Amino Acids (2014) 46:2681–2691
DOI 10.1007/s00726-014-1823-y

ORIGINAL ARTICLE

Metabolomic analysis of amino acid and fat metabolism in rats with L-tryptophan supplementation

Zheng Ruan · Yuhui Yang · Yanmei Wen · Yan Zhou · Xiaofang Fu · Sheng Ding · Gang Liu · Kang Yao · Xin Wu · Zeyuan Deng · Guoyao Wu · Yulong Yin

Received: 5 May 2014 / Accepted: 31 July 2014 / Published online: 20 August 2014

Abstract Tryptophan (TRP) is an important precursor for several neurotransmitters and metabolic regulators, which play a vital role in regulating nutrient metabolism. The purpose of this study was to investigate the effects of tryptophan supplementation on the biochemical profiles, intestinal structure, liver structure and serum metabolome in rats. Rats received daily intragastric administration of either tryptophan at doses of 200 mg/kg body weight per day or saline (control group) for 7 days. TRP supplementation had a tendency to decrease the body weight of rats ($P > 0.05$). The levels of urea and CHO in serum were decreased in the TRP-supplemented group rats compared with control group rats ($P < 0.05$). TRP supplementation increased the villus height and the ratio of villus height to crypt depth in the jejunum compared to control group rats ($P < 0.05$). Metabolic effects of tryptophan supplementation include: (1) increases in the serum concentrations of lysine, glycine, alanine, glutamate, glutamine, citrulline, methionine, tyrosine, 1-methylhistidine, and albumin, and decreases in the concentrations of serum branched-chain amino acid (isoleucine, valine and leucine); (2) decreases in the serum concentrations of formate and nitrogenous products (trimethylamine, TMAO, methylamine and dimethylamine), and in the contraction of trimethylamine in feces; (3) decreases in serum levels of lipids, low density lipoprotein, very low density lipoprotein, together with the elevated ratio of acetoacetate to β-hydroxybutyrate. The results indicate that tryptophan supplementation reduced the catabolism of dietary amino acids and promoted protein synthesis in rats, promoted the oxidation of fatty acid and reduced fat deposition in the body of rats.

Keywords Tryptophan · Animo acids · Metabolism · Metabolomic · Nuclear magnetic resonance spectroscopy

Abbreviations

TRP	Tryptophan
PC	Principal components
PCA	Principal component analysis
NMR	Nuclear magnetic resonance

Introduction

Tryptophan (TRP) is not only one of the limiting dietary indispensable amino acids in protein metabolism, but also an important precursor for melatonin, serotonin, *N*-acetylserotonin and anthranilic acid (Biggio et al. 1974; Kim et al. 2007; Wu 2007). It was shown that the plasma levels of melatonin, serotonin, *N*-acetylserotonin and anthranilic acid were

Z. Ruan · Y. Yang · Y. Wen · Y. Zhou · Z. Deng · Y. Yin (✉)
State Key Laboratory of Food Science and Technology, College of Life Science and Food Engineering, Nanchang University, Rm325, 235 Nanjing Street, Nanchang 330047, China
e-mail: yinyulong@isa.ac.cn

X. Fu
Analytic Center for Hubei Entry-Exit Inspection and Quarantine Bureau, Wuhan 430031, China

S. Ding
Institute of Nutrition and Food Safety, Center for Disease Control and Prevention of Jiangxi Province, Nanchang 330029, China

G. Liu · K. Yao · X. Wu · Y. Yin
Hunan Engineering and Research Center of Animal and Poultry Science, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha 410125, China

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

increased when tryptophan was intragastrically administered (Esteban et al. 2004; Platten et al. 2005; Sánchez et al. 2008; Sugden et al. 1985). Previous studies have suggested that tryptophan and its metabolites can affect both innate and acquired immunity systems (Esteban et al. 2004; Perianayagam et al. 2005; Platten et al. 2005; Shi et al. 2004). Melatonin formed from tryptophan in gastrointestinal tract could be considered as a protective agent because of its recognized biological effects, including antioxidant, anti-inflammatory properties (Kolli et al. 2013a, b; Sewerynek et al. 1996). Tryptophan exerts this effect by scavenging free radicals with melatonin, by stimulating mucosal prostaglandin release and increasing the gastric blood flow (Brzozowski et al. 1997; Bülbüller et al. 2003). In addition, tryptophan plays an important role in regulating nutrient metabolism (Li et al. 2007; Moehn et al. 2012; Yao et al. 2010). Dietary tryptophan supplementation significantly increased in hepatic protein synthesis in vivo (Sidransky et al. 1968), and resulted in significant decreases in hepatic lipids and triglyceride (Adamstone and Spector 1950; Akiba et al. 1992). However, there is lack of data concerning the effect of dietary tryptophan supplementation on amino acid metabolism.

Metabonomics has been identified as a powerful top-down systems biological tool to assess functionalities of nutrients and foods via the simultaneous measurement of multiple metabolites in complex organisms. It can aid the understanding of how metabolic balances may be disturbed by nutritional interventions (Noguchi et al. 2003; Rezzi et al. 2007). Nuclear magnetic resonance (NMR) spectroscopy is one of the major techniques used in metabonomic studies (Wang et al. 2009a). On the basis of NMR analysis, He et al. (2009) demonstrated that dietary arginine supplementation decreases fat deposition, enhances protein synthesis in skeletal muscle, and modulates intestinal microbial metabolism in growing pigs. Liu et al. (2011) found that chronic cysteamine supplementation results in perturbation of energy metabolism in rats using an NMR technique. An NMR-based metabonomic approach was applied to evaluate the effects of intervention with a high-fat, high-cholesterol diet in Syrian golden hamsters (Jiang et al. 2013). In these previous studies, metabonomics was shown to be very useful for exploring the complex relationship between nutritional intervention and metabolism in order to clarify the role of dietary components in maintaining health and the development of disease (He et al. 2011a).

In the present study, the global metabolic profile of serum samples after dietary tryptophan supplementation in rats was screened by a ^{1}H NMR-based metabolomic approach. Our objective was to provide insight into the effects of tryptophan supplementation on internal metabolic processes. Metabolic profiles of tryptophan supplementation in rats can improve the current understanding of the relationship between metabolites and tryptophan supplementation and establish fundamental data for future experimental study on tryptophan. Growth performance, serum biochemical profiles, small intestine index, liver index, and the contents of dimethylamine, and trimethylamine in rat feces were measured, as well as histological analysis of jejunum and liver tissue were performed to achieve our study objective.

