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ORIGINAL ARTICLE

Dietary supplementation with Chinese herbal powder enhances ileal digestibilities and serum concentrations of amino acids in young pigs

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Abstract This study was designed to determine the effect of ultra-fine Chinese herbal powder as a dietary additive on serum concentrations and apparent ileal digestibilities (AID) of amino acids (AA) in young pigs. In Experiment 1, 60 Duroc × Landrace × Yorkshire piglets weaned at 21 days of age were randomly assigned to one of three treatments, representing supplementation with 0 or 2 g/kg of the powder, or 0.2 g/kg of colistin (an antibiotic) to corn- and soybean meal-based diets ($n = 20$ per group). Blood samples from five piglets per group were collected on days 7, 14, and 28 to determine serum AA concentrations. In Experiment 2, 12 barrows with an average initial body weight of 7.64 kg were randomly assigned to one of the three dietary treatments, followed by surgical placement of a simple T-cannula at the terminal ileum. All of the diets contained 0.1% titanium oxide as a digestibility marker. The samples of terminal ileal digesta were collected on day 7 for determining AID of AA. Results show that dietary supplementation with the herbal powder increased ($P < 0.05$) serum concentrations and AID of most AA by 10–50% and 10–16%, respectively. As an indicator of improved intestinal function, AID values of calcium were also enhanced in piglets supplemented with the herbal powder. Dietary supplementation of colistin increased serum concentrations and AID values of some AA by 8–44% and 10–15%, respectively, in comparison with the non-supplemented group. These novel findings demonstrate that the herbal powder can enhance the digestibility of dietary protein and the intestinal absorption of AA into the systemic circulation in post-weaning pigs, therefore providing a new mechanism for its growth- and immunity-promoting efficacy.

Keywords Ultra-fine Chinese herbal powder · Amino acids · Digestion · Piglets

Abbreviations

AA	Amino acids
AID	Apparent ileal digestibilities
CP	Crude protein
NO	Nitric oxide
UCH	Ultra-fine Chinese herbal

Introduction

Natural weaning of piglets is a gradual process and occurs over several weeks or months. However, in modern

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intensive swine production systems, piglets are weaned earlier, between 15 and 28 days of age to maximize the whole herd production (Smith et al. 2008). The abrupt changes in feed composition and environment at weaning dramatically alter intestinal metabolism and digestive function, which often results in intestinal malabsorption of nutrients (Wu 1998). Additionally, reduced provision of immunologically important factors from sow's milk (Wu and Knabe 2004) impairs immune function (Touchette et al. 2002), therefore increasing the susceptibility of piglets to the gram-negative bacterial infection and disease incidence. Further, weaning, as a major stress factor in swine production, reduces feed intake and efficiency and alters the composition of the gut microbiota (Namkung et al. 2004). Therefore, weaning is associated with growth retardation, as well as an increase in both morbidity and mortality in piglets (Smith et al. 2008).

Antimicrobials have traditionally been used as the first choice in prevention and treatment of diseases induced by weaning stress (Shuford and Patel 2005). However, they potentially impact human health due to the veterinary drug residues and emergence of antimicrobial resistant strains of zoonotic microorganisms in food animals (Shuford and Patel 2005). These public concerns have prompted European Union and other nations to ban the use of dietary antimicrobials (WHO 2002). Therefore, novel antimicrobial alternatives with low residues and low resistance are now being encouraged and under active investigation in many countries. Among potential antimicrobial alternatives, traditional Chinese herbal medicine is one of the most promising candidates (Deng et al. 2007; Ng et al. 2004; Yin et al. 2008a).

Ultra-fine Chinese herbal (UCH) powder with an average granule diameter of 30 μm, as a phytochemical dietary additive, was composed of *Acanthopanax senticosus* (AS), *Astragalus membranaceus* (AM), *Codonopsis pilosula* (COP), *Crataegus pinnatifida* (CRP), *Salvia miltiorrhiza* (SM), and chitosan (Kong et al. 2007a, b). As one of major components in the herbal power, AS is highly effective in treating allergies (Yi et al. 2002), stress-induced pathophysiologic changes (Fujikawa et al. 1996), and inflammation (Jung et al. 2003). The AS extract also enhances immune responses (Kong et al. 2007c) and physiological development of the gut microflora (Yin et al. 2008b) in weaned piglets. AM is known for its effect on stimulating energy metabolism, tissue regeneration, and immunity in the body (Cho et al. 2007). COP possesses immunomodulatory, antioxidant, free-radical scavenging, and antiulcer activities, and is commonly employed for treatment of dyspepsia, poor appetite, and psychoneurosis (Wang et al. 1996). Traditionally, CRP has a strong antibacterial activity against pathogenic bacteria (Kao et al. 2007), whereas SM is mainly used for treatment of infectious and inflammatory diseases (Kathrin et al. 2004). In addition, dietary supplementation of chitosan improves growth performance, feed efficiency, and the immune response in weaned piglets (Huang et al. 2007; Tang et al. 2005; Yin et al. 2008c).

Previous studies have demonstrated that the UCH powder is safe and effective in preventing intestinal dysfunction, improving growth performance (Kong et al. 2007a), as well as exerting beneficial effects on immune responses (Kong et al. 2007b) and gut microbiota development (He et al. 2008) in weanling piglets. However, the underlying mechanisms are largely unknown. Considering that most of composition herbs in the UCH powder are commonly employed for treatment of dyspepsia and poor appetite, and because amino acids (AA) are not only building blocks for protein synthesis but also serve as regulators of key metabolic pathways (Hu et al. 2008; Jobgen et al. 2006; Phang et al. 2008) and the immune response (Li et al. 2007), we hypothesized that the UCH powder may affect the digestion of dietary protein, the intestinal absorption of AA, and circulating levels of AA in weanling piglets. This hypothesis was tested in the present study using dietary supplementation with the herbal powder, as well as measuring the apparent ileal digestibilities (AID) and serum concentrations of AA. As another indicator of intestinal function, the AID of dry matter, energy, crude protein, crude ash, calcium, and phosphorus were also determined in weanling piglets.

Materials and methods

Preparation of the UCH powder

Herbs of the UCH powder were purchased from Changsha Pharmaceutical Co. (Hunan, China), and chitosan was provided by Dalian Institute of Chemical Physics, the Chinese Academy of Sciences (Dalian, China). The mixture was crumbled to an ultra-fine powder with an average granule diameter of 30 μm, packed in hermetical plastic bags, and stored at room temperature before use. Contents of total polysaccharide, flavone and organic acid in the powder, which were determined by the vitriol-anthracene ketone test, rutin test and base titration with 0.1 M NaOH (Kong et al. 2004, 2007c), were 2.61, 0.71, and 2.00%, respectively. Contents (%) of AA in the powder, analyzed by ion-exchange chromatography (Li et al. 2008), were as follows: alanine 0.309, arginine 0.335, aspartate plus asparagine 0.670, cysteine 0.162, glutamate plus glutamine 0.817, glycine 0.249, histidine 0.116, isoleucine 0.172, leucine 0.551, lysine 0.343, phenylalanine 0.425, proline 0.223, serine 0.328, threonine 0.242, tyrosine 0.036, valine 0.077, and total AA, 12.803.

