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ORIGINAL ARTICLE

Dietary supplementation with *Astragalus* polysaccharide enhances ileal digestibilities and serum concentrations of amino acids in early weaned piglets

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Abstract Two experiments were conducted to evaluate the effects of dietary supplementation with *Astragalus* polysaccharide (APS) on growth performance, apparent ileal digestibilities (AID) of amino acids (AA), and their serum concentrations in early weaned piglets. In Exp. 1, 60 pigs were weaned at 21 days of age (BW 7.35 ± 0.23 kg) and allocated to three treatments (20 pigs/treatment), representing supplementing 0.0% (control), 0.02% colistin (antibiotic), or 0.1% APS to a corn- and soybean meal-based diet. Average daily gain (ADG), average daily feed intake (ADFI), and feed/gain ratio (F/G) were measured weekly. Blood samples were obtained from five pigs selected randomly from each treatment for the measurement of serum free AA concentrations on days 7, 14, and 28. In Exp. 2, 12 pigs were weaned at 21 day of age (BW 7.64 ± 0.71 kg), assigned to three treatment groups as in Exp. 1, and surgically fitted with a simple T-cannula at the terminal ileum. Ileal digesta samples were obtained for the measurement of AID of AA on days 7, 14 and 28. Dietary APS did not affect ADFI, but enhanced ($P < 0.05$) ADG by 11 and 4.4%, and improved F/G by 5.6 and 8.4%, respectively, compared with the control and antibiotic groups. Addition of APS to the diet increased AID and serum concentrations of most nutritionally essential and non-essential AA (including arginine, proline, glutamate, lysine, methionine, tryptophan, and threonine) on days 14 and 28. Circulating levels of total AA were affected by the age of pigs and treatment × time interaction. Collectively, these findings indicate that APS may ameliorate the digestive and absorptive function and regulate AA metabolism to beneficially increase the entry of dietary AA into the systemic circulation, which provide a mechanism to explain the growth-promoting effect of APS in early weaned piglets.

Keywords Amino acids · *Astragalus* polysaccharide · Digestibilities · Performance · Pig

Abbreviations

AA	Amino acid
AM	*Astragalus mongholicus*
APS	*Astragalus* polysaccharide
AID	Apparent ileal digestibilities
ADG	Average daily gain
ADFI	Average daily feed intake
F/G	Feed/gain ratio

Introduction

Early weaned pigs have an underdeveloped immune system (Li et al. 2007a, b; Huang et al. 2007) and compromised digestive function (Hampson 1986).

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Therefore, antibiotics have been supplemented to the diet for weanling piglet over a half century to prevent infectious disease (Schwarz et al. 2001; Frydendahl 2002). While this practice has also been highly effective in enhancing growth performance of the neonates, it has led to the emergence of the drug-resistance in humans and livestock (Monroe and Polk 2000), as well as antibiotic-residues in animal products (Schwarz et al. 2001). Thus, there is a growing need worldwide to explore alternative dietary additives (Hayes et al. 2002; Wu et al. 2007; Yin et al. 2008).

Some polysaccharide phytochemicals can profoundly affect the immune system (Lee and Jeon 2005) and intestinal function (Kong et al. 2007a, b). Such work raised an attractive possibility that these natural substances may be highly effective to ameliorate the problems of weaning-associated gut dysfunction and growth retardation syndrome in pigs. In this regard, it is noteworthy that polysaccharide fractions of *Astragalus mongholicus* (AM) and *Astragalus* polysaccharide (APS) have been reported to reduce fatigue, the loss of appetite, and the incidence of diarrhea in animals (Bedir et al. 2000; Cui et al. 2003; Shao et al. 2004). Additionally, there is evidence that dietary supplementation with APS can improve growth performance in early weaned pig (Li et al. 2007a, b). However, the underling mechanisms are largely unknown.

Amino acids are not only building blocks for tissue proteins (major components of animal growth) but also key regulators of immune responses and metabolic pathways that regulate nutrient utilization (Jobgen et al. 2006; Hu et al. 2008; Wang et al. 2008; Yao et al. 2008). We hypothesized that dietary APS supplementation may stimulate the digestion of dietary protein and the absorption of resultant amino acids, therefore improving growth performance in early weaned piglets. This hypothesis was tested by determining apparent ileal digestibilities (AID) of amino acids (AA) and their serum concentrations in the piglets.