Table 1 The composition and nutrient of experiment diet

Ingredients	Content (%)	Chemical composition	Content
Wheat	14.0	Digestible energy (Mcal/kg)	3.4
Corn	43.0	Dry matter (%)	90.0
Soybean meal	24.0	Crude protein (%)	21.0
Full fat soybean extruded	8.0	Crude fat (%)	4.5
Soybean oil	1.4	Calcium (%)	1.0
Whey powder	3.0	Total phosphate (%)	0.7
Fish meal	3.2	Sodium (%)	0.3
Limestone	1.3	Met + Cys (%)	0.78
Dicalcium phosphate	1.1	Lys (%)	1.35
Vitamin-mineral premix[a]	1.0	Thr (%)	0.88

The nutrient levels of the diets were based on China General Quality Standards for Animal Feed (GB14924.1-2001)

[a] The vitamin–mineral premix provided (per kilogram feed): vitamin A, 14,000 IU; vitamin D_3, 1,500 IU; vitamin E, 5 mg; vitamin K, 5 mg; thiamine, 13 mg; riboflavin, 12 mg; pyridoxine, 12 mg; vitamin B_{12}, 0.022 mg; niacin, 60 mg; pantothenic acid, 24 mg; biotin, 0.2 mg; folic acid, 6 mg; choline chloride, 350 mg; Fe (as iron sulfate), 120 mg; Cu (as copper oxide), 10 mg; Mn (as manganous oxide), 75 mg; Zn (as zinc oxide), 30 mg; I (as ethylenediamine dihydroiodide), 0.5 mg; and Se (as sodium selenite), 0.2 mg

Materials and methods

Rats, diets and experimental design

Experimental protocols used in this study were approved by the Nanchang University Animal Experiment Ethics Committee. The experimental rats were used in compliance with the Chinese guidelines for the Laboratory Animals Care (Ruan et al. 2007). In the experiments, 16 adult male Sprague–Dawley rats (weighting 220 ± 10 g) were obtained from SLAC laboratory Animal CO., Ltd (Changsha, China). Afterward, they were transferred to individual metabolic cages after acclimatization for 7 days and maintained on a light–dark cycle of 12 h light and 12 h dark. Free access to standard rodent diet (Table 1) (Ruan et al. 2014) and drinking water was provided. The temperature between 22 and 25 °C, and humidity (50 ± 10 %) were maintained throughout the study (Ren et al. 2012, 2013).

Rats were randomly divided into two equal groups, two treatments were as follows: (a) control group, which received oral administration of physiological saline solution (0.9 % NaCl, at a dose of 2 mL) per day for 1 week; (b) TRP-supplemented group, which was given oral administration of L-TRP (dissolved in saline solution at doses of 200 mg/kg body weight) (Aladdin, China) per day for 1 week. The dosage selected for this study was based on previous reports (Brzozowski et al. 1997; Bülbüller et al. 2005).

Growth performance and sample collection

The body weight of each rat was recorded everyday during the 7 days of experimental period (Ren et al. 2014).

At the end of the experiments, all rats were fasted overnight to avoid a postprandial effect on serum metabolites after the last TRP administration (Xiao et al. 2013). And blood samples (~4 mL) were collected from postcava under anaesthesia (intraperitoneal injection of sodium pentobarbital). Small intestine and liver samples were quickly collected (Liu et al. 2012). The weight of small intestine and liver were recorded, and the length of the small intestine was measured. Serum were obtained by centrifugation at 4,000 rpm and 4 °C for 15 min and stored at −80 °C until ready for NMR spectroscopy analysis and other biochemical analyses (He et al. 2011b).

The feces of each rat was collected at 0–24 h after the last oral administration of L-TRP. The samples were stored at −80 °C until ready for the determination of the contents of dimethylamine and trimethylamine (Tan et al. 2011).

Conventional biochemical measurements

Serum biochemical metabolites included alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose (GLU), lactate dehydrogenase (LDH), total protein (TP), blood ammonia (AMM), creatine kinase (CK), serum creatinine (CRE), urea, cholesterol (CHO), triglyceride (TG), low density lipoprotein (LDL), high density lipoprotein (HDL), serum cholinesterase (CHE). All assays were performed using a CX-4 Auto-Blood Biochemical Analyzer (Beckman Inc., USA) according to the manufacturer's protocol (Nanjing Jiancheng Technology Co., Ltd, Nanjing, China).

Small intestine index and histological analysis

Small intestine index (%) was calculated as: (small intestine weight/body weight) × 100 (Castro et al. 1975).

The small intestines were cut and separated using the following protocol: the first 10-cm segment being duodenum; the last 20-cm segment being ileum; the rest of the small intestine segment being jejunum. A portion of mid-jejunum (~3 cm) was collected for subsequent analysis of morphology (Chen et al. 2003). Intestinal tissue sections from jejunum were washed with cold phosphate buffered saline, fixed by immersion in 10 % neutral-buffered formalin. Samples were embedded in paraffin, sectioned at a 5 μm thickness and stained with hematoxylin and eosin as described by Nabuurs et al. (1993). The height of the villi and the depth of the crypts were evaluated using Image-Pro Plus 6.0 image processing and analysis system. For each sample, 10 fields were measured and the mean value was calculated. The method was according to the description of van Beers-Schreurs et al. (1998). The histological analysis was performed by an investigator who was unaware of the origin of tissue sections.

Liver index and histological analysis

Liver index (%) was calculated as: (liver weight/body weight) × 100 (Mahaffey et al. 1981).

Histological analysis and hematoxylin–eosin staining were performed as previously reported (Zou et al. 1998). Liver tissue was fixed in 10 % neutral-buffered formalin, and then processed into paraffin section by routine procedure. The sections of 5 μm thickness were cut and affixed to glass slides, deparaffinized, and stained with hematoxylin and eosin.

Determination of dimethylamine and trimethylamine production

Each feces sample (2 g) was homogenized in 8 mL of 7.5 % cold trichloroacetic acid (TCA) using a homogenizer and the homogenate was centrifuged at 4,000 rpm and 4 °C for 15 min. The residue was re-extracted twice with 4 mL of 5 % TCA and treated as above. All supernatants were combined and it was made up to 50 mL with deionized water. The contents of dimethylamine and trimethylamine in rat feces were determined by spectrophotometer 722E (Shanghai Spectrum, China). The methods were according to the description of Benjakul et al. (2004).

^{1}H NMR spectroscopy of plasma samples

For ^{1}H NMR spectroscopy experiments, serum samples were prepared by mixing 350 μL of serum with 350 μL of saline (0.9 % NaCl was dissolved in D_2O) in a microcontainer. D_2O for field frequence lock purposes. The resulting solution was centrifuged at 13,000 rpm for 10 min to remove precipitates. A 550 μL aliquot of the supernatant was transferred into 5-mm NMR tubes. ^{1}H NMR measurements of serum were acquired on a Bruker Avance DRX-600 spectrometer (Bruker Biospin, Rheinstetten, Germany) at a temperature of 298 K, operating at a proton frequency of 295.0 MHz, using a cryogenic high-resolution probe. The measurement

of ^{1}H NMR spectra for each serum sample, a Carr–Purcell–Meiboom–Gill (CPMG) spin-echo pulse sequence was used to emphasize resonances from low molecular-weight metabolites (Liu et al. 2011; Wang et al. 2008; He et al. 2009, 2011c). A total of 64 transients were collected into 32,000 data points using a spectral width of 8,417.5 Hz, a relaxation delay of 3.0 s, and an acquisition time of 3.9 s.

Multivariate analysis and data processing

All NMR spectra from serum samples were Fourier-transformed, phase adjusted and baseline corrected using MNova-6.1.1 (MestreLab, Santiago de Compostela, Spain). The chemical shift was compared to the reference of the lactate doublet at δ1.33 (Ruan et al. 2013). Each spectrum was segmented into contiguous segments having an equal width (0.01 ppm) and integrated over the region from δ8.5 to 0.8. The region δ4.66–5.20 was removed to avoid the influence of the water signal. The integral of each region was determined. Resultant data sets were then imported into SIMCA-P 12.0 (Umetrics, Sweden) for multivariate statistical analysis. In order to discern the presence of inherent similarities of spectral profiles, an unsupervised PR method, principal component analysis (PCA), was applied to all data sets from serum samples (Wei et al. 2008). The resulting data of PCA were visualized by the PC score plots and loading plots. Each point in the score plots represents an individual spectrum of a sample, and each point in the loading plots represents a single NMR spectral region or chemical shifts. Therefore, the loading plots indicate the spectral variables of metabolites that most strongly influence patterns in the score plots. Similarities and differences between samples can be observed in the scores plots, while spectral regions responsible for the differences can be viewed in the corresponding loading plots (Liu et al. 2011).

