Received: 21 July 2019 | Revised: 7 September 2019 | Accepted: 25 September 2019

DOI: 10.1111/jpn.13244

SPECIAL ISSUE ARTICLE

Pigs

Journal of Animal Physiology and Animal Nutrition | WILEY

Effects of astragalus and ginseng polysaccharides on growth performance, immune function and intestinal barrier in weaned piglets challenged with lipopolysaccharide

Kangli Wang[1] | Haoran Zhang[1] | Qianjie Han[1] | Junhong Lan[1] | Guangyong Chen[1] | Guangtian Cao[2] | Caimei Yang[1]

[1]Key Laboratory of Applied Technology on Green-Eco-Healthy Animal Husbandry of Zhejiang Province, The Zhejiang Provincial Engineering Laboratory for Animal Health and Internet Technology, College of Animal Science and Technology, Zhejiang A & F University, Hangzhou, China

[2]College of Standardization, China Jiliang University, Hangzhou, China

Correspondence
Caimei Yang, Key Laboratory of Applied Technology on Green-Eco-Healthy Animal Husbandry of Zhejiang Province, The Zhejiang Provincial Engineering Laboratory for Animal Health and Internet Technology, College of Animal Science and Technology, Zhejiang A & F University, Hangzhou, China.
Email: yangcaimei2012@163.com

Funding information
Key Research Project of Zhejiang Province, Grant/Award Number: 2017C02005; Zhejiang Provincial Key Research and Development Program, Grant/Award Number: 2019C02051; National Natural Science Foundation of China, Grant/Award Number: 31501985

Abstract

This experiment was conducted to evaluate the effects of astragalus polysaccharides (Aps) and ginseng polysaccharide (Gps) on growth performance, liver function, immune function, TLR4 signalling pathways and intestinal barrier in weaned piglets challenged with lipopolysaccharide (LPS). In an experiment spanning 28 days, 180 weaned piglets were randomly divided into three treatment groups: basal diet (Con), basal diet supplemented with 800 mg/kg Gps (Gps) and basal diet supplemented with 800 mg/kg Aps (Aps). At the end of the experiment, 12 piglets of each group were selected; half (n = 6) were intraperitoneally injected with LPS and half with normal saline. Dietary supplementation with Aps and Gps significantly increased (p < .05) the average daily gain and feed conversion rate. Lipopolysaccharide challenge increased (p < .05) expression of serum urea nitrogen (BUN), alanine aminotransferase (ALT), aspartate aminotransferase (AST), interleukin-1β (IL-1β) and tumour inflammatory factor-α (TNF-α), but decreased (p < .05) serum superoxide dismutase (SOD) level, total antioxidant capacity (T-AOC) and immunoglobulin A (IgA) expression. Lipopolysaccharide-challenged piglets fed with Aps or Gps had lower (p < .05) BUN, ALT, AST, IL-1β and TNF-α levels and greater (p < .05) SOD, T-AOC and IgA levels. Lipopolysaccharide challenge increased (p < .05) the expression of TLR4, MyD88 and NF-κB, and LPS-challenged piglets fed diets supplemented with Aps or Gps increased TLR4 and MyD88 and decreased NF-κB expression. Lipopolysaccharide challenge reduced (p < .05) the jejunal villus height, and piglets fed with Aps or Gps had increased (p < .05) jejunal villus height. Supplementation with Aps or Gps enhanced the expression of occludin and claudin in challenged or unchallenged piglets. In conclusion, dietary supplementation with Aps or Gps enhanced piglet growth performance, alleviated liver dysfunction and reduced immunological stress caused by LPS, as well as increased the intestinal barrier function.

KEYWORDS

astragalus polysaccharides, ginseng polysaccharide, immune function, intestinal barrier, lipopolysaccharide, weaning piglet

Wang and Zhang are contributed equally to this work.

1 | INTRODUCTION

Antibiotics have been used in feed since 1950s to improve body gain and feed conversion, and reduce diarrhoea and the mortality rate in pigs (Cromwell, 2002; Zimmerman, 1986). However, antibiotic residues and drug-resistance caused by antibiotics have increased awareness and promoted the development of antibiotic substitutes (Ferket, Lyons, & Jacques, 2004; Patterson, Chapman, Hegedus, Barchia, & Chin, 2005). In recent years, plant polysaccharides, as a kind of immunopotentiator and metabolic regulator, have been attracting increasing attention. Yan, Meng, Lee, Wang, and Kim (2011) reported that ginseng polysaccharide (Gps) altered the diversity and composition of gut microbiota in mice with antibiotic-associated diarrhoea, balanced metabolic processes and promoted the recovery of mucosa in broiler chickens. Zhou et al. (2017) reported that an herbal formulation containing astragalus polysaccharides (Aps) significantly improved the expression of TNF-α, IFN-γ and IL-2 in mice. Liu, Zhao, and Luo (2017) reported that oxidant stress can be inhibited via the up-regulation of antioxidant factors by extract of the dried root of *Astragalus membranaceus* in a myocardial ischaemic rat model.

Lipopolysaccharides (LPS) are the primary constituent of the outer membrane surface of almost all Gram-negative bacteria and are essential for the physical integrity and biological function of the bacterial outer membrane (Xia et al., 2018). When *Escherichia coli* multiply or die, LPS are released from the bacterial membrane surface, leading to natural or innate immune responses of the body, resulting in an increase in the concentration of inflammatory cytokines as well as a reduction in the immune function of the body (Alexander & Rietschel, 2001; Medzhitov, 2002; Tang et al., 2013). At present, studies have shown that polysaccharides can alleviate body damage induced by LPS. Lentinan can ameliorate intestinal inflammation though inhibiting inflammatory signalling pathways (Toll-like receptor 4 and nucleotide-binding oligomerization domain protein) and pro-inflammatory cytokines (tumour necrosis factor-α, interleukin-1β and interleukin-6) expression (Wang, Wang, et al., 2019). Wang et al. (2015) showed that the appropriate dose of *Astragalus polysaccharide* promoted growth and immunomodulate effect in LPS-infected broiler chicks. As well as, *Astragalus polysaccharide* may inhibit depressive-like behaviours and inflammatory response by regulating NF-kappa B and MAPK signalling pathways in LPS-induced rats (Li et al., 2018).

Pharmacological and clinical studies have shown that plant polysaccharides have many functions, such as immune regulation, anti-tumour activity, anti-inflammatory activity and anti-viral functions (Yin, Zhang, & Li, 2019). However, the reports about plant polysaccharides are mainly focused on the immune function studied in vitro or in mice, but little is known about the effects of plant polysaccharides in weaned piglets. The current study was conducted to assess the effects of Aps and Gps on growth performance, liver function, immune function, TLR4 signalling pathways and intestinal barrier function in weaned piglets. This study will provide a theoretical basis for the development of ASP and GSP into a new type of resistance product.

2 | MATERIALS AND METHODS

2.1 | Animals and treatments

The experimental scheme was approved by the Ethics Committee of Zhejiang A & F University, Hangzhou, China. A total of 180 weaned piglets (Duroc × Landrace × Yorkshire) were randomly divided into three treatment groups, with six pens in each group and 10 piglets per pen. The experiment lasted for 28 days. The three groups were as follows: control group (Con), fed a basal diet; Aps group, fed the basal diet supplemented with 800 mg/kg Aps; and Gps group, fed the basal diet supplemented with 800 mg/kg Gps.

On d 29, 12 healthy piglets were selected from each group (totalling 36 piglets) and divided into two treatments. Six piglets were intraperitoneally injected with LPS at 25 μg/kg body weight, and the remaining six piglets were intraperitoneally injected with normal saline (Wang, Chen, et al., 2019). The LPS treatments were labelled LPS-Con, LPS-Aps and LPS-Gps, respectively, and the normal saline treatment was labelled NS-Con, NS-Aps and NS-Gps respectively.