Animal, housing, and treatment

This study was carried out in accordance with the Chinese guidelines for animal welfare and experimental protocol (Yin et al. 2004).

Experiment 1

A total of 60 castrated Duroc × Landrace × Yorkshire piglets with an average body weight of 5.64 kg were weaned at 21 days of age and randomly assigned to one of three treatments in a randomized complete block design, representing dietary supplementation with 0 or 2 g/kg of the UCH powder, or 0.2 g/kg of colistin, on the basis of body weight and sex, as described by Kong et al. (2007a, b). Colistin is an antibiotic commonly used for preventing and treating diarrhea in weanling piglets, and its dose of 0.2 g/kg was chosen according to the manufacturer's instructions. There were 20 piglets (10 barrows and 10 gilts) per treatment group, with one pig per pen. Each 0.6 m × 1.2 m pen was equipped with hard plastic completely slotted flooring, a single-hole feeder and a water nipple to allow ad libitum consumption of feed and water. The temperature and relative humidity were maintained at 28 ± 2°C and 65–75%, respectively, in an air-controlled nursery room. The ingredients and composition of the diets are summarized in Tables 1 and 2. On days 7, 14, and 28 after initiation of the dietary supplementation with the UCH powder, jugular venous blood samples (5 ml per piglet) were withdrawn randomly from five piglets per treatment group by venepuncture into plastic uncoated tubes between 8:00 and 10:00 a.m. Sera were obtained by centrifugation at 750 ×*g* and 4°C for 20 min and stored at −20°C until analysis for AA. One milliliter of the serum sample and 2.5 ml of 7.5% trichloracetic acid solution were mixed thoroughly and centrifuged at 12,000 ×*g* and 4°C for 15 min. The supernatant fluid was collected and determined for AA by an ion-exchange AA analyzer (Hitachi L-8800 Auto-Analyzer, Tokyo, Japan) as described by Li et al. (2008).

Experiment 2

Twelve castrated barrows with an average body weight of 7.64 kg were randomly assigned into one of three dietary treatments, as described in "Experiment 1". Each piglet was surgically fitted with a simple T-cannula at the terminal ileum according to the procedures of Yin et al. (1991). A detailed description of pre- and post-operative care was previously described by Yin et al. (1993). The

Table 1 Dietary ingredients and nutrient levels in diets

Dietary ingredients (%)	Dietary supplementation		
	Ultra-fine Chinese herbal powder	Colistin	None
Corn (8.24% CP)	66.55	66.55	66.55
Soybean meal (44.72% CP)	24.00	24.00	24.00
Fish meal (CP 65.05%)	6.00	6.00	6.00
Acidifier	1.00	1.00	1.00
Corn starch	0	0.18	0.20
$Ca(H_2PO_4)_2$	0.60	0.60	0.60
$CaCO_3$	0.74	0.74	0.74
Vitamin premix[a]	0.04	0.04	0.04
Choline chloride (50%)	0.08	0.08	0.08
Trace element premix[b]	0.15	0.15	0.15
Salt	0.25	0.25	0.25
Flavor	0.05	0.05	0.05
L-Lysine HCl	0.25	0.25	0.25
L-Methionine	0.06	0.06	0.06
L-Threonine	0.03	0.03	0.03
Ultra-fine Chinese herbal powder	0.20	0	0
Colistin	0	0.02	0
Nutrient levels			
Metabolic energy (MJ/kg)	13.91	13.89	13.82
Crude protein	20.77	19.55	20.26
Calcium	0.93	0.72	0.87
Phosphorus	0.54	0.54	0.53

[a] Providing the following vitamins per kg diet: 20,000 IU vitamin A, 400,000 IU vitamin D_3, 3,000 mg vitamin E, 300 mg vitamin K, 700 mg vitamin B_2, 200 mg vitamin B_6, 3 mg vitamin B_{12}, 8 mg biotin, 800 mg folic acid, and 2400 mg nicotinic acid

[b] Providing the following minerals per kg diet: 165 mg Zn ($ZnSO_4$), 165 mg Fe ($FeSO_4$), 33 mg Mn ($MnSO_4$), 16.5 mg Cu ($CuSO_4$), 297 μg CaI_2, and 297 μg Se (Na_2SeO_3)

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

Item	Dietary supplementation		
	UCH powder	Colistin	None
Alanine	0.96	1.16	1.07
Arginine	0.85	1.07	1.00
Aspartate[a]	1.53	2.01	1.82
Cysteine	0.23	0.23	0.26
Glutamate[b]	2.97	3.82	3.49
Glycine	0.79	0.94	0.85
Histidine	0.41	0.53	0.49
Isoleucine	0.60	0.79	0.70
Leucine	1.73	1.81	1.64
Lysine	0.93	1.41	1.18
Methionine	0.03	0.03	0.07
Phenylalanine	0.98	1.18	1.09
Serine	0.81	1.00	0.92
Proline	1.25	1.32	1.29
Threonine	0.65	0.82	0.75
Tyrosine	0.17	0.20	0.20
Valine	0.71	0.91	0.82

[a] Including aspartate plus asparagine

[b] Including glutamate plus glutamine

cannulas were prepared from Tygon tubing (Shao Guang Plastics, Changsha, Hunan, China). The piglets were returned to the metabolic crates immediately after surgery. Additionally, the size of metabolism crates could be changed by a moveable lateral wall when necessary. During a 7-day recovery period, the piglets had free access to drinking water and the basal diet (the same control diet as in "Experiment 1"). The temperature and relative humidity were the same as in "Experiment 1". Following recovery, the piglets were fed the diets as described in "Experiment 1", except that all of the diets contained 0.1% titanium oxide as a digestibility marker. During the experimental period, the skin around the cannula was cleaned with lukewarm water several times daily and dried, followed by application of a skin protecting paste (Stomahesive Paste, Convatec, Princeton, USA). Additionally, foamed material was placed between the retaining ring and the skin to absorb leaking digesta and prevent inflammation or ulcer.

Samples of ileal digesta were collected, from 08:00 to the next 08:00 on day 7, into plastic bags tied to the barrel of the cannula. The bags were removed and replaced as soon as they were filled with digesta. Ileal digesta were stored immediately at 4°C after collection. At the end of the experiment, ileal digesta were pooled within the same pig and homogenized. A subsample of each homogenate was freeze-dried and ground through a 1-mm mesh screen for analysis. Dry matter, crude ash, energy, crude protein, calcium, and phosphorus contents of diets and ileal digesta were analyzed according to AOAC (2003) procedures. AA compositions of ileal digesta samples were determined by an ion-exchange AA analyzer after hydrolysis in 6 N HCl in sealed vacuum tubes at 110°C for 24 h according to the method of Li et al. (2008). Titanium oxide contents in the feed and digesta were determined according to the method described by Yin et al. (2000). The AID of AA was calculated in the following manner:

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

Where $\mathrm{AA_f}$ is the AA content in diet (Table 2), $\mathrm{AA_d}$ is the AA content in digesta, $\mathrm{TiO_{2f}}$ is the analyzed titanium oxide content in diet, and $\mathrm{TiO_{2d}}$ is the analyzed titanium oxide content in digesta (Fan et al. 2005).