Statistical analysis

All experimental results were statistically analyzed using the software of SPSS 16.0 (SPSS Inc., Chicago, USA), and data were presented as mean ± standard error (SE). The independent samples *t* test was conducted to examine significant differences between the two groups (Gao et al. 2013). Values of $P < 0.05$ were regarded as significant statistical significance.

Results

The effects of TRP supplementation on body weight in rats

The effect of TRP supplementation on the body weight of rats was shown in Fig. 1. At the beginning of the study, the body weight of rats in the TRP-supplemented group was the same as in the control group. At the end of the experiments, TRP supplementation had a tendency to decrease the body weight of rats, but there was no statistically significant difference between the two groups ($P > 0.05$).

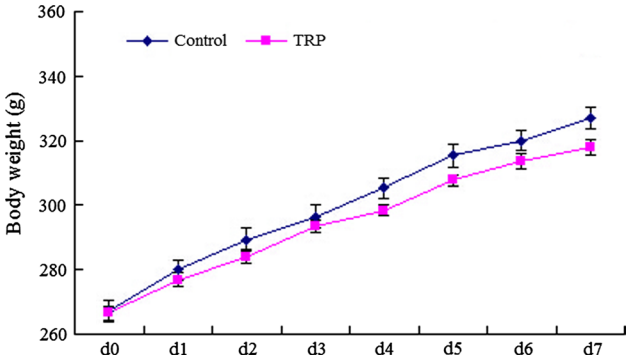

Fig. 1 The effects of TRP supplementation on the body weight in rats. Data from each group ($n = 8$) were averaged and presented as mean ± SE. The rats were treated with TRP (at doses of 200 mg/kg body weight) for 7 days, and control group rats received an isovolumetric amount of physiological saline solution in the same manner. The body weight of each rat was recorded everyday during the 7 days of experimental period

Table 2 The effects of TRP supplementation on serum biochemical profiles in rats

Parameters	Control group	TRP-supplemented group	*P* values
ALP (U/L)	263.20 ± 6.54	243.60 ± 9.90	0.14
ALT (U/L)	22.50 ± 1.04	23.40 ± 1.25	0.61
AST (U/L)	88.25 ± 3.86	84.00 ± 4.45	0.51
GLU (mmol/L)	7.74 ± 0.41	8.98 ± 0.43	0.06
LDH (U/L)	499.50 ± 43.56	455.60 ± 53.22	0.56
TP (g/L)	53.64 ± 0.96	55.24 ± 1.41	0.41
AMM (μmol/L)	34.83 ± 1.21	31.64 ± 2.84	0.30
CK (U/L)	573.60 ± 43.83	512.80 ± 61.95	0.45
CRE (μmol/L)	37.72 ± 2.28	39.10 ± 4.31	0.76
Urea (mmol/L)	6.85 ± 0.10	5.97 ± 0.34	0.03
CHO (mmol/L)	1.52 ± 0.05	1.31 ± 0.08	0.04
TG (mmol/L)	0.85 ± 0.05	0.75 ± 0.04	0.20
LDL (mmol/L)	0.16 ± 0.01	0.15 ± 0.01	0.17
HDL (mmol/L)	1.09 ± 0.02	1.08 ± 0.04	0.76
CHE (U/L)	154.40 ± 7.78	151.00 ± 6.04	0.74

Data from each group ($n = 8$) were averaged and presented as mean ± SE

P values less than 0.05 were regarded as significant statistical significance

The effects of TRP supplementation on conventional biochemical indices in serum

Serum biochemical indices in control and TRP-supplemented group rats were shown in Table 2. The serum level

of GLU was greater, and the level of LDL was lower in the TRP-supplemented group rats than in the control group rats ($P > 0.05$). These data were consistent with those on the relative signal integrals using ^{1}H-NMR-based metabonomics method (Table 4). In addition, the serum level of urea and CHO were decreased in the TRP-supplemented group rats compared with control group rats ($P < 0.05$).

Small intestine index and histological analysis

Representative figures for the jejunum stained with hematoxylin and eosin taken from the control and TRP-supplemented group rats are shown in Fig. 2a, b. There are no significant difference for small intestine length and index between control and TRP group ($P > 0.05$) (Fig. 2c, d). The villus height (Fig. 2e) and the ratio of villus height to crypt depth (Fig. 2g) in the jejunum were higher in the TRP-supplemented group rats than in control group rats ($P < 0.05$).

Liver index and histological analysis

The effects of TRP supplementation on liver index and morphology in rats were depicted in Fig. 3. TRP supplementation had no significant effects on liver weight, liver index ($P > 0.05$) and morphology in TRP-supplemented group rats compared with control group rats.

The effects of TRP supplementation on the contents of dimethylamine and trimethylamine in rat feces

The effects of TRP supplementation on the contents of dimethylamine and trimethylamine in rat feces were showed in Table 3. Compared with control group, TRP-supplemented group showed a significant decrease in the content of trimethylamine in rat feces ($P < 0.05$). TRP supplementation had a tendency to decrease the content of dimethylamine in rat feces, but there was no statistically significant difference between the two groups ($P > 0.05$).

^{1}H NMR spectroscopic analysis of serum samples

Typical 600 MHz ^{1}H NMR spectra obtained from rats serum were shown in Fig. 4. From these spectra, a total of 41 metabolites were unambiguously assigned in the ^{1}H NMR spectra of serum samples. Their chemical shifts, peak multiplicity and the corresponding ^{1}H NMR signal multiplicities are displayed in Table 4. Assignment of metabolites was made by comparison with the basis of existing literature (Jiang et al. 2013; Liao et al. 2007; Nicholson and Wilson 1989; Zira et al. 2013).

To detect more subtle treatment-related metabolic differences, pattern recognition techniques were applied. PCA was performed on the ^{1}H NMR spectra of serum samples

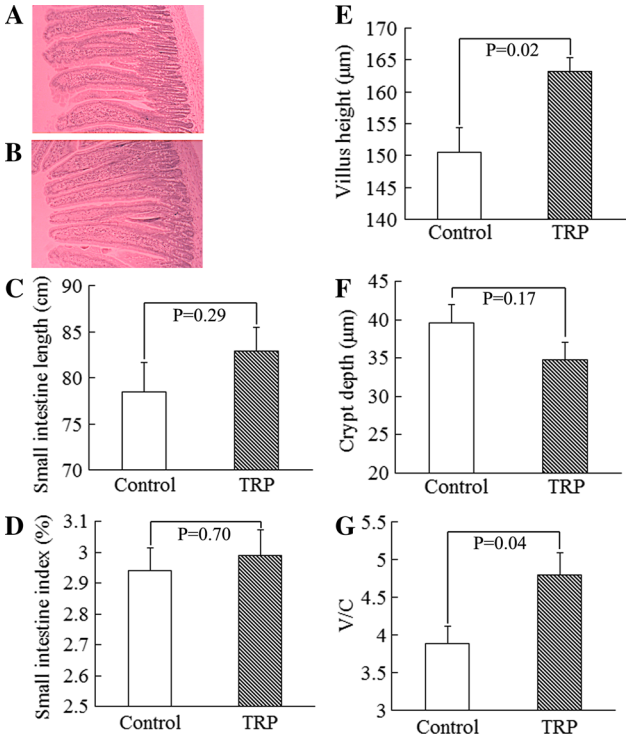

Fig. 2 The effects of TRP supplementation on small intestine length, small intestine index and the morphology of jejunum in rats. Histological features of jejunum sections stained with H&E, typical images (original magnification ×100) were chosen from control group rats (**a**) and TRP-supplemented group rats (**b**); the effects of TRP supplementation on small intestine length (**c**) and small intestine index (**d**) in rats; the effects of TRP supplementation on villus height (**e**), crypt depth (**f**) and ratio of villous height to crypt depth (**g**) of jejunum in rats. Data from each group ($n = 8$) were averaged and presented as mean ± SE

between control group and TRP-supplemented group. The PCA score plot of the ^{1}H NMR serum data was shown in Fig. 5a. This plot displayed the first two PCs and accounts for 73.1 % of the variation in the samples. And the corresponding loading plot is shown in Fig. 5b, which suggested that increased serum ^{1}H NMR peaks of acetoacetate and decreased peaks of isoleucine and trimethylamine are the major contributors to the separate clustering of the control group and TRP-supplemented group (Table 5).

