

Effect of dried roots of *Astragalus membranaceus* in the diets of young growing pigs on growth performance and immune function*

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ABSTRACT

The experiment was conducted to investigate the effect of *Astragalus* powder prepared by using different comminution techniques on growth performance and immune function of pigs. Duroc × Landrace × Yorkshire young growing pigs (n=90, female, 60 days of age, liveweight 21.88 ± 1.26 kg) were randomly allocated to three treatments. Each treatment had three replicates with ten pigs per pen. The basal diets were not supplemented or supplemented (5 g/kg) with 80 mesh *Astragalus* (180 µm), or micron *Astragalus* (6.32 µm), respectively; the feeding experiment lasted 30 days. After completion of the feeding experiment, three animals from each treatment were chosen to determine the effect of *Astragalus* on immune function. Results showed that supplementation with micron *Astragalus* significantly increased (P<0.05) average daily gain (ADG), both Concanavalin (ConA) and lipopolysaccharide (LPS)-induced splenocyte and peripheral lymphocyte proliferation, and significantly increased (P<0.05) serum IgG, IgA, IL-1α and IL-2 concentrations compared with the control groups. Supplementation with 80 mesh *Astragalus* only increased (P<0.05) serum IgA, IL-1α and IL-2 concentrations compared with control. The results indicated that micron *Astragalus* was more effective than 80 mesh *Astragalus* in improving growth performance and enhancing immune function of pigs.

KEY WORDS: *Astragalus*, growth performance, immunity, pigs

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INTRODUCTION

Extensive use of antibiotics results in residual medicament in animal products, and bacterial antibiotic resistance and tolerance (Hamilton-Miller, 2004). This represents a considerable risk to human health. Therefore, it has become urgent to develop a non-antibiotic immunopotentiator with high efficiency, low toxicity and extensive resource. Many Chinese herbal medicines have been reported to have the effect of immune enhancement (Liu et al., 1998; Xie and Song, 2000) and have a great potential in practical application. *Astragalus membranaceus* is one of the oldest and most commonly used Chinese herbal medicines in China, Korea, Japan and other Asian countries, and is well known to strengthen the host defense system as a tonic (Zhao et al., 1990; Hong et al., 1992; Jin and Kurashige, 1996). Pharmacological tests showed that it possessed well-documented hepatoprotective, antioxidative, antiviral, antihypertensive and immunostimulant activities (Zee-Cheng, 1992; Bedir et al., 2000; Song et al., 2000).

At present, many researchers have studied the effective ingredient content, powder liquidity, bulk density, solubilization, granularity, pharmacological function, etc. of Chinese herbal medicine powder prepared by using superfine and conventional milling (Sheng et al., 2003; Li et al., 2004; Zou, 2005). Up to now, no studies on the effect of *Astragalus* powder prepared by using different comminution techniques on growth performance and immune function of young growing pigs have been done. The current experiment was conducted to evaluate the effect of 80 mesh *Astragalus* and micron *Astragalus* (5 g/kg diet) as additives in pig diets on growth performance and immune function.

MATERIAL AND METHODS

Chinese herbal medicine

Roots of *Astragalus membranaceus* was purchased from Huadong Pharmaceutical Company (Hangzhou, China), washed with water, then dried at 40°C and processed to ultramicro-powder by using QYF fluidized-bed pneumatic jet mill (Kunshan Miyou Industrial Co., Ltd, Kunshan, China) or to a conventional fine powder by using SFY-K-2-type conventional mill (Kunshan Miyou Industrial Co., Ltd, Kunshan, China), respectively, in our Institute of Feed Science. The conventional fine powder was ground through 80 mesh sieve. The particle sizes were determined by Mastersizer Laser Particle Size Analyzer (Malvern, UK). The average particle sizes of 80 mesh *Astragalus* and micron *Astragalus* used in the current study were 180 and 6.32 µm, respectively.

Animals and experimental design

This experiment was approved by the Institutional Animal Care and Use Committee at Zhejiang University and was conducted in accordance with the National Institutes of Health guidelines for the care and use of experimental animals. The feeding trial was carried out in the Swine Research and Teaching Farm at Zhejiang University. Duroc×Landrace×Yorkshire female pigs (n=90), 60 days of age and weighing 21.88 ± 1.26 kg, were randomly assigned to three treatments. The pigs had been weaned at 36 days after birth. Each of these groups consisted of three replications (i.e. pens) with ten animals per replicate. All pigs received the same basal diet, either not supplemented or supplemented (5 g/kg) with 80 mesh *Astragalus* or micron *Astragalus*, respectively. Diets were formulated to meet or exceed NRC (1998) requirements for 20-50 kg pigs. No antibiotic was included in diets (Table 1). The feeding trial lasted for 30 days after seven days of adaptation period. All pigs were housed in an open-front pig barn with concrete floor pens 350×350 cm. A wet feeders with two waterers were allocated in each pen for pigs. During the 30-day feeding trial, all pigs were given *ad libitum* access to feed and water. Feed consumption per pen was recorded daily, and pig weight was measured at 15-day intervals. Each day, feed refusals per pen were measured and subtracted from the total feed intake.