Statistical analysis

Data were expressed as the mean ± SEM. Results were statistically analyzed using one-way ANOVA (SAS Institute, Cary, NC). The Duncan's multiple range test was used to compare differences among the treatment groups. A P value of less than 0.05 was taken to indicate statistical significance.

Results

Serum concentrations of AA in piglets

On day 7 of the feeding trial ("Experiment 1"), total concentrations of serum AA in piglets fed the UCH powder-supplemented diet were lower ($P < 0.05$) than those in colistin-supplemented piglets but did not differ ($P > 0.05$) from the values in the non-supplemented group (Table 3). The UCH-powder treatment increased ($P < 0.05$) serum concentrations of asparagine and phenylalanine, while decreasing ($P < 0.05$) those of cysteine, glycine and lysine, when compared with the non-supplemented group. Serum concentrations of lysine, phenylalanine and tyrosine in piglets fed the UCH powder-supplemented diet were higher ($P < 0.05$), but serum concentrations of glutamine and methionine were lower ($P < 0.05$) than those in the colistin-supplemented group. Interestingly, colistin supplementation decreased ($P < 0.05$) serum concentrations of cysteine, glycine, lysine, and tyrosine but increased ($P < 0.05$) serum concentrations of asparagine and glutamine, compared with the non-supplemented group.

On day 14 of the feeding trial, total concentrations of serum AA in piglets fed the UCH powder-supplemented diet were higher ($P < 0.05$) than those in the non-supplemented

Table 3 Serum concentrations (μg/mL) of amino acids in weaned piglets on day 7 after initiation of dietary supplementation with ultra-fine Chinese herbal powder

Item	Dietary supplementation		
	UCH powder	Colistin	None
Alanine	92.1 ± 5.7	98.2 ± 13.5	98.8 ± 11.5
Arginine	44.3 ± 10.8	46.6 ± 4.5	40.3 ± 3.8
Aspartate[A]	10.2 ± 0.5[a]	9.7 ± 1.6[a]	6.9 ± 2.0[b]
Cysteine	3.8 ± 0.4[b]	3.5 ± 0.4[b]	4.9 ± 0.2[a]
Glutamate[B]	110 ± 9.0[b]	136 ± 11.0[a]	117 ± 14.0[b]
Glycine	89 ± 6.0[b]	102 ± 15.0[b]	129 ± 17.0[a]
Histidine	17.9 ± 2.2	15.9 ± 2.8	20.6 ± 3.9
Isoleucine	23.3 ± 3.0	23.8 ± 1.6	19.8 ± 2.5
Leucine	40.2 ± 6.7	39.8 ± 0.8	37.5 ± 5.5
Lysine	66.1 ± 4.5[b]	54.3 ± 6.1[c]	78.6 ± 8.3[a]
Methionine	9.9 ± 2.3[b]	13.0 ± 1.8[a]	11.6 ± 1.0[ab]
Phenylalanine	30.3 ± 1.4[a]	24.3 ± 1.3[b]	24.7 ± 1.8[b]
Serine	26.5 ± 4.9	25.4 ± 4.0	26.9 ± 3.1
Threonine	70.2 ± 14.9	49.9 ± 18.8	54.6 ± 6.7
Tyrosine	31.0 ± 2.9[a]	22.1 ± 4.5[b]	28.4 ± 2.9[a]
Valine	35.7 ± 4.5	31.9 ± 5.5	35.4 ± 2.8
Total AA	641 ± 66[b]	759 ± 61[a]	729 ± 19[ab]

Data are expressed as means ± SEM, $n = 5$. Means with different superscripts in a row differ ($P < 0.05$)

AA amino acids

[A] Including aspartate plus asparagine

[B] Including glutamate plus glutamine

Table 4 Serum concentrations (μg/mL) of amino acids in weaned piglets on day 14 after initiation of dietary supplementation with ultra-fine Chinese herbal powder

Item	Dietary supplementation		
	UCH powder	Colistin	None
Alanine	99.2 ± 10.3[a]	88.2 ± 8.2[ab]	76.3 ± 9.2[b]
Arginine	42.7 ± 2.4	38.9 ± 10.6	35.0 ± 4.9
Aspartate[A]	10.0 ± 0.5[a]	8.0 ± 1.2[b]	7.1 ± 1.0[b]
Cysteine	4.5 ± 0.6	4.9 ± 0.7	3.9 ± 0.8
Glutamate[B]	115 ± 14[a]	111 ± 14[a]	77.0 ± 4.0[b]
Glycine	113 ± 13	96 ± 10	100 ± 13
Histidine	11.7 ± 1.7	13.1 ± 2.5	14.4 ± 0.9
Isoleucine	22.3 ± 2.2	21.5 ± 3.3	18.6 ± 3.5
Leucine	31.8 ± 3.1	35.0 ± 3.5	30.5 ± 8.2
Lysine	44.5 ± 3.6	50.1 ± 8.8	43.4 ± 1.4
Methionine	11.9 ± 0.8[a]	7.7 ± 0.7[b]	8.4 ± 2.5[b]
Phenylalanine	25.1 ± 1.8	24.8 ± 4.6	24.2 ± 2.7
Serine	27.7 ± 2.7[a]	25.1 ± 2.7[ab]	22.8 ± 2.7[b]
Threonine	55.7 ± 3.3[a]	39.7 ± 4.6[b]	44.0 ± 4.9[b]
Tyrosine	27.1 ± 2.6	29.3 ± 4.3	31.9 ± 6.8
Valine	28.8 ± 3.3	29.9 ± 4.0	27.0 ± 2.6
Total amino acid	693 ± 28[a]	623 ± 93[ab]	575 ± 89[b]

Data are expressed as means ± SEM, $n = 5$. Means with different superscripts in a row differ ($P < 0.05$)

[A] Including aspartate plus asparagine

[B] Including glutamate plus glutamine

group but did not differ ($P > 0.05$) from the values for colistin-supplemented piglets (Table 4). The UCH powder increased ($P < 0.05$) serum concentrations of alanine, glutamine, and serine compared with the non-supplemented group, as well as serum concentrations of asparagine, methionine, and threonine compared with the other two groups. Serum concentration of glutamine in piglets fed the colistin-supplemented diet was higher ($P < 0.05$) than that in the non-supplemented group (Table 4).