Discussion

Tryptophan is the precursor of several neurotransmitters and metabolic regulators, which plays an important role in regulating nutrient metabolism (Moehn et al. 2012; Peters 1991). In the present study, ^{1}H nuclear magnetic resonance spectroscopy was used to demonstrate that the effect of tryptophan supplementation on the metabolome in serum of rats. The results from both PCA and system statistical metabolic

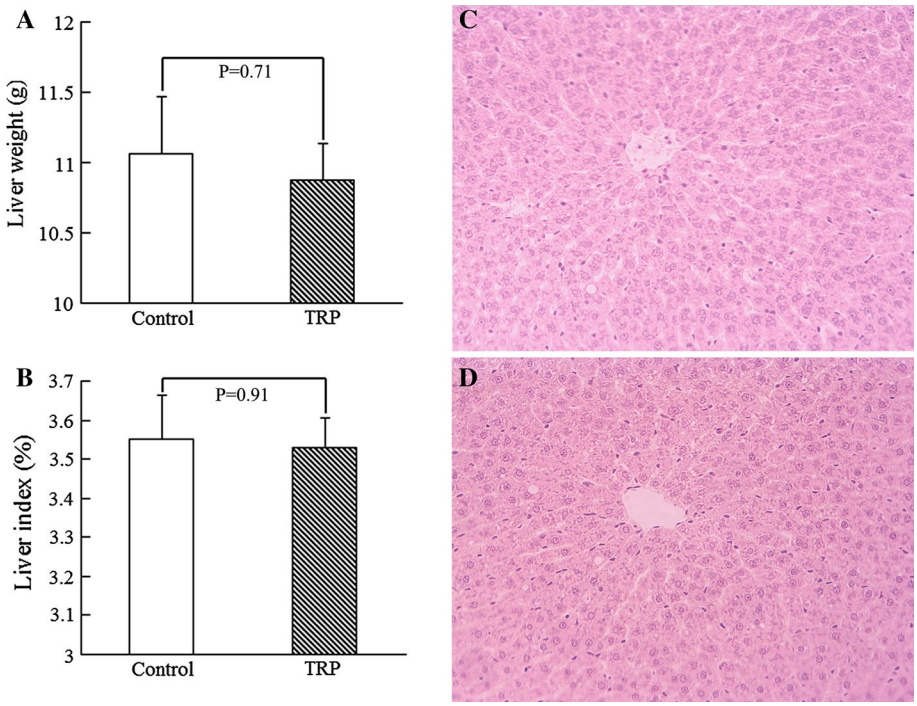

Fig. 3 The effects of TRP supplementation on liver index and the morphology of liver in rats. **a** Liver weight; **b** liver index; histological features of liver sections stained with H&E, typical images (original magnification ×200) were chosen from the control group rats (**c**) and TRP-supplemented group rats (**d**). Data from each group ($n = 8$) were averaged and presented as mean ± SE

Table 3 The effects of TRP supplementation on the contents of dimethylamine and trimethylamine in rat feces

Parameters	Control group	TRP-supplemented group	*P* values
Dimethylamine (mg/kg)	60.78 ± 5.61	48.58 ± 5.11	0.14
Trimethylamine (mg/kg)	167.23 ± 7.38	141.97 ± 6.04	0.02

Data from each group ($n = 8$) were averaged and presented as mean ± SE

P values less than 0.05 were regarded as significant statistical significance

correlation analysis probed the global disturbances in the metabolic profiles, including metabolites involved in amino acid metabolism, intestinal bacteria-related metabolism and lipid metabolism reflected the molecular mechanisms of the response of rats to tryptophan supplementation.

A novel and unexpected finding from this study was that tryptophan supplementation affected amino acid metabolism, and promoted protein synthesis in the body. Tryptophan is one of the amino acids essential for protein synthesis (Jessen and Sheikh 1992). Previous research has shown that dietary tryptophan deficiency decreased muscle and liver protein synthesis rates (Cortamira et al. 1991), and tryptophan supplementation significantly increased in hepatic protein synthesis in vivo (Sidransky et al. 1968). Sidransky (2010) reported that dietary tryptophan deficiency reduced plasma protein concentration, and tryptophan deficiency resembled protein deficiency. In this study, we found that tryptophan supplementation led to increases in the levels of many amino acids, including lysine, glycine, alanine, glutamate, glutamine, citrulline, methionine, tyrosine, 1-methylhistidine and albumin, together with the increases in serum concentrations of TP through traditional clinical chemistry method, implying that tryptophan supplementation promoted protein synthesis in the body (Li

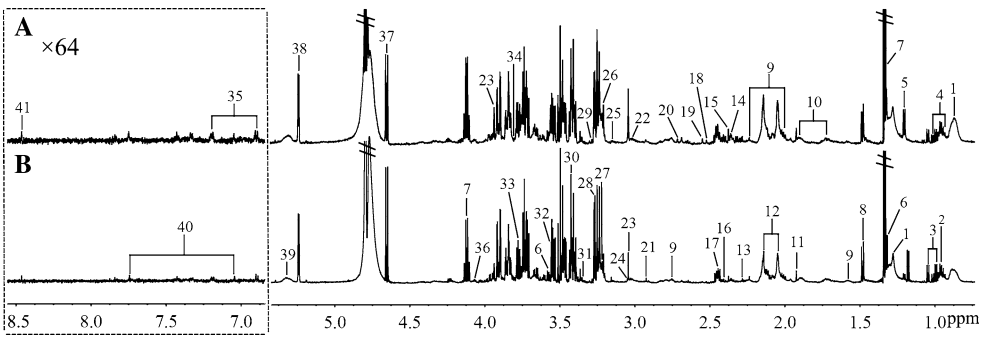

Fig. 4 Typical 600 MHz ^{1}H NMR spectra of serum taken from rats with the control group (**a**) and TRP-supplemented group (**b**)

Table 4 Changes in relative concentrations of serum metabolites in TRP-supplemented rats on the basis of chemical shifts relative to lactate at δ1.33

