Amino Acids (2009) 37:199–208
DOI 10.1007/s00726-008-0192-9

ORIGINAL ARTICLE

Metabolomic analysis of the response of growing pigs to dietary L-arginine supplementation

Qinghua He · Xiangfeng Kong · Guoyao Wu · Pingping Ren · Huiru Tang · Fuhua Hao · Ruilin Huang · Tiejun Li · Bie Tan · Peng Li · Zhiru Tang · Yulong Yin · Yongning Wu

Received: 15 September 2008 / Accepted: 5 October 2008 / Published online: 7 November 2008

Abstract Arginine plays an important role regulating nutrient metabolism, but the underlying mechanisms are largely unknown. This study was conducted to determine the effect of dietary arginine supplementation on the metabolome in serum of growing pigs using ^{1}H nuclear magnetic resonance spectroscopy. Sixteen 120-day-old pigs (48 ± 1 kg) were randomly assigned to one of two groups, representing supplementation with 0 or 1.0% L-arginine to corn- and soybean meal-based diets. Serum was collected after a 46-day period of treatment. Dietary arginine supplementation decreased fat deposition and increased protein accretion in the body. Principal component analysis showed that serum concentrations of low density lipoprotein, very low density lipoprotein, and urea were lower, but concentrations of creatinine, tricarboxylic acid cycle metabolites, ornithine, lysine and tyrosine were greater in arginine-supplemented than in control pigs. Additionally, the arginine treatment affected serum concentrations of nitrogenous and lipid signaling molecules (glycerophosphorylcholine and myo-inositol) and intestinal bacterial metabolites (formate, ethanol, methylamine, dimethylamine, acetate, and propionate). These novel findings suggest that dietary arginine supplementation alters the catabolism of fat and amino acids in the whole body, enhances protein synthesis in skeletal muscle, and modulates intestinal microbial metabolism in growing pigs.

Keywords Arginine · Metabolomics · Serum · Pigs · Nuclear magnetic resonance spectroscopy

Abbreviations

COSY	Correlated spectroscopy
CPMG	Carr–Purcell–Meiboom–Gill
GPC	Glycerophosphorylcholine
LDL	Low density lipoprotein
NMR	Nuclear magnetic resonance
PCA	Principal component analysis
TCA	Tricarboxylic acid cycle
TMAO	Trimethylamine-*N*-oxide
TOCSY	Total correlation spectroscopy
VLDL	Very low density lipoprotein

Q. He · X. Kong · Y. Yin (✉)
State Key Laboratory of Food Science and Technology, Nanchang University, 330047 Nanchang, Jiangxi, China
e-mail: yinyulong@isa.ac.cn

Q. He · X. Kong · G. Wu · R. Huang · T. Li · B. Tan · P. Li · Z. Tang · Y. Yin
Laboratory of Animal Nutritional Physiology and Metabolism, Institute of Subtropical Agriculture, The Chinese Academy of Sciences, 410125 Changsha, Hunan, China

Q. He · Y. Wu (✉)
National Institute for Nutrition and Food Safety, Chinese CDC, 100050 Beijing, China
e-mail: wuyn@public.bta.net.cn

G. Wu · P. Li
Department of Animal Science, Texas A&M University, College Station, TX 77843-2471, USA

P. Ren · H. Tang · F. Hao
State Key Laboratory of Magnetic Resonance and Atomic and Molecular Physics, Wuhan Centre for Magnetic Resonance, Wuhan Institute of Physics and Mathematics, The Chinese Academy of Sciences, 430071 Wuhan, Hubei, China

Introduction

Arginine is not only a building block for proteins but also an essential precursor for the synthesis of biologically

important metabolites, including nitric oxide, polyamines, and creatine (Galli 2007; Grillo and Colombatto 2007; Wu and Morris 1998). In addition, arginine plays an important role in regulating nutrient metabolism and immune function in animals (Fu et al. 2005; Jobgen et al. 2006; Kim and Wu 2008; Li et al. 2008; Tan et al. 2008a), therefore enhancing the efficiency of feed utilization for protein accretion. In support of this notion, muscle protein synthesis (Yao et al. 2008) and body weight gain (Kim and Wu 2004) were enhanced in arginine-supplemented piglets. Additionally, dietary arginine supplementation reduced fat accretion and improved the metabolic profile in Zucker diabetic fatty rats (Fu et al. 2005; Wu et al. 2007c). At present, the underlying mechanisms for the action of arginine remain largely unknown.

Metabolomics, defined as the analysis of metabolome (a complete set of small molecule metabolites in a biological sample), has emerged as a powerful discovery tool in nutrition research (Wang et al. 2007; Wang et al. 2008b). The two widely used detection methods are nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (Nicholson and Wilson 2003; Nicholson et al. 2004). Metabolic fingerprints can be obtained by NMR to detect changes in concentrations of metabolites in accessible physiological fluids (e.g., plasma and urine) (Bertram et al. 2005; Dumas et al. 2006; Lenz et al. 2004; Rezzi et al. 2007b). Alterations in the metabolome can provide an insight into end points of metabolic fluxes in cells, tissues, or the whole body (Wang et al. 2007).

In view of the foregoing, we hypothesized that enteral arginine provision may alter metabolites of amino acids, glucose and fatty acids in serum. This hypothesis was tested with the pig [a widely used animal model for studying human nutrition and metabolism (Ou et al. 2007; Suryawan et al. 2008; Van Goudoever et al. 2000)] using high-resolution ^{1}H NMR spectroscopy in conjunction with multivariate data analysis.

Materials and methods

Pigs, diets, housing and experimental design

Sixteen healthy castrated male pigs (Duroc × Landrace × Large Yorkshire strain) were obtained from a local commercial swine herd and had free access to the corn- and soybean meal-based diet (Table 1; Tianke Company, Guangzhou, China) for 1 week before being fed experimental diets. The basal diet without antibiotics was formulated to meet or exceed the nutrient recommendations of National Research Council (1998). At 120 days of age, pigs were randomly assigned to one of two groups, representing supplementation with 2.05% L-alanine (isonitrogenous control) or 1.0% L-arginine to the basal diet ($n = 8$ pigs per group). L-Alanine and L-arginine were obtained from Ajinomoto Inc. (Tokyo, Japan) and added to the basal diet at the expense of wheat bran. At the beginning of amino acid supplementation, the initial body weights of pigs in the control and arginine groups were 48.0 ± 4.1 and 48.0 ± 3.3 kg (mean ± SD), respectively. Pigs were housed individually in an environmentally controlled facility (23.5°C; 55% relative humidity; 12-h dark and 12-h light cycle) with hard-plastic completely slotted flooring, and had free access to diets and drinking water. The experiment was carried out in accordance with the Chinese guidelines for animal welfare and experimental protocol and approved by the Animal Care and Use Committee of The Chinese Academy of Sciences (Yin et al. 2004).