On day 28 of the feeding trial, total concentrations of AA in piglets fed the UCH powder- and colistin-supplemented diets were higher ($P < 0.05$) when compared with piglets in the non-supplemented group, but did not differ from the value for colistin-supplemented pigs (Table 5). The UCH powder increased ($P < 0.05$) serum concentrations of phenylalanine, leucine, isoleucine, valine, alanine, lysine, methionine, and histidine, compared with the non-supplemented group, as well as those of threonine and histidine compared with the colistin-supplemented pigs. In addition, the colistin treatment increased ($P < 0.05$) serum concentrations of tyrosine, leucine, isoleucine, valine, alanine, histidine, and lysine compared with the non-supplemented group (Table 5). Serum concentrations of other AA did not differ ($P > 0.05$) among the three treatments.

AID of crude protein, energy, dry matter, minerals, and AA

The AID of crude protein, energy, dry matter, or total minerals did not differ ($P > 0.05$) among the three groups of pigs (Table 6). However, the AID of calcium was higher ($P < 0.05$) but the AID of phosphorus was lower ($P < 0.05$) in pigs fed the UCH powder-supplemented diet than that in the non-supplemented group (Table 6). The AID of calcium did not differ ($P > 0.05$) between the UCH powder- and colistin-supplemented pigs.

Table 7 summarizes the AID of AA in pigs on day 7 after initiation of UCH powder supplementation. Both of the UCH powder and colistin enhanced ($P < 0.05$) the AID values of histidine, isoleucine, methionine, phenylalanine and threonine, when compared with the non-supplemented group. In addition, the AID of arginine in piglets fed the UCH powder-supplemented diet was higher ($P < 0.05$) than those in the other two groups. Further, dietary colistin supplementation increased ($P < 0.05$) the AID of leucine

Table 5 Serum concentrations (μg/mL) of amino acids in weaned piglets on day 28 after initiation of dietary supplementation with ultra-fine Chinese herbal powder

Item	Dietary supplementation		
	UCH powder	Colistin	None
Alanine	97.5 ± 14.3[a]	91.1 ± 8.4[a]	65.5 ± 7.2[b]
Arginine	46.7 ± 14.5	47.4 ± 5.9	42.2 ± 4.7
Aspartate[A]	11.9 ± 2.7	11.8 ± 0.8	9.8 ± 1.5
Cysteine	3.9 ± 0.7	3.4 ± 0.3	3.7 ± 0.9
Glutamate[B]	122 ± 22	116 ± 13	107 ± 13
Glycine	108 ± 17	120 ± 11	97 ± 19
Histidine	18.8 ± 0.6[a]	16.1 ± 1.2[b]	13.1 ± 2.2[c]
Isoleucine	22.9 ± 2.3[a]	22.9 ± 2.6[a]	16.5 ± 1.6[b]
Leucine	39.6 ± 2.9[a]	39.0 ± 4.1[a]	27.1 ± 2.6[b]
Lysine	56.4 ± 7.3[a]	48.8 ± 5.5[a]	37.6 ± 3.6[b]
Methionine	12.0 ± 1.6[a]	10.4 ± 1.1[ab]	8.1 ± 2.0[b]
Phenylalanine	26.2 ± 4.9[a]	23.9 ± 2.5[ab]	20.7 ± 2.2[b]
Serine	24.7 ± 2.7[ab]	25.5 ± 3.4[a]	20.0 ± 3.4[b]
Threonine	67.1 ± 14.4[a]	38.7 ± 5.5[b]	51.5 ± 15.9[ab]
Tyrosine	30.6 ± 9.2[ab]	37.9 ± 4.5[a]	27.8 ± 4.9[b]
Valine	33.0 ± 4.2[a]	31.9 ± 4.9[a]	24.1 ± 3.4[b]
Total AA	746 ± 39[a]	731 ± 68[a]	545 ± 35[b]

Data are expressed as mean ± SEM, $n = 5$. Means with different superscripts in a row differ ($P < 0.05$)

AA amino acids

[A] Including aspartate plus asparagine

[B] Including glutamate plus glutamine

Table 6 Apparent ileal digestibilities (%) of nutrients in weaned piglets after initiation of dietary supplementation with ultra-fine Chinese herbal powder

Apparent ileal digestibility	Dietary supplementation		
	UCH powder	Colistin	None
Dry matter	87.7 ± 0.47	86.7 ± 0.90	87.8 ± 0.38
Crude ash	97.8 ± 0.21	97.7 ± 0.14	97.6 ± 0.18
Energy	87.5 ± 1.0	87.9 ± 0.43	87.0 ± 1.7
Crude protein	86.1 ± 3.8	87.3 ± 1.6	87.0 ± 1.1
Calcium	71.6 ± 4.2[a]	71.4 ± 4.0[a]	65.1 ± 6.1[b]
Phosphorus	47.1 ± 5.8[b]	55.6 ± 5.0[a]	54.6 ± 3.6[a]

Data are expressed as means ± SEM, $n = 4$. Means with different superscripts in a row differ ($P < 0.05$)

and tyrosine, in comparison with the non-supplemented group. The AID values of other AA did not differ ($P > 0.05$) among the three groups of pigs.

The AID values of AA in pigs on day 14 are shown in Table 8. Dietary supplementation with the UCH powder or colistin increased ($P < 0.05$) the AID of arginine, glutamate plus glutamine, lysine, and valine, compared with the non-supplemented group. In addition, the UCH powder

Table 7 Apparent ileal digestibilitis (%) of amino acids in weaned piglets on day 7 after initiation of dietary supplementation with ultra-fine Chinese herbal powder

Item	Dietary supplementation		
	UCH powder	Colistin	None
Alanine	70 ± 2.4	73 ± 1.2	71 ± 1.4
Arginine	70 ± 2.0[a]	65 ± 1.3[b]	64 ± 2.0[b]
Aspartate[A]	72 ± 2.6	73 ± 2.0	70 ± 3.7
Cysteine	63 ± 0.0	65 ± 2.1	62 ± 2.0
Glutamate[B]	75 ± 1.9	75 ± 1.6	73 ± 1.9
Glycine	71 ± 2.4	71 ± 2.3	72 ± 2.4
Histidine	64 ± 1.3[a]	65 ± 1.1[a]	60 ± 1.1[b]
Isoleucine	70 ± 2.2[a]	70 ± 1.1[a]	65 ± 1.5[b]
Leucine	75 ± 1.1[ab]	79 ± 2.2[a]	70 ± 3.4[b]
Lysine	66 ± 1.4	67 ± 2.0	65 ± 2.3
Methionine	68 ± 2.0[a]	70 ± 2.8[a]	61 ± 2.0[b]
Phenylalanine	72 ± 1.2[a]	72 ± 1.1[a]	70 ± 0.9[b]
Serine	66 ± 1.9	67 ± 2.9	65 ± 3.0
Threonine	65 ± 2.1[a]	65 ± 2.0[a]	59 ± 2.3[b]
Tyrosine	65 ± 2.2[ab]	68 ± 2.0[a]	60 ± 2.1[b]
Valine	65 ± 3.3	62 ± 2.1	64 ± 2.3

Data were expressed as means ± SEM, $n = 4$. Means with different superscripts in a row differ ($P < 0.05$)

[A] Including aspartate plus asparagine

[B] Including glutamate plus glutamine

enhanced ($P < 0.05$) the AID of leucine and serine, as well as colistin enhancing phenylalanine and histidine, in comparison with the non-supplemented pigs.