Materials and methods

Preparation of APS

Astragalus polysaccharide was isolated from AM, as previously described (Shao et al. 2004). Briefly, sliced rhizomes of AM grown in Liaoning Province of China were extracted three times with boiling water. The supernatant was applied to a DEAE-Sephacel (2.6 × 100 cm) column, and bound materials were eluted with a linear gradient of 0–2 mM NaCl. The fractions containing carbohydrates were pooled and precipitated three times with ethanol. The resultant polysaccharide extract was dialyzed against several changes of water and then lyophilized. The final product contained 95% carbohydrate but no detectable protein or nuclear acids, as measured at 280 and 260 nm wavelengths (Kong et al. 2007a). The molecular weight of the extract was approximately 3.5×10^3 to 1.55×10^6, as determined by the gel filtration method.

Animals, experimental design and diets

This study involved a growth trial (Exp. 1) and a digestibility experiment (Exp. 2). The protocol was approved by the Animal Care and Use Committee of the Institute of Subtropical Agriculture, The Chinese Academy of Sciences.

Exp. 1 (growth trial). Sixty crossed piglets [(Landrace × Yorkshire) × Duroc] with an initial average BW of 7.35 ± 0.23 kg were used in the 28-day growth trial. The pigs were weaned at 21 days of age and allocated randomly on the basis of body weight and litter origin to three treatments in a randomized complete block design. The dietary treatments were: the control group (basal diet), the antibiotics group (basal diet + 0.02% colistin), and the APS group (basal diet + 0.1% APS). There were 20 pigs (ten barrows and ten gilts) in each treatment, with one pig per pen. Each 0.6 × 1.2 m pen was equipped with a single-hole feeder and a water nipple to allow ad libitum consumption of feed and water. Feed was added to the feeders three times daily (0800, 1600, and 2400 hours). The temperature was kept at 28 ± 2°C, and relative humidity was maintained at 65–75%. Feed intake was determined weekly. Pigs were weighed 1 h prior to feeding in the morning at the beginning of the experiment (day 0), day 7, 14, 21 and the end of the experiment (day 28). Average daily gain (ADG), average daily feed intake (ADFI) and feed/gain ratio (F/G) were calculated.

All diets were formulated according to National Research Council (NRC 1998)-recommended requirements of nutrients by swine. Vitamins and minerals were supplemented to meet or exceed NRC (1998) standards for pigs with body weights of 10–20 kg. Ingredients and AA composition of the diets are summarized in Tables 1 and 2, respectively. On days 7, 14, and 28, pigs were weighed at 1 h prior to feeding in the morning. After weighing, blood samples were collected from the jugular vein of five pigs, randomly selected from each treatment, into 10-mL heparin-free vacutainer tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA). Blood samples were centrifuged at 3,000 rpm (Heraeus Biofuge 22R Centrifuge, Hanau, Germany) for 10 min at 4°C. The supernatant fluid (serum) was stored at −20°C for AA analysis.

Exp. 2 (digestibility experiment). Twelve barrows with an initial average BW of 7.64 ± 0.71 kg were assinged randomly into one of three dietary treatments groups, as described in Exp. 1. Each pig was surgically fitted with a

Table 1 Ingredient and chemical composition of the experimental diets (as-fed basis)

Item	Treatment		
	APS	Colistin	Control
Ingredient composition (%)			
Corn	64.00	64.00	64.00
Soybean meal	24.00	24.00	24.00
Fish meal	8.05	8.05	8.05
Acidifier[a]	1.00	1.00	1.00
Corn starch	0.00	0.08	0.10
$Ca(H_2PO_4)_2$	0.60	0.60	0.60
$CaCO_3$	0.74	0.74	0.74
Vitamin premix[b]	0.04	0.04	0.04
Choline chloride (50%)	0.08	0.08	0.08
Mineral premix[c]	0.15	0.15	0.15
Salt	0.30	0.30	0.30
Flavor	0.10	0.10	0.10
L-Lysine-HCl	0.65	0.65	0.65
DL-Methionine	0.06	0.06	0.06
L-Threonine	0.03	0.03	0.03
Titanium-oxide	0.10	0.10	0.10
APS	0.10	0.00	0.00
Colistin	0.00	0.02	0.00
Analyzed composition			
DE (MJ/kg)	14.22	14.21	14.25
DM (%)	90.12	89.92	90.20
CP (%)	22.92	22.88	22.91
Ca (%)	0.93	0.90	0.92
P (%)	0.54	0.54	0.53