Table 1. Composition of experimental diets, as-fed basis

Ingredients, g/kg		Analysed content, g/kg	
Maize	587	Dry matter	887
Soyabean meal	250	Crude protein	195
Wheat middlings	50	Calcium	8
Yeast	10	Total P	6.6
Fish meal	40	Lysine	13.5
Limestone	10	Methionine	3.3
Monocalcium phosphate	20		
Sodium chloride	3		
Vitamin-mineral premix ¹	30		
DE ² , MJ/kg	14.46		

¹ provided the following amounts of minerals/vitamins per kg of feed in experiment diets, IU: vit. A, 2200; vit. D₁, 500; mg: Fe, 100; Cu, 6; Mn, 4; Zn, 100; I, 0.14; Se, 0.3; 16; vit. E, 16; vit. K, 0.5; vit. B₁, 1.5; vit. B₂, 4; vit. B₆, 2; vit. B₁₂, 0.02; niacin, 22; D-pantothenic acid, 12; biotin 0.08; folic acid, 0.3; ² DE based on calculated values

Sample collection and assay

Sampling procedure. At the end of the feeding trial, twelve pigs were slaughtered at a commercial slaughterhouse (Zhengning, Ningbo, China). The

pigs were fasted 12 h and euthanized by bleeding after intramuscular injection of sodium pentobarbital solution before slaughter. Samples from the spleen were obtained for cell culture. Blood samples (15 ml per pig) were drawn in collection tubes by venipuncture of the anterior vena cava of pigs at approximately 8.00 a.m. before slaughter. Five ml blood samples per pig were transferred immediately into aseptic capped tubes with sodium heparin. Other 10 ml blood samples per pig were allowed to clot at 37°C for 2 h prior to collect serum.

Physical structure assay of 80 mesh Astragalus and micron Astragalus. Five g samples of 80 mesh *Astragalus* and micron *Astragalus* were examined by Philips XL30 environmental scanning electron microscope ESEM. Their physical structures were observed on low magnification (25×) and high magnification (1000×), respectively.

Preparation and proliferation assay of splenocyte and peripheral lymphocyte. Mitogen-induced splenocyte and peripheral lymphocytes proliferation were examined by MTT assay (Kong et al., 2004; Zhou et al., 2005). Spleen samples were gently smashed by pressing with the flat surface of a syringe plunger against stainless steel sieve (200 mesh). The splenocytes were washed twice and then resuspended in RPMI 1640 (GIBCO BRL, USA) supplemented with benzyl-penicillin (100 IU/ml), streptomycin (100 IU/ml) and 10% foetal bovine serum. Blood samples with sodium heparin were diluted with an equal volume of Hanks' solution and carefully layered on the surface of lymphocyte separation medium. The lymphocytes' band was collected and washed twice with RPMI 1640 without foetal bovine serum. The splenocytes and peripheral lymphocytes were adjusted to 2×10^6 /ml and 6×10^6 /ml respectively with RPMI 1640 media and incubated in 96-well tissue culture plates with 100 µl/well, adding either 100 µl of ConA (25 µg/ml, SIGMA, USA), LPS (100 µg/ml, SIGMA, USA) or complete medium (controls). After 44-h incubation at 37°C in 5% CO₂ humid incubator, 20 µl MTT (5 mg/ml, Amresco, Solon, USA) were added into each well incubating for another 4 h and then 100 µl of DMSO were added into each well and shaken for 10 min to dissolve the precipitation completely. The light absorbance was measured at 570 nm with Enzyme-linked Immunosorbent Assay Reader (Model BIO-RAD-550, USA). The stimulation index (SI) was calculated as the absorbance of mitogen-stimulated cells divided by the absorbance of unstimulated, control (media only) cells.

Immune indexes analysis of serum. Concentrations of IL-1α and IL-2 in the serum were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits (Pharmingen, CA, USA) according to the manufacturer's instructions. The concentration of serum IgG, IgA was measured by agar gel simple diffusion in two dimensions (Yang and Zheng, 1992).

