REVIEW ARTICLE

Emerging technologies for amino acid nutrition research in the post-genome era

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Abstract Amino acids (AA) are not only the building blocks of proteins but are also key regulators of metabolic pathways in cells. However, the mechanisms responsible for the effects of AA are largely unknown. With the completion of human and other mammalian genome projects, revolutionary technologies in life sciences characterized by high throughput, high efficiency, and rapid computation are now available for AA nutrition research. These advanced tools include genetics (the genomic variety), epigenetics (stable and heritable changes in gene expression or cellular phenotype that occurs without changes in DNA sequence), transcriptomics (alternative mRNA splicing, microRNAs, and gene transcription), proteomics (protein expression and interactions), metabolomics (metabolite profiles in cells and tissues), and bioinformatics (analysis of metabolic pathways using systems biology approach). These robust, powerful methods can be employed for the analysis of DNA, RNA, protein, and low-molecular-weight metabolites, whose expression and concentration are affected by the interaction between genes and dietary AA. With the omics and other advanced methodologies, we expect that the molecular actions of AA on target tissues can be defined and that optimal dietary recommendations for these nutrients can be devised for individual humans (personalized nutrition) and animals (targeted feeding) in response to changes in physiological and pathological conditions.

Keywords Omics technology · Amino acid · Nutrition · Post-genome era

Abbreviations
AA	Amino acids
miRNA	microRNA
NMR	Nuclear magnetic resonance
RNAi	RNA interference
SAGE	Serial analysis of gene expression
siRNA	Small interference RNA
SNPs	Single nucleotide polymorphisms

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Introduction

Amino acids (AA) are precursors for the synthesis of protein, other nitrogenous substances (e.g., nitric oxide, urea, uric acid, polyamines, dopamine, thyroxine, heme, and glutathione), glucose, and fatty acids (Galli 2007; Grillo and Colombatto 2007; Li et al. 2007; Sugita et al. 2007). In addition, increasing evidence shows that AA regulate cellular signaling pathways, thereby impacting key metabolic pathways (Jobgen et al. 2006; Kim et al. 2007b; Wang et al. 2008b; Wu et al. 2007a). Thus, the interaction between genes and dietary AA is a complex process involving multi-organ physiology and all levels of regulation (genes, chromatin structure, gene expression, proteins, and metabolites).

The traditional approaches to study AA nutrition include digestibility trials, nitrogen balance, assessments of growth

and reproductive performance, isotope tracer techniques (quantification of protein turnover, as well as AA synthesis, catabolism and flux), determination of AA metabolites and their function using various analytical methods, northern and western blots, and evaluation of health status (Fang et al. 2008; Han et al. 2008; Stoll and Burrin 2006; Suryawan et al. 2008; Wu et al. 2008). While these approaches have played a historically important role in protein and AA nutrition research and remain irreplaceable today, they are limited in scope. With the completion of human and other mammalian genome projects, revolutionary technologies in life sciences characterized by high throughput, high efficiency, and rapid computation have been developed. These advanced tools, such as genetics, epigenetics, transcriptomics, proteomics and metabolomics, are now available to determine how AA in the diet may affect both the expression levels and biological activities of DNA, RNA, protein, and low-molecular weight metabolites (Forde and McCutchen-Maloney 2002; Hu et al. 2008a, b; Zhan et al. 2008) in humans and animals (Fig. 1).

Omics techniques in the post-genome era

Nutrigenetics

It is well known that different individuals (humans or animals) respond differently to the same diet, as indicated by differences in the susceptibility to disease, as well as the efficiency of nutrient absorption and utilization (Kim 2005). Genetic variability is most likely responsible for the diverse biological outcomes. Among genomic variety, single nucleotide polymorphisms (SNPs) are considered to be the major genetic source of phenotypic variability that differentiates individuals within a given species (Gorringe et al. 2007). At the same time, other types of genetic variability
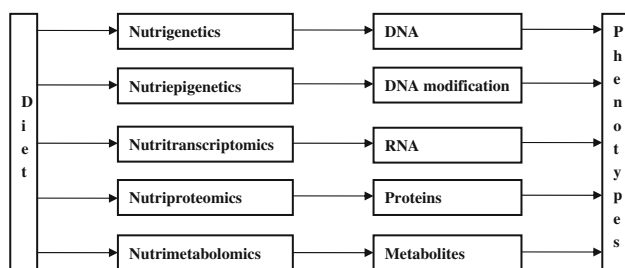

Fig. 1 Omics technologies for nutrition research in the post-genome era. With the completion of human and other mammalian genome projects, revolutionary technologies in life sciences are now available for amino acid nutrition research. These advanced tools include genetics, epigenetics, transcriptomics, proteomics, and metabolomics. These powerful methods can be employed for the analysis of DNA, RNA, protein, and low-molecular-weight metabolites in cells and tissues

(e.g., insertions or deletions of nucleotides and variability in copy number of repeated sequences) also make some contributions to phenotypic changes (Sanders et al. 2008). To address this important issue, a term "nutrigenetics" has been coined, which can be defined as the relationship between diet and genotype as well as its impact on health and disease.