APS Astragalus polysaccharide

[a] Guangzhou Tianke Industry Co., Guangzhou, Guangdong, China

[b] Supplied per kilogram of diet: vitamin A, 20,000 IU; vitamin D_3, 4,000 IU; vitamin E, 30 mg; vitamin K, 3 mg; vitamin B_2, 27 mg; vitamin B_6, 2 mg; vitamin B_{12}, 30 μg; biotin, 80 μg; folic acid, 8 mg; nicotinic acid, 24 mg

[c] Supplied per kilogram of diet: Zn ($ZnSO_4$), 165 mg; Fe ($FeSO_4$), 165 mg; Mn ($MnSO_4$), 33 mg; Cu ($CuSO_4$), 165 mg, I (CaI_2), 297 μg; Se (Na_2SeO_3), 297 μg

simple T-cannula at the terminal ileum according to the procedures described by Yin et al. (1991). The pre- and post-operative care of pigs was performed as described by Yin et al. (2004). The cannulas were prepared from Tygon tubing (Norton Performance Plastics, Wayne, NJ). The pigs were returned to the metabolic crates immediately after surgery. Additionally, the size of metabolic crates could be changed by adjusting a moveable lateral wall when needed. Each crate was equipped with a suspended water line fitted with a low-pressure nipple and wire flooring. During the 7-day period of recovery, the pigs received the basal diet ad libitum. Drinking water was freely available. The temperature and relative humidity were the same as in Exp.1.

Table 2 Analyzed amino acid composition of the experimental diets (%, as-fed basis)

Item	Treatment		
	APS	Colistin	Control
Crude protein	22.9	22.9	22.9
Indispensable amino acids			
Arginine	1.23	1.27	1.23
Cystine	0.29	0.32	0.33
Histidine	0.52	0.51	0.52
Isoleucine	0.80	0.82	0.82
Leucine	2.07	2.11	2.05
Lysine	1.28	1.27	1.36
Methionine	0.30	0.30	0.30
Phenylalanine	1.24	1.17	1.25
Threonine	0.83	0.84	0.88
Tryptophan	0.59	0.59	0.59
Tyrosine	0.23	0.23	0.23
Valine	1.03	1.06	1.03
Dispensable amino acids			
Alanine	1.21	1.28	1.35
Aspartate	1.94	1.93	1.92
Glutamate	3.76	3.37	3.44
Glycine	1.00	1.02	0.99
Proline	1.82	1.89	1.82
Serine	1.02	1.02	1.07

APS Astragalus polysaccharide

Following recovery, the pigs were fed the diet as described in Exp 1. All diets contained 1 g/kg titanium-oxide as a digestion marker. During the experimental period, the skin around the cannula was cleaned with lukewarm water several times daily. Additionally, foamed material was placed between the retaining ring and the skin to absorb any leaking digesta and prevent infection. At 0800 hours on days 7, 14 and 28, ileal digesta samples were collected for 24 h into plastic bags tied to the barrel of the cannula. The bags were removed and replaced when they were filled with the digesta. Ileal digesta samples were stored immediately at −20°C. At the end of the experiment, all the ileal digesta samples were thawed, pooled within pig and period, and homogenized. A subsample of each homogenate was freeze-dried and ground through a 1-mm mesh screen for chemical analysis.