The basal diet was designed to meet the nutritional requirements of NRC (1998) and contained no antibiotics (Table 1). Water and feed were provided ad libitum. All pigs were housed in a room with the temperature controlled between 25°C and 28°C.

2.2 | Preparation of Aps and Gps

The polysaccharide had a purity of 80% and a molecular weight of 20,000–60,000. Aps consisted of hexanoic acid, glucose, fructose,

TABLE 1 Composition and nutrient levels of the basal diet

Ingredients	Content, %	Nutrient level	Content
Corn	55.00	DE, MJ/Kg	14.17
Wheat midding	3.50	CP, %	20.35
Phospholipid	2.00	Lys, %	1.34
Whey powder	5.00	Met + Cys, %	0.77
Extruded soybean	7.30	Thr, %	0.80
Soybean meal	18.50	Ca, %	0.95
Fish meal	5.00	TP, %	0.65
Dicalcium phosphate	1.00	AP, %	0.48
Limestone	1.10		
NaCl	0.1		
L-Lysine HCl	0.35		
DL-Methionine	0.15		
Vitamin–mineral Premix[a]	1.00		
Total	100		

[a]Supplied the following per kg of diet: vitamin A, 10,000 IU; vitamin D_3, 400 IU; vitamin E, 10 mg; pantothenic acid, 15 mg; vitamin B_6, 2 mg; biotin, 0.3 mg; folic acid, 3 mg; vitamin B_{12}, 0.009 mg; ascorbic acid, 40 mg; Fe, 150 mg; Cu, 130 mg; Mn, 60 mg; Zn, 120 mg; I, 0.3 mg; and Se, 0.25 mg.

TABLE 2 Effects of the Aps and Gps on growth performance in weaned piglets

Item	Treatment[1]			SEM[2]	p value
	Con	Aps	Gps		
Initial BW, kg	8.74	9.08	8.94	0.70	.50
Final BW, kg	17.52[b]	18.78[a]	18.61[a]	3.39	.04
ADG, g	325.16[b]	359.26[a]	358.29[a]	4.44	.01
ADFI, g	640.40[a]	614.69[b]	643.41[a]	16.16	<.01
F:G	1.98[a]	1.76[c]	1.83[b]	6.16	.01

Note: In the same row, values with no letter or the same letter superscripts mean no significant difference ($p > .05$), while with different letter superscripts mean significant difference ($p < .05$).
[1]Con, Aps and Gps represent the piglets supplemented with basal diet, piglets supplemented with the astragalus polysaccharides and piglets supplemented with ginseng polysaccharide respectively.
[2]Pooled *SEM*; $n = 6$ per treatment.

rhamnose, arabinose and galacturonan. Gps consisted of rhamnolipid, xylose, glucose and galactose. Aps and Gps were supplied by Vegamax Biotechnology.

2.3 | Growth performance

According to our previous operations (Yang et al., 2018), piglets were weighed individually at the beginning and on d 28 of the experiment. Daily intake was recorded. Average daily feed intake (ADFI), average daily gain (ADG) and feed to gain (F:G) for each pen were calculated.

2.4 | Sample collection

At 1.5 hr and 3 hr after the LPS challenge, all 36 piglets were used for blood sample collection. Samples were taken from the front cavity vein and centrifuged (3,000 *g*, 10 min) at 4°C; then, the serum was separated and promptly stored at −20°C for further analysis. All 36 piglets were slaughtered after LPS challenge 4 hr. Jejunum segments were collected and preserved in 4% paraformaldehyde and stored at 4°C. The jejunum mucosa was collected in Eppendorf tubes and stored at −80°C.

2.5 | Serum parameter analysis

Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and serum urea nitrogen (BUN) were determined using kits following the manufacturer's instructions. Expression of immunoglobulin A (IgA), immunoglobulin M (IgM), immunoglobulin G (IgG), interleukin-1β (IL-1β) and TNF-α (tumour necrosis factor-α) was assayed using immunoturbidimetry kits. All kits were purchased from the Jiancheng Biological Engineering Research Institute, Nanjing, China.

2.6 | Morphological analysis of the jejunum

The jejunum was fixed in 10% formaldehyde solution, routinely sampled, dehydrated, paraffin-embedded, sliced (4 μm thick), stained with haematoxylin–eosin and observed under an optical microscope, and the main descriptive parts were photographed (Microscope: Nikon Eclipse ci; imaging system: Nikon digital sight DS-FI2). The villus height and crypt depth of each intestinal sample were measured at 10x visual fields, and the villus height/crypt depth ratio (VCR) was calculated for each treatment group. Pathological changes were also observed in the same field of vision.

2.7 | Jejunal immunohistochemical analysis

Immunohistochemical analyses to determine TLR4, MyD88 and NF-κB expression were performed on each specimen. Three 200× visual fields were randomly selected for photography. Three areas of uniform dimension and contrast were taken as standard in all photographs for analysis using the Image-Pro 6.0 software. By analysing the circular areas in each photograph, the cumulative optical density of each positive circle was obtained, and the average value was calculated.

2.8 | Intestinal barrier function analysis

The expression of tight junction proteins (occludin and claudin) in the jejunal mucosa was determined by Western blotting (WB). Total protein was obtained from dissolved cells and tissues. The equivalent number of lysates was submitted to SDS polyacrylamide gel electrophoresis and transferred to the PVDF membrane (MiLople). After 2 hr of sealing with 5% skimmed milk, the membrane was incubated at 4°C, overnight with corresponding antibodies, and then incubated with the two antibodies at 25°C for 2 hr. The expected protein bands were detected using Image Quant™ LAS 4000 (GE Healthcare Life Sciences). The relative abundance of target protein (normalized to β-actin) was analysed using the Image-Pro Plus 6.0 software.

2.9 | Statistical analysis

One-way ANOVA was performed using SPSS Statistics 21.0 (SPSS), and the GraphPad Prism 6 (GraphPad Software) was used to make histograms. The data of growth performance are presented as least square means plus pooled *SEM*. *p* value of <.05 was considered statistically significant.