Table 1 Composition and nutrient levels of the basal diet (g/kg)

Items	Content
Ingredients	
Corn	633.2
Soybean meal	253
Wheat bran	50
Soybean oil	34
Salt	3.0
Calcium hydrogen phosphate	7.0
Calcium carbonate	9.8
Vitamin-mineral premix[a]	10
Nutrients[b]	
Crude protein	172.3
Total phosphorus	5.2
Total calcium	7.5
L-Arginine	10.7
L-Lysine	9.7
Digestible energy, (kJ/g)	14.28

[a] Supplying the following (mg/kg diet): Cu (as $CuSO_4$), 15; Zn (as $ZnSO_4$), 104; Fe (as $FeSO_4$), 100; Mn (as $MnSO_4$), 19; vitamin A, 10,000 IU; vitamin D, 1,000 IU; vitamin E, 40 IU; vitamin K, 2.5; choline, 570; pantothenic acid, 16; riboflavin, 5; folic acid, 2; niacin, 25; thiamine, 1.6; vitamin B6, 1.8; biotin, 0.2; vitamin B12, 0.25; choline chloride (50%), 1,000; calcium propionate, 1,000; ethoxyquine, 10; and wheat bran, 6,590

[b] Calculated values according to NRC (National Research Council 1998)

Serum collection and storage

At the end of a 46-day period of supplementation, blood samples (10 mL) were collected by venipuncture of the jugular vein between 08:00 and 10:00 hours following a 12-h period of feed deprivation to avoid a postprandial effect on serum metabolites (Kong et al. 2008; Yin et al. 2008). Sera were separated from whole blood by

centrifugation at 1000*g* and 4°C for 10 min and stored in 2-mL aliquots at −80°C until NMR analysis and other biochemical analyses.

^{1}H NMR spectroscopic measurement of serum

One hundred microlitres of 0.9% NaCl (saline) in D_2O was mixed with 500 μL serum (D_2O was added for locking signal). Proton NMR measurements of serum were recorded on a Bruker Avance DRX-600 spectrometer (Bruker Biospin, Rheinstetten, Germany) at 298 K, operating at a ^{1}H frequency of 600.11 MHz and equipped with a triple-resonance, high-resolution probe. A total of 10 min was allowed for the temperature to reach equilibration for each sample before a spectrum was acquired. The 90° pulse length (∼10.0 μs) was adjusted individually for each sample. A total of 32 transients were collected into 32k data points for each spectrum with a spectral width of 20 ppm and a recycle delay (RD) of 2.0 s.

Three ^{1}H NMR spectra were acquired for each sample. (1) A standard one-dimensional NMR spectrum (NOESY) which is a general representation of the total biochemical composition, was acquired using the first increment of the NOESY pulse sequence to achieve water presaturation [90°-t_1-90°-t_m-90°-acq] (Nicholson et al. 1995). The interpulse delay t_1 was 3 μs, the mixing time t_m was 100 ms, and irradiation of the water resonance was used during t_m and RD. (2) A Carr–Purcell–Meiboom–Gill (CPMG) spin-echo pulse experiment designed to attenuate interfering signals from macromolecules with short spin–spin relaxation times was acquired using the CPMG pulse sequence [90°-(T-180°-T)$_n$-acq] (Meiboom and Gill 1958). A total spin–spin relaxation delay ($2n\tau$) of 200 ms and water peak irradiation was applied during RD. (3) A diffusion-edited NMR spectrum, which selectively measures large macromolecules, was acquired using the bipolar-pair longitudinal-eddy-current (BPP-LED) pulse sequence [RD-90°-G_1-τ-180°-G_2-τ-90°-Δ-90°-G_3-τ-180°-G_4-τ-90°-T_e-90°-acq] (Wu et al. 1995). Duration of 2.5 ms was used for diffusion-edited spectra, followed by a delay (τ) of 400 μs to allow for the decay of eddy currents. A diffusion time (Δ) of 100 ms and a delay T_e of 5 ms were used together with water peak irradiation during RD. For resonance assignment purposes, two-dimensional (2D) ^{1}H–^{1}H correlation spectroscopy (COSY) (Hurd 1990) and total correlation spectroscopy (TOCSY) (Bax and Davis 1985) were also acquired for selected serum samples.

Conventional biochemical measurements

Serum biochemical metabolites included glucose, low density lipoprotein (LDL), very low density lipoprotein (VLDL), high density lipoprotein (HDL); triglycerides, total cholesterol, total protein, albumin, and blood urea nitrogen. All assays were performed using a CX-4 Auto-Blood Biochemical Analyzer (Beckman Inc., Fullerton, CA, USA) according to the manufacturer's instructions (Beijing Leadman Biochemistry Technology Co. Ltd, Beijing, China).

Growth performance and carcass composition

Body weights and feed intakes were measured and recorded at the beginning of the trial and weekly thereafter. The average daily gain and the average daily feed consumption were calculated for the period of arginine supplementation (Tan et al. 2008b). On the last day of the feeding trial, immediately after blood collection was completed, all pigs were immediately anesthetized with sodium pentobarbital (50 mg/kg body weight) and killed by jugular puncture and eviscerated (Tan et al. 2008b). The head and skin were removed. Then the carcass was split longitudinally. Each right carcass side was weighed and then physically dissected into muscle, fat and bone.

Data analysis

Free induction decays were multiplied by an exponential window function of 1.0 Hz prior to Fourier transformation and corrected for phase and baseline distortions using TopSpin 2.0 (Bruker). Chemical shift was referenced to the peak of the methyl proton of L-lactate at δ1.33.