Key	Metabolites	Moieties	δ^1H (ppm) and multiplicity	Tryptophan	*P* values
1	LDL/VLDL	CH_3,CH_2CH_2C=	0.88(m), 1.28(m)	↓	0.25
2	Leucine	αCH,δCH_3,δCH_3	0.91(d), 0.96(d), 3.72(t)	↓	0.33
3	Valine	αCH_3,βCH,γCH_3	0.99(d), 1.04(d)	↓	0.15
4	Isoleucine	γCH_3,δCH_3	0.94(t), 1.01(d)	↓	0.01
5	β-Hydroxybutyrate	γCH_3	1.22(d)	↓	0.17
6	Threonine	αCH,βCH,γCH_3	1.32(d), 4.25(m), 3.58(d)	–	0.51
7	Lactate	αCH,βCH_3	1.33(d), 4.11(q)	↑	0.26
8	Alanine	αCH,βCH_3	3.77(q), 1.48(d)	↑	0.32
9	Lipids	CH_2*CH_2CO,CH_2–C=C	1.58(m), 2.04(m)	↓	0.41
		CH_2–C=O,CH–O–CO	2.24(m), 2.75(m)	↓	0.07
10	Lysine	αCH,βCH_2,γCH_2,δCH_2	3.77(t), 1.89(m), 1.73(m)	↑	0.19
11	Acetate	CH_2–C=O	1.92(s)	↓	0.28
12	Glycoprotein	CH_3–C=O	2.05(s), 2.08(m), 2.15(s)	–	0.75
13	Acetoacetate	CH_3,CH_2	2.29(s), 3.49(s)	↑	0.04
14	Glutamate	αCH,βCH_2,γCH_2	3.75(m), 2.08(m), 2.37(m)	↑	0.42
15	Pyruvate	CH_3	2.37(s)	–	0.89
16	Succinate	α,βCH_2	2.41(s)	–	0.57
17	Glutamine	αCH,βCH_2,γCH_2	3.68(t), 2.15(m), 2.45(m)	↑	0.43
18	Citrate	CH_2	2.52(d), 2.70(d)	–	0.71
19	Methylamine	CH_3	2.54(s)	↓	0.37
20	Dimethylamine	CH_3	2.71(s)	↓	0.39
21	Trimethylamine	CH_3	2.92(s)	↓	0.04
22	Albumin	Lysyl-CH_2	3.02(s)	↑	0.21
23	Creatine	N–CH_3,CH_2	3.04(s), 3.93(s)	↑	0.26
24	Creatinine	CH_3,CH_2	3.05 (s), 4.05(s)	↑	0.47
25	Citrulline	αCH_2,γCH_2,δCH_2	3.70(m), 1.58(m), 3.15(t)	↑	0.38
26	Choline	N–$(CH_3)_3$,αCH_2,βCH_2	3.20(s), 4.05(t), 3.51(t)	–	0.69
27	GPC	N–$(CH_3)_3$,OCH_2,NCH_2	3.22(s), 4.32(t), 3.51(t)	–	0.56
28	TMAO	CH_3	3.26(s)	↓	0.24
29	Betaine	CH_3,CH_2	3.28(s), 3.90(s)	↓	0.19
30	Taurine	N–CH_2,S–CH_2	3.27(t), 3.43(t)	↑	0.48
31	Proline	βCH_2,γCH_2,δCH_2	2.02–2.33(m), 2.00(m), 3.35(t)	↓	0.35
32	Glycine	CH_2	3.56(s)	↑	0.22
33	Methionine	αCH,βCH_2,γCH_2,δCH_3	3.78(m), 2.16(m), 2.6(dd), 2.14(s)	↑	0.29
34	Ornithine	CH_2,αCH	3.80(s), 3.79(t)	–	0.87
35	Tyrosine	αCH,CH_2	7.19(d), 6.89(d)	↑	0.43
36	Myo-Inositol	5-CH,4,6-CH,2-CH	3.30(t), 3.63(t), 4.06(t)	–	0.71
37	β-Glucose	2-CH,1-CH	3.25(dd), 4.65(d)	↑	0.07
38	α-Glucose	1-CH	5.24(d)	↑	0.09
39	Unsaturated lipids	=C–CH_2–C=,–CH=CH–	5.19(m), 5.31(m)	↓	0.14
40	1-Methylhistidine	4-CH,2-CH	7.05(s), 7.77(s)	↑	0.48
41	Formate	CH	8.45(s)	↓	0.42

"↓" and "↑" indicates the metabolite levels are lower or higher, respectively, compared with the control group; "–"indicates the metabolite levels are the same as the control group

s singlet, *d* doublet, *t* triplet, *q* quartet, *m* multiplet, *dd* doublet of doublets, *GPC* glycerophosphoryl choline, *TMAO* trimethylamine-*N*-oxide

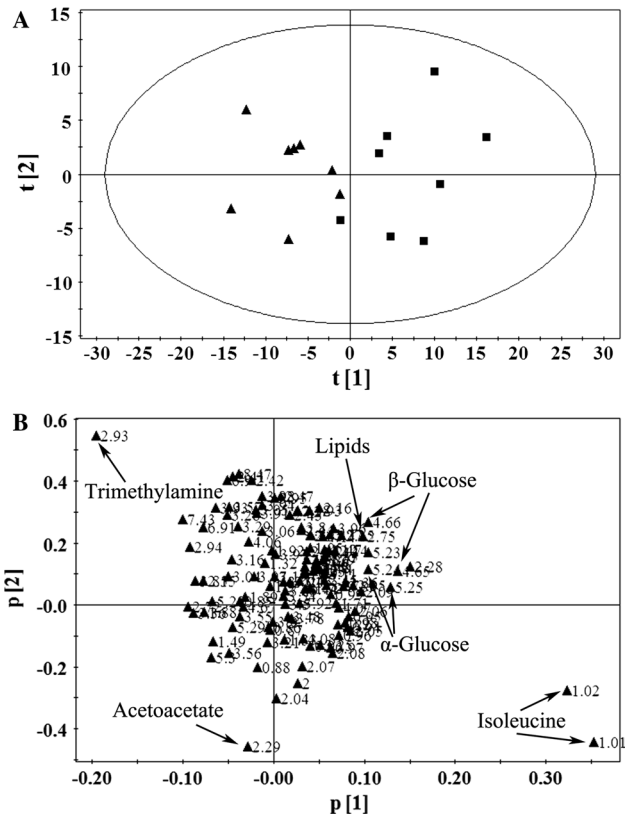

Fig. 5 PCA score plot (**a**) and loading plot (**b**) from ^{1}H NMR spectra of serum obtained from control group (*filled triangle*) and TRP-supplemented group (*filled square*) rats

Table 5 Changes in relative concentrations of serum metabolites in TRP-supplemented rats compared to control group rats

Metabolites	NMR chemical shift (δ)	Change in direction	*P* values
LDL/VLDL	0.86(m)	↓	0.25
β-Hydroxybutyrate	1.22(d)	↓	0.17
Acetoacetate	2.29(s)	↑	0.04
Lipids	2.75(m)	↓	0.07
Unsaturated lipids	5.31(m)	↓	0.14
β-Glucose	4.65(d)	↑	0.07
α-Glucose	5.24(d)	↑	0.09
Lactate	1.33(d)	↑	0.26
Isoleucine	1.01(d)	↓	0.01
Valine	1.04(d)	↓	0.15
Albumin	3.02(s)	↑	0.21
Lysine	1.89(m)	↑	0.19
Glycine	3.56(s)	↑	0.22
Trimethylamine	2.93(s)	↓	0.04
TMAO	3.26(s)	↓	0.24

"↑" indicates a relative increase, and "↓" indicates a relative decrease in the integral value for the region containing the identified metabolite

P values are significant at the <0.05 level

et al. 2009). The increased concentrations of these amino acids in serum may be explained by tryptophan supplementation increasing the absorption of amino acids. Tryptophan was transformed into serotonin in gut, and serotonin played an important role in promoting gastrointestinal motility (Brown 1995; Jin et al. 1999), and intestinal secretion (Siriwardena and Kellum 1993), which possibly led to an increase in the digestion of dietary protein and the absorption of amino acids from the lumen of the small intestine. In support of this notion, tryptophan supplementation led to an increase in villus height and the ratio of villus height to crypt depth, compared with the control group. The villus height, crypt depth and the ratio of villus height to crypt depth can be considered as a criterion that reflects the development and the absorption capacity of small intestine (Montagne et al. 2003; Pluske et al. 1996). Thus, an increase in villus height, villus/crypt ratio or a decrease in crypt depth means a promotion in the digestion of protein and absorption of amino acids (Hou et al. 2010). The branched-chain amino acid (isoleucine, valine and leucine) cannot be synthesized by animals, but are essential nutrients that must be obtained from foods (Harper et al. 1984). And that they are primarily metabolized in skeletal muscle (Buse and Reid 1975; Shinnick and Harper 1976). Skeletal muscle takes up branched-chain amino acid from the arterial blood, synthesizes other amino acids and proteins (Ferrando et al. 1995; Wu 2007). In the present study, the decrease in serum concentrations of branched-chain amino acid in TRP-supplemented group suggested that tryptophan supplementation enhanced protein synthesis in skeletal muscle (Platell et al. 2000), and led to an increase of branched-chain amino acid absorption from serum.