Chemical analysis

Dry matter, crude protein, calcium, and phosphorus contents of diets and ileal digesta samples were analyzed according to AOAC (2003) procedures. Serum AA concentrations were determined using Hitachi L-8800 Amino Acid Analyzer (Tokyo, Japan), as previously described

(Yao et al. 2008). For AA analysis in diets and ileal digesta, samples were hydrolyzed in 6 N HCl at 110°C for 24 h, and sulfur amino acids were measured after performic acid oxidation, and tryptophan content was determined after alkaline hydrolysis (AOAC 2003). Titanium-oxide concentrations in feed and digesta were determined according to the method described by Yin et al. (2000). AID of AA was calculated using the following equation:

$$AID = (AA_f - AA_d) \times (TiO_{2f}/TiO_{2d})/AA_f$$

Where AA_f is AA concentration in diet, AA_d is AA concentration in digesta, TiO_{2f} is titanium-oxide concentration in diet, and TiO_{2d} is titanium-oxide concentration in digesta (Fan et al. 2005).

Statistical analysis

The data on growth performance were analyzed by one-way analysis of variance using the GLM procedure of SAS for a randomized complete block design (SAS Inst. Inc., Cary, NC). The data on serum-free AA concentrations and AID of AA were analyzed as a split-plot design for repeated measures using the GLM procedure of SAS. The statistical model included the effect of treatment as the main plot (tested by the animal within treatment variance) and effects of time and the treatment × time interaction as the subplot. Comparisons among treatments within sampling times were made when a significant F test ($P < 0.05$) for the treatment × time interaction was observed. The Duncan's multiple comparison test was used to determine differences among the means of treatment groups. $P < 0.05$ was taken to indicate statistical significance.

Results

Growth Performance (Exp 1)

All pigs were healthy and grew well throughout the entire experimental period. The final average body-weights of pigs in the APS, antibiotics, and control groups were 18.3, 17.6, and 17.2 kg, respectively. Data on growth performance of pigs are summarized in Table 3. The addition of APS reduced ($P < 0.05$) ADFI by 11% in week 2 but increased ($P < 0.05$) ADFI by 14% in week 4, compared with the control group. The overall ADFI in the 28-day experimental period did not differ between the APS-supplemented and control pigs. In comparison with the control group, APS supplementation did not affect ADG in weeks 1 and 2 but enhanced ($P < 0.05$) ADG in weeks 3 and 4 by 21 and 18%, respectively, resulting in an overall 11% increase of ADG during the 4-week period. At weeks 1, 3, and 4, the F/G ratio was 8.6, 9.8, and 6.0% lower in APS-supplemented pigs than in control pigs with an overall improvement of feed efficiency by 5.6% in 4 weeks. During the 28-day experimental period, ADFI did not differ between APS- and colistin-supplemented pigs, but ADG was 4.4% higher and F/G ratio was 8.4% lower in the APS group.

Table 3 Growth performance of piglets fed the experimental diets (Exp. 1)

Phase	Item	Treatment			Pooled SEM
		APS	Colistin	Control	
Week 1	ADFI (g)	277[b]	317[a]	289[ab]	4.2
Week 2		437[b]	499[a]	493[a]	8.6
Week 3		644	659	671	16
Week 4		927[a]	845[ab]	810[b]	13
Overall		617	624	578	13
Week 1	ADG (g)	226	229	211	4.4
Week 2		320	314	316	7.4
Week 3		439[a]	338[b]	363[b]	11
Week 4		612[a]	558[b]	520[b]	8.9
Overall		403[a]	386[a]	364[b]	6.3
Week 1	F/G	1.38[b]	1.43[ab]	1.51[a]	0.02
Week 2		1.56	1.57	1.56	0.02
Week 3		1.57[c]	1.64[b]	1.74[a]	0.03
Week 4		1.57[b]	1.65[a]	1.67[a]	0.02
Overall		1.53[b]	1.67[a]	1.62[a]	0.03

The experiment lasted 28 days; $n = 20$

[a, b, c] Values within a row with different letters differ ($P < 0.05$)

APS Astragalus polysaccharide, *SEM* standard error of the mean, *ADFI* average daily feed intake, *ADG* average daily gain, *F/G* feed/gain ratio