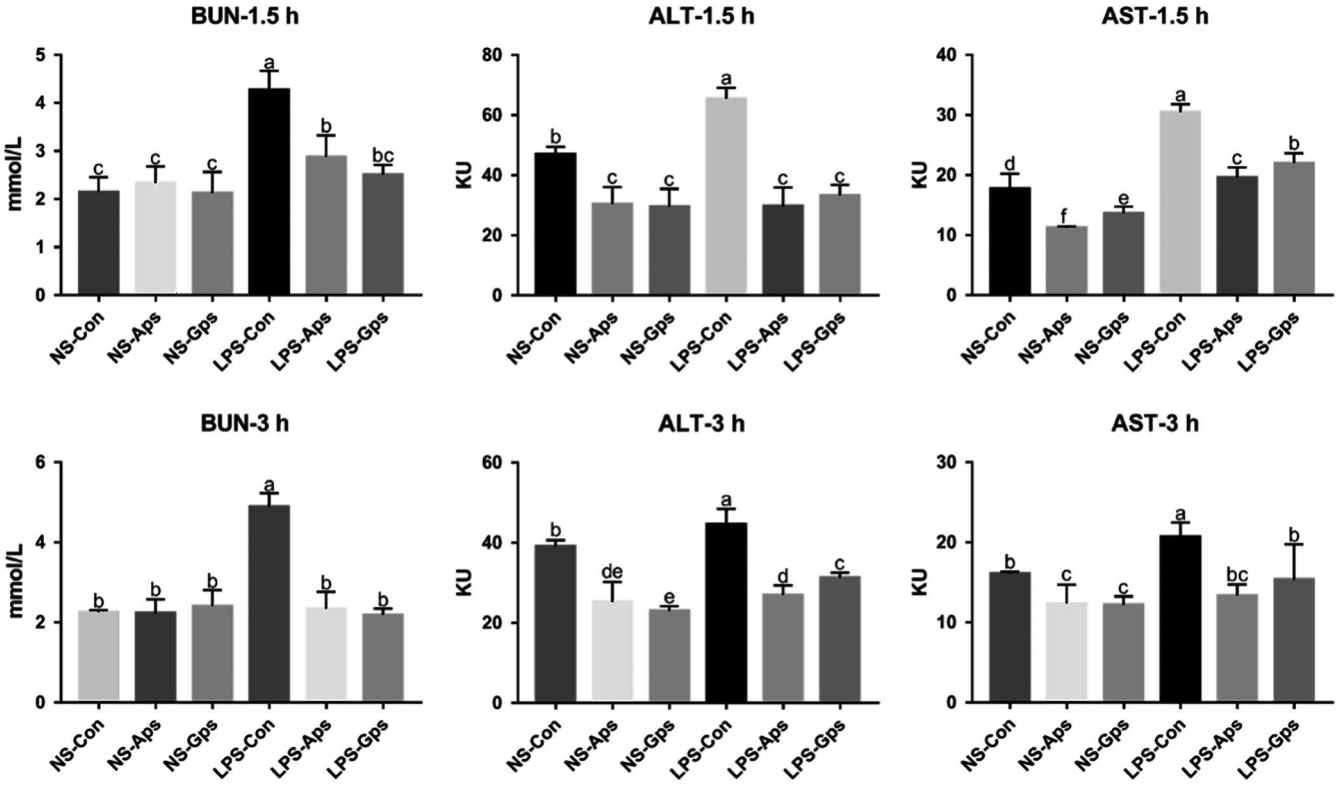

FIGURE 1 Effects of Aps and Gps on liver function in weaned piglets challenged with LPS involved 1.5 hr and 3 hr. NS-Con represents the control piglets treated with normal saline; NS-Aps represents the piglets supplemented with Aps and treated with normal saline; NS-Gps represents the piglets supplemented with Gps and treated with normal saline; LPS-Con represents the control piglets challenged with LPS; LPS-Aps represents the piglets supplemented with Aps and challenged with LPS; LPS-Gps represents the piglets supplemented with Gps and challenged with LPS; and small superscript letter indicates differences ($p < .05$). Values mean $n = 6$ for the analysis

3 | RESULTS

3.1 | Growth performance

The effects of Aps and Gps on growth performance in weaned piglets are listed in Table 2. Piglets fed diets supplemented with Aps or Gps had higher ($p < .05$) final body weight and ADG when compared with control group piglets. The F:G was lower ($p < .05$) in piglets fed diets supplemented with Aps or Gps than that in the control group piglets.

3.2 | Serum biochemical index

Piglets fed diets supplemented with Aps and Gps had lower ($p < .05$) ALT and AST levels than did control group piglets under normal saline treatment (Figure 1). Lipopolysaccharide challenge

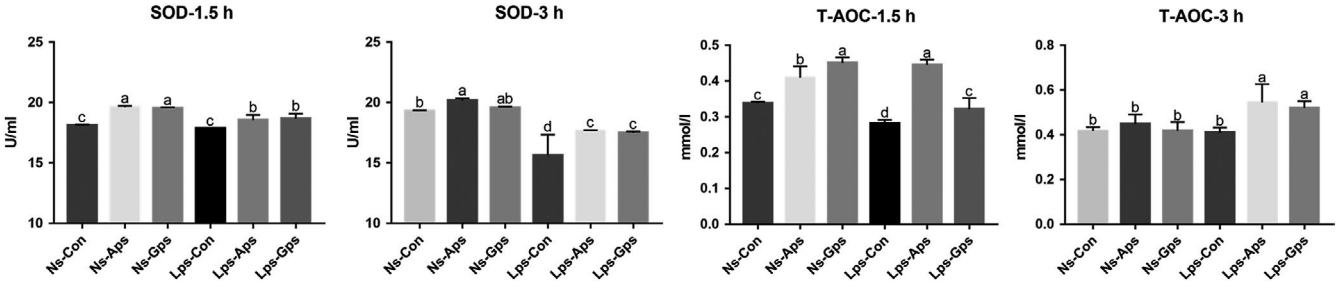

FIGURE 2 Effects of Aps and Gps on SOD and T-AOC in weaned piglets challenged with LPS involved 1.5 hr and 3 hr. NS-Con represents the control piglets treated with normal saline; NS-Aps represents the piglets supplemented with Aps and treated with normal saline; NS-Gps represents the piglets supplemented with Gps and challenged with normal saline; LPS-Con represents the control piglets challenged with LPS; LPS-Aps represents the piglets supplemented with Aps and challenged with LPS; LPS-Gps represents the piglets supplemented with Gps and challenged with LPS; and small superscript letter indicates differences ($p < .05$). Values mean $n = 6$ for the analysis

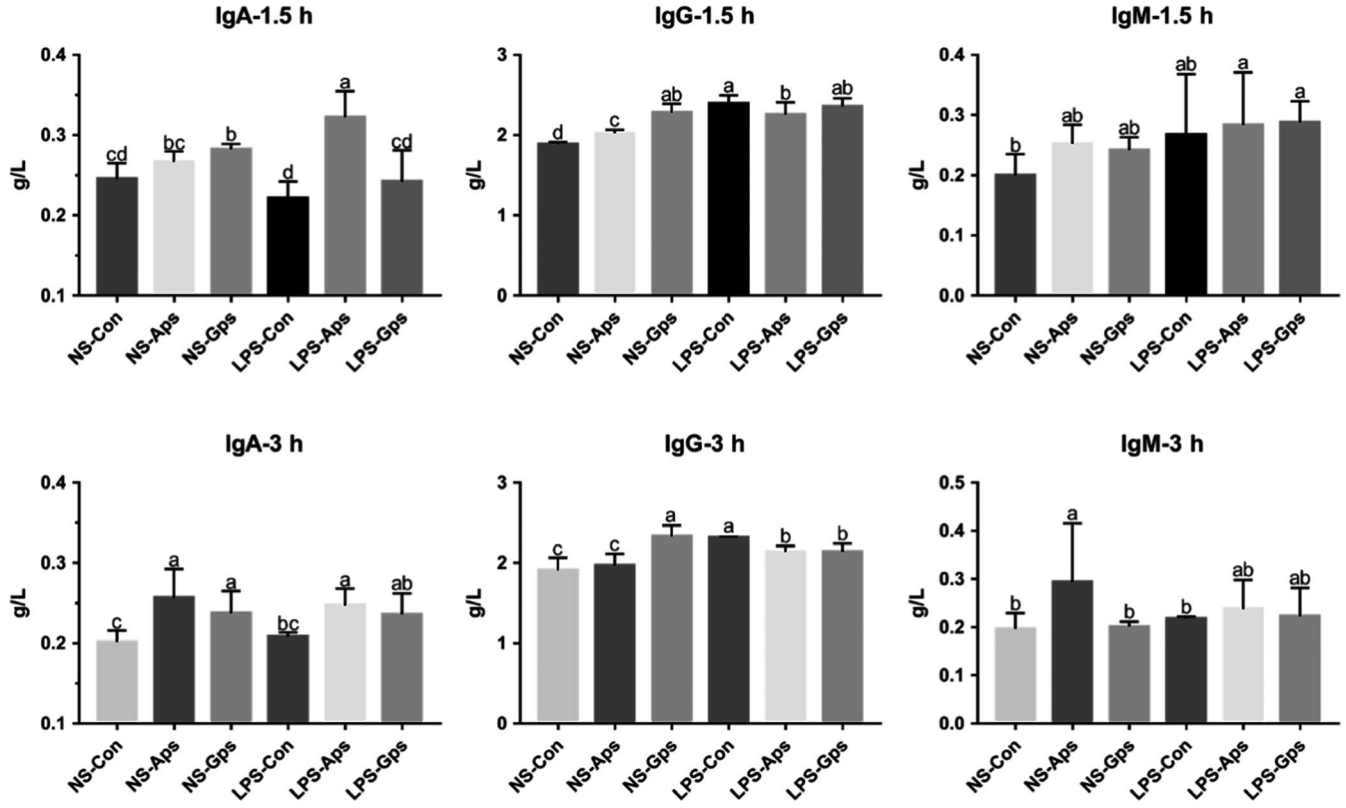

FIGURE 3 Effects of Aps and Gps on immunoglobulin in weaned piglets challenged with LPS involved 1.5 hr and 3 hr. NS-Con represents the control piglets treated with normal saline; NS-Aps represents the piglets supplemented with Aps and challenged with normal saline; NS-Gps represents the piglets supplemented with Gps and challenged with normal saline; LPS-Con represents the control piglets challenged with LPS; LPS-Aps represents the piglets supplemented with Aps and challenged with LPS; LPS-Gps represents the piglets supplemented with Gps and challenged with LPS; and small superscript letter indicates differences ($p < .05$). Values mean $n = 6$ for the analysis

increased ($p < .05$) the serum BUN, ALT and AST levels at 1.5 hr and 3 hr when compared with the situation under normal saline treatment. At 3 hr of challenge with LPS, both LPS-Aps and LPS-Gps treatment piglets had lower ($p < .05$) serum levels of BUN, ALT and AST than did LPS-Con piglets.