Another novel and unexpected finding from this study is that tryptophan supplementation modulates intestinal microbial metabolism. The serum concentrations of nitrogenous products (trimethylamine, TMAO, methylamine and dimethylamine), formate, as well as short-chain fatty acids (acetate) were decreased in TRP-supplemented group rats. Trimethylamine, TMAO, methylamine and dimethylamine, acetate and formate are microbial metabolites of carbohydrates and amino acids (Dumas et al. 2006; Louis et al. 2007; Smith et al. 1994; Walker et al. 2005), and they are likely produced in the lumen of the small intestine and large intestine. The decrease in the serum levels of these metabolites may be explained by a reduced number and/or altered activity of intestinal microflora. In addition, tryptophan supplementation decreased the contents of trimethylamine and dimethylamine in rat feces. These results also support the view that tryptophan supplementation modulates intestinal microbial metabolism. Bülbüller et al. (2005) found that tryptophan supplementation (at doses of

200 mg/kg body weight) significantly reduced the ability of intestinal bacteria. A decrease in the number of intestinal bacteria may reduce the catabolism of dietary amino acids in the gut, therefore contributing to an increase in the entry of dietary amino acids into the portal venous blood (Wang et al. 2009b). In support of this notion, plasma concentrations of lysine, glycine, alanine, glutamate, glutamine, citrulline, methionine and tyrosine were higher in TRP-supplemented group than in control group rats. The ammonia generated from intestinal amino acids catabolism, and either enters the portal venous blood or is utilized locally for urea synthesis (Wu 1995). The presence of a functional urea cycle in enterocytes serves as the first line of defense against ammonia toxicity in mammals (Wu 2007). Therefore, the reduction of intestinal bacterial populations can beneficially decrease the production of ammonia and urea in serum due to the reduced catabolism of amino acids in gut. The result is consistent with the decrease in concentrations of serum ammonia and urea through traditional clinical chemistry method.

We found that tryptophan supplementation affected lipid metabolism, leading to reduced fat deposition in the body of TRP-supplemented group, compared with the control group. Both acetoacetate and β-hydroxybutyrate are products of fatty acid oxidation in liver (Gaou et al. 2001; Wojtczak 1968), and the ratio of acetoacetate to hydroxybutyrate is an indicator of cytosolic and mitochondrial redox states (Pérez et al. 2004; Yamamoto et al. 1980). In this study, tryptophan supplementation increased the level of acetoacetate and decreased the level of β-hydroxybutyrate in serum (Table 5), thus elevated the ratio of acetoacetate to β-hydroxybutyrate. The result suggested that tryptophan supplementation promoted the oxidation of fatty acid. So it would lead to a reduced availability of fatty acids for the synthesis of lipids, LDL and VLDL in the body. In support of this view, tryptophan supplementation decreased the serum concentrations of lipids, unsaturated lipids, LDL and VLDL in TRP-supplemented group rats compared to the control group rats. In addition, we found that the changes in serum conventional biochemical indices (the decreased levels of CHO, TG, and LDL) also supported the notion that tryptophan supplementation promoted lipid metabolism and reduced fat accumulation in the body. Previous studies have suggested that tryptophan-deficient diet led to heavy accumulation of fat in the liver, and increasing with the duration of the experiment, but tryptophan supplementation remarkably reduced the amount of fat in liver (Adamstone and Spector 1950). Akiba et al. (1992) also confirmed that dietary L-tryptophan supplementation resulted in significant decreases in hepatic lipids, in particular triglyceride. Özer et al. (2004, 2007) revealed that tryptophan supplementation (intraperitoneal injection, at doses of 100 mg/kg body weight, for 7 days) significantly decreased in the body weight of mice. This is in agreement with the result of our study that TRP supplementation also led to the decreased body weight in rats. The result also demonstrated that tryptophan supplementation reduced fat accumulation in the body.

In conclusion, the results of our study indicate that tryptophan supplementation affects the serum metabolome in rats. The changes in the serum levels of amino acids and related metabolites, nitrogenous products and short-chain fatty acids, lipids and related metabolites suggested that tryptophan supplementation enhanced protein accretion and reduced fat deposition and modulated intestinal microbial metabolism in the body of rats.

Acknowledgments This research was financially supported by National Natural Science Foundation of China (31330075, 31360552, 31201813, and 31001014) and the Research Foundation (SKLF-TS-201108 and SKLF-TS-200817) and the Open Project Program (SKLF-KF-201005 and SKLF-KF-201216) of State Key Laboratory of Food Science and Technology, Nanchang University, and Texas A&M AgriLife Research (H-8200). G. Wu was supported by the Chinese Academy of Sciences Visiting Professorship for Senior International Scientists Grant (No. 2011T2S15).

Conflict of interest The authors declare that they have no conflict of interests.

References

Adamstone FB, Spector H (1950) Tryptophan deficiency in the rat. Histologic changes induced by forced feeding of an acid-hydrolyzed casein diet. Arch Pathol 49:173–184

Akiba Y, Takahashi K, Horiguchi M, Ohtani H, Saitoh S, Ohkawara H (1992) L-tryptophan alleviates fatty liver and modifies hepatic microsomal mixed function oxidase in laying hens. Comp Biochem Physiol A Physiol 102:769–774

Benjakul S, Visessanguan W, Tanaka M (2004) Induced formation of dimethylamine and formaldehyde by lizardfish (*Saurida micropectoralis*) kidney trimethylamine-*N*-oxide demethylase. Food Chem 84(2):297–305

Biggio G, Fadda F, Fanni P, Tagliamonte A, Gessa GL (1974) Rapid depletion of serum tryptophan, brain tryptophan, serotonin and 5-hydroxyindoleacetic acid by a tryptophan-free diet. Life Sci 14:1321–1329

Brown DR (1995) Mucosal protection through active intestinal secretion: neural and paracrine modulation by 5-hydroxytryptamine. Behav Brain Res 73:193–197

Brzozowski T, Konturek PC, Konturek SJ, Pajdo R, Bielanski W, Brzozowska I, Stachura J, Hahn EG (1997) The role of melatonin and L-tryptophan in prevention of acute gastric lesions induced by stress, ethanol, ischemia, and aspirin. J Pineal Res 23:79–89

Bülbüller N, Akkuş MA, Ilhan YS, Baysal F, Ozercan I, Aygen E, Kirkil C (2003) The effects of L-tryptophan and pentoxiphylline on stress ulcer. Ulus Travma Derg 9:90–95

Bülbüller N, Pektaş B, Özdarendeli A, Doğru O, Aygen E, Akpolat N (2005) The effect of L-tryptophan on hemorrhagic shock induced bacterial translocation. J Surg Res 123:194–199