Serum-free AA concentration

Concentrations of AA in pig serum are summarized in Table 4. All AA, except for valine, were affected ($P < 0.05$) by treatment as well as time and treatment × time interaction. Consequently, comparisons of the means among treatments within sampling time were made. On day 7, concentrations of tryptophan, glutamate, and glycine were higher ($P < 0.05$) but concentrations of arginine, cystine, isoleucine, lysine, methionine, and phenylalanine were lower ($P < 0.05$) in the serum of APS-supplemented pigs than in control pigs. On day 14, serum concentrations of all AA except for threonine, valine, alanine, and aspartate were higher ($P < 0.05$) in the APS group, compared with the control group; serum concentrations of threonine, valine, alanine, and aspartate did not differ between these two groups of pigs. Similar results were observed on day 28, except that serum concentrations of phenylalanine, threonine, valine, and aspartate were higher ($P < 0.05$) in

Table 4 Serum concentrations of amino acids in piglets fed the experimental diets (μg/ml)

Item	Time									Pooled SEM	Time effect, *P* value
	Day 7			Day 14			Day 28				
	APS	Colistin	Control	APS	Colistin	Control	APS	Colistin	Control		
Nutritionally indispensable amino acids											
Arginine	32.5^{c}	46.6^{a}	40.1^{b}	44.4^{a}	38.5^{b}	35.1^{b}	53.4^{a}	47.5^{b}	42.0^{c}	1.4	<0.01
Cystine	3.8^{b}	3.5^{c}	5.0^{a}	6.9^{a}	4.6^{b}	3.9^{b}	3.6	3.4	3.7	0.22	<0.01
Histidine	21.3^{b}	25.7^{a}	20.5^{b}	17.5^{a}	13.0^{b}	14.2^{b}	17.9^{a}	16.1^{b}	13.2^{c}	0.64	<0.01
Isoleucine	18.4^{c}	23.5^{a}	19.9^{b}	23.8^{a}	21.3^{b}	18.8^{b}	18.8^{b}	22.3^{a}	16.4^{c}	0.57	0.01
Leucine	34.5^{b}	40.1^{a}	37.0^{ab}	35.5^{a}	35.2^{a}	30.3^{b}	39.7^{a}	39.3^{a}	27.3^{b}	0.88	<0.01
Lysine	38.4^{c}	54.4^{a}	47.8^{b}	61.2^{a}	50.5^{b}	43.5^{c}	46.4^{b}	48.5^{a}	37.6^{c}	0.99	<0.01
Methionine	24.1^{c}	41.0^{a}	36.0^{b}	41.1^{a}	34.4^{b}	31.7^{b}	47.4^{a}	42.5^{b}	35.5^{c}	1.2	<0.01
Phenylalanine	17.4^{b}	24.3^{a}	24.6^{a}	25.3	24.6	24.1	23.6^{a}	23.9^{a}	20.6^{b}	0.51	<0.01
Threonine	36.4^{c}	49.9^{a}	45.0^{b}	52.0^{a}	39.6^{c}	44.0^{b}	69.7^{a}	38.4^{c}	51.4^{b}	0.94	<0.01
Tryptophan	33.3^{b}	39.8^{a}	34.0^{b}	38.4^{a}	33.0^{b}	30.6^{b}	45.4^{a}	40.5^{b}	36.6^{c}	0.91	<0.01
Valine	25.4	31.0	25.4	29.3	29.5	27.7	27.9	31.5	24.4	0.88	0.09
Nutritionally dispensable amino acids											
Alanine	68.1^{b}	97.9^{a}	98.6^{a}	98.6^{a}	88.2^{b}	76.7^{b}	84.4^{b}	91.1^{a}	65.2^{c}	0.68	<0.01
Aspartate	6.9^{b}	9.6^{a}	6.9^{b}	11.3^{a}	8.1^{b}	7.5^{b}	11.2^{a}	11.6^{a}	9.9^{b}	0.54	<0.01
Glutamate	114^{b}	137^{a}	116^{b}	104^{b}	112^{b}	76.8^{c}	119^{b}	116^{b}	110^{b}	3.4	<0.01
Glycine	99.4^{b}	110^{a}	115^{a}	94.1^{b}	95.5^{b}	103^{b}	101^{b}	120^{a}	96.5^{b}	2.6	<0.01
Proline	23.0^{c}	39.3^{a}	33.6^{b}	38.3^{a}	34.4^{a}	29.0^{b}	44.6^{a}	39.7^{b}	35.3^{c}	1.4	<0.01
Serine	23.3^{b}	25.5^{ab}	26.6^{a}	27.6^{a}	25.1^{ab}	22.9^{b}	21.6^{b}	25.7^{a}	20.4^{c}	1.0	0.04
Tyrosine	74.1	73.2	70.9	64.4^{b}	73.4^{a}	53.3^{c}	77.4^{a}	80.7^{a}	72.1^{b}	1.1	<0.01