3.3 | Antioxidation indexes

Lipopolysaccharide injection decreased ($p < .05$) the serum SOD level at 1.5 hr and 3 hr, and serum T-AOC at 1.5 hr when compared with the situation under normal saline injection (Figure 2). The NS-Aps and NS-Gps piglets had higher ($p < .05$) serum SOD and T-AOC levels than did NS-Con piglets at 1.5 hr. Furthermore, both LPS-Aps and LPS-Gps treatment piglets had higher SOD and T-AOC levels at 1.5 hr and 3 hr when compared with the LPS-Con treatment piglets.

3.4 | Immunoglobulin

Effects of dietary supplementation with Aps and Gps on serum immunoglobulins are depicted in Figure 3. The NS-Aps piglets had a higher ($p < .05$) IgG level at 1.5 hr and higher ($p < .05$) IgA and IgM levels at 3 hr when compared with NS-Con piglets. The LPS-Aps piglets had a higher ($p < .05$) IgA level at 1.5 hr and 3 hr when compared with LPS-Con piglets.

3.5 | Serum inflammatory factors

Effects of dietary supplementation with Aps and Gps on serum cytokines are shown in Figure 4. Piglets in NS-Aps or NS-Gps treatments showed a higher ($p < .05$) concentration of IL-1β and TNF-α ($p < .05$) at 3 hr than did NS-Con piglets. The LPS challenge sharply increased IL-1β and TNF-α levels. Lipopolysaccharide-challenged piglets fed diets supplemented with Aps or Gps had decreased ($p < .05$) IL-1β and TNF-α levels when compared with LPS-Con piglets.

3.6 | Expression of jejunal TLR4, MyD88 and NF-κB

Compared with the piglets under the NS-Con treatment, the NS-Aps and NS-Gps piglets had higher ($p < .05$) expression of TLR4, MyD88 and NF-κB (Figure 5). Lipopolysaccharide challenge sharply increased ($p < .05$) the expression of TLR4, MyD88 and NF-κB. Lipopolysaccharide-challenged piglets fed diets supplemented with Aps or Gps had decreased ($p < .05$) TLR4, MYD88 and NF-κB expression when compared with LPS-Con piglets.

3.7 | Jejunal morphology analysis

Compared with the piglets under the NS-Con treatment, the NS-Aps or NS-Gps piglets had increased ($p < .05$) villus height and VCR in

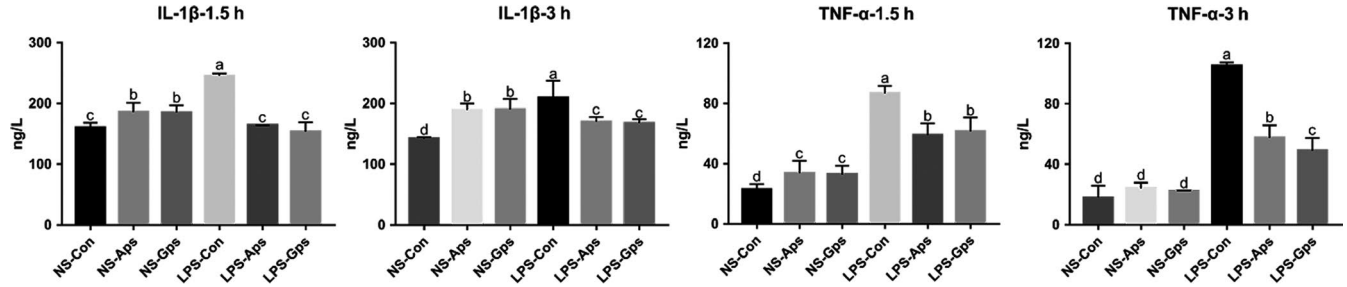

FIGURE 4 Effects of Aps and Gps on serum inflammatory factors in weaned piglets challenged with LPS involved 1.5 hr and 3 hr. NS-Con represents the control piglets treated with normal saline; NS-Aps represents the piglets supplemented with Aps and challenged with normal saline; NS-Gps represents the piglets supplemented with Gps and challenged with normal saline; LPS-Con represents the control piglets challenged with LPS; LPS-Aps represents the piglets supplemented with Aps and challenged with LPS; LPS-Gps represents the piglets supplemented with Gps and challenged with LPS; and small superscript letter indicates differences ($p < .05$). Values mean $n = 6$ for the analysis

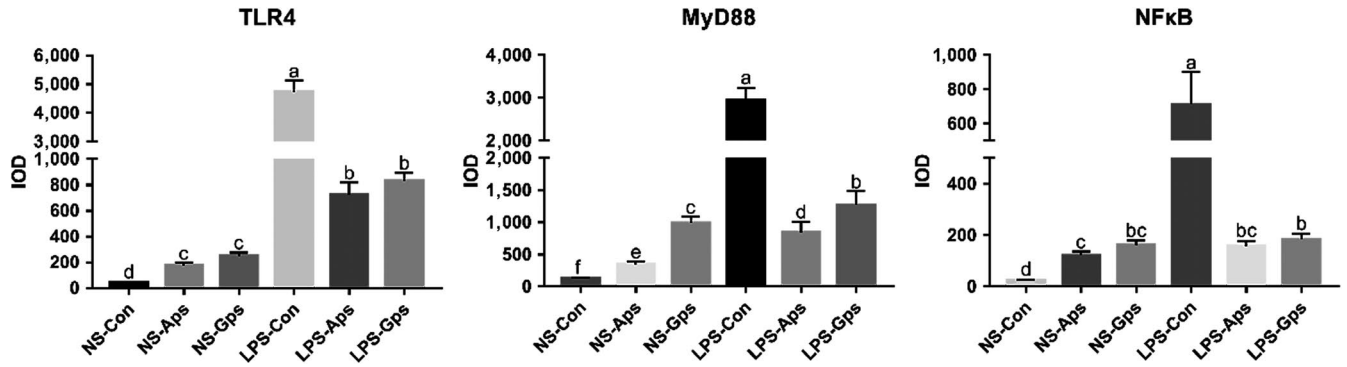

FIGURE 5 Effects of Aps and Gps on jejunal about TLR4, MyD88 and NFκB in weaned piglets challenged with LPS involved 1.5 hr and 3 hr. NS-Con represents the control piglets treated with normal saline; NS-Aps represents the piglets supplemented with Aps and challenged with normal saline; NS-Gps represents the piglets supplemented with Gps and challenged with normal saline; LPS-Con represents the control piglets challenged with LPS; LPS-Aps represents the piglets supplemented with Aps and challenged with LPS; LPS-Gps represents the piglets supplemented with Gps and challenged with LPS; and small superscript letter indicates differences ($p < .05$). Values mean $n = 6$ for the analysis