Water-suppressed NMR spectra were binned and automatically integrated with TopSpin 2.0 (Bruker). The integrals of these bins covered the region δ0.5–8.5 at spectra CMPG, NOESY and BPP-LED. The integral data were input as variables for principal component analysis (PCA). The region δ4.66–5.20 was removed to avoid the effects of imperfect water suppression. The region δ4.08–4.20 and δ1.30–1.40 was also removed due to the presence of peaks from L-lactate whose serum concentrations increased in the process of blood collection. Consequently, the spectra over the range δ0.5–1.30, 1.40–4.08, 4.20–4.66 and δ5.20–8.5 were selected, and reduced to 3,671 regions each 0.002 ppm wide. Each integral region was normalized to the sum of all integral regions for each spectrum prior to pattern recognition analyzes. PCA was performed on all NMR spectra of this cohort using mean-centered scaling with the software Simca-P 10.0 (Umetrics, Sweden). Data were visualized with the PC scores plots, where each point represents an individual spectrum of a sample, and loadings plots, where each point represents a single NMR spectral region or chemical shifts. From the score and loading plots, classification of samples and the biochemical components responsible for the classification, respectively, can be listed (Yang et al. 2007).

Relative integrals from some selected metabolites contributing to the classification of control and arginine-supplemented pigs, as well as data on other serum biochemical metabolites, are presented as mean ± SD. Results were statistically analyzed using one-way ANOVA (SAS Institute, NC, USA). Duncan's multiple range test was used to compare differences among the treatment groups. Growth performance, carcass measurements and serum biochemical metabolites measured by conventional method were evaluated by Student's *t* test. A *P*-value of less than 0.05 was taken to indicate statistical significance.

Results

^{1}H NMR spectrum of serum samples

Examples of a typical one-dimensional ^{1}H NMR CPMG (Fig. 1a), NOESY (Fig. 1b) and BPP-LED (Fig. 1c) spectrum from a representative control pig fed the basal diet are illustrated in Fig. 1. From these spectra, 49 metabolites were unambiguously assigned. Their chemical shifts and peak multiplicity are given in Table 2 along with the corresponding ^{1}H NMR chemical shifts and signal multiplicities. Assignment of metabolites was made by comparison with the published literature (Liao et al. 2007; Nicholson and Wilson 1989; Nicholson et al. 1995) and confirmed by 2D ^{1}H–^{1}H COSY and TOCSY methods (data not shown).

The spectra from all serum samples contained resonances from amino acids, organic acids, lipoproteins, lipids, unsaturated lipids, and glycoprotein, as well as choline, myo-inositol, and urea. Tricarboxylic acid (TCA) cycle metabolites, including succinate, citrate and fumarate, were also detected by ^{1}H NMR spectroscopy.

Visual inspection of the ^{1}H NMR spectra showed visible differences in overall composition between control and arginine-supplemented pigs (data not shown). However, these qualitative observations were all by visual inspection and inter-pig variation would make accurate data interpretation difficult. Therefore, a more formal spectral comparison was performed using PCA.

Principal component analysis

PCA on serum CPMG spectra between control and arginine-supplemented pigs showed clear clustering (Fig. 2a). PCA on serum NOESY and BPP-LED spectra were not listed because they had the similar trend with CPMG spectra. Based on the PCA loadings, a list of metabolites whose changes in serum led to the clustering is presented in Table 2.

Figure 2 shows the PCA results as score and loading plots for the first two principal components from the CPMG spectra of control and arginine-supplemented pigs. The clustering is obvious for the two groups of pigs, and PC 1 and PC 2 explained 72.7% of the total variances within the data (Fig. 2a). The differences between the control and arginine-supplemented pigs were clearly associated with differences in lipid composition. Based on the assignment of chemical shifts of lipids, the concentrations of LDL, VLDL, lipids, unsaturated lipids, glycoprotein, choline and glycerophosphorylcholine were lower in serum from arginine-supplemented pigs compared with control pigs (Fig. 2b). These changes were associated

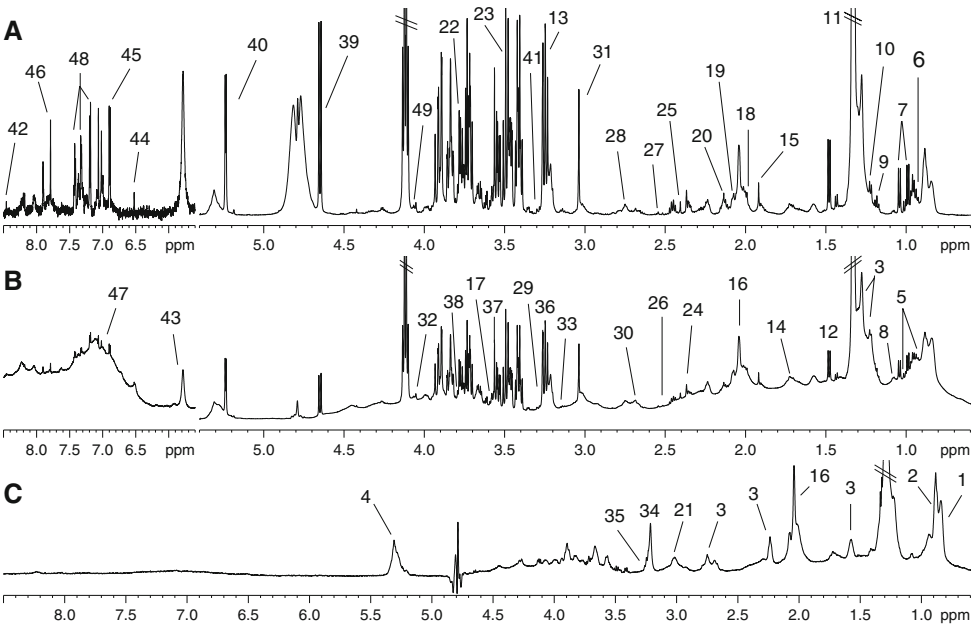

Fig. 1 Typical 600 MHz ^{1}H NMR CPMG (**a**), NOESY (**b**) and BPP-LED (**c**) spectrum of serum taken from a control pig. The spectra in the aromatic region (δ5.6–8.5) were magnified 16 times (**a**) or four times (**b**) compared to the aliphatic region (δ0.6–5.4). About 49 metabolites were unambiguously assigned. Their chemical shifts and peak multiplicity are given in Table 2. s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets

Table 2 Changes in relative concentrations of serum metabolites in arginine-supplemented pigs on the basis of chemical shifts relative to the methyl group of lactate at δ1.33