[a, b, c] Within the same age groups, values in a row sharing different superscript letters differ ($P < 0.05$); $n = 5$

APS Astragalus polysaccharides, *SEM* standard error of the mean

APS-supplemented pigs than in control pigs but serum concentrations of cystine, alanine, and proline did not differ between the two groups of pigs. On days 7, 14, and 28, dietary supplementation with colistin increased ($P < 0.05$) serum concentrations of most AA in pigs compared with the control group. On days 14 and 28, serum concentrations of arginine, histidine, isoleucine, lysine, methionine, and glycine were higher ($P < 0.05$) in APS- than in colistin-supplemented pigs, whereas serum concentrations of leucine, phenylalanine, threonine, tryptophan, tyrosine, valine, alanine, aspartate, glutamate, and proline did not differ between the two groups of pigs. The overall valine concentration in APS- and colistin-supplemented pigs were 27.53 μg/ml and 30.67 g/ml, and were higher ($P < 0.05$) than in control pigs (25.82 μg/ml), respectively.

AID of AA (Exp 2)

In Exp. 2, all pigs remained healthy and completely consumed their meals. At the end of the experiment, the pigs were euthanized. Examination of the cannulation site and gastrointestinal tract revealed no abnormalities. The AID values of all AA, except for arginine, were affected ($P < 0.05$) by treatment, time, and treatment × time interaction. Consequently, comparisons among treatments within sampling times were made (Table 5). Compared with the control group, the APS supplementation enhanced the AID of the following AA: (1) histidine, leucine, methionine, phenylalanine, tryptophan, glutamate, and glycine on day 7, (2) cystine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, tryptophan, tyrosine, alanine, glutamate, glycine, proline and serine on day 14, and (3) all AA, except for arginine, isoleucine, leucine, methionine, phenylalanine, alanine, and proline, on day 28. In comparison with pigs fed the control diet, dietary supplementation with colistin increased ($P < 0.05$) the AID of most AA, including: (1) cystine, histidine, leucine, methionine, phenylalanine, tryptophan, glutamate, and glycine on day 7, (2) all AA, except for leucine, aspartate, glutamate, and glycine on day 14, and (3) cystine, histidine, lysine, threonine, tryptophan, tyrosine, valine, glycine, and serine on day 28. On days 14 and 28, AID values of threonine, tryptophan, and alanine were higher in APS- than in colistin-supplemented pigs. The overall AID of arginine in

Table 5 Apparent ileal amino acid digestibility coefficients (%) in piglets fed the experimental diets