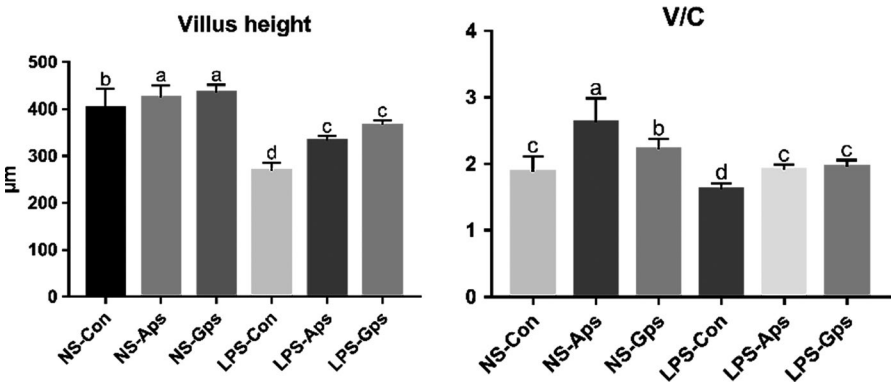

FIGURE 6 Effects of Aps and Gps on jejunal morphology in weaned piglets challenged with LPS involved 1.5 hr and 3 hr. NS-Con represents the control piglets treated with normal saline; NS-Aps represents the piglets supplemented with Aps and challenged with normal saline; NS-Gps represents the piglets supplemented with Gps and challenged with normal saline; LPS-Con represents the control piglets challenged with LPS; LPS-Aps represents the piglets supplemented with Aps and challenged with LPS; LPS-Gps represents the piglets supplemented with Gps and challenged with LPS; and small superscript letter indicates differences ($p < .05$). Values mean $n = 6$ for the analysis

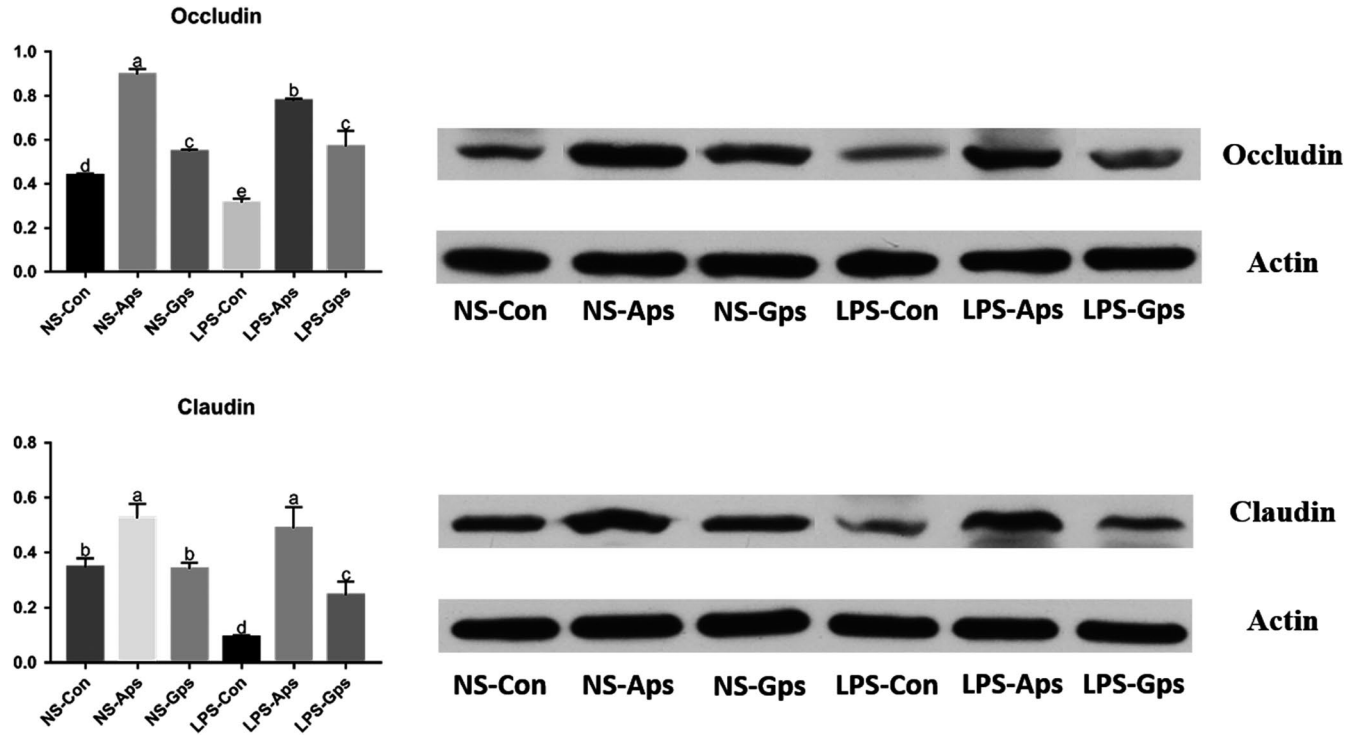

FIGURE 7 Effects of Aps and Gps on occludin and claudin of jejunal in weaned piglets challenged with LPS involved 1.5 hr and 3 hr. NS-Con represents the control piglets treated with normal saline; NS-Aps represents the piglets supplemented with Aps and treated with normal saline; NS-Gps represents the piglets supplemented with Gps and treated with normal saline; LPS-Con represents the control piglets challenged with LPS; LPS-Aps represents the piglets supplemented with Aps and challenged with LPS; LPS-Gps represents the piglets supplemented with Gps and challenged with LPS; and small superscript letter indicates differences ($p < .05$). Values mean $n = 6$ for the analysis of liver function indexes

jejunum (Figure 6). Lipopolysaccharide challenge decreased ($p < .05$) villus height and VCR. In addition, both LPS-Aps and LPS-Gps treatments increased ($p < .05$) villus height and VCR when compared with the LPS-Con treatment.

3.8 | Expression of jejunal occludin and claudin

The effects of Aps and Gps on occludin and claudin expression are shown in Figure 7. Compared with the NS-Con piglets, the Aps piglets had higher ($p < .05$) expression of occludin and claudin. Lipopolysaccharide challenge decreased ($p < .05$) the expression of occludin and claudin, and both LPS-Aps and LPS-Gps piglets showed higher ($p < .05$) occludin and claudin expression than did LPS-Con piglets.

4 | DISCUSSION

Plant polysaccharides have attracted a lot of attention because of their bioactivities, such as antioxidant activity, radioprotection effect, and hypolipidemic and immunomodulatory activities (Xie et al., 2015). Dietary supplementation with Aps was reported to increase growth performance in Nile tilapia (Zahran, Risha, AbdelHamid, Mahgoub, & Ibrahim, 2014). Wild-ginseng adventitious root meal was found to improve body weight gain and feed intake in chicken (Yan et al., 2009). Aps and sulfated Aps displayed dose-dependent growth-promoting and immunomodulatory effect in LPS-infected broiler chicks (Wang et al., 2014). Yin et al. (2008) reported that Aps promoted growth performance, ameliorated amino acid metabolism and increased the entry of dietary amino acid into the systemic circulation in early weaned piglets. Largemouth bass fed diets supplemented with Aps and chitosan oligosaccharides, alone or in combination, showed significantly increased final weight and specific growth rate, as well as increased feed conversion ratio (Lin et al., 2017). Furthermore, juvenile broilers fed diets supplemented with Aps had higher body weight gains and feed conversion ratio (Wu, 2018). In our study, we also observed that dietary supplementation with Aps and Gps enhanced growth performance and feed conversion ratio in weaned piglets.

Aps has been shown to exert protective effects on liver damage induced by cyclophosphamide, docetaxel and epirubicin in mice (Liu et al., 2014). The polysaccharide extracted from Angelica and Astragalus (AAP) significantly decreased the activities of AST and ALT and enhanced the activity of SOD in CCl4-treated (carbon tetrachloride) mice (Pu et al., 2015). Further research found that, the early weaned piglets fed chitosan and galacto-mannan-oligosaccharide, BUN level was reduced whereas serum total protein concentration was increased, in addition to improvement in liver function and antioxidant capacity (Tang et al., 2005). According to our results, LPS-challenged piglets fed diets supplemented with Aps or Gps had

lower AST and ALT levels than did LPS-Con piglets, which indicated that the supplementation of Aps or Gps alleviated liver damage induced by LPS.