On day 28 after initiation of dietary supplementation, both the UCH powder and colistin increased ($P < 0.05$) the AID values of most AA, including aspartate plus asparagines, cysteine, glycine, histidine, leucine, lysine, phenylalanine, serine, threonine, and valine compared with the non-supplemented group (Table 9). In addition, the AID of arginine was higher ($P < 0.05$) in the UCH powder-supplemented pigs than in the other two groups. The AID values of other AA did not differ ($P > 0.05$) among the three groups of pigs.

Discussion

There has been growing interest in recent years in the use of Chinese herbal medicines or their ingredients as new, alternative growth promoters for livestock and poultry (Guo et al. 2004; Kong et al. 2007a; Yin et al. 2008a). This study determines serum concentrations of AA, as well as the AID of AA, other organic nutrients (including crude protein and energy) and minerals (including crude ash, calcium and phosphorus) in weaned piglets supplemented with either a UCH powder or an antibiotic (colistin). The

Table 8 Apparent ileal digestibilities (%) of amino acids in weaned piglets on day 14 after initiation of dietary supplementation with ultra-fine Chinese herbal powder

Item	Dietary supplementation		
	UCH powder	Colistin	None
Alanine	78 ± 2.4	77 ± 3.4	77 ± 2.4
Arginine	82 ± 3.1[a]	83 ± 2.1[a]	76 ± 2.1[b]
Aspartate[A]	80 ± 3.5	81 ± 3.4	80 ± 3.3
Cysteine	77 ± 3.1	76 ± 3.0	74 ± 3.1
Glutamate[B]	85 ± 2.7[a]	84 ± 2.5[a]	79 ± 2.4[b]
Glycine	78 ± 2.5	80 ± 3.3	77 ± 4.4
Histidine	74 ± 2.2[ab]	78 ± 2.2[a]	70 ± 2.1[b]
Isoleucine	79 ± 3.1	81 ± 3.3	78 ± 2.4
Leucine	81 ± 3.4[a]	78 ± 2.4[ab]	76 ± 2.2[b]
Lysine	73 ± 2.3[a]	74 ± 3.3[a]	67 ± 1.5[b]
Methionine	76 ± 3.3	75 ± 4.1	74 ± 3.5
Phenylalanine	78 ± 2.9[ab]	81 ± 1.7[a]	74 ± 1.9[b]
Serine	81 ± 2.3[a]	79 ± 2.4[ab]	75 ± 2.3[b]
Threonine	77 ± 2.4	80 ± 2.3	78 ± 3.7
Tyrosine	80 ± 3.1	79 ± 2.3	81 ± 2.4
Valine	83 ± 2.2[a]	84 ± 3.4[a]	78 ± 2.2[b]

Data are expressed as means ± SEM, $n = 4$. Means with different superscripts in a row differ ($P < 0.05$)

[A] Including aspartate plus asparagine

[B] Including glutamate plus glutamine

Table 9 Apparent ileal digestibilities (%) of amino acids in weaned piglets on day 28 after initiation of dietary supplementation with ultra-fine Chinese herbal powder

Item	Dietary supplementation		
	UCH powder	Colistin	None
Alanine	82 ± 3.1	82 ± 3.9	80 ± 4.0
Arginine	88 ± 2.2[a]	80 ± 2.1[b]	81 ± 3.1[b]
Aspartate[A]	88 ± 1.2[a]	87 ± 2.0[a]	76 ± 2.2[b]
Cysteine	80 ± 2.2[a]	82 ± 2.3[a]	75 ± 2.3[b]
Glutamate[B]	90 ± 1.6	89 ± 3.2	90 ± 3.4
Glycine	82 ± 1.9[a]	83 ± 2.2[a]	75 ± 2.0[b]
Histidine	81 ± 2.0[a]	83 ± 2.1[a]	72 ± 2.0[b]
Isoleucine	83 ± 3.2	86 ± 3.1	84 ± 4.0
Leucine	88 ± 2.5[a]	89 ± 2.4[a]	82 ± 2.9[b]
Lysine	82 ± 2.1[a]	82 ± 2.1[a]	75 ± 2.1[b]
Methionine	80 ± 2.2	81 ± 3.2	82 ± 2.3
Phenylalanine	83 ± 2.2[a]	84 ± 2.3[a]	76 ± 2.4[b]
Serine	89 ± 3.2[a]	90 ± 2.2[a]	83 ± 2.1[b]
Threonine	80 ± 2.1[a]	83 ± 2.2[a]	75 ± 2.3[b]
Tyrosine	79 ± 2.3	80 ± 3.1	77 ± 3.1
Valine	79 ± 3.1[a]	80 ± 3.2[a]	74 ± 2.3[b]

Note: Data were expressed as means ± SEM, $n = 4$. Means with different superscripts in a row differ ($P < 0.05$)

[A] Including aspartate plus asparagine

[B] Including glutamate plus glutamine

results show that dietary supplementation with the UCH powder increased the circulating levels (Tables 3, 4, 5) and AID values (Tables 7, 8, 9) of most AA by 15–25% in piglets weaned at 21 days of age. Further, supplementing the UCH powder to the piglet diet improved the AID of calcium by ~10% in the animals (Table 6). In addition, dietary supplementation with colistin enhanced serum concentrations and AID values of some AA by 8–44% and 10–15%, respectively, as well as the AID of calcium by ~10%. These findings are novel and important because they indicate that the bioavailabilities of dietary AA were greater in UCH powder-supplemented pigs, an agriculturally important species and an established animal model for studying human nutrition and metabolism (Ou et al. 2007; Suryawan et al. 2008). The results are consistent with our previous report that dietary supplementation with the UCH powder increased daily weight gain in weaned pigs by 18% (Kong et al. 2007a).

AA regulate key metabolic pathways that are crucial for maintenance, health, and growth of animals (Hu et al. 2008; Jobgen et al. 2006; Wu et al. 2004). An increase in the amounts of nutrients (particularly AA) that enter the portal vein from the small intestine can be sufficient to promote tissue protein synthesis in piglets (Wu et al. 2007). Consistent with this view, dietary supplementation with the UCH powder-enhanced serum concentrations and AID of most AA in 21- to 49-day-old weaned piglets, which resulted in the growth- promoting efficacy of the UCH powder in weanling piglets (Kong et al. 2007a). Additionally, the UCH-powder treatment increased ADG and ADFI, and improved the gain: feed ratio by 11% on day 21 after the supplementation compared with the non-supplemented group (Kong et al. 2007a). At the end of the 28-day trial, the body weight of the powder-supplemented piglets was 13% greater than the non-supplemented group (Kong et al. 2007a). Therefore, the Chinese herbs can stimulate the digestion and absorption of dietary nutrients (particularly protein or AA), and may also directly regulate the metabolism of absorbed nutrients through signal transduction mechanisms, including AA, nitric oxide (NO) and pyrroline-5-carboxylate signaling (Galli 2007; Grillo and Colombatto 2007; Hu et al. 2007; Jobgen et al. 2006).