Item	Time									Pooled SEM	Time effect, *P* value
	Day 7			Day 14			Day 28				
	APS	Colistin	Control	APS	Colistin	Control	APS	Colistin	Control		
Nutritionally indispensable amino acids											
Arginine	88.2	89.7	84.7	89.3	87.4	83.4	86.5	87.6	83.3	0.87	0.07
Cystine	72.3^{b}	76.6^{a}	70.7^{b}	68.7^{a}	69.4^{a}	66.1^{b}	76.2^{b}	82.2^{a}	79.4^{c}	0.92	<0.01
Histidine	83.0^{a}	83.6^{a}	80.2^{b}	75.6^{a}	74.3^{a}	71.5^{b}	82.4^{a}	82.5^{a}	74.4^{b}	0.84	<0.01
Isoleucine	76.3	76.7	75.4	75.4^{a}	71.4^{b}	66.5^{c}	77.3	79.6	77.5	0.90	<0.01
Leucine	83.4^{a}	83.0^{a}	80.5^{b}	73.4^{a}	71.7^{ab}	68.3^{b}	79.5	82.5	81.2	0.99	<0.01
Lysine	77.2^{b}	79.5^{a}	79.3^{a}	74.7^{a}	73.6^{a}	68.5^{b}	77.5^{a}	78.7^{a}	73.7^{b}	0.92	<0.01
Methionine	74.4^{a}	75.4^{a}	69.6^{b}	76.6^{a}	75.2^{a}	63.4^{b}	75.4	74.3	76.1	1.0	<0.01
Phenylalanine	78.3^{a}	76.3^{a}	73.7^{b}	71.6^{a}	72.3^{a}	69.3^{b}	77.5	78.5	80.6	1.2	<0.01
Threonine	76.5	77.5	75.5	60.4^{b}	66.6^{a}	60.6^{b}	73.4^{a}	73.5^{a}	70.4^{b}	0.99	<0.01
Tryptophan	71.7^{a}	70.7^{a}	66.7^{b}	81.4^{a}	83.5^{a}	73.4^{b}	70.4^{a}	71.7^{a}	62.1^{b}	0.98	<0.01
Tyrosine	74.1	73.2	70.9	64.4^{b}	73.4^{a}	53.3^{c}	77.4^{a}	80.7^{a}	72.1^{b}	1.1	<0.01
Valine	81.1^{a}	77.0^{b}	78.4^{ab}	68.5^{b}	74.0^{a}	67.3^{b}	77.6^{a}	78.5^{a}	74.4^{b}	0.91	<0.01
Nutritionally dispensable amino acids											
Alanine	74.6	74.6	72.6	68.8^{a}	67.2^{a}	64.2^{b}	75.5^{b}	78.3^{a}	76.5^{ab}	0.77	<0.01
Aspartate	75.6	74.1	76.6	70.6	72.5	69.2	79.4^{a}	77.8^{ab}	74.5^{b}	1.1	<0.01
Glutamate	54.4^{a}	48.9^{b}	43.3^{c}	72.6^{a}	70.6^{ab}	67.2^{b}	76.3^{a}	70.6^{c}	73.3^{b}	1.2	<0.01
Glycine	68.7^{a}	68.4^{a}	60.6^{b}	72.4^{a}	67.2^{b}	68.3^{b}	72.5^{a}	70.3^{a}	58.3^{b}	0.99	0.02
Proline	75.6	76.6	74.8	76.3^{a}	78.5^{a}	72.6^{b}	73.4	70.7	69.5	1.1	<0.01
Serine	67.3	68.3	67.6	72.1^{a}	74.6^{a}	66.5^{b}	80.1^{a}	80.8^{a}	69.5^{b}	1.2	<0.01
Tyrosine	24.3a	22.1b	21.8b	35.6^{a}	29.5^{b}	30.9^{b}	33.7^{b}	37.9^{a}	27.6^{c}	0.84	<0.01

[a, b, c] Within the same age groups, values in a row sharing different superscript letters differ ($P < 0.05$); $n = 5$

APS Astragalus polysaccharides, *SEM* standard error of the mean

APS- and colistin-supplemented pigs were 88.00 and 88.09%, and were higher ($P < 0.05$) than in control pigs (83.41%), respectively.

Discussion

Weaning is a critical event in the postnatal growth and development of mammals. To increase the productivity of sows and reduce the incidence of infectious disease in herds, piglets are normally weaned at approximately 21 days of age on many swine farms (Tang et al. 2005; Kong et al. 2007a; Niekamp et al. 2007). However, early weaned piglets exhibit intestinal atrophy and dysfunction, which results in impaired digestion of dietary proteins and diminished absorption of the resultant small peptides and amino acids (Hampson 1986; Wu et al. 1996). Results of this work indicate for the first time that dietary supplementation with APS increased ileal digestibilities and serum concentrations of most AA (both nutritionally essential and nonessential) in pigs weaned at 21 days of age. These findings may provide a new strategy to ameliorate the weaning-associated wasting syndrome in piglets.