Juvenile broilers fed with *Astragalus membranaceus* polysaccharide demonstrated higher serum IgG, IgM and IgA levels in comparison with control group (Wu, 2018). However, excessive *Astragalus membranaceus* polysaccharide dose could not improve its efficiency of growth performance and immunity further. Furthermore, the sEPS, compound of Aps and sulfated epimedium polysaccharide, have been reported to overcome cytasters-induced immunosuppression, significantly enhance T-lymphocyte proliferation and increase IgG and IgM levels (Guo et al., 2012). Abdullahi, Kallon, Yu, Zhang, and Li (2016) have also reported that Astragalus and Ginseng polysaccharide increase the expression of cytokines and immunoglobulin in broilers. We found that adding Aps significantly increased IgA levels in unchallenged piglets. Stimulation of LPS decreased IgA level, while the addition of Aps and Gps maintained the level of IgA, which improved the immune function of pigs under stress.

Astragalus membranaceus extract can inhibit advanced glycation end products-induced inflammatory cytokine production (IL-1β and TNF-α) to down-regulate macrophage-mediated inflammation via p38 MAPK and NF-κB signalling pathways (Qin et al., 2012). Aps alleviated LPS-induced immunological stress response in chicken. Aps supplementation ameliorated LPS-induced increase in the mRNA abundance of TLR4, NF-κB, IL-1, IL-6, avian uncoupling protein, α-1-acid glycoprotein, hemopexin and y + LAT2 (Liu et al., 2015). Besides, 200 mg/kg Aps significantly enhanced percentages of peripheral blood ANAE + T lymphocytes, CD4+, CD8+ cells and CD4+/CD8+ ratio and the content of INF-γ and IL-2 (Qiu et al., 2010). Aps injection up-regulated the expression of IL-1β and TNF-α in fish head and kidney in a dose-dependent manner (Yuan et al., 2008). Xi et al. (2016) reported that Gps improved IL-2 and TNF-α levels in sow serum and milk, which may be further beneficial to piglet health and growth through biological transmission effects during late pregnancy and lactation. Both Aps and Gps increased level of IL-2, IL-10, I FN-γ and TNF-α in chicken (Abdullahi et al., 2016). Our results showed that LPS increased IL-1β and TNF-α levels significantly. Furthermore, Aps and Gps reduced LPS-induced immune stress.

Aps significantly up-regulated gene expression of TNF-α, IL-6 and iNOS, rapidly activated TLR4-related MAPKs and induced translocation of NF-κB as well as degradation of IκB-α of RAW264.7 cell (Wei et al., 2016). In vivo, mice treated with Aps had induced TLR4 expression (Yin et al., 2010). TLR4 plays a key role in LPS-induced intestinal inflammation (Liu et al., 2012). Further in vitro and in vivo research found that Aps may modulate immunity of host organisms by stimulating the key nodes in the TLR4-MyD88-dependent signalling pathway, including TLR4, MyD88 and NF-κB (Zhou, Liu, Long, Zhou, & Bao, 2018). Our results showed that Aps and Gps improved the expression TLR4, MyD88 and NF-κB in unchallenged piglets. Furthermore, Aps and Gps alleviated the abnormal expression of TLR4, MyD88 and NF-κB, which were induced by LPS.

Phytosterols have been reported to decrease diarrhoea rate and serum cholesterol levels, reduce lipid peroxidation and increase the villus height/crypt depth ratio of the duodenum and jejunum in piglets (Hu, Li, Zhang, Zhuo, & Feng, 2017). The polysaccharide extracted from *Acanthopanax senticosus* ameliorated LPS-induced deterioration of digestive ability in LPS-challenged mice by improving villus height and decreasing crypt depth in the jejunum (Han et al., 2016). Wang et al. (2014) reported that Aps and sulfated Aps increased BWG and jejunal villus height and elevated the number of jejunal intraepithelial lymphocytes in LPS-infected broiler chicken, indicating that Aps is a potential growth regulator in early LPS challenge. The early LPS challenge has been found to delay intestinal growth and impair small intestinal structure and absorptive function in broiler chicken (Hu et al., 2011). Current results showed that both dietary supplementation with Aps and Gps increased villus height and the ratio of jejunum villus height to crypt depth the in LPS-challenged and unchallenged piglets, which indicated that Aps or Gps can protect intestinal development and promote the maintenance of normal intestinal function after stimulation by LPS.

Claudin and occludin are tight junction proteins; tight junctions are important structures for maintaining the mechanical barrier and permeability of mucosal epithelium. Lipopolysaccharide treatment has been shown to down-regulate the mRNA expression of occludin and claudin-1 in the jejunum (Liu et al., 2012; Wang et al., 2014). And in post- and pre-addition Aps groups, the expression of ZO-1 and occludin has been shown to significantly increase (Wang, Li, Yang, & Yao, 2013). Sun et al. (2016) reported that fermented Yupingfeng polysaccharides up-regulated the mRNA expression of occludin, claudin and ZO-1 in the jejunum and ileum of weaning rex rabbits. The mRNA expression of claudin and ZO-1 in the ileum of the Yupingfeng polysaccharides-treated animals was significantly higher than that in the control group animals. Our results showed that Aps and Gps maintained the intestinal barrier function by improving the expression of occludin and claudin, even after stimulation by LPS.

5 | CONCLUSION

In conclusion, dietary supplementation with Aps or Gps could improve the growth performance, liver function and intestinal villus morphology. It may regulate immune functioning in piglets by activating the TLR4-mediated MyD88-dependent signalling pathway and enhance the expression of tight junction proteins. Plant polysaccharides could reduce intestinal stress to promote the healthy growth of piglets and alleviate the drug residues caused by antibiotics to some extent.

ACKNOWLEDGEMENTS

The present research was supported by the Key Research Project of Zhejiang Province (No. 2017C02005), Zhejiang Provincial Key Research and Development Program (No. 2019C02051) and the National Natural Science Foundation of China (No. 31501985). We also acknowledge Vegamax Biotechnology for providing Astragalus polysaccharide products and Ginseng polysaccharide products.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Kangli Wang https://orcid.org/0000-0002-9442-7157

REFERENCES

Abdullahi, A. Y., Kallon, S., Yu, X., Zhang, Y., & Li, G. (2016). Vaccination with astragalus and ginseng polysaccharides improves immune response of chickens against H5N1 avian influenza virus. *BioMed Research International*, *2016*, 1–8. https://doi.org/10.1155/2016/1510264

Alexander, C., & Rietschel, E. T. (2001). Invited review: Bacterial lipopolysaccharides and innate immunity. *Journal of Endotoxin Research*, *7*, 167–202. https://doi.org/10.1177/09680519010070030101

Cromwell, G. L. (2002). Why and how antibiotics are used in swine production. *Animal Biotechnology*, *13*, 7–27. https://doi.org/10.1081/ABIO-120005767

Ferket, P. R., Lyons, T. P., & Jacques, K. A. (2004). Alternatives to antibiotics in poultry production: Responses, practical experience and recommendations. In *Nutritional biotechnology in the feed & food industries alltechs symposium: Re-imagining the feed industry* (Vol. *27*(3), pp. 57–67).