Key	Metabolites	Moieties	δ^1H (ppm) and multiplicity	Arginine
1	LDL	$CH_3^*(CH_2)_n$	0.84 (t)	↓
2	VLDL	$CH_3^*CH_2CH_2$ C=	0.88 (t)	↓
3	Lipids (triglycerides and fatty acids)	$CH_3(CH_2)_n$,$(CH_2)_n$,	1.22 (m),1.29 (m),	↓
		$CH_2^*CH_2CO$, CH_2–C=C, CH_2–C=O, CH–O–CO	1.58 (m), 2.04 (m),	
			2.24 (m), 2.75 (m)	
4	Unsaturated lipids	=C–CH_2–C=, –CH=CH–	5.19 (m), 5.31 (m)	↓
5	Isoleucine	γCH_3, δCH_3	1.01 (d), 0.94 (t)	↓
6	Leucine	αCH, δCH_3, δCH_3	3.72 (t), 0.91 (d), 0.96 (d)	↓
7	Valine	αCH, βCH, γCH_3	3.61 (d), 2.26 (m), 0.99 (d), 1.04 (d)	↓
8	Propionate	CH_2,CH_3	2.19 (q), 1.06 (t)	↓
9	Ethanol	CH_2,CH_3	3.65 (q), 1.18 (t)	↓
10	β-Hydroxybutyrate	γCH_3	1.22 (d)	↓
11	Lactate	αCH, βCH_3	4.11 (q), 1.33 (d)	
12	Alanine	αCH, βCH_3	3.77 (q), 1.48 (d)	↓
13	Arginine	αCH, βCH_2, γCH_2, δCH_2	3.76 (t), 1.89 (m), 1.63 (m), 3.25 (t)	↓
14	Lysine	αCH, βCH_2, γCH_2, δCH_2	3.77 (t), 1.89 (m), 1.73 (m), 1.47 (m)	↑
15	Acetate	CH_2–C=O	1.92 (s)	↓
16	Glycoprotein	CH_3–C=O	2.05 (s), 2.08 (s), 2.15 (s)	↓
17	Threonine	αCH, βCH, γCH_3	3.58 (d), 4.25 (m), 1.32 (d)	↑
18	Proline	βCH_2, γCH_2, δCH_2	2.02–2.33 (m), 2.00 (m), 3.35 (t)	–
19	Glutamate	αCH, βCH_2, γCH_2	3.75 (m), 2.08 (m), 2.37 (m)	↑
20	Glutamine	αCH, βCH_2, γCH_2	3.68 (t), 2.15 (m), 2.45 (m)	↑
21	Albumin	Lysyl-CH_2	3.02	↓
22	Methionine	αCH, βCH_2, γCH_2, δCH_3	3.78 (m), 2.16 (m), 2.6 (dd), 2.14 (s)	↓
23	Acetoacetate	CH_3,CH_2	2.29 (s), 3.49 (s)	↑
24	Pyruvate	CH_3	2.37 (s)	↑
25	Succinate	α, βCH_2	2.41 (s)	↑
26	Citrate	CH_2	2.52 (d), 2.70 (d)	↓
27	Methylamine	CH_3	2.54 (s)	↑
28	Dimethylamine	CH_3	2.71 (s)	↓
29	TMAO	CH_3	3.26 (s)	↓
30	Aspartic acid	αCH, βCH_2	3.89 (m), 2.68 (m), 2.82 (m)	↓
31	Creatine	N–CH_3, CH_2	3.04 (s), 3.93 (s)	↑
32	Creatinine	CH_3,CH_2	3.05 (s), 4.05(s)	↑
33	Citrulline	αCH_2, γCH_2, δCH_2	3.70 (m), 1.58 (m), 3.15 (t)	–
34	Choline	N–$(CH_3)_3$, αCH_2, βCH_2	3.2 (s), 4.05 (t), 3.51 (t)	↓
35	GPC	N–$(CH_3)_3$, OCH_2, NCH_2	3.22 (s), 4.32 (t), 3.68 (t)	↓
36	Taurine	N–CH_2, S–CH_2	3.26 (t), 3.41 (t)	↑
37	Glycine	CH_2	3.56 (s)	↓
38	Ornithine	CH_2, αCH	3.80 (s), 3.79 (t)	↑
39	β-Glucose	2-CH, 1-CH	3.25 (dd), 4.65 (d)	↑
40	α-Glucose	1-CH	5.24 (d)	↑
41	Betaine	CH_3,CH_2	3.28 (s), 3.90 (s)	↑
42	Formate	CH	8.45 (s)	↓
43	Urea	NH_2	5.78 (s)	↓
44	Fumarate	CH	6.52(s)	↑
45	Tyrosine	CH,CH	7.19 (m), 6.89 (m)	↑
46	1-Methylhistidine	4-CH, 2-CH	7.05 (s), 7.77 (s)	↓

Table 2 continued

Key	Metabolites	Moieties	δ^1H (ppm) and multiplicity	Arginine
47	3-Methylhistidine	4-CH, 2-CH	7.00 (s), 7.60 (s)	↑
48	Phenylalanine	2,6-CH, 3,5-CH, 4-CH	7.33 (m), 7.38 (m), 7.42 (m)	–
49	Myo-Inositol	5-CH, 4,6-CH, 2-CH	3.30 (t), 3.63 (t), 4.06 (t)	↑

s singlet; *d* doublet; *t* triplet; *q* quartet; *m* multiplet; *dd* doublet of doublets;*GPC* glycerophosphorylcholine; *LDL* low density lipoprotein; *VLDL* very low density lipoprotein; *TMAO* trimethylamine-*N*-oxide

↑ and ↓, the metabolite levels are higher or lower, respectively, compared with that of the control. −, the metabolite levels are the same as the control

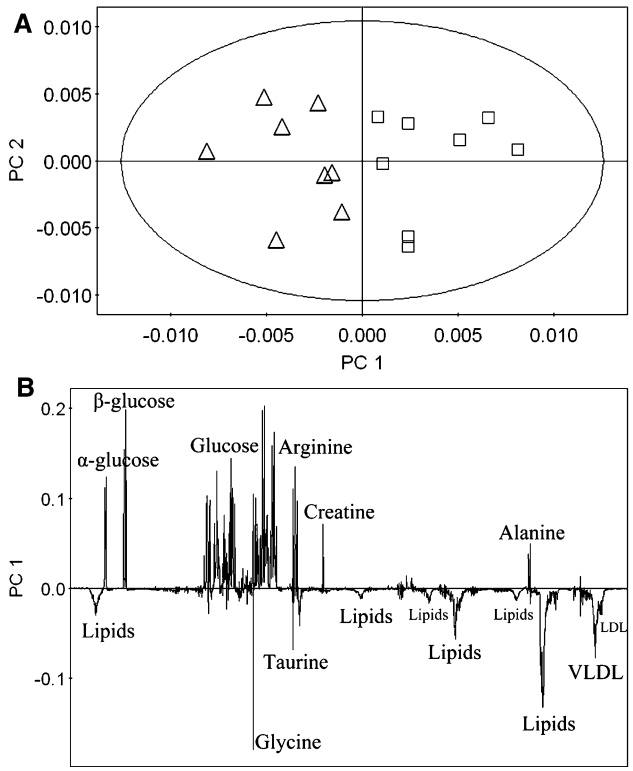

Fig. 2 PCA to compare the metabolome of the control (*open triangle*) and arginine-supplemented (*open square*) pig groups based on the CPMG spectra. Panel **a** is scores plot for pig sera from the PC1 and PC2 (R^2 = 72.7%), which shows a separate clustering of profiles from PC1. Panel **b** is complementary PCA loading plot, which shows the metabolites with large intensities contributed to the clustering