The ileum is the major site for the terminal digestion of dietary protein, as well as the absorption of small peptides and free AA (Libao-Mercado et al. 2006). A novel and important result from the current study is that digestibilities of most AA were greater in response to dietary supplementation with APS or colistin (Table 5). Additionally, AID values of two limiting AA (threonine and tryptophan) were higher in APS- than in colistin-supplemented pigs. Increased digestibilities of AA would result in increased absorption of AA into enterocytes. Although branched-chain amino acids, aspartate, glutamate, glutamine, proline, and arginine are extensively catabolized by enterocytes of post-weaning pigs (Wu et al. 1994; Wu 1997); degradation of other AA is absent or negligible in these cells (Chen et al. 2007). Thus, serum concentrations of these essential AA would rise (Table 4) when their absorption into the small intestine was enhanced (Table 5). Because muscle protein synthesis is very sensitive to the circulating levels of AA in young pigs (Davis et al. 1998; Frank et al. 2007)

via mTOR and perhaps other signaling pathways (Davis et al. 2000; Jobgen et al. 2006), an increase in serum concentrations of AA would promote protein accretion and thus growth performance in early weaned pigs (Table 3). Notably, as reported by Li et al. 2007a, b, a consistent effect of supplementing APS to the diet for weanling pigs is an increase in body-weight gain and enhancement of feed efficiency (Table 3). This is likely explained by the increased availability of AA for anabolic reactions in response to the APS treatment.

An interesting observation from the present study is that although dietary APS supplementation had no effect on ileal digestibility of arginine in pigs on days 7, 14, and 28 (Table 5), serum concentrations of arginine were markedly increased in ASP-supplemented pigs than in the control groups on all days of measurement (Table 4). Because whole-body protein deposition in pigs was augmented in response to the APS treatment (Table 3), the elevated level of serum arginine must result from endogenous synthesis of arginine. Both metabolic and enzymological studies have established that the small intestine plays a major role in the synthesis of arginine from glutamine/glutamate and proline (Wu and Morris 1998). Of particular interest, ileal digestibilities of glutamine/glutamate and proline on day 14 and of proline on day 28 were markedly increased by dietary APS supplementation (Table 5), therefore providing more substrates for the synthesis of arginine in enterocytes (Wu 1997). Recent studies have revealed that arginine stimulates muscle protein synthesis in young pigs via increasing the mTOR signaling activity (Yao et al. 2008). This likely contributes to the improvement of growth performance in the pigs (Table 3). In view of a critical role for arginine in metabolism and growth in young pigs (Wu et al. 2004), future studies are warranted to determine whether APS increases arginine synthesis in enterocytes as some bacteria-derived polysaccharides (Wu and Brosnan 1992) and to elucidate the underlying mechanisms.

Another novel finding of the current work is that although ileal digestibilities of threonine and valine on day 14 or ileal digestibilities of leucine, isoleucine, methionine, and phenylalanine on day 28 did not differ between APS-supplemented pigs and the control group (Table 5), serum concentrations of these essential AA were higher in APS-supplemented pigs (Table 4). Because these AA cannot be synthesized in enterocytes or extra-intestinal cells of pigs (Wu and Knabe 1995; Wu 1998) but can be extensively degraded by intestinal luminal bacteria (Nabuurs 1995; Chen et al. 2007), an increase in their serum concentrations in APS-treated pigs may result from a reduction in their catabolism by the gut microorganisms. This raised a possibility that APS may beneficially modulate the number, population, and activity of intestinal microbes to favor the entry of dietary AA into the portal circulation. Therefore, this phytochemical may be classified as a prebiotic to replace antibiotics in swine diets. Further research is necessary to test this important hypothesis.

In summary, although the precise mechanisms responsible for immuno-modulator and growth promoter of APS remains to be explored more, it was indicated from our current study that supplementing APS to the diet for early weaned pigs increased ileal digestibilities and serum concentrations of most AA, as well as body-weight gain and feed efficiency. Our results also indicate that APS may regulate AA metabolism in enterocytes and intestinal luminal microorganisms to beneficially increase the entry of dietary AA into the systemic circulation. We suggest that this phytochemical may be an effective, as well as a useful alternative of antibiotics in swine production.

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