Statistical analysis

All data measured in the study were analysed by comparing means according to least significant difference test, using the general linear model procedure of SAS (version 6.12). Pens were considered the experimental unit for growth performance analysis, the individual pig served as the experimental unit for other data analysis. Effects were considered significant at $P < 0.05$.

RESULTS

Physical structure of 80 mesh Astragalus and micron Astragalus

ESEM images of 80 mesh *Astragalus* and micron *Astragalus* at low magnification (25×) (Figure 1) showed that the micron *Astragalus* powder particles were more uniform in shape and smaller in size than 80 mesh *Astragalus* powder particles. The ESEM images at high magnification (1000×) (Figure 1) showed that nearly all plant cells of micron *Astragalus* were disrupted, the intracellular contents had been released and formed an amorphous mass. For 80 mesh *Astragalus*, the cell wall was only partially disrupted and intracellular contents were incompletely released.

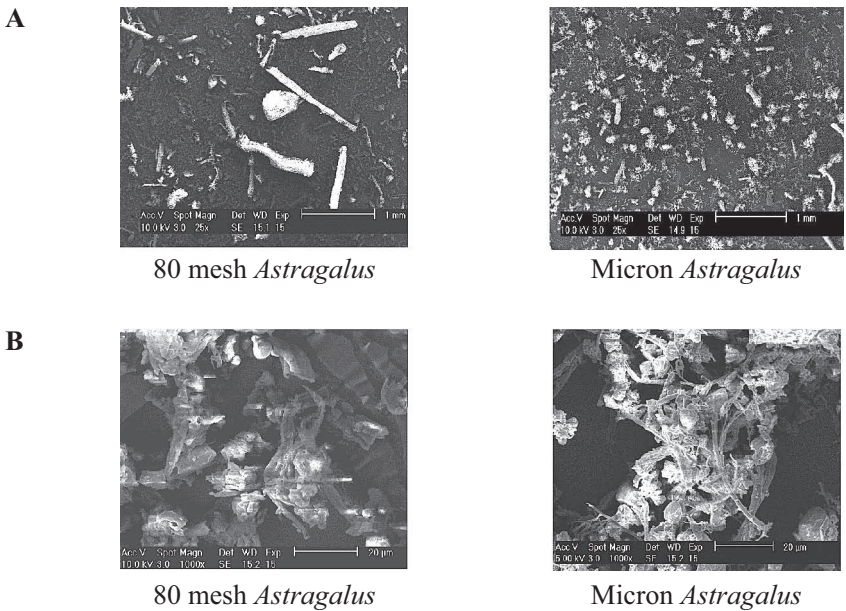


Figure 1. Environmental scanning electron microscope (ESEM) images of micron *Astragalus* and 80 mesh *Astragalus*. Images A, B of 80 mesh *Astragalus* (a, c) and Micron *Astragalus* (b, d) were obtained in ESEM on low magnification (25×) and high magnification (1000×), respectively

Effect of Astragalus and micron Astragalus on growth performance

Pigs fed with micron *Astragalus* (Table 2) had higher ($P<0.05$) ADG than those fed other diets. However, supplementation with 80 mesh *Astragalus* had no ($P>0.05$) effect on growth performance when compared with the control groups. Feed intake and feed conversion ratio were not affected by diet.

Table 2. Effect of micron or 80 mesh *Astragalus* on growth performance of pigs¹

	Control	<i>Astragalus</i>		SEM	P-value
		80 mesh	micron		
Initial weight, kg	21.94	22.02	21.82	0.696	0.959
Final liveweight, kg	45.13	45.90	47.93	0.815	0.340
Average daily gain, g	773.1 ^b	798.1 ^b	870.3 ^a	20.60	0.008
Average daily feed intake, kg	1.57	1.60	1.66	0.043	0.121
Gain/feed	0.49	0.50	0.53	0.077	0.868

means within a row with different letters (a,b) differ significantly ($P<0.05$)

¹ values represent the means of three replications (pens) per treatment. Treatments lasted 30 days

Effect of Astragalus and micron Astragalus on immune function

Supplementation with micron *Astragalus* (Table 3) significantly increased ($P<0.05$) both ConA and LPS-induced splenocyte and peripheral lymphocyte

Table 3. Effect of micron or 80 mesh *Astragalus* on splenocyte and peripheral lymphocyte proliferation (SI) of pigs¹