with the decreases in concentrations of citrate, β-hydroxybutyrate, dimethylamine, TMAO, ethanol, formate, acetate, propionate, albumin, urea, some amino acids (including alanine, leucine, valine, glutamate, glutamine, aspartic acid, glycine, 1-methylhistidine and methionine). The loadings plot (Fig. 2b) also showed that concentrations of arginine, lysine, threonine, tyrosine, 3-methylhistidine, ornithine, acetoacetate, and myo-inositol were greater in the serum of arginine-supplemented pigs, compared with the control group. In addition, elevation of α-glucose, β-glucose, pyruvate, succinate, fumarate, creatine, creatinine, methylamine, betaine and taurine were observed in the serum of arginine-supplemented pigs (Table 2).

Table 3 Relative integrals from selected serum metabolites in control and arginine-supplemented pigs

Metabolites	Control	Arginine	*P* values
α-Glucose (δ5.24)	2.08 ± 0.13	2.34 ± 0.31	0.04
β-Glucose (δ4.65)	2.72 ± 0.16	2.96 ± 0.23	0.03
Creatine (δ3.04)	0.70 ± 0.21	0.69 ± 0.21	0.91
Creatinine (δ4.05)	0.183 ± 0.028	0.177 ± 0.023	0.67
Urea (δ5.78)	0.516 ± 0.116	0.403 ± 0.082	0.17
LDL (δ0.84)	1.94 ± 0.56	1.74 ± 0.34	0.42
VLDL (δ0.86)	3.33 ± 0.42	2.65 ± 0.28	0.01
Lipid (δ1.30)	6.57 ± 1.14	5.14 ± 0.64	0.01
Unsaturated lipid (δ5.30)	1.88 ± 0.47	1.50 ± 0.23	0.15

Normalized integral of metabolites in spectrum (normalized to 100, chemical shift region over the ranges of δ0.5–1.30, 1.40–4.08, 4.20–4.66, and 5.20–8.50

Data are mean ± SD, $n = 8$

LDL low density lipoprotein; *VLDL* very low density lipoprotein

Relative signal integrals of metabolites

Table 3 summarizes relative signal integrals for some metabolites that contributed to the clustering of serum from control and arginine-supplemented pigs as noted above. Both serum α-glucose and β-glucose in arginine-supplemented pigs were higher when compared with control pigs ($P < 0.05$). However, VLDL and lipid of serum from arginine-supplemented pigs were lower than those from control pigs ($P < 0.01$).

Conventional biochemical assays for metabolites in serum

Serum biochemical metabolites in control and arginine-supplemented pigs are shown in Table 4. Concentrations of glucose were greater, but concentrations of urea were lower in the serum of arginine-supplemented pigs than in the

Table 4 Serum biochemical metabolites of pigs analyzed using conventional methods

Parameters	Control	Arginine	*P* values
Glucose (mmol/L)	8.07 ± 1.41	8.62 ± 1.59	0.47
LDL (mmol/L)	1.69 ± 0.27	1.52 ± 0.35	0.29
VLDL (mmol/L)	0.212 ± 0.023	0.181 ± 0.018	0.03
HDL (mmol/L)	1.27 ± 0.09	1.38 ± 0.14	0.09
Triglycerides (mmol/L)	0.65 ± 0.13	0.54 ± 0.07	0.04
Total cholesterol (mmol/L)	2.94 ± 0.43	2.62 ± 0.39	0.15
Total protein (g/L)	82.1 ± 5.9	76.3 ± 7.7	0.11
Albumin (g/L)	33.8 ± 4.3	31.9 ± 2.2	0.29
Urea (mmol/L)	2.73 ± 0.31	2.42 ± 0.24	0.04

Data are mean ± SD, $n = 8$

LDL low density lipoprotein; *VLDL* very low density lipoprotein; *HDL* high density lipoprotein

control group. These data were consistent to those on the relative signal integrals of glucose. In addition, concentrations of VLDL and triglycerides were reduced in arginine-supplemented pigs compared with control pigs ($P < 0.05$).

Growth performance and carcass measurements

Feed intakes during the 46-day experimental period did not differ between control and arginine-supplemented pigs (2,403 ± 165 vs 2,460 ± 172 g/day). At the end of amino acid supplementation, the final body weights of pigs in the control and arginine groups were 85.6 ± 4.9 and 86.4 ± 3.1 kg, respectively. Average daily gain was 817 ± 71 and 834 ± 64 g/day ($P = 0.06$) for control and arginine-supplemented pigs, respectively. The content of skeletal muscle in the carcass was 8.1% greater (63.1 ± 2.8 vs 58.4 ± 2.09%, $n = 8$/group; $P = 0.002$), but the content of fat mass in the carcass was 8.0% lower (20.8 ± 1.1 vs 22.6 ± 1.4%, $n = 8$/group; $P = 0.013$), in arginine-supplemented pigs than in control pigs.

Discussion

Metabolomics provides a useful systems approach to understanding global changes of metabolites in animals in response to alterations in nutrition, genetics, environments, and gut microbiota (Nicholson and Wilson 2003; Nicholson et al. 2004; Wang et al. 2007). The NMR spectroscopy is sensitive to simultaneously detect changes in concentrations of low-molecular weight substances in physiological fluids (Nicholson and Wilson 2003; Nicholson et al. 2004; Van Dorsten et al. 2006). Importantly, the analysis of serum is a noninvasive technology that can aid in elucidating the mechanisms responsible for the actions of dietary nutrients (Wang et al. 2008b). Results of the present study demonstrate for the first time that dietary arginine supplementation reduced concentrations of VLDL and lipids in the serum of growing pigs, which was associated with reduced fat accretion in the carcass. The arginine treatment also decreased serum concentrations of intestinal microbial metabolites (formate, ethanol, short-chain fatty acids, dimethylamine, and trimethylamine-*N*-oxide), but increasing serum concentrations of β-glucose (Tables 2, 3), which indicate altered metabolism of both amino acids and carbohydrates in the gut microflora. Furthermore, skeletal muscle content in the carcass was enhanced in arginine-supplemented pigs. Thus, arginine favorably modulates the metabolism of fat and protein for lean tissue gain in growing pigs.