Identifying key SNPs that may influence the nutritional response and health of an individual has been a primary driving force in the development of most nutrigenetic approaches to date (Hu et al. 2008a, b). High-throughput genotyping methods for large-scale association studies include TaqMan®SNP Genotyping Assay®, single-base extension-based assays, mass spectrometry-based methods (e.g., the Sequenom® MassARRAY genotyping system), the Invader assay, Pyrosequencing, and gene chip methods (e.g., the new Affymetrix 500 K SNPChip Array) (Jurinke et al. 2005; Sanders et al. 2008). Accordingly, statistical methods have been developed to analyze this type of experimental data (Fu et al. 2008).

Gene copy number variants, which may be an important source of changes in gene expression that are responsible for much of the variability in an organism's response to diets, have been recognized (Hu et al. 2008b; Sanders et al. 2008). Thus, future studies will be conducted beyond the consideration of only variability between SNPs, and will also take into account the other types of genetic variability. Genome-wide copy number detection using microarray technologies has been one of the recent research topics on large-scale variations in genomes. Affymetrix GeneChip and Copy Number Analyzer for Affymetrix GeneChip v2.0 allow for not only accurate and high-resolution copy number estimations, but also the analysis of allelic imbalances, thereby providing a powerful platform to explore the complexities of genomes (Ogawa et al. 2007).

Nutriepigenetics

Epigenetics is the study of stable and heritable changes in gene expression or cellular phenotype that occurs without changes in DNA sequence. DNA methylation and covalent and noncovalent modifications of chromatin are well-known epigenetic mechanisms. Noncoding RNA-mediated gene regulation at the levels of DNA and chromatin (e.g., transcriptional gene silencing and activation) is also considered as an epigenetic mechanism. The epigenetic state of the genome is established in early development and can be modified during pregnancy. The fetal genome is generally thought to affect the later life of the organism, which is defined as fetal programming (Kim 2005; Wolff et al. 1998). Nutrition is one of the most important players in the epigenetic repertoire (Wu et al. 2004a). Gene-nutrient interactions can alter embryonic or fetal development, which sets the stage for metabolic regulation, susceptibility

to diseases, behavioral adaptation, and nutrient utilization in the adulthood (Wu et al. 2006). Therefore, epigenetic studies aid in explaining how nutrition affects the offspring genome and why individuals respond differently to environmental signals in an inter-generational effect.

Methods commonly used in an epigenetic study are those that map DNA methylation and histone modifications (e.g., bisulfite DNA modification), chromatin immunoprecipitation assay, as well as RNA interference (RNAi). The Methylamp™ Global DNA Methylation Quantification Kit is now developed for detecting global DNA methylation status using genomic DNA (http://www.epigentek.com).

Nutritranscriptomics

The genome is organized in chromosomes within the nucleus of the cell, and is transcriptionally heterogenous. Available evidence shows that transcription of genes is controlled by nutrients (including AA), hormones, and other regulatory factors (Curi et al. 2005; Hu et al. 2008b; Jobgen et al. 2006).

Precursor mRNA splicing is a critical process by which introns are removed and exons are joined together to form a protein-coding mature RNA. There are general four types of alternative splicings: (1) exon skipping, (2) alternative 5′ss selection, (3) 3′ss selection, and (4) intron retention (Kim et al. 2007b). Because of alternative splicing, a single primary transcript can encode multiple protein isoforms. Consequently, more diverse animal proteomes are generated for distinct biological functions.

Short noncoding RNAs in animals also play regulatory roles in gene expression. Most abundant noncoding RNAs are microRNAs (miRNAs), which are small regulatory RNA molecules with 19–25 nucleotides in length (Cullen 2006). Since the discovery of lin-4 and let-7 in *Caenorhabditis elegans* by Lee et al. (1993), miRNAs have emerged as essential regulators of gene expression in recent years, including the degradation of target mRNA (Giraldez et al. 2006; Zamore et al. 2000), mRNA translation (Lee and Ambros 2001), heterochromatin structure remodeling (Reinhart and Bartel 2002), and transposon silencing (Aravin et al. 2007). Each miRNA has hundreds of evolutionary conserved targets and perhaps more non-conserved targets (Bentwich et al. 2005). Based on a four-genome analysis of 3′-UTRs, approximately 30% of all human genes may be regulated by miRNAs (Lewis et al. 2005). Therefore, miRNAs have regulatory roles in biology and pathophysiology. In addition, miRNA modulation of physiological processes has important implications for nutrition.

Transcriptomics aims at quantifying the levels of expression of all or a selected subset of genes based on the amounts of RNA present in a sample. This technology is useful for assessing simultaneously the relative abundance of transcripts for thousands of genes under various experimental conditions. Transcriptome profiling generally involves DNA, cDNA, oligonucleotide arrays, as well as serial analysis of gene expression (SAGE), and provides a powerful tool to determine effects of nutrients (e.g., AA) on gene expression in cells and tissues (Hu et al. 2008b; Kim 2005). High-throughput technologies, including deep sequencing and splicing-sensitive microarrays, are regular methods for alternative splicing analysis (Ben-Dov et al. 2008). Integration of these technologies with immunoprecipitation and bioinformatic methods will shed light on the regulatory gene network. At the same time, changes in expression of miRNAs can only be detected at the RNA level due to the nature of non-coding miRNAs. In contrast to capillary sequencing, recently available "next generation" sequencing technologies provide inexpensive increases in throughput, thereby offering a more comprehensive view of the miRNA transcriptome.

Most functional genomics experiments, such as gene expression profiling and proteomics, can help us identify genes that are potentially associated with certain biological processes of interest (Hu et al. 2008b). However, reverse genetic approach using RNAi, which was first discovered in *C. elegans* (Fire et al. 1998) and plants (Jorgensen et al. 1996), has provided a promising method to experimentally test the association of phenotype with genotype of silenced genes. RNAi is post-transcriptional silencing of gene utilizing a homologous double-stranded RNA (dsRNA) (Lehner et al. 2004) and can be delivered in different ways. A small interference ($\sim$21 nucleotide) RNA (siRNA) could be synthesized and transfected into cells. To avoid the relatively high cost and transient silencing of this process (Myers et al. 2003), vectors that express short hairpin RNAs have been developed. Subsequently, the nanoparticles generated from cyclodextrin-containing polycations and siRNA were successfully delivered in vivo using the nano technology (Hu-Lieskovan et al. 2005). RNAi has proven to be a powerful method to study gene function and it is expected that cellular mechanism of transcriptional regulation can be further investigated using an RNAi-mediated approach (Kim and Rossi 2008).

Nutriproteomics

Proteins are the final products of gene expression and have important structural and regulatory functions in cells (Forde and McCutchen-Maloney 2002; Liao et al. 2008). Thus, proteomic technology has emerged as a revolutionary discovery tool to study how dietary AA can alter the proteomes of organisms. Using proteomics, researchers can simultaneously display and determine thousands of proteins in a study sample and identify their changes in response to physiological, pathological, and nutritional

alterations (Hu et al. 2008a; Wang et al. 2006, 2008c). Currently, the most commonly used proteomics technologies are based on either the specific digestion of proteins by a protease (usually trypsin) or the direct analysis of intact proteins. The protein hydrolysis method, also known as the bottom-up approach, involves 2-dimensional polyacrylamide gel electrophoresis and multidimensional protein identification. In contrast, the analysis of intact proteins requires their chromatographic separation and identification by mass spectrometry (e.g., surface-enhanced laser desorption ionization) (Wang et al. 2006). Recently, several quantitative proteomic techniques have been developed, such as 2D DIGE (difference gel electrophoresis), ICAT (isotope-coded affinity tag), iTRAQ (isobaric tags for relative and absolute quantification) (Chen et al. 2007), and proteolytic O-18- labeling strategies (Miyagi and Rao 2007). Finally, a promising approach for proteomics is the protein microarray technology, which can be used to detect changes in the expression and post-translational modifications of hundreds or even thousands of proteins in a parallel way (Chen et al. 2007). Identification of different phosphoproteins and their phosphorylation sites can be accomplished by combining proteomics with radioactive labeling, phospho-specific staining, immunoprecipitation, or immunoblotting, as well as metal affinity chromatography (Vercauteren et al. 2007; Yan and He 2008). This approach provides informational insights into signaling pathways triggered by nutrients and other factors.

Nutrimetabolomics

Gene expression or protein concentration may only indicate the potential for physiological or metabolic changes in cells, tissues and organs, but does not represent real endpoints of the complex regulatory processes in organisms. Nutrimetabolomics, defined as the analysis of effects of diet on the metabolome (a complete set of small-molecule metabolites in a biological sample), is at its infancy in nutrition research. However, this technology has already provided novel and important insights into understanding the metabolic responses of humans or animals to dietary interventions (e.g., He et al. 2008). Currently, the widely used methods for nutrimetabolomics studies involve proton nuclear magnetic resonance (NMR) technology (Bertram et al. 2007) and mass spectrometry (MS). LC-MS, which is complementary to NMR, offers superb sensitivity, but is limited by the essentially nonquantitative nature of the method and the requirement of internal standardization.

Bioinformatics

With the exceedingly large amounts of genome data generated from advanced post-genomics era, great opportunities and challenges subsequently come along the way. Bioinformatics (analysis of metabolic pathways using systems biology approach) plays an essential role in the interface of molecular biology and computational analysis. Biological problems can be approached from cellular and organism levels by studying individual genes and proteins or a gene network using whole genome data (Ideker et al. 2001). Temporal and spatial gene expression changes under different physiological status could also be investigated. Bioinformatics method can help us utilize data on high-throughput DNA sequences, mRNA profile, protein expression, and metabolites to elucidate the mechanisms that regulate the structure and functions of genes, biochemical pathways, and physiological processes.

Application of omics techniques to AA nutrition research

Polymorphism and genetic background related to AA nutrition

Humans or animals in different regions of the world may have different genetic backgrounds. Thus, it is possible that AA metabolism differs among the diverse individuals. Recently studies have been conducted to evaluate the metabolic variability between Swedish and British populations (Lenz et al. 2004), or among American, Chinese and Japanese (Dumas et al. 2006). Cross-population differences were detected in urinary metabolites of AA owing to genetic and cultural differences. The results suggest that polymorphism of a specific gene might result in different patterns of AA utilization among human subjects. In another investigation, Kim (2007) reported that dietary levels of folate and methionine, as well as their interaction with alcohol intake, affected the absorption and availability of dietary methyl donors and, consequently, the development of colorectal cancer. Interestingly, a relatively common polymorphism (C677T) within the MTHFR gene is protective in most of the human population. However, population with this kind of polymorphism may be at increased risk for colorectal cancer when the intake of methyl donors from the diet is low (Kim 2007).

Polymorphism of the genes for AA transporters may affect AA absorption and utilization, and, thus, health, growth and development (Wang et al. 2008d). This is particularly important for the small intestine and kidneys, because they absorb AA from the intestinal lumen and renal tubules into the circulation, respectively, thereby regulating AA homeostasis in plasma. The expression profile of AA transporters was analyzed in the murine duodenum, jejunum, ileum, and colon using high-density microarrays (Anderle et al. 2005). The findings indicated

differences in the absorption of carrier-mediated nutrients (including AA) among the different intestinal segments. The most pronounced differences were detected between the adjoining segments of the ileum and colon, reflecting much lower rates of nutrient transport in the colon than in the small intestine. Additionally, the gene profile analysis revealed different expression levels for intestinal AA transporters between animals and humans (Kim et al. 2007a). For example, mice exhibited abundant mRNA levels for peptide transporter HPT1, AA transporters CSNU3, CT1, and ASC1, whereas rats had high expression levels for peptide transporter PEPT1, AA transporters CSNU1, and 4F2HC, in the small intestine (Anderle et al. 2005). In contrast, the highly expressed genes in the human small intestine were peptide transporter HPT1, AA LAT3, 4F2HC, and PROT (Anderle et al. 2005; Kim et al. 2007a). These data are consistent with the known difference in dietary AA intakes per kg body weight among various species (Wu et al. 2007b). Finally, genetic studies established that defects in AA absorption by epithelial cells of the small intestine result in inherited disorders, such as cystinuria, lysinuric protein intolerance, hartnup disorder, iminoglycinuria, dicarboxylic aminoaciduria, hyperammonemia, and hypertension (Bröer 2008). This critical knowledge has led to successful development of effective therapeutic treatments for the affected individuals.

AA nutrition and epigenetics

DNA methylation is an important epigenetic determinant in gene expression, the maintenance of DNA integrity and stability, chromosomal modifications, and the development of mutations (Kim 2005). Early life nutrition has the potential to change chromatin structure, alter gene expression, and affect health throughout the life cycle (Wu et al. 2006). Emerging evidence suggests that alterations in epigenetic marking of the genome may be a key mechanism by which nutritional exposure in utero can influence gene expression, and therefore, phenotype (Wang et al. 2008a; Wu et al. 2006). In addition, maternal nutrition can affect the phenotype of offspring by influencing the degree of CpG methylation (Wu et al. 2004a).

The methyl groups that participate in DNA methylation reactions are ultimately derived from methionine. Since levels of methionine in plasma are influenced by diet, epidemiological data suggest that changes in dietary contents of methyl donors could alter DNA methylation, as well as specific and global gene expression. A recent study with ewes reveals that restricting the dietary provision of methionine and folate during the periconceptional period can lead to widespread epigenetic alterations to DNA methylation in offspring and modify adult health-related phenotypes (Sinclair et al. 2007). In addition, a folate- and methyl-deficient diet can alter the expression of DNA methyltransferases and methyl CpG binding proteins involved in epigenetic gene silencing in livers of F344 rats (Ghoshal et al. 2006). Dietary interventions with AA and other nutrients can affect S-adenosylmethionine availability (Grillo and Colombatto 2007), which can facilitate many crucial methylation reactions in pregnant mice to modulate the epigenetic DNA methylation of specific genes (Waterland 2006). These very complex effects suggest that adequate intake of methionine from the diet can maintain optimal DNA methylation in cells. Similarly, methionine supplementation can induce hypermethylation of DNA in specific genomic regions. When methionine content is low in the diet, it is important that dietary supplementation with this AA can support therapeutic maintenance of DNA methylation without causing excessive or potentially adverse locus-specific hypermethylation (Waterland 2006). For example, feeding pregnant black a/a mice a diet supplemented with methyl group donors may alter epigenetic regulation of agouti expression in their offspring, as indicated by increased agouti/black mottling in the direction of the pseudoagouti phenotype (Waterland 2006). Of particular interest, epigenetic phenotypes are also maternally heritable (Wolff et al. 1998). Furthermore, the inherent change of the epigenomic marks that are established early in life through behavioral programming is potentially reversible in the adult in response to dietary supplementation with methyl group donors (Weaver et al. 2005).

AA nutrition and transcriptomics

Transcriptomics is a powerful tool in discovery research regarding the effect of AA on global gene expression. For example, Fu et al. (2005) reported that dietary arginine supplementation selectively reduced white-fat mass in Zucker-diabetic fatty rats (Fu et al. 2005). Similarly, supplementing arginine to the diet for growing-finishing pigs decreased body white-fat, while increasing muscle protein gain (Tan et al. 2008). At the molecular level, microarray analysis indicated that the arginine treatment increased expression of AMP-activated protein kinase, nitric oxide synthase-1, and peroxisome proliferator-activated receptor γ coactivator-1alpha (a master regulator of mitochondrial biogenesis) by 123, 145, and 500%, respectively, in the adipose tissue of Zucker-diabetic fatty rats (Fu et al. 2005). In addition, AMP-activated protein kinase triggers the oxidation of fatty acids and glucose in skeletal muscle, heart, liver, and adipose tissue, whereas cGMP, whose production from GTP is increased by nitric oxide, stimulates the mitochondrial oxidation of acetyl-CoA in cells (Jobgen et al. 2006). All of these changes are expected to promote the mitochondrial oxidation of energy substrates,

thereby decreasing the availability of acetyl-CoA for fatty acid synthesis.

Glutamine is the most abundant free AA in the body and plays a regulatory role in physiology and nutrition at gene and protein levels (Curi et al. 2005). The underlying mechanisms involve changes at metabolism (e.g., oxidative fuel, gluconeogenic precursor and lipogenic precursor), cell integrity (survival, cell proliferation), protein synthesis and degradation, redox potential, respiratory burst, insulin resistance, insulin secretion, and extracellular matrix synthesis (Curi et al. 2005). Dietary supplementation with glutamine has been shown to regulate expression of many genes related to metabolism, signal transduction, cell defense, and repair and to activate intracellular signaling pathways (Wang et al. 2008a). Particularly, the glutamine treatment increased intestinal expression of genes that are necessary for cell growth and removal of oxidants, while reducing expression of genes that promote oxidative stress and immune activation (Wang et al. 2008a). Functionally, glutamine supplementation enhanced intestinal oxidative-defense capacity, prevented jejunal atrophy, and promoted small intestine growth and body weight gain in weaned piglets (Wang et al. 2008a; Wu et al. 1996b).

High-throughput technologies, including deep sequencing and splicing-sensitive microarrays, have provided much evidence that alternative splicing is an important process for gene regulation (Ben-Dov et al. 2008). For example, Drosophila sex determination (McIntyre et al. 2006) and mammalian synaptic transmission (Ule et al. 2005) have been reported to be associated with alternative splicing process. Seventy-five percent of alternative splicing events have been related to subtle AA substitutions, removal of protein motif, or protein truncations of coding regions (Blencowe 2006; Matlin et al. 2005).

AA nutrition and proteomics

Comparative functional proteomics enables the characterization of differentially expressed proteins in intestinal cells under apoptotic conditions (Lenaerts et al. 2006). For example, addition of glutamine to culture medium affected expression of 28 proteins in the human epithelial intestinal cell line HCT-8 (Deniel et al. 2007). Interestingly, 34, 17, and 13% of them are involved in cell cycle and apoptosis, in signal transduction, and in cytoskeleton organization, respectively. Furthermore, glutamine has been reported to regulate expression of several proteins (e.g., p47phox, p22phox, gp91phox, alpha-actin and fibronectin, ASK1, c-myc, c-jun and p70s6 k) in animal cells (Curi et al. 2005).

Methionine restriction can inhibit the growth of cancer cells, which cannot grow in a methionine-depleted medium supplemented with homocysteine, a toxic metabolite of methionine (Perta-Kajan et al. 2007). Comparison of the

2-DE patterns of SGC7901 cells cultured in M + H- or M-H + medium revealed ten differentially expressed proteins in gastric cancer cells SGC7901 (Xin et al. 2007). These proteomics data are invaluable for defining the molecular mechanisms by which methionine restriction can induce cell cycle arrest and apoptosis in human gastric cancer (Xin et al. 2007).

L-Arginine has a beneficial role in angiogenesis and the cardiovascular system (Montanez et al. 2008). Using the proteomics technology, we found that adding a physiological level of arginine (0.1 mM) to an arginine-deficient medium increased expression of structural proteins (vimentin and tropomyosin) and mitochondrial cytochrome bc_1 complex iii- chain A (a component of the electron transport chain) but decreased expression of stress-related proteins (PDZ domain containing-3) in coronary vascular endothelial cells (Lei et al. 2008). The analysis of real-time PCR and western blotting confirmed elevated mRNA and protein levels for vimentin in arginine-treated cells. These novel findings aid in elucidating the mechanisms responsible for the beneficial effect of physiological levels of L-arginine on the circulatory system. Additionally, proteomic analysis revealed that arginine deprivation in Caco-2 cells decreased cell proliferation and heat-shock-protein expression, while enhancing the susceptibility of cells to apoptosis (Lenaerts et al. 2007).

AA nutrition and metabolomics

Metabolomics has emerged as a biomarker discovery tool for metabolic profiling in nutritional research. The metabolome in body fluids or tissues may provide a more comprehensive view of cellular metabolic regulation and an opportunity of identifying surrogate markers for nutritional or disease status. For example, an NMR-based metabolomic study was conducted to investigate different biochemical effects of a short-term high intake of milk or meat protein on children (Bertram et al. 2007). The results indicated that the milk diet increased the urinary excretion of hippurate, while the meat diet increased the urinary excretion of creatine, histidine, and urea. This reflects a higher amount of creatine and histidine in meat than in milk, as well as the difference in AA composition between milk and meat proteins. Additionally, the NMR analysis indicated that the milk diet changed the lipid profile of serum, but the meat diet had no effect (Bertram et al. 2007).

Some metabolomics studies have also evaluated the effect of proteins of animal versus plant origin on metabolic profiles in humans. Higher urinary levels in creatine, creatinine, carnitine, acetylcarnitine, taurine, trimethylamine-N-oxide, and glutamine are the metabolic signatures of the high-meat diet (Rezzi et al. 2007). In contrast, higher urinary excretion of p-hydroxyphenylacetate and a lower

Table 1 Roles of the omics technologies as discovery tools in amino acid nutrition research

Composition of proteins, peptides, AA, and nitrogenous metabolites in the diet, tissue fluid, and whole body

Digestion of dietary protein and transport of AA from intestinal lumen into portal vein

Polymorphisms of AA synthesis and catabolism in organisms

Regulation of gene transcription, mRNA stability, mRNA translation, and post-translational protein modifications by AA

Modulation of signal transduction, nutrient metabolism, oxidative stress, immune function, reproduction, and health by AA

Cell- and species differences in AA metabolism

Individualized requirements of AA by humans and animals

Biomarkers for optimal needs of nutrients, as well as development and diagnosis of disease

level of N, N, N-trimethyllysine are associated with the vegetarian diet (Rezzi et al. 2007). Thus, meat intake may promote higher production of ammonia, but consumption of vegetable protein may reduce intestinal microbial activity (Yin et al. 2008).

Using ^{1}H-NMR spectroscopy, He et al. (2008) determined the effect of dietary arginine supplementation on the serum metabolome in growing pigs. Principal component analysis indicated 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 AA in the whole body, enhances protein synthesis in skeletal muscle, and modulates intestinal microbial nitrogen metabolism in growing pigs.

The developmental change of metabolites in biological fluid may provide important clues about their nutritional significance (Kong et al. 2008; Li et al. 2008). For example, AA profiling has revealed that arginine (an essential AA for neonates) is remarkably deficient in sow's milk (Wu and Knabe 1994) and present in reduced levels in plasma of 7- to 21-day-old piglets compared with newborn piglets (Wu et al. 2004b). In contrast, arginine is highly abundant in porcine allantoic fluid during early gestation (Wu et al. 1996a). Based on these findings, a strategy of arginine supplementation was developed to enhance the growth of milk-fed young piglets (Kim and Wu 2004, 2008; Wu et al. 2004b) and improve the reproductive performance of gilts (Mateo et al. 2007, 2008; Wu et al. 2008) and rats (Zeng et al. 2008).

Thus, analysis of AA and their metabolites as a metabolomic subset, together with the correlation-based analyses, can help identify pathways responsible for the effects of excessive intakes of AA from the diet (Noguchi et al. 2003). In contrast to the conventional single

metabolite studies (Dekaney et al. 2008; Ou et al. 2007), comprehensive analysis of biomarkers can offer an unbiased strategy for global assessment of AA metabolism and requirement by humans and animals (Ptolemy et al. 2007). In light of such findings, optimal dietary recommendations for AA can be devised for individual humans (personalized nutrition) and animals (targeted feeding) in response to changes in physiological and pathological conditions.

Conclusion and perspectives

To date, the study of biochemical mechanisms that regulate dietary AA metabolism and requirements has been largely based on a black-box approach. Importantly, the recent developments of omics techniques are transforming AA nutrition research in the post-genome era. These powerful discovery tools allow for studies of the function of AA and enable in-depth investigation of the regulatory roles for dietary AA in gene and protein expression (Table 1). Thus, omics and other advanced methodologies potentially play an important role in refining optimal individual requirements of AA for health and production benefits, but they are currently not yet widely applied to nutrition research because of relatively high costs and a lack of the necessary knowledge. There are a very large number of proteins and metabolites, as well as their exceedingly dynamic ranges, diverse physicochemical properties, and complex interactions in cells (Liu et al. 2008; Wang et al. 2008e; Zu et al. 2007). Therefore, integrating various omics technologies and bioinformatics with the traditional techniques will provide comprehensive information about AA metabolism and nutrition in organisms. Such a revolutionary approach is expected to solve major nutrition-associated problems in humans and animals (including obesity, diabetes, cardiovascular disease, cancer, aging, and intrauterine growth retardation).

References

Anderle P, Sengstag T, Mutch DM, Rumbo M, Praz V, Mansourian R, Delorenzi M, Williamson G, Roberts MA (2005) Changes in the transcriptional profile of transporters in the intestine along the anterior–posterior and crypt-villus axes. BMC Genomics 6:69

Aravin AA, Sachidanandam R, Girard A, Fejes-Toth K, Hannon GJ (2007) Developmentally regulated piRNA clusters implicate MILI in transposon control. Science 316:744–747

Ben-Dov C, Hartmann B, Lundgren J, Valcárcel J (2008) Genome-wide analysis of alternative pre-mRNA splicing. J Biol Chem 283:1229–1233

Bentwich I, Avniel A, Karov Y, Aharonov R, Gilad S, Barad O, Barzilai A, Einat P, Einav U, Meiri E, Sharon E, Spector Y, Bentwich Z (2005) Identification of hundreds of conserved and nonconserved human microRNAs. Nat Genet 37:766–770

Bertram HC, Hoppe C, Petersen BO, Duus JO, Molgaard C, Michaelsen KF (2007) An NMR-based metabonomic investigation on effects of milk and meat protein diets given to 8-year-old boys. Br J Nutr 97:758–763

Blencowe BJ (2006) Alternative splicing: new insights from global analyses. Cell 126:37–47

Bröer S (2008) Amino acid transport across mammalian intestinal and renal epithelia. Physiol Rev 88:249–286

Chen X, Sun LW, Yu YB, Xue Y, Yang PY (2007) Amino acid-coded tagging approaches in quantitative proteomics. Expert Rev Proteomics 4:25–37

Cullen BR (2006) Viruses and microRNAs. Nat Genet 38:S25–S30

Curi R, Lagranha CJ, Doi SQ, Sellitti DF, Procopio J, Pithon-Curi TC, Corless M, Newsholme P (2005) Molecular mechanisms of glutamine action. J Cell Physiol 204:392–401

Dekaney CM, Wu G, Yin YL, Jaeger LA (2008) Regulation of ornithine aminotransferase gene expression and activity by all-trans retinoic acid in Caco-2 intestinal epithelial cells. J Nutr Biochem 19:674–681

Deniel N, Marion-Letellier R, Charlionet R, Tron F, Leprince J, Vaudry H, Ducrotté P, Déchelotte P, Thébault S (2007) Glutamine regulates the human epithelial intestinal HCT-8 cell proteome under apoptotic conditions. Mol Cell Proteomics 6:1671–1679

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 1H 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

Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC (1998) Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 391:806–811

Forde CE, McCutchen-Maloney SL (2002) Characterization of transcription factors by mass spectrometry and the role of SELDI-MS. Mass Spectrom Rev 21:419–439

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

Fu WJ, Stromberg AJ, Viele K, Carroll RJ, Wu G (2008) Statistics and bioinformatics in nutritional sciences: analysis of complex data in the era of systems biology. J Nutr Biochem (in press)

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

Ghoshal K, Li X, Datta J, Bai S, Pogribny I, Pogribny M, Huang Y, Young D, Jacob ST (2006) A folate- and methyl-deficient diet alters the expression of DNA methyltransferases and methyl CpG binding proteins involved in epigenetic gene silencing in livers of F344 rats. J Nutr 136:1522–1527

Giraldez AJ, Mishima Y, Rihel J, Grocock RJ, Van Dongen S, Inoue K, Enright AJ, Schier AF (2006) Zebrafish MiR-430 promotes deadenylation and clearance of maternal mRNAs. Science 312:75–79

Gorringe KL, Jacobs S, Thompson ER, Sridhar A, Qiu W, Choong DY, Campbell IG (2007) High-resolution single nucleotide polymorphism array analysis of epithelial ovarian cancer reveals numerous microdeletions and amplifications. Clin Cancer Res 13:4731–4739

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

He QH, Kong XF, Wu G, Ren PP, Tang HR, Hao FH, Huang RL, Li TJ, Tan BE, Li P, Tang ZR, Yin YL, Wu YN (2008) Metabolomic analysis of the response of growing pigs to dietary L-arginine supplementation. Amino Acids. doi:10.1007/s00726-008-0192-9

Hu CA, Khalil S, Zhaorigetu S, Liu Z, Tyler M, Wan G, Valle D (2008a) Human Δ^1-pyrroline-5-carboxylate synthase: function and regulation. Amino Acids 35:665–672

Hu CA, Williams DB, Zhaorigetu S, Khalil S, Wan GH, Valle D (2008b) Functional genomics and SNP analysis of human genes encoding proline metabolic enzymes. Amino Acids 35:655–664

Hu-Lieskovan S, Heidel JD, Bartlett DW, Davis ME, Triche TJ (2005) Sequence-specific knockdown of EWS-FLI1 by targeted, nonviral delivery of small interfering RNA inhibits tumor growth in a murine model of metastatic Ewing's sarcoma. Cancer Res 65:8984–8992

Ideker T, Galitski T, Hood L (2001) A new approach to decoding life: systems biology. Annu Rev Genomics Hum Genet 2:343–372

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

Jorgensen RA, Cluster PD, English J, Que Q, Napoli CA (1996) Chalcone synthase cosuppression phenotypes in petunia flowers: comparison of sense vs. antisense constructs and single-copy vs. complex T-DNA sequences. Plant Mol Biol 31:957–973

Jurinke C, Denissenko MF, Oeth P, Ehrich M, van den Boom D, Cantor CR (2005) A single nucleotide polymorphism based approach for the identification and characterization of gene expression modulation using MassARRAY. Mutat Res 573:83–95

Kim YI (2005) Nutritional epigenetics: impact of folate deficiency on DNA methylation and colon cancer susceptibility. J Nutr 135:2703–2709

Kim DH (2007) The interactive effect of methyl-group diets and polymorphism of methylenetetrahydrofolate reductase on the risk of colorectal cancer. Mutat Res 622:14–18

Kim D, Rossi J (2008) RNAi mechanisms and applications. Biotechniques 44:613–616

Kim SW, Wu G (2004) Dietary arginine supplementation enhances the growth of milk-fed young piglets. 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

Kim HR, Park SW, Cho HJ, Chae KA, Sung JM, Kim JS, Landowski CP, Sun D, Abd El-Aty AM, Amidon GL, Shin HC (2007a) Comparative gene expression profiles of intestinal transporters in mice, rats and humans. Pharmacol Res 56:224–236

Kim E, Magen A, Ast G (2007b) Different levels of alternative splicing among eukaryotes. Nucleic Acids Res 35:125–131

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

Lee RC, Ambros V (2001) An extensive class of small RNAs in *Caenorhabditis elegans*. Science 294:862–864

Lee RC, Feinbaum RL, Ambros V (1993) The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75:843–854

Lehner B, Fraser AG, Sanderson CM (2004) Technique review: how to use RNA interference. Brief Funct Genomic Proteomic 3:68–83

Lei XQ, Wu G, Meininger CJ, Wang FL, Wang JJ (2008) Proteomics analysis reveals enhanced expression of structural proteins in microvascular endothelial cells treated with L-arginine. Amino Acids (in press)

Lenaerts K, Mariman E, Bouwman F, Renes J (2006) Glutamine regulates the expression of proteins with a potential health-promoting effect in human intestinal Caco-2 cells. Proteomics 6:2454–2464

Lenaerts K, Renes J, Bouwman FG, Noben JP, Robben J, Smit E, Mariman EC (2007) Arginine deficiency in preconfluent intestinal Caco-2 cells modulates expression of proteins involved in proliferation, apoptosis, and heat shock response. Proteomics 7:565–577

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

Lewis BP, Burge CB, Bartel DP (2005) Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 120:15–20

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

Li P, Kim SW, Li XL, Datta S, Pond WG, Wu G (2008) Dietary supplementation with cholesterol and docosahexaenoic acid affects concentrations of amino acids in tissues of young pigs. Amino Acids. doi:10.1007/s00726-008-0196-5

Liao XH, Majithia A, Huang XL, Kimmel AR (2008) Growth control via TOR kinase signaling, an intracellular sensor of amino acids and energy availability, with crosstalk potential to proline metabolism. Amino Acids 35:761–770

Liu ZP, Wu LY, Wang Y, Zhang XS, Chen LN (2008) Bridging protein local structures and protein functions. Amino Acids 35:627–650

Mateo RD, Wu G, Bazer FW, Park JC, Shinzato I, Kim SW (2007) Dietary L-arginine supplementation enhances the reproductive performance of gilts. J Nutr 137:652–656

Mateo RD, Wu G, Moon HK, Carroll JA, Kim SW (2008) Effects of dietary arginine supplementation during gestation and lactation on the performance of lactating primiparous sows and nursing piglets. J Anim Sci 86:827–835

Matlin AJ, Clark F, Smith CW (2005) Understanding alternative splicing: towards a cellular code. Nat Rev Mol Cell Biol 6:386–398

McIntyre LM, Bono LM, Genissel A, Westerman R, Junk D, Telonis-Scott M, Harshman L, Wayne ML, Kopp A, Nuzhdin SV (2006) Sex-specific expression of alternative transcripts in Drosophila. Genome Biol 7:R79

Miyagi M, Rao KC (2007) Proteolytic O-18-labeling strategies for quantitative proteomics. Mass Spectrom Rev 26:121–136

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

Myers JW, Jones JT, Meyer T, Ferrell JE Jr (2003) Recombinant Dicer efficiently converts large dsRNAs into siRNAs suitable for gene silencing. Nat Biotechnol 21:324–328

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

Ogawa S, Nanya Y, Yamamoto G (2007) Genome-wide copy number analysis on GeneChip platform using copy number analyzer for affymetrix GeneChip 2.0 software. Methods Mol Biol 396:185–206

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

Perta-Kajan J, Twardowski T, Jakubowski H (2007) Mechanisms of homocysteine toxicity in humans. Amino Acids 32:561–572

Ptolemy AS, Lee R, Britz-McKibbin P (2007) Strategies for comprehensive analysis of amino acid biomarkers of oxidative stress. Amino Acids 33:3–18

Reinhart BJ, Bartel DP (2002) Small RNAs correspond to centromere heterochromatic repeats. Science 297:1831

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

Sanders MA, Verhaak RG, Geertsma-Kleinekoort WM, Abbas S, Horsman S, van der Spