng, M. M., Yang, F., Zhang, Y. Z., Li, P., Gong, J., Wu, G. Y., Fan, M. Z., Liu, Y. L., Hou, Y. Q. and Yin, Y. L. (2009). Dietary supplementation of *Acanthopanax senticosus* extract enhances

gut health in weanling piglets. *Livestock Science* **123**, 268–275.

He, Q. H., Kong, X. F., Hou, Y. Q., Yin, Y. L., Yin, F. G., Liu, H. J., Li, T. J., Huang, R. L., Yu, H. & Gong, J. H. (2008). Effects of Chinese herbal ultra-fine powder as a dietary additive on gut microbiota in early-weaned piglets. In *Recent Progress in Medicinal Plants* (Multi-Volume Series), pp. 453–465. Houston, TX: Studium Press, LLC.

Huang, R. L., Deng, Z. Y., Yang, C. B., Yin, Y. L., Xie, M. Y., Wu, G. Y., Li, T. J., Li, L. L., Tang, Z. R., Kang, P., Hou, Z. P., Deng, D., Xiang, H., Kong, X. F. & Guo, Y. M. (2007). Dietary oligochitosan supplementation enhances immune status of broilers. *Journal of Science of Food and Agriculture* **87**, 153–159.

Jobgen, W. S., Fried, S. K., Fu, W. J., Meininger, C. J. & Wu, G. (2006). Regulatory role for the arginine–nitric oxide pathway in metabolism of energy substrates. *Journal of Nutritional Biochemistry* **17**, 571–588.

Kong, X. F., Hu, Y. L., Rui, R., Wang, D. Y. & Li, X. R. (2004). Effects of Chinese herbal medicinal ingredients on peripheral lymphocyte proliferation and serum antibody titer after vaccination in chicken. *International Journal of Immunopharmacology* **4**, 975–982.

Kong, X. F., Hu, Y. L., Yin, Y. L., Wu, G. Y., Rui, R., Wang, D. Y. & Yang, C. B. (2006). Chinese herbal ingredients are effective immune stimulators for chickens infected with the Newcastle disease virus. *Poultry Science* **85**, 2169–2175.

Kong, X. F., Wu, G. Y., Liao, Y. P., Hou, Z. P., Liu, H. J., Yin, F. G., Li, T. J., Huang, R. L., Zhang, Y. M., Deng, D., Xie, M. Y., Deng, Z. Y., Xiong, H., Ruan, Z., Kang, P., Yang, C. B., Yin, Y. L. & Fan, M. Z. (2007*a*). Dietary supplementation with Chinese herbal ultra-fine powder enhances cellular and humoral immunity in early-weaned piglets. *Livestock Science* **108**, 94–98.

Kong, X. F., Yin, Y. L., Wu, G. Y., Liu, H. J., Yin, F. G., Li, T. J., Huang, R. L., Ruan, Z., Xiong, H., Deng, Z. Y., Xie, M. Y., Liao, Y. P. & Kim, S. W. (2007*b*). Dietary supplementation with *Acanthopanax senticosus* extract modulates cellular and humoral immunity in weaned piglets. *Asian-Australian Journal of Animal Science* **20**, 1453–1461.

Kong, X. F., Wu, G. Y., Liao, Y. P., Hou, Z. P., Liu, H. J., Yin, F. G., Li, T. J., Huang, R. L., Zhang, Y. M., Deng, D., Kang, P., Wang, R. X., Tang, Z. R., Yang, C. B., Deng, Z. Y., Xiong, H., Chu, W. Y., Ruan, Z., Xie, M. Y. & Yin, Y. L. (2007*c*). Effects of Chinese herbal ultra-fine powder as a dietary additive on growth performance, serum metabolites and intestinal health in early-weaned piglets. *Livestock Science* **108**, 272–275.

Kong, X. F., Yin, Y. L., He, Q. H., Yin, F. G., Liu, H. J., Li, T. J., Huang, R. L., Geng, M. M., Ruan, Z., Deng, Z. Y., Xie, M. Y. & Wu, G. (in press). Dietary supplementation with Chinese herbal powder enhances ileal digestibilities and serum concentrations of amino acids in young pigs. *Amino Acids*.

Kong, X. F., Yin, F. G., He, Q. H., Liu, H. J., Li, T. J., Huang, R. L., Fan, M. Z., Liu, Y. L., Hou, Y. Q., Li, P., Ruan, Z., Deng, Z. Y., Xie, M. Y., Xiong, H. & Yin, Y. L. (2009). *Acanthopanax senticosus* extract as a dietary additive enhances the apparent ileal digestibility of amino acids in weaned piglets. *Livestock Science* **123**, 261–267.

Li, L. L., Zhang, B., Zhu, N. S., Li, A. K., Zhang, F. S., Yin, Y. L., Peng, H. Z. & Zhang, P. (2007). Effects of *Atractylodes macrocephala koidz* polysaccharides on partial immuno-logic functions in early-weaned piglet. *Agricultural Modernization Research* **28**, 487–489.

Liu, Y. W. & Liu, N. (1992). The interleukin's functions and clinical applications. *Chinese Journal of Veterinary Science and Technology* **12**, 46–47.

National Research Council. (1998). *Nutrient Requirements for Swine*, 10th edn. Washington, DC: National Academy Press.

Poussier, P. & Julius, M. (1994). Thymus-independent T-cell development and selection in the intestinal epithelium. *Annual Review of Immunology* **12**, 521–553.

Vente-Spreeuwenberga, M. A. M., Verdonkb, J. M. A. J., Koninkxc, J. F. J. G., Beynend, A. C. & Verstegene, M. W. A. (2004). Dietary protein hydrolysates vs the intact proteins do not enhance mucosal integrity and growth performance in weaned piglets. *Livestock Production Science* **85**, 151–164.

Wang, S. R. (1991). Advances in interleukin. *Journal of Southwest University for Nationalities: Natural Science* **17**, 83–86.

Wen, G. H. (2006). *Effects of polysaccharide of* Atractylodes macrocephala *Koidz on growth performance and immune function of Cherry Valley ducks*. PhD Thesis, Hunan Agricultural University, Hunan, P.R. China.

Wu, G., Meier, S. A. & Knabe, D. A. (1996). Dietary glutamine supplementation prevents jejunal atrophy in weaned pigs. *Journal of Nutrition* **126**, 2578–2584.

Wu, G., Flynn, N. E., Knabe, D. A. & Jaeger, L. A. (2000). A cortisol surge mediates the enhanced polyamine synthesis in porcine enterocytes during weaning. *American Journal of Physiology* **279**, R554–R559.

Yin, Y. L., Tang, Z. R., Sun, Z. H., Liu, Z. Q., Li, T. J., Huang, R. L., Ruan, Z., Deng, Z. Y., Gao, B., Chen, L. X., Wu, G. Y. & Kim, S. W. (2008*a*). Effect of galacto-mannan-oligosaccharides or chitosan supplementation on cytoimmunity and humoral immunity in early-weaned piglets. *Asian-Australian Journal of Animal Science* **21**, 723–731.

Yin, F. G., Yin, Y. L., Kong, X. F., Liu, Y. L., He, Q. H., Li, T. J., Huang, R. L., Hou, Y. Q., Shu, X. G., Tan, L. X., Chen, L. X., Gong, J. H., Kim, S. W. & Wu, G. Y. (2008*b*). Dietary supplementation with *Acanthopanax senticosus* extract modulates gut microflora in weaned piglets. *Asian-Australian Journal of Animal Science* **21**, 1330–1338.