Item	Peripheral lymphocyte proliferation		Splenocyte proliferation	
	ConA	LPS	ConA	LPS
	induced			
Control	1.84 ^b	1.50 ^b	1.06 ^b	1.11 ^b
80 mesh <i>Astragalus</i>	1.87 ^b	1.55 ^b	1.11 ^b	1.18 ^{ab}
Micron <i>Astragalus</i>	2.54 ^a	2.02 ^a	1.27 ^a	1.26 ^a
SEM ⁺	0.041	0.032	0.034	0.057
P-value	0.000	0.000	0.002	0.100

means within a column with different letters (a,b) differ significantly ($P<0.05$)

SI=OD_{570nm} of experimental value/OD_{570nm} of control value

¹ values are means of three replicates of three pigs per replicate

proliferation when compared with the control groups. Supplementation with 80 mesh *Astragalus* had no ($P>0.05$) effect on splenocyte or peripheral lymphocyte proliferation. Supplementation with micron *Astragalus* significantly increased ($P<0.05$; Table 4) IgG, IgA, IL-1 α and IL-2 concentrations in serum compared with the

control, supplementation with 80 mesh *Astragalus* also significantly increased (P<0.05) IgA, IL-1α and IL-2 concentrations in serum compared with the control, but the effect of micron *Astragalus* was more marked than 80 mesh *Astragalus*.

Table 4. Effect of micron or 80 mesh *Astragalus* on immune indexes in serum of pigs¹

	Control	<i>Astragalus</i>		SEM	P-value
		80 mesh	micron		
IgG, g/l	4.41 ^b	4.69 ^{ab}	4.81 ^a	0.143	0.075
IgA, g/l	0.53 ^b	0.62 ^a	0.66 ^a	0.020	0.005
IL-1, α, pg/ml	8.48 ^c	15.45 ^b	19.33 ^a	0.819	0.000
IL-2, pg/ml	13.95 ^c	20.10 ^b	27.28 ^a	0.638	0.000

means within a row with different letters differ significantly (P<0.05)

¹ values are means of three replicates of three pigs per replicate

DISCUSSION

The results of growth performance showed that supplementation with micron *Astragalus* significantly increased ADG of young growing pigs. Supplementation with 80 mesh *Astragalus* had no effect on growth performance. The feeding value of *Astragalus* may be affected by the addition concentration, preparation technique, sanitary condition, level performance, diet composition, and so on. Sun and Liu (2004) determined the effect of different levels (10, 15, 20 and 25 g/kg) of *Astragalus* on the growth performance of piglets and found that effect of adding 20 g/kg *Astragalus* was the best. Their results showed that *Astragalus* had a positive dose dependent effect on growth performance. The current results suggested that *Astragalus* had effect in improving growth performance and the effect had been affected by the preparation technique.

Splenocyte and peripheral blood lymphocyte proliferation and serum IgA, IgG, IL-1α and IL-2 levels reflect the immunity function of pigs. Previous *in vivo* and *in vitro* results have shown that *Astragalus* could promote ConA and LPS-induced splenocyte and peripheral lymphocyte proliferation (Chen et al., 2003; Kong et al., 2004). *Astragalus* and its active ingredients significantly increased IL-1 activity and promoted IL-2 release (Li et al., 2002; Erdem et al., 2005). Cytokines play an important role in the regulation of immune response (Park et al., 2000; Lee et al., 2005). In the present study we also found that supplementation with micron *Astragalus* significantly enhanced immune function. Supplementation with 80 mesh *Astragalus* had some effect on enhancing immune function of pigs, but the effect was less than micron *Astragalus*.

The principal finding of the current study was that micron *Astragalus* was more effective than 80 mesh *Astragalus* in improving growth performance and immune function of young growing pigs. Many researchers confirmed

the efficacy of super-fine comminution-treated Chinese herbal medicine (Du and He, 2000; Li et al., 2004; Xu et al., 2004). The possible mechanisms were explained in two points. The first mechanism was that the plant cell walls of Chinese herbal medicine prepared by using super-fine comminution were highly disrupted, which allows access of digestive enzymes to the released intracellular contents the other one was that due to fine particle and large surface area, its adhesive function was enhanced, time of settling and releasing of medicine in body was prolonged. These were beneficial to improvement of absorption rate and bioavailability.

CONCLUSIONS

Our findings suggested that micron *Astragalus* had better effect in improving growth performance and immune function of pigs than 80 mesh *Astragalus*. It may be that superfine comminution treatment improved absorption rate and bioavailability of *Astragalus* through disrupting the plant cell structure and improving its physical-chemical properties. Further research should be conducted to investigate the effects and possible mechanism of action of *Astragalus* powder by using different preparation techniques. Ongoing experiments in our laboratory will thoroughly address these questions in order to develop a non-antibiotic immunopotentiator with high efficiency to be used in the pig industry.

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