A deficiency of dietary protein or AA has long been known to impair immune function and increase the susceptibility of animals to infectious disease (Li et al. 2007). Increasing evidence shows that dietary supplementation of specific AA to animals with malnutrition, infectious disease, or weaning-associated stress enhances the immune status, thereby reducing morbidity and mortality (Li et al. 2007; Tan

et al. 2008). In the present study, we found that the UCH-powder treatment consistently increased serum concentrations of arginine on days 7, 14, and 28 after initiation of the dietary supplementation (Tables 3, 4, 5). Our finding raised a possibility that both protein digestion and endogenous arginine synthesis may be up-regulated by certain phytochemicals. Arginine is one of the most versatile AA in metabolism and is utilized by multiple pathways (Wu and Morris 1998). Of particular interest, this AA is the common substrate for the synthesis of NO and polyamines, which have enormous biological importance (Wu and Meininger 2002). Also, arginine may activate the mTOR signaling pathway for protein synthesis (Yao et al. 2008), therefore promoting the proliferation and differentiation of cells in the immune and other systems. Early in vitro studies have identified that arginine is required for the proliferation of rodent and human T lymphocytes in response to mitogens and the killing of tumor cells by activated macrophages (Hibbs et al. 1987). The mediating molecules are now known to be polyamines and NO, respectively (Li et al. 2007). In our previous study, dietary supplementation with 0.2% UCH powder to weaned piglets enhanced the production of cytokines and antibodies, as well as lymphocyte proliferating activity (Kong et al. 2007b). Its immunity-enhancing efficacy may be contributable to the increased NO production, because the UCH-powder treatment increased serum levels of NO metabolites (nitrite and nitrate) in young pigs (Kong unpublished data). Therefore, AA (especial arginine) and NO production by iNOS are of most relevance to the stimulatory effect of the UCH powder on the immune response in piglets. An improvement in immune function brought about by dietary supplementation with the UCH powder can also play an important role in minimizing the use of glucose and AA by cells of the immune system (Field et al. 2002), therefore directing these nutrients towards tissue protein synthesis.

Another novel and important finding from this study is that serum concentration and AID of glutamine were increased on day 14 after initiation of the UCH-powder supplementation (Table 4). Glutamine plays a vital role in the homeostasis of the immune system, acid–base balance, intestinal function, redox status, and muscle protein accretion (Li et al. 2007; Wang et al. 2008; Wu et al. 2007). Interestingly, plasma and muscular concentrations of glutamine exhibit a marked decline in response to catabolic states (e.g., fasting, inflammation, and early stage of lactation) and intensive exercise (Newsholme and Calder 1997). As an essential precursor for the synthesis of purine and pyrimidine nucleotides, glutamine is required for proliferation of lymphocytes, and as a major energy substrate for cells of the immune system (Field et al. 2002), glutamine is necessary for mounting a successful immune response to an immunological challenge. The UCH-powder treatment also increased serum concentrations of alanine and asparagine [two metabolites of glutamine (Wu et al. 2007)] in pigs, compared with the non-supplemented group (Tables 4 and 5). These two AA also regulate T lymphocyte proliferation and antibody production by B cells (Li et al. 2007). Additionally, alanine is a major substrate for the hepatic synthesis of glucose (a significant energy substrate for lymphocytes and macrophages), which in turn influences immune function (Li et al. 2007).

It is noteworthy that dietary UCH-powder supplementation enhanced the circulating levels of many essential AA (Tables 3, 4, 5) whose carbon skeletons cannot be synthesized in pigs but which must be adequately provided from the diet to support maximal growth (Wu et al. 2007). For example, serum concentrations of phenylalanine on days 7 and 28 after initiation of the UCH-powder supplementation were increased (Tables 3, 5) and its AID values on days 7, 14, and 28 were also enhanced (Tables 7, 8, 9) in response to the herbal treatment, compared with the non-supplemented group. Besides serving as an substrate for protein synthesis, an important function of phenylalanine is to up-regulate GTP cyclohydrolase I activity, which is the first and rate-controlling enzyme for the synthesis of tetrahydrobiopterin (an essential cofactor for NO synthase) (Shi et al. 2004). In addition, the UCH powder-supplemented treatment increased serum concentrations of serine on days 14 and 28 after initiation of the supplementation, as well as the AID of serine on days 14 and 28 (Tables 8, 9). The multiple pathways for serine utilization include one-carbon unit metabolism, the hepatic and renal synthesis of glucose, and the synthesis of glycine, ceramide, and phosphatidylserine as structural components and signaling molecules of cells, including T and B lymphocytes (Jones et al. 1999). Indeed, phosphatidylserine has been implicated in the regulation of IL-2 production and T lymphocyte activation in response to immunological challenge (Pelassy et al. 1991). Further, on both days 14 and 28, the AID and circulating levels of threonine (a major component of intestinal mucosal protein) were approximately 10 and 30% higher in UCH powder-supplemented pigs than in the non-supplemented pigs. Moreover, on day 28, serum concentrations of leucine [an AA that can activate the mTOR signaling pathway (Liao et al. 2008)] were enhanced in response to dietary UCH-powder supplementation (Table 5). Collectively, these novel findings help explain why the UCH powder can promote the growth performance and immunity in weaned piglets.

At present, it is not clear how dietary UCH-powder supplementation can improve AA digestibilities in pigs. However, it is known that the UCH powder increased the growth of beneficial lactobacillus (e.g., *bifidobacteria* and *lactobacilli*) and suppressed of bacterial pathogens (*E. coli*) (He et al. 2008), which suggested that the UCH powder could effectively promote the development of the normal

gut microbiota and healthy intestinal environment in the weaned piglets. These lactobacillus are also beneficial for maintaining the integrity and function of the small intestine (Yang et al. 2007), which then promotes the absorption and transport of glucose, AA, calcium and other nutrients across the intestinal epithelium into the portal vein (Wu 1998). Furthermore, the UCH powder may affect the metabolism of nutrients (particularly AA) in the lumen of the small intestine by altering the growth and metabolism of gut microbiota, therefore resulting in changes in the amounts of AA (free and protein-bound) in the ileal digesta. The variation of AID values for different AA may be explained by the different actions of microbes on metabolism in the lumen of the small intestine (Fuller and Redes 1998).

In summary, dietary supplementation with the UCH powder enhances the AID and absorption of dietary AA in weaned piglets. The efficacy of the herbal powder is more potent than colistin, a widely used antibiotic. These findings aid in explaining the growth- and immunity-promoting effect of the UCH powder. Because the underlying mechanisms are likely to be complex and multifactorial, future studies are warranted to determine the effects of active components of the UCH powder on the digestion and absorption of dietary nutrients.