Arginine can be synthesized from glutamine and proline in humans (Hu et al. 2008) and pigs (Wu et al. 2007b). Previous studies have shown that dietary supplementation with arginine reduced plasma concentrations of urea in pigs (Kim and Wu 2004) and obese rats (Fu et al. 2005; Wu et al. 2007c), while increasing plasma levels of arginine, ornithine, and creatine in pigs (Kim and Wu 2004; Wu and Morris 1998). Such effects of arginine were confirmed in the present study, indicating the robust of the NMR-based metabolomic analysis. Although the half-life of arginine in plasma of 8- to 180-day-old pigs was 1.6–1.8 h (Wu et al. 2007a), long-term dietary supplementation with arginine was effective in increasing its concentration in the serum of growing pigs after 12-h food deprivation. Additionally, we found that dietary supplementation with arginine reduced serum concentrations of VLDL, triglycerides, glycerophosphorylcholine, choline, citrate, 1-methylhistidine, glycoprotein, dimethylamine, and trimethylamine-*N*-oxide, but increased serum concentrations of 3-methylhistidine, betaine, methylamine, pyruvate, ketone bodies, and succinate (Tables 2, 3). The changes in serum lipids and related metabolites further support the notion that arginine and its products play an important role in the metabolism of energy substrates (Jobgen et al. 2006; Montanez et al. 2008; Shantz and Levin 2007). On the basis of current knowledge about the role for arginine in regulating nutrient utilization in pigs (Wu et al. 2007b), changes in these serum metabolites could not be expected. However, these novel data from the metabolomic study provide crucial insight into cellular metabolic changes in response to dietary arginine supplementation and the underlying possible mechanisms.

Data on serum metabolites and body compositions suggest coordinate regulation of nutrient metabolism that favors oxidation of fatty acids and net protein synthesis in growing pigs. Both acetoacetate and β-hydroxybutyrate are products of fatty acid oxidation in liver, and their ratios are

useful indicators of the mitochondrial redox state (Wu et al. 1991). Interestingly, arginine supplementation increased concentrations of acetoacetate but decreased concentrations of β-hydroxybutyrate in serum (Table 2), therefore enhancing the ratio of acetoacetate to β-hydroxybutyrate. This suggests a more oxidized state in cells, which could be brought about by intensive oxidation of fatty acids. In support of this view, we found that serum concentrations of succinate, an intermediate of the TCA cycle, were elevated in arginine-supplemented pigs (Table 2). An outcome would be a reduced availability of fatty acids for the tissue-specific synthesis of triglycerides, LDL and VLDL in the body (Fu et al. 2005).

A novel and unexpected finding from this work is that serum concentrations of formate, ethanol, and short-chain fatty acids (acetate and propionate), as well as nitrogenous products (dimethylamine and TMAO) were decreased in arginine-supplemented pigs (Table 2). Notably, they are microbial metabolites of carbohydrates and amino acids (Rezzi et al. 2007a; Sugita et al. 2007; Tannock 2004), which are likely produced in the lumen of the small intestine and large intestine. Changes in these metabolites may result from a reduced number and/or altered activity of intestinal microorganisms (Cornell and Stelmasiak 2007; Li et al. 2007). The findings raise an important question regarding a role for arginine in regulating nutrient metabolism and the ecology of the gut microbiota. Increased concentrations of luminal arginine are expected to enhance nitric oxide generation by both intestinal epithelial cells and luminal bacteria (Wu and Meininger 2002; Wu et al. 2008). Nitric oxide, in turn, can kill microorganisms (Han et al. 2008), therefore reducing their number and metabolic activity. This action of arginine would be analogous to that of antibiotics that have been used as feed additives for over a half century to improve the growth performance of pigs (Yen et al. 1985). Intestinal bacteria have been suggested to play a quantitatively important role in degrading dietary amino acids, particularly essential amino acids (Chen et al. 2007; Van Goudoever et al. 2000; Li et al. 2008). A decrease in the number of intestinal bacteria may limit the catabolism of luminal amino acids, therefore contributing to an increase in the entry of dietary amino acids into the portal vein (Wang et al. 2008a). In support of this notion, plasma concentrations of lysine were greater in arginine-supplemented than in control pigs (Table 2). Additionally, intestinal microbes convert dietary nondigestible fibers into short-chain fatty acids, ethanol, and other nutrients that can be used by the mammalian host as energy sources and as precursors for fatty acid synthesis (Backhed et al. 2004; Fang et al. 2008; Ley et al. 2006). Interestingly, a reduction in the number of intestinal bacteria is expected to be associated with decreased fat accretion (Turnbaugh et al. 2006), although the underlying mechanisms are not known. Alternatively, arginine itself can modulate metabolic pathways in intestinal microorganisms, as recently reported for mammalian cells (Fu et al. 2005; Orlando et al. 2008; Satriano 2007; Suenaga et al. 2008). Further studies are warranted to test these novel hypotheses.

Another new and intriguing observation from the present study is that dietary arginine supplementation altered concentrations of lipid signaling molecules in pig serum (Table 2). Glycerophosphorylcholine (GPC) is synthesized from choline by both mammalian and bacterial cells (Burt and Ribolow 1994). Accordingly, a reduction in serum concentrations of choline was associated with a decrease in circulating levels of GPC in arginine-supplemented pigs (Table 2). GPC is an inhibitor of lysophospholipase (Burt and Ribolow 1994), which catalyzes the hydrolysis of physiologically important lysophospholipids (including lysophosphatidic acid and sphingosine 1-phosphate). These lipids play an important role in cellular signaling and, therefore, the regulation of nutrient metabolism (Tokumura 2004). In addition, serum concentrations of myo-inositol were elevated in response to dietary arginine supplementation. This carbocyclic polyol provides the structural basis for a number of secondary messengers (including inositol phosphates, phosphatidylinositol, and phosphatidylinositol phosphate) in eukaryotic cells (Di Paolo and De Camilli 2006). Therefore, inositol is involved in the regulation of intracellular calcium concentrations, insulin signal transduction, gene expression, and oxidation of fatty acids (Di Paolo and De Camilli 2006; Siddiqui et al. 2008). Collectively, we suggest that GPC and myo-inositol may mediate the beneficial action of dietary arginine supplementation on enhancing protein accretion and reducing fat deposition in growing pigs.

In conclusion, results of this study indicate that dietary arginine supplementation affected the serum metabolome in growing pigs. A novel and unexpected observation was that serum concentrations of intestinal microbial nitrogenous metabolites were lower in arginine-supplemented than in control pigs. Additionally, concentrations of nitrogenous and lipid signaling molecules in serum were altered in response to the arginine treatment, in association with enhanced protein accretion and reduced fat deposition in the body. The findings indicate that a NMR-based metabolomic approach was useful to comprehensively investigate the changes of serum metabolites in pigs.

Acknowledgments We thank Dr. Darrell Knabe and Dr. Junjun Wang for helpful comments on the manuscript. This research was jointly supported by grants from the Chinese Academy of Sciences and Knowledge Innovation Project (KSCX2-YW-N-051, KZCX3-SW-441, YW-N-022, and KSCX2-SW323), K·C. Wong Education Foundation (Hong Kong), National Basic Research Program of China (2004CB117502), NSFC (30528006, 30671517, 30700581, 30771558 and 30371038), the Outstanding Overseas Chinese Scholars Fund of

the Chinese Academy of Sciences (2005-1-4 and 2005-1-7), Fund of Agricultural Science and Technology outcome application (2006GB24910468), National Scientific and Technological Support Project (2006BAD12B07 and 2006BAD12B02-5-2), Guang Dong Province and Guangzhou City Project (2006B200330005), Program for Gangjiang Scholars and Innovative University Research Team (65292 and IRT0540), Hunan Key Projects 2007FJ1003,06FJ3046), Texas AgriLife Research (H-8200), and National Research Initiative Competitive Grant (2008-35206-18764) from the USDA Cooperative State Research, Education, and Extension Service.

References

Backhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, Semenkovich CF, Gordon JI (2004) The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci USA 101:15718–15723

Bax A, Davis DG (1985) MLEV-17-based two-dimensional homonuclear magnetization transfer spectroscopy. J Magn Reson 65:355–360

Bertram HC, Kristensen NB, Malmendal A, Nielsen NC, Bro R, Andersen HJ, Harmon DL (2005) A metabolomic investigation of splanchnic metabolism using ^{1}H NMR spectroscopy of bovine blood plasma. Anal Chim Acta 536:1–6

Burt CT, Ribolow H (1994) Glycerol phosphorylcholine (GPC) and serine ethanolamine phosphodiester (SEP): evolutionary mirrored metabolites and their potential metabolic roles. Comp Biochem Physiol Biochem Mol Biol 108:11–20

Chen LX, Yin YL, Jobgen WJS, Jobgen SC, Knabe DA, Hu WX, Wu GY (2007) In vitro oxidation of essential amino acids by jejunal mucosal cells of growing pigs. Livest Sci 109:19–23

Cornell HJ, Stelmasiak T (2007) A unified hypothesis of coeliac disease with implications for management of patients. Amino Acids 33:43–49

Di Paolo G, De Camilli P (2006) Phosphoinositides in cell regulation and membrane dynamics. Nature 443:651–657

Dumas ME, Maibaum EC, Teague C, Ueshima H, Zhou B, Lindon JC, Nicholson JK, Stamler J, Elliott P, Chan Q, Holmes E (2006) Assessment of analytical reproducibility of ^{1}H NMR spectroscopy based metabonomics for large-scale epidemiological research: the INTERMAP Study. Anal Chem 78:2199–2208

Fang ZF, Luo J, Qi ZL, Huang FR, Zhao SJ, Liu MY, Jiang SW, Peng J (2008) Effects of 2-hydroxy-4-methylthiobutyrate on portal plasma flow and net portal appearance of amino acids in piglets. Amino acids. doi: 10.1007/s00726-008-0110-1

Fu WJ, Haynes TE, Kohli R, Hu J, Shi W, Spencer TE, Carroll RJ, Meininger CJ, Wu G (2005) Dietary L-arginine supplementation reduces fat mass in Zucker diabetic fatty rats. J Nutr 135:714–721

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

Han J, Liu YL, Fan W, Chao J, Hou YQ, Yin YL, Zhu HL, Meng GQ, Che ZQ (2008) Dietary L-arginine supplementation alleviates immunosuppression induced by cyclophosphamide in weaned pigs. Amino Acids. doi: 10.1007/s00726-008-0184-9

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 35:665–672

Hurd RE (1990) Gradient-enhanced spectroscopy. J Magn Reson 87:422–428

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

Kim SW, Wu G (2004) Dietary arginine supplementation enhances the growth of milk-fed young pigs. J Nutr 134:625–630

Kim SW, Wu G (2008) Regulatory role for amino acids in mammary gland growth and milk synthesis. Amino Acids. doi: 10.1007/s00726-008-0151-5

Kong XF, Yin YL, He QH, Yin FG, Liu HJ, Li TJ, Huang RL, Geng MM, Ruan Z, Deng ZY, Xie MY, Wu G (2008) Dietary supplementation with Chinese herbal powder enhances ileal digestibilities and serum concentrations of amino acids in young pigs. Amino Acids. doi: 10.1007/s00726-008-0176-9

Lenz EM, Bright J, Wilson ID, Hughes A, Morrisson J, Lindberg H, Lockton A (2004) Metabonomics, dietary influences and cultural differences: a ^{1}H NMR-based study of urine samples obtained from healthy British and Swedish subjects. J Pharm Biomed 36:841–849

Ley RE, Turnbaugh PJ, Klein S, Gordon JI (2006) Microbial ecology: human gut microbes associated with obesity. Nature 444:1022–1023

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

Li P, Mai K, Trushenski J, Wu G (2008) New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds. Amino Acids. doi: 10.1007/s00726-008-0171-1

Liao PQ, Wei L, Zhang XY, Li XJ, Wu HF, Wu YJ, Ni JZ, Pei FK (2007) Metabolic profiling of serum from gadolinium chloride-treated rats by ^{1}H NMR spectroscopy. Anal Biochem 364:112–121

Meiboom S, Gill D (1958) Modified spin-echo method for measuring nuclear relaxation times. Rev Sci Instrum 29:688–691

Montanez R, Rodriguez-Caso C, Sanchez-Jimenez F, Medina MA (2008) In silico analysis of arginine catabolism as a source of nitric oxide or polyamines in endothelial cells. Amino Acids 34:223–229

National Research Council (1998) Nutrient requirements of swine, 10th edn. National Academic Press, Washington, DC

Nicholson JK, Wilson ID (1989) High resolution proton magnetic resonance spectroscopy of biological fluids. Prog Nucl Magn Reson Spectrosc 21:449–501

Nicholson JK, Wilson ID (2003) Opinion: understanding 'global' systems biology: metabonomics and the continuum of metabolism. Nat Rev Drug Discov 2:668–676

Nicholson JK, Foxall PJ, Spraul M, Farrant RD, Lindon JC (1995) 750 MHz ^{1}H and ^{1}H–^{13}C NMR spectroscopy of human blood plasma. Anal Chem 67:793–811

Nicholson JK, Holmes E, Lindon JC, Wilson ID (2004) The challenges of modeling mammalian biocomplexity. Nat Biotechnol 22:1268–1274

Orlando GF, Wolf G, Engelmann M (2008) Role of neuronal nitric oxide synthase in the regulation of the neuroendocrine stress response in rodents: insights from mutant mice, Amino Acids 35:17–27

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

Rezzi S, Ramadan Z, Fay LB, Kochhar S (2007a) Nutritional metabonomics: applications and perspectives. J Proteome Res 6:513–525

Rezzi S, Ramadan Z, Martin FP, Fay LB, van Bladeren P, Lindon JC, Nicholson JK, Kochhar S (2007b) Human metabolic phenotypes link directly to specific dietary preferences in healthy individuals. J Proteome Res 6:4469–4477

Satriano J (2007) Kidney growth, hypertrophy and the unifying mechanism of diabetic complications. Amino Acids 33:331–339

Shantz LM, Levin VA (2007) Regulation of ornithine decarboxylase during oncogenic transformation: mechanisms and therapeutic potential. Amino Acids 33:213–223

Siddiqui RA, Havey KA, Zaloga GP (2008) Modulation of enzymatic activities by n−3 polyunsaturated fatty acids to support cardiovascular health. J Nutr Biochem 19:417–437

Suenaga R, Tomonaga S, Yamane H, Kurauchi I, Tsuneyoshi Y, Sato H, Denbow DM, Furuse M (2008) Intracerebroventricular injection of L-arginine induces sedative and hypnotic effects under an acute stress in neonatal chicks. Amino Acids 35:139–146

Sugita Y, Takao K, Toyama Y, Shirahata A (2007) Enhancement of intestinal absorption of macromolecules by spermine in rats. Amino Acids 33:253–260

Suryawan A, O'Connor PMJ, 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 B, Li XG, Kong X, Huang R, Ruan Z, Yao K, Deng Z, Xie M, Shinzato I, Yin Y, Wu G (2008a) Dietary L-arginine supplementation enhances the immune status in early-weaned piglets. Amino Acids. doi: 10.1007/s00726-008-0155-1

Tan B, Yin Y, Liu Z, Li X, Xu H, Kong X, Huang R, Tang W, Shinzato I, Smith SB, Wu G (2008b) Dietary L-arginine supplementation increases muscle gain and reduces body fat mass in growing–finishing pigs. Amino Acids. doi: 10.1007/s00726-008-0148-0

Tannock GW (2004) A special fondness for lactobacilli. Appl Environ Microbiol 70:3189–3194

Tokumura A (2004) Metabolic pathways and physiological and pathological significances of lysolipid phosphate mediators. J Cell Biochem 92:869–881

Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 444:1027–1031

Van Dorsten FA, Daykin CA, Mulder TP, Van Duynhoven JP (2006) Metabonomics approach to determine metabolic differences between green tea and black tea consumption. J Agric Food Chem 54:6929–6938

Van Goudoever JB, Stoll B, Henry JF, Burrin DG, Reeds PJ (2000) Adaptive regulation of intestinal lysine metabolism. Proc Natl Acad Sci USA 97:11620–11625

Wang Y, Lawler D, Larson B, Ramadan Z, Kochhar S, Holmes E, Nicholson JK (2007) Metabonomic investigations of aging and caloric restriction in a life-long dog study. J Proteome Res 6:1846–1854

Wang WW, Qiao SY, Li DF (2008a) Amino acids and gut function. Amino Acids. doi: 10.1007/s00726-008-0152-4

Wang JJ, Wu G, Zhou HJ, Wang FL (2008b) Emerging technologies for amino acid nutrition research in the post-genome era. Amino Acids. doi:10.1007/s00726-008-0193-8

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

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

Wu GY, Gunasekara A, Brunengraber H, Marliss EB (1991) Effects of extracellular pH, CO_2, and HCO_3^- on ketogenesis in perfused rat liver. Am J Physiol Endocrinol Metab 261:E221–E226

Wu DH, Chen AD, Johnson CS (1995) An improved diffusion-ordered spectroscopy experiment incorporating bipolar-gradient pulses. J Magn Reson Ser A 115:260–264

Wu G, Bazer FW, Cudd TA, Jobgen WS, Kim SW, Lassala A, Li P, Matis JH, Meininger CJ, Spencer TE (2007a) Pharmacokinetics and safety of arginine supplementation in animals. J Nutr 137:1673S–1680S

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

Wu G, Collins JK, Perkins-Veazie P, Siddiq M, Dolan KD, Kelly KA, Heaps CL, Meininger CJ (2007c) Dietary supplementation with watermelon pomace juice enhances arginine availability and ameliorates the metabolic syndrome in Zucker diabetic fatty rats. J Nutr 137:2680–2685

Wu G, Bazer FW, Datta S, Johnson GA, Li P, Satterfield MC, Spencer TE (2008) Proline metabolism in the conceptus: implications for fetal growth and development. Amino Acids 35:691–702

Yang Y, Li C, Nie X, Feng X, Chen W, Yue Y, Tang H, Deng F (2007) Metabonomic studies of human hepatocellular carcinoma using high-resolution magic-angle spinning ^{1}H NMR spectroscopy in conjunction with multivariate data analysis. J Proteome Res 6:2605–2614

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

Yen JT, Nienaber JA, Pond WG, Varel VH (1985) Effect of carbadox on growth, fasting metabolism, thyroid function and gastrointestinal tract in young pigs. J Nutr 115:970–979

Yin YL, Deng ZY, Huang HL, 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 Y (2008) 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