Buse MG, Reid SS (1975) Leucine. A possible regulator of protein turnover in muscle. J Clin Invest 56:1250–1261

Castro GA, Copeland EM, Dudrick SJ, Johnson LR (1975) Intestinal disaccharidase and peroxidase activities in parenterally nourished rats. J Nutr 105:776–781

Chen J, Lin H, Hu M (2003) Metabolism of flavonoids via enteric recycling: role of intestinal disposition. J Pharmacol Exp Ther 304(3):1228–1235

Cortamira NO, Seve B, Lebreton Y, Ganier P (1991) Effect of dietary tryptophan on muscle, liver and whole-body protein synthesis in weaned piglets: relationship to plasma insulin. Br J Nutr 66:423–435

Dumas ME, Barton RH, Toye A, Cloarec O, Blancher C, Rothwell A, Fearnside J, Tatoud R, Blanc V, Lindon JC, Mitchell SC, Holmes E, McCarthy MI, Scott J, Gauguier D, Nicholson JK (2006) Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice. Proc Natl Acad Sci 103:12511–12516

Esteban S, Nicolaus C, Garmundi A, Rial RV, Rodríguez AB, Ortega E, Ibars CB (2004) Effect of orally administered L-tryptophan on serotonin, melatonin, and the innate immune response in the rat. Mol Cell Biochem 267:39–46

Ferrando AA, Williams BD, Stuart CA, Lane HW, Wolfe RR (1995) Oral branched-chain amino acids decrease whole-body proteolysis. J Parent Enter Nutr 19:47–54

Gao HC, Zhu H, Song CY, Lin L, Xiang Y, Yan ZH, Bai GH, Ye FQ, Li XK (2013) Metabolic changes detected by ex vivo high resolution ^{1}H NMR spectroscopy in the striatum of 6-OHDA-induced Parkinson's rat. Mol Neurobiol 47:123–130

Gaou I, Malliti M, Guimont MC, Lettéron P, Demeilliers C, Peytavin G, Degott C, Pessayre D, Fromenty B (2001) Effect of stavudine on mitochondrial genome and fatty acid oxidation in lean and obese mice. J Pharmacol Exp Ther 297:516–523

Harper AE, Miller RH, Block KP (1984) Branched-chain amino acid metabolism. Annu Rev Nutr 4:409–454

He Q, Kong X, Wu G, Ren P, Tang H, Hao F, Huang R, Li T, Tan B, Li P, Tang Z, Yin Y, Wu Y (2009) Metabolomic analysis of the response of growing pigs to dietary L-arginine supplementation. Amino Acids 37:199–208

He Q, Tang H, Ren P, Kong X, Wu G, Yin Y, Wang Y (2011a) Dietary supplementation with L-arginine partially counteracts serum metabonome induced by weaning stress in piglets. J Proteome Res 10:5214–5221

He Q, Ren P, Zhao F, Kong XF, Wu GY, Yin YL (2011b) Metabonomics and its role in amino acid nutrition research. Front Biosci 16:2451–2460

He Q, Ren P, Kong X, Xu W, Tang H, Yin Y and Wang Y (2011c) Intrauterine growth restriction alters the metabonome of the serum and jejunum in piglets. Mol BioSyst 7:2147–2155

He Q, Ren P, Kong X, Wu Y, Wu G, Li P, Hao F, Tang H, Yin Y (2012) Comparison of serum metabolite compositions between obese and lean growing pigs. J Nutr Biochem 23:133–139

Hou YQ, Wang L, Ding B, Liu Y, Zhu H, Liu J, Li Y, Wu X, Yin Y, Wu G (2010) Dietarya-ketoglutarate supplementation ameliorates intestinal injury in lipopolysaccharide-challenged piglets. Amino Acids 39:555–564

Jessen H, Sheikh MI (1992) L-tryptophan uptake by segment-specific membrane vesicles from the proximal tubule of rabbit kidney. Biochem J 286:103–110

Jiang CY, Yang KM, Yang L, Miao ZX, Wang YH, Zhu HB (2013) A ^{1}H NMR-based metabonomic investigation of time-related metabolic trajectories of the plasma, urine and liver extracts of hyperlipidemic hamsters. PLoS One 8:e66786

Jin JG, Foxx-Orenstein AE, Grider JR (1999) Propulsion in guinea pig colon induced by 5-hydroxytryptamine (HT) via 5-HT_4 and 5-HT_3 receptors. J Pharmacol Exp Ther 288:93–97

Kim SW, Mateo RD, Yin Y, Wu G (2007) Functional amino acids and fatty acids for enhancing production performance of sows and piglets. Asian Australas J Anim Sci 20:295–306

Kolli VK, Abraham P, Isaac B, Kasthuri N (2013a) Preclinical efficacy of melatonin to reduce methotrexate-induced oxidative stress and small intestinal damage in rats. Dig Dis Sci 58:959–969

Kolli VK, Kanakasabapathy I, Faith M, Ramamoorthy H, Isaac B, Natarajan K, Abraham P (2013b) A preclinical study on the protective effect of melatonin against methotrexate-induced small intestinal damage: effect mediated by attenuation of nitrosative stress, protein tyrosine nitration, and PARP activation. Cancer Chemother Pharmacol 71:1209–1218

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

Li P, Mai K, Trushenski J, Wu G (2009) New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds. Amino Acids 37:43–53

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

Liu G, Wang Y, Wang Z, Cai J, Lv X, Zhou A (2011) Nuclear magnetic resonance (NMR)-based metabolomic studies on urine and serum biochemical profiles after chronic cysteamine supplementation in rats. J Agric Food Chem 59:5572–5578

Liu XD, Wu X, Yin YL, Liu YQ, Geng MM, Yang HS, Francois B, Wu GY (2012) Effects of dietary L-argininеor N-carbamylglutamate supplementation during late gestation of sows on the miR-15b/16, miR-221/222, VEGFA and eNOS expression in umbilicalvein. Amino Acids 42:2111–2119

Louis P, Scott KP, Duncan SH, Flint HJ (2007) Understanding the effects of diet on bacterial metabolism in the large intestine. J Appl Microbiol 102:1197–1208

Mahaffey KR, Capar SG, Gladen BC, Fowler BA (1981) Concurrent exposure to lead, cadmium, and arsenic. Effects on toxicity and tissue metal concentrations in the rat. J Lab Clin Med 98:463–481

Moehn S, Pencharz PB, Ball RO (2012) Lessons learned regarding symptoms of tryptophan deficiency and excess from animal requirement studies. J Nutr 142:2231S–2235S

Montagne L, Pluske JR, Hampson DJ (2003) A review of interactions between dietary fibre the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. Anim Feed Sci Technol 108:95–117

Nabuurs MJA, Hoogendoorn A, Van der Molen EJ, Van Osta ALM (1993) Villus height and crypt depth in weaned and unweaned pigs, reared under various circumstances in the Netherlands. Res Vet Sci 55:78–84

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

Noguchi Y, Sakai R, Kimura T (2003) Metabolomics and its potential for assessment of adequacy and safety of amino acid intake. J Nutr 133:2097S–2100S

Özer Ç, Gönül B, Take G, Erdoğan D, Tong E, Ercan ZS (2004) Tryptophan administration increase contractility and change the ultrastructure of mice duodenum. Amino Acids 27:215–220

Özer Ç, Gönül B, Ercan ZS, Take G, Erdoğan D (2007) The effect of tryptophan administration on ileum contractility and oxidant status in mice. Amino Acids 32:453–458

Pérez MJ, Sánchez-Medina F, Torres M, Gil A, Suárez A (2004) Dietary nucleotides enhance the liver redox state and protein synthesis in cirrhotic rats. J Nutr 134:2504–2508

Perianayagam MC, Oxenkrug GF, Jaber BL (2005) Immune-modulating effects of melatonin, N-acetylserotonin, and *N*-acetyldopamine. Ann NY Acad Sci 1053:386–393

Peters JC (1991) Tryptophan nutrition and metabolism: an overview. In: Kynurenine and serotonin pathways, vol 294. Springer, New York, pp 345–358

Platell C, Kong SE, McCauley R, Hall JC (2000) Branched-chain amino acids. J Gastroenterol Hepatol 15:706–717

Platten M, Ho PP, Youssef S, Fontoura P, Garren H, Hur EM, Gupta R, Lee LY, Kidd BA, Robinson WH, Sobel RA, Selley ML,

Steinman L (2005) Treatment of autoimmune neuroinflammation with a synthetic tryptophan metabolite. Science 310:850–855

Pluske JR, Williams IH, Aherne FX (1996) Maintenance of villous height and crypt depth in piglets by providing continuous nutrition after weaning. Anim Sci 62:131–144

Ren WK, Yin YL, Liu G, Yu XL, Li YH, Yang G, Li TJ, Wu GY (2012) Effect of dietary arginine supplementation on reproductive performance of mice with porcine circovirus type 2 (PCV2) infection. Amino Acids 42:2089–2094

Ren WK, Luo W, Wu MM, Liu G, Yu XL, Fang J, Li TJ, Yin YL, Wu GY (2013) Dietary L-glutamine supplementation improves pregnancy outcome in mice infected with type-2 porcine circovirus. Amino Acids 45:479–488

Ren W, Chen S, Yin J, Duan J, Li T, Liu G, Feng Z, Tan B, Yin Y, Wu G (2014) Dietary arginine supplementation of mice alters the microbial population and activates intestinal innate immunity. J Nutr 144:568–579

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

Ruan Z, Zang G, Yin YL, Li TJ, Huang RL, Kim SW, Wu GY, Deng ZY (2007) Dietary requirement of true digestible phosphorus and total calcium for growing pigs. Asian-Australas J Anim Sci 20:1236–1242

Ruan Z, Lv Y, Fu X, He Q, Deng Z, Liu W, Yu Y, Wu X, Wu G, Wu X, Yin Y (2013) Metabolomic analysis of amino acid metabolism in colitic rats supplemented with lactosucrose. Amino Acids 45:877–887

Ruan Z, Yang Y, Zhou Y, Wen Y, Ding S, Liu G, Wu X, Liao P, Deng Z, Assaad H, Wu G, Yin Y (2014) Metabolomic analysis of amino acid and energy metabolism in rats supplemented with chlorogenic acid. Amino Acids. doi:10.1007/s00726-014-1762-7

Sánchez S, Sánchez CL, Paredes SD, Rodriguez AB, Barriga C (2008) The effect of tryptophan administration on the circadian rhythms of melatonin in plasma and the pineal gland of rats. J Appl Biomed 6:177–186

Sewerynek E, Reiter RJ, Melchiorri D (1996) Oxidative damage in the liver induced by ischemia reperfusion: protection by melatonin. Hepatogastroenterology 43:898–905

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

Shinnick FL, Harper AE (1976) Branched-chain amino acid oxidation by isolated rat tissue preparations. Biochimica et Biophysica Acta 437:477–486

Sidransky H (2010) Tryptopha: biochemical and health implications. CRC Press, Boca Raton

Sidransky H, Sarma DSR, Bongiorno M, Verney E (1968) Effect of dietary tryptophan on hepatic polyribosomes and protein synthesis in fasted mice. J Biol Chem 243:1123–1132

Siriwardena MDA, Kellum MDJM Jr (1993) A 5-HT_2 receptor mediates serotonin-induced electrolyte transport in rat left colon. J Surg Res 55:323–329

Smith JL, Wishnok JS, Deen WM (1994) Metabolism and excretion of methylamines in rats. Toxicol Appl Pharmacol 125: 296–308

Sugden D, Namboodiri MAA, Klein DC, Grady RK, Mefford IN (1985) Ovine pineal indoles: effects of L-tryptophan or L-5-hydroxytryptophan administration. J Neurochem 44:769–772

Tan BE, Yin YL, Liu ZQ, Tang WJ, Xu HJ, Kong XF, Li XG, Yao K, Gu WT, Smith SB, Wu GY (2011) Dietary L-arginine supplementation differentially regulates expression of fat-metabolic genes in porcine adipose tissue and skeletal muscle. J Nutr Biochem 22:441–445

van Beers-Schreurs HM, Nabuurs MJ, Vellenga L, Kalsbeek-van der Valk HJ, Wensing T, Breukink HJ (1998) Weaning and the weanling diet influence the villous height and crypt depth in the small intestine of pigs and alter the concentrations of short-chain fatty acids in the large intestine and blood. J Nutr 128:947–953

Walker AW, Duncan SH, Leitch ECM, Child MW, Flint HJ (2005) pH and peptide supply can radically alter bacterial populations and short-chain fatty acid ratios within microbial communities from the human colon. Appl Environ Microbiol 71:3692–3700

Wang JJ, Chen LX, Li P, Li XL, Zhou HJ, Wang FL, Li DF, Yin YL and Wu GY (2008) Gene Expression is Altered in Piglet Small Intestine by Weaning and Dietary Glutamine supplementation. J Nutr 138:1025–1032

Wang J, Wu G, Zhou H, Wang F (2009a) Emerging technologies for amino acid nutrition research in the post-genome era. Amino Acids 37:177–186

Wang WW, Qiao SY, Li DF (2009b) Amino acids and gut function. Amino Acids 37:105–110

Wei L, Liao P, Wu H, Li X, Pei F, Li W, Wu Y (2008) Toxicological effects of cinnabar in rats by NMR-based metabolic profiling of urine and serum. Toxicol Appl Pharmacol 227:417–429

Wojtczak AB (1968) Control of acetoacetate and β-hydroxybutyrate production in rat liver mitochondria. Biochem Biophys Res Commun 31:634–640

Wu G (1995) Urea synthesis in enterocytes of developing pigs. Biochem J 312:717–723

Wu GY, Bazer FW, Davis TA, Johnson GA, Kim SW, Knabe DA, Spencer TE, Yin YL (2007) Important roles for arginine-family amino acids in swine nutrition and production. Livest Sci 122:8–22

Xiao H, Wu MM, Tan BE, Yin YL, Li TJ, Xiao DF, Li L (2013) Effects of composite antimicrobial peptides in weanling piglets challenged with deoxynivalenol: II. Intestinal morphology and function. J Anim Sci 4750–4756

Yamamoto M, Tanaka J, Ozawa K, Tobe T (1980) Significance of acetoacetate/β-hydroxybutyrate ratio in arterial blood as an indicator of the severity of hemorrhagic shock. J Surg Res 28:124–131

Yao K, Fang J, Yin YL, Feng ZM, Tang ZR, Wu G (2010) Tryptophan metabolism in animals: important roles in nutrition and health. Front Biosci (Schol Ed) 3:286–297

Zira A, Kostidis S, Theocharis S, Sigala F, Engelsen SB, Andreadou I, Mikros E (2013) ^{1}H NMR-based metabonomics approach in a rat model of acute liver injury and regeneration induced by CCl_4 administration. Toxicology 303:115–124

Zou YR, Kottmann AH, Kuroda M, Taniuchi I, Littman DR (1998) Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. Nature 393:595–599