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References

Cho WCS, Leung KN (2007) In vitro and in vivo immunomodulating and immunorestorative effects of *Astragalus membranaceus*. J Ethnopharmacol 113:132–141

Deng ZY, Zhang JW, Wu GY, Yin YL, Ruan Z, Li TJ, Chu WY, Kong XF, Zhang YM, Fan YW, Liu R, Huang RL (2007) Dietary supplementation with polysaccharides from *semen cassiae* enhances immunoglobulin production and interleukin gene expression in early-weaned piglets. J Sci Food Agric 87: 1868–1873

Fan MZ, Li TJ, Yin YL, Fang RJ, Tang ZR, Hou ZP, Huang RL, Deng ZY, Zhong HY, Zhang RG, Zhang J, Wang B, Schulze H (2005) Effect of phytase supplementation with two levels of phosphorus diets on ileal and faecal digestibilities of nutrients and phosphorus, calcium, nitrogen and energy balances in growing pigs. Anim Sci 81:67–75

Field CJ, Johnson IR, Schley PD (2002) Nutrients and their role in host resistance to infection. J Leukoc Biol 71:16–32

Fujikawa T, Yamaguchi A, Morita I, Takeda H, Nishibe S (1996) Protective effects of *Acanthopanax senticosus* harms from *Hokkaido* and its components on gastric ulcer in restrained cold water stressed rats. Biol Pharmaceut Bull 19:1227–1230

Fuller MF, Redes PJ (1998) Nitrogen cycling in the gut. Annu Rev Nutr 18:385–411

Galli F (2007) Amino acid and protein modification by oxygen and nitrogen species. Amino Acids 32:497–499

Grillo MA, Colombatto S (2007) S-Adenosylmethionine and radical-based catalysis. Amino Acids 32:197–202

Guo FC, Kwakkel RP, Soede J, Williams BA, Verstegen MWA (2004) Effect of a Chinese herb medicine formulation, as an alternative for antibiotics, on performance of broilers. Br Poult Sci 6:793–797

He QH, Kong XF, Hou YQ, Yin YL, Yin FG, Liu HJ, Li TJ, Huang RL, Yu H, Gong JH, Nyachoti CM (2008) Effects of Chinese herbal ultra-fine powder as a dietary additive on gut microflora in early-weaned piglets. Recent Prog Med Plants 24:453–465

Hibbs HB, Taintor RR, Vavrin Z (1987) Macrophage cytotoxicity: Role for L-arginine deiminase and imino nitrogen oxidation to nitrite. Science 235:473–476

Hu CA, Donald SP, Yu J, Lin WW, Liu ZH, Steel G, Obie C, Valle D, Phang JM (2007) Overexpression of proline oxidase induces proline-dependent and mitochondria-mediated apoptosis. Mol Cell Biochem 295:85–92

Hu CA, Khalil S, Zhaorigetu S, Liu Z, Tyler M, Wan G, Valle D (2008) Human Δ^1-pyrroline- 5-carboxylate synthase: function and regulation. Amino Acids. doi:10.1007/S00726-008-0075-0

Huang RL, Yin YL, Li MX, Wu G, Li TJ, Li LL, Yang CB, Zhang J, Wang B, Deng ZY, Zhang YG, Tang ZR, Kang P, Guo YM (2007) Dietary oligochitosan supplementation enhances immune status of broilers. J Sci Food Agric 87:153–159

Jobgen WS, Fried SK, Fu WJ, Meininger CJ, Wu G (2006) Regulatory role for the arginine-nitric oxide pathway in energy-substrate metabolism. J Nutr Biochem 17:571–588

Jones DR, Gonzalez-Garcia A, Diez E, Martinez AC, Carrera AC, Merida I (1999) The identification of phosphatidylinositol 3, 5-bisphosphate in T-lymphocytes and its regulation by interleukin-2. J Biol Chem 274:18407–18413

Jung HJ, Park HJ, Kim RG, Shin KM, Ha J, Choi JW, Kim HJ, Lee YS, Lee KT (2003) In vivo anti-inflammatory and anti-nociceptive effects of liriodendrin isolated from the stem bar of *Acanthopanax senticosus*. Planta Med 69:610–616

Kao ES, Wang CJ, Lin WL, Chu CY, Tseng TH (2007) Effects of polyphenols derived from fruit of *Crataegus pinnatifida* on cell transformation, dermal edema and skin tumor formation by phorbol ester application. Food Chem Toxicol 45:1795–1804

Kathrin D, Clarissa G, Karin K, Matthias H (2004) HPLC-based activity profiling of *salvia miltiorrhiza* for MAO A and iNOS inhibitory activities. Planta Med 70:909–913

Kong XF, Hu YL, Rui R, Wang DY, Li XR (2004) Effects of Chinese herbal medicinal ingredients on peripheral lymphocyte proliferation and serum antibody titer after vaccination in chicken. Int J immunopharmacol 4:975–982

Kong XF, Wu GY, Liao YP, Hou ZP, Liu HJ, Yin FG, Li TJ, Huang RL, Zhang YM, Deng D, Kang P, Wang RX, Tang ZY, Yang CB, Deng ZY, Xiong H, Chu WY, Ruan Z, Xie MY, Yin YL (2007a) Effects of Chinese herbal ultra-fine powder as a dietary additive on growth performance, serum metabolites and intestinal health in early-weaned piglets. Livest Sci 108:272–275

Kong XF, Wu GY, Liao YP, Hou ZP, Liu HJ, Yin FG, Li TJ, Huang RL, Zhang YM, Deng D, Xie MY, Deng ZY, Xiong H, Ruan Z, Kang P, Yang CB, Yin YL, Fan MZ (2007b) Dietary supplementation with

Chinese herbal ultra-fine powder enhances cellular and humoral immunity in early-weaned piglets. Livest Sci 108:94–98

Kong XF, Yin YL, Wu GY, Liu HJ, Yin FG, Li TJ, Huang RL, Ruan Z, Xiong H, Deng ZY, Xie MY, Liao YP, Kim SW (2007c) Dietary supplementation with the *acanthopanax senticosus* extract enhances cellular and humoral immunities in early-weaned piglets. Asian-Aust J Anim Sci 20:1453–1461

Li P, Yin YL, Li DF, Kim SW, Wu G (2007) Amino acids and immune function. Br J Nutr 98:237–252

Li TJ, Dai QZ, Yin YL, Zhang J, Huang RL, Ruan Z, Deng Z, Xie M (2008) Dietary starch sources affect net portal appearance of amino acids and glucose in growing pigs. Animal 2:723–729

Liao XH, Majithia A, Huang XL, Kimmel AR (2008) Growth control via TOR kinase signaling, an intracellular sensor of amino acids and energy availability, with crosstalk potential to proline metabolism. Amino Acids. doi:10.1007/s00726-008-0100-3

Namkung H, Li M, Gong J, Yu H, Cottrill M, de Lange CFM (2004) Impact of feeding blends of organic acids and herbal extracts on growth performance, gut microbiota and digestive function in newly weaned pigs. Can J Anim Sci 84:697–704

Newsholme EA, Calder PC (1997) The proposed role of glutamine in some cells of the immune system and speculative consequences for the whole animal. Nutrition 13:728–730