Guo, L., Liu, J., Hu, Y., Wang, D., Li, Z., Zhang, J., … Nguyen, T. L. (2012). Astragalus polysaccharide and sulfated epimedium polysaccharide synergistically resist the immunosuppression. *Carbohydrate Polymers*, *90*, 1055–1060. https://doi.org/10.1016/j.carbpol.2012.06.042

Han, J., Xu, Y., Yang, D., Yu, N., Bai, Z., & Bian, L. (2016). Effect of polysaccharides from acanthopanax senticosus on intestinal mucosal barrier of escherichia coli lipopolysaccharide challenged mice. *Asian-Australasian Journal of Animal Sciences*, *29*, 134–141. https://doi.org/10.5713/ajas.15.0534

Hu, Q., Li, S., Zhang, Y., Zhuo, Z., & Feng, J. (2017). Phytosterols on growth performance, antioxidant enzymes and intestinal morphology in weaned piglets. *Journal of the Science of Food and Agriculture*, *97*, 4629–4634. https://doi.org/10.1002/jsfa.8333

Hu, X., Guo, Y., Li, J., Yan, G., Bun, S., & Huang, B. (2011). Effects of an early lipopolysaccharide challenge on growth and small intestinal structure and function of broiler chickens. *Canadian Journal of Animal Science*, *91*, 379–384. https://doi.org/10.4141/cjas2011-008

Li, C., Wang, Y., Qu, J., Chen, B., Dai, G., Zhang, X., … Sun, H. (2018). Astragalus polysaccharide inhibits lipopolysaccharide-induced depressive-like behaviors and inflammatory response through regulating NF-kappa B and MAPK signaling pathways in rats. *International Journal of Clinical and Experimental Medicine*, *11*, 2361–2370.

Lin, S.-M., Jiang, Y., Chen, Y.-J., Luo, L., Doolgindachbaporn, S., & Yuangsoi, B. (2017). Effects of Astragalus polysaccharides (APS) and chitooligosaccharides (COS) on growth, immune response and disease resistance of juvenile largemouth bass, *Micropterus salmoides*. *Fish & Shellfish Immunology*, *70*, 40–47. https://doi.org/10.1016/j.fsi.2017.08.035

Liu, L., Shen, J., Zhao, C., Wang, X., Yao, J., Gong, Y., & Yang, X. (2015). Dietary Astragalus polysaccharide alleviated immunological stress in broilers exposed to lipopolysaccharide. *International Journal of Biological Macromolecules*, *72*, 624–632. https://doi.org/10.1016/j.ijbiomac.2014.08.057

Liu, P., Zhao, H., & Luo, Y. (2017). Anti-aging implications of *Astragalus membranaceus* (Huangqi): A well-known Chinese tonic. *Aging and Disease*, *8*, 868. https://doi.org/10.14336/AD.2017.0816

Liu, W., Gao, F. F., Li, Q., Lv, J. W., Wang, Y., Hu, P. C., … Wei, L. (2014). Protective effect of astragalus polysaccharides on liver injury induced by several different chemotherapeutics in mice. *Asian Pacific Journal of Cancer Prevention*, *15*, 10413–10420. https://doi.org/10.7314/APJCP.2014.15.23.10413

Liu, Y., Chen, F., Odle, J., Lin, X., Jacobi, S. K., Zhu, H., … Hou, Y. (2012). Fish oil enhances intestinal integrity and inhibits TLR4 and NOD2 signaling pathways in weaned pigs after LPS challenge. *The Journal of Nutrition*, *142*(11), 2017–2024. https://doi.org/10.3945/jn.112.164947

Medzhitov, R. (2002). Decoding the patterns of self and nonself by the innate immune system. *Science*, *296*, 298–300. https://doi.org/10.1126/science.1068883

Patterson, J., Chapman, T., Hegedus, E., Barchia, I., & Chin, J. (2005). Selected culturable enteric bacterial populations are modified by diet acidification and the growth promotant Tylosin. *Letters in Applied Microbiology*, *41*, 119–124. https://doi.org/10.1111/j.1472-765X.2005.01743.x

Pu, X., Fan, W., Yu, S., Li, Y., Ma, X., Liu, L., … Zhang, W. (2015). Polysaccharides from Angelica and Astragalus exert hepatoprotective effects against carbon-tetrachloride-induced intoxication in mice. *Canadian Journal of Physiology and Pharmacology*, *93*, 39–43. https://doi.org/10.1139/cjpp-2014-0331

Qin, Q., Niu, J., Wang, Z., Xu, W., Qiao, Z., & Gu, Y. (2012). *Astragalus membranaceus* inhibits inflammation via phospho-P38 mitogen-activated protein kinase (MAPK) and nuclear factor (NF)-κB pathways in advanced glycation end product-stimulated macrophages. *International Journal of Molecular Sciences*, *13*, 8379–8387. https://doi.org/10.3390/ijms13078379

Qiu, H., Cheng, G., Xu, J., Zhang, N., Liu, F., Zhu, X., … Zhang, Y. (2010). Effects of astragalus polysaccharides on associated immune cells and cytokines in immunosuppressive dogs. *Procedia in Vaccinology*, *2*, 26–33. https://doi.org/10.1016/j.provac.2010.03.006

Sun, H., Ni, X., Song, X., Wen, B., Zhou, Y., Zou, F., … Wang, P. (2016). Fermented Yupingfeng polysaccharides enhance immunity by improving the foregut microflora and intestinal barrier in weaning rex rabbits. *Applied Microbiology and Biotechnology*, *100*, 8105–8120. https://doi.org/10.1007/s00253-016-7619-0

Tang, J., Jiang, Y., Tang, Y., Chen, B., Sun, X., Su, L., & Liu, Z. (2013). Effects of propofol on damage of rat intestinal epithelial cells induced by heat stress and lipopolysaccharides. *Brazilian Journal of Medical and Biological Research*, *46*, 507–512. https://doi.org/10.1590/1414-431x20132785

Tang, Z.-R., Yin, Y.-L., Nyachoti, C. M., Huang, R.-L., Li, T.-J., Yang, C., … Fan, M. Z. (2005). Effect of dietary supplementation of chitosan and galacto-mannan-oligosaccharide on serum parameters and the insulin-like growth factor-I mRNA expression in early-weaned piglets. *Domestic Animal Endocrinology*, *28*, 430–441. https://doi.org/10.1016/j.domaniend.2005.02.003

Wang, K. L., Chen, G. Y., Cao, G. T., Xu, Y. L., Wang, Y. X., & Yang, C. M. (2019). Effects of *Clostridium butyricum* and *Enterococcus faecalis* on growth performance, intestinal structure and inflammation in lipopolysaccharide-challenged weaned piglets. *Journal of Animal Science*, *97*(10), 4140–4151. https://doi.org/10.1093/jas/skz235

Wang, X., Li, Y., Shen, J., Wang, S., Yao, J., & Yang, X. (2015). Effect of Astragalus polysaccharide and its sulfated derivative on growth performance and immune condition of lipopolysaccharide-treated broilers. *International Journal of Biological Macromolecules*, *76*, 188–194. https://doi.org/10.1016/j.ijbiomac.2015.02.040

Wang, X., Li, Y., Yang, X., & Yao, J. (2013). Astragalus polysaccharide reduces inflammatory response by decreasing permeability of LPS-infected Caco2 cells. *International Journal of Biological Macromolecules*, *61*, 347–352. https://doi.org/10.1016/j.ijbiomac.2013.07.013

Wang, X., Shen, J., Li, S., Zhi, L., Yang, X., & Yao, J. (2014). Sulfated Astragalus polysaccharide regulates the inflammatory reaction in LPS-infected broiler chicks. *International Journal of Biological Macromolecules*, *69*, 146–150. https://doi.org/10.1016/j.ijbiomac.2014.05.004

Wang, X., Wang, W., Wang, L., Yu, C., Zhang, G., Zhu, H., … Liu, Y. (2019). Lentinan modulates intestinal microbiota and enhances barrier integrity in a piglet model challenged with lipopolysaccharide. *Food & Function*., *10*, 479–489. https://doi.org/10.1039/c8fo02438c

Wei, W., Xiao, H.-T., Bao, W.-R., Ma, D.-L., Leung, C.-H., Han, X.-Q., … Han, Q.-B. (2016). TLR-4 may mediate signaling pathways of Astragalus polysaccharide RAP induced cytokine expression of RAW264.7 cells. *Journal of Ethnopharmacology*, *179*, 243–252. https://doi.org/10.1016/j.jep.2015.12.060

Wu, S. (2018). Effect of dietary *Astragalus membranaceus* polysaccharide on the growth performance and immunity of juvenile broilers. *Poultry Science*, *97*, 3489–3493. https://doi.org/10.3382/ps/pey220

Xi, Q., Jiang, Y., Zhao, S., Zeng, B., Wang, F., Wang, L.-N., … Zhang, Y.-L. (2016). Effect of ginseng polysaccharides on the immunity and growth of piglets by dietary supplementation during late pregnancy and lactating sows. *Animal Science Journal*, *88*, 863–872. https://doi.org/10.1111/asj.12678

Xia, B., Meng, Q., Feng, X., Tang, X., Jia, A., Feng, J., … Zhang, H. (2018). Probing the molecular regulation of lipopolysaccharide stress in piglet liver by comparative proteomics analysis. *Electrophoresis*, *39*, 2321–2331. https://doi.org/10.1002/elps.201700467

Xie, J.-H., Jin, M.-L., Morris, G. A., Zha, X.-Q., Chen, H.-Q., Yi, Y., … Xie, M.-Y. (2015). Advances on bioactive polysaccharides from medicinal plants. *Critical Reviews in Food Science and Nutrition*, *56*, S60–S84. https://doi.org/10.1080/10408398.2015.1069255

Yan, F., Zhang, Q.-Y., Jiao, L., Han, T., Zhang, H., Qin, L.-P., & Khalid, R. (2009). Synergistic hepatoprotective effect of Schisandrae lignans with Astragalus polysaccharides on chronic liver injury in rats. *Phytomedicine*, *16*, 805–813. https://doi.org/10.1016/j.phymed.2009.02.004

Yan, L., Meng, Q. W., Lee, J. H., Wang, J. P., & Kim, I. H. (2011). Effects of dietary wild-ginseng adventitious root meal on growth performance, blood profiles, relative organ weight and meat quality in broiler chickens. *Asian-Australasian Journal of Animal Sciences*, *24*, 258–263. https://doi.org/10.5713/ajas.2011.10222

Yang, C. M., Zhang, L. L., Cao, G. T., Feng, J., Yue, M., Xu, Y. L., … Guo, X. Q. (2018). Effects of dietary supplementation with essential oils and organic acids on the growth performance, immune system, faecal volatile fatty acids and microflora community in weaned piglets. *Journal of Animal Science*, https://doi.org/10.1093/jas/sky426

Yin, F. G., Liu, Y. L., Yin, Y. L., Kong, X. F., Huang, R. L., Li, T. J., … Hou, Y. (2008). Dietary supplementation with Astragalus polysaccharide enhances ileal digestibilities and serum concentrations of amino acids in early weaned piglets. *Amino Acids*, *37*, 263–270. https://doi.org/10.1007/s00726-008-0142-6

Yin, M., Zhang, Y., & Li, H. (2019). Advances in research on immunoregulation of macrophages by plant polysaccharides. *Frontiers in Immunology*, *10*, 145. https://doi.org/10.3389/fimmu.2019.00145

Yin, X., Chen, L., Liu, Y., Yang, J., Ma, C., Yao, Z., … Li, M. (2010). Enhancement of the innate immune response of bladder epithelial cells by Astragalus polysaccharides through upregulation of TLR4 expression. *Biochemical and Biophysical Research Communications*, *397*, 232–238. https://doi.org/10.1016/j.bbrc.2010.05.090

Yuan, C., Pan, X., Gong, Y., Xia, A., Wu, G., Tang, J., & Han, X. (2008). Effects of Astragalus polysaccharides (APS) on the expression of immune response genes in head kidney, gill and spleen of the common carp, *Cyprinus carpio* L. *International Immunopharmacology*, *8*, 51–58. https://doi.org/10.1016/j.intimp.2007.10.009

Zahran, E., Risha, E., AbdelHamid, F., Mahgoub, H. A., & Ibrahim, T. (2014). Effects of dietary Astragalus polysaccharides (APS) on growth performance, immunological parameters, digestive enzymes, and intestinal morphology of Nile tilapia (*Oreochromis niloticus*). *Fish & Shellfish Immunology*, *38*, 149–157. https://doi.org/10.1016/j.fsi.2014.03.002

Zhou, L., Liu, Z., Wang, Z., Yu, S., Long, T., Zhou, X., & Bao, Y. (2017). Astragalus polysaccharides exerts immunomodulatory effects via TLR4-mediated MyD88-dependent signaling pathway in vitro and in vivo. *Scientific Reports*, *7*, https://doi.org/10.1038/srep44822

Zhou, X., Liu, Z., Long, T., Zhou, L., & Bao, Y. (2018). Immunomodulatory effects of herbal formula of astragalus polysaccharide (APS) and polysaccharopeptide (PSP) in mice with lung cancer. *International Journal of Biological Macromolecules*, *106*, 596–601. https://doi.org/10.1016/j.ijbiomac.2017.08.054

Zimmerman, D. R. (1986). Role of subtherapeutic levels of antimicrobials in pig production. *Journal of Animal Science*, *62*, 6–17. https://doi.org/10.2527/1986.62Supplement_36s

How to cite this article: Wang K, Zhang H, Han Q, et al. Effects of astragalus and ginseng polysaccharides on growth performance, immune function and intestinal barrier in weaned piglets challenged with lipopolysaccharide. *J Anim Physiol Anim Nutr*. 2019;00:1–10. https://doi.org/10.1111/jpn.13244

浙江农林大学图书馆收录证明报告

《SCI-Expanded》检索结果（收录情况）

WEB OF SCIENCE™

Web of Science™ Core Collection

经检索《Web of Science™ Core Collection》，王康莉 发表的下述 1 篇论文被《SCI－Expanded》收录。（检索时间：2019 年 12 月 4 日）

第 1 条，共 1 条

标题: Effects of astragalus and ginseng polysaccharides on growth performance, immune function and intestinal barrier in weaned piglets challenged with lipopolysaccharide
作者: Wang, KL (Wang, Kangli); Zhang, HR (Zhang, Haoran); Han, QJ (Han, Qianjie); Lan, JH (Lan, Junhong); Chen, GY (Chen, Guangyong); Cao, GT (Cao, Guangtian); Yang, CM (Yang, Caimei)
来源出版物: JOURNAL OF ANIMAL PHYSIOLOGY AND ANIMAL NUTRITION DOI: 10.1111/jpn.13244 提前访问日期: NOV 2019
Web of Science 核心合集中的 "被引频次": 0
被引频次合计: 0
入藏号: WOS:000496279900001
PubMed ID: 31724241
地址: [Wang, Kangli; Zhang, Haoran; Han, Qianjie; Lan, Junhong; Chen, Guangyong; Yang, Caimei] Zhejiang A&F Univ, Coll Anim Sci & Technol, Zhejiang Prov Engn Lab Anim Hlth & Internet Techn, Key Lab Appl Technol Green Ecohlth Anim Husb Zhej, Hangzhou, Zhejiang, Peoples R China.
[Cao, Guangtian] China Jiliang Univ, Coll Standardizat, Hangzhou, Zhejiang, Peoples R China.
通讯作者地址: Yang, CM (通讯作者)，Zhejiang A&F Univ, Coll Anim Sci & Technol, Zhejiang Prov Engn Lab Anim Hlth & Internet Techn, Key Lab Appl Technol Green Ecohlth Anim Husb Zhej, Hangzhou, Zhejiang, Peoples R China.
电子邮件地址: yangcaimei2012@163.com
ISSN: 0931-2439
eISSN: 1439-0396

注：以上检索结果均得到被检索人的确认。

浙江农林大学图书馆 论文查收引证专用章

《SCI-Expanded》检索结果（收录情况）
浙江农林大学图书馆
检索人：桑宇芳
2019 年 12 月 4 日