Ng TB, Liu F, Wang HX (2004) The antioxidant effects of aqueous and organic extracts of *Panax quinquefolium*, *Panax notoginseng*, *Codonopsis pilosula*, *Pseudostellaria heterophylla* and *Glehnia littoralis*. J Ethnopharmacol 93(2–3):285–288

Ou DY, Li DF, Cao YH, Li XL, Yin JD, Qiao SY, Wu G (2007) Dietary supplementation with zinc oxide decreases expression of the stem cell factor in the small intestine of weanling pigs. J Nutr Biochem 18:820–826

Pelassy C, Breittmayer JP, Mary D, Aussel C (1991) Inhibition of phosphatidylserine synthesis by phosphatidic acid in the Jurkat T cell line: role of calcium ions released from intracellular stores. J Lipid Mediat 4:199–209

Phang JM, Donald SP, Pandhare J, Liu Y (2008) The metabolism of proline, as a stress substrate, modulates carcinogenic pathways. Amino Acids. doi:10.1007/s00726-008-0063-4

Shi W, Meininger CJ, Haynes TE, Hatakeyama K, Wu G (2004) Regulation of tetrahy- drobiopterin synthesis and bioavailability in endothelial cells. Cell Biochem Biophys 41:415–433

Shuford JA, Patel R (2005) Antimicrobial growth promoter use in livestock-implications for human health. Rev Med Microbiol 16:17–24

Smith AL, Stalder KJ, Serenius TV, Baas TJ, Mabry JW (2008) Effect of weaning age on nursery pig and sow reproductive performance. J Swine Health Prod 16:131–137

Suryawan A, O'Connor PMJO, Bush JA, Nguyen HV, Davis TA (2008) Differential regulation of protein synthesis by amino acids and insulin in peripheral and visceral tissues of neonatal pigs. Amino Acids. doi:10.1007/s00726-008-0149-z

Tan BE, Li XG, Kong XF, Huang RL, Ruan Z, Yao K, Deng ZY, Xie MY, Shinzato I, Yin YL, Wu G (2008) Dietary L-arginine supplementation enhances the immune status in early-weaned piglets. Amino Acids. doi:10.1007/s00726-008-0155-1

Tang ZR, Yin YL, Nyachoti CM, Huang RL, Li TJ, Yang CB, Yang XJ, Gong JH, Peng J, Qi DS, Xing JJ, Sun ZH, Fan MZ (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. Dom Anim Endocrinol 28:430–441

Touchette KJ, Carroll JA, Allee GL, Matteri RL, Dyer CJ, Beausang LA, Zannelli ME (2002) Effect of spray-dried plasma and lipopolysaccharide exposure on weaned pigs: I. Effects on the immune axis of weaned pigs. J Anim Sci 80:494–501

Wang ZT, Ng TB, Yeung HW, Xu GJ (1996) Immunomodulatory effect of a polysaccharide-enriched preparation of *Codonopsis pilosula* roots. Gen Pharmacol 27:1347–1350

Wang JJ, Chen LX, Li P, Li XL, Zhou HJ, Wang FL, Li DF, Yin YL, Wu G (2008) Gene expression is altered in piglet small intestine by weaning and dietary glutamine supplementation. J Nutr 138: 1025–1032

WHO (2002) Antimicrobial resistance. http://www.who.int/mediacentre/factsheets/fs194/en/print.html. Accessed 1 May 2007

Wu G (1998) Intestinal mucosal amino acid catabolism. J Nutr 128:1249–1252

Wu G, Meininger CJ (2002) Regulation of nitric oxide synthesis by dietary factors. Annu Rev Nutr 22:61–86

Wu G, Morris SM Jr (1998) Arginine metabolism: nitric oxide and beyond. Biochem J 336:1–17

Wu G, Fang YZ, Yang S, Lupton JR, Turner ND (2004) Glutathione metabolism and its implications for health. J Nutr 134:489–492

Wu GY, Bazer FW, Davis TA, Jaeger LA, Johnson GA, Kim SW, Knabe DA, Meininger CJ, Spencer TE, Yin YL (2007) Important roles for the arginine family of amino acids in swine nutrition and production. Livest Sci 112:8–22

Yang F, Wang JJ, Li XJ, Ying TY, Qiao SY, Li DF, Wu G (2007) Two-dimensional gel electrophoresis and mass spectrometry analysis of interactions between *Lactobacillus Fermentum* I5007 and intestinal epithelial cells. Electrophoresis 28:4330–4339

Yao K, Yin YL, Chu WY, Liu ZQ, Deng D, Li TJ, Huang RL, Zhang JS, Tan BE, Wang W, Wu G (2008) Dietary arginine supplementation increases mTOR signaling activity in skeletal muscle of neonatal pigs. J Nutr 138:867–872

Yi JM, Hong SH, Kim JH, Kim HK, Song HJ, Kim HM (2002) Effect of *Acanthopanax senticosus* stem on mast cell-dependent anaphylaxis. J Ethnopharmacol 79:347–352

Yin YL, Huang RL, Zhong HY, Chen CM, Dai H (1991) Influence of different cannulation techniques on the pre-cecal digestibility of protein, amino acids and cell wall constituents from diets, containing different protein meal, in pigs. Anim Feed Sci Tech 35:271–281

Yin YL, Huang RL, Zhong HY (1993) Comparison of the ileorectal anastomsis and conventional method for the measurement of ileal digestibility of protein sources and mixed diets in growing pigs. Anim Feed Sci Tech 42:297–308

Yin YL, McEvoy J, Schulze H, McCracken KJ (2000) Studies on cannulation method and alternative indigestible markers and the effects of food enzyme supplementation in barley-based diets on ileal and overall apparent digestibility in growing pigs. Anim Sci 70:63–72

Yin YL, Deng ZY, Huang RL, Li TJ, Zhong HY (2004) The effect of arabinoxylanase and protease supplementation on nutritional value of diets containing wheat bran or rice bran in growing pig. J Anim Feed Sci 13:445–461

Yin FG, Liu YL, Yin YL, Kong XF, Huang RL, Li TJ, Wu GY, Hou YQ (2008a) Dietary supplementation with *Astragalus polysaccharide* enhances ileal digestibilities and serum concentrations of amino acids in early weaned piglets. Amino Acids. doi: 10.1007/s00726-008-0142-6

Yin FG, Yin YL, Kong XF, Liu HJ, Yang CB, Wu GY, He QH, Li TJ, Huang RL (2008b) Dietary supplementation with *Acanthopanax senticosus* extract modulates gut microflora in weaned piglet. Asian-Aust J Anim Sci (in press)

Yin YL, Tang ZR, Sun ZH, Liu ZQ, Li TJ, Huang RL, Ruan Z, Deng ZY, Gao B, Chen LX, Wu GY, Kim SW (2008c) Effect of galacto-mannan-oligosaccharides or chitosan supplementation on cytoimmunity and humoral immunity response in early-weaned piglets. Asian-Aust J Anim Sci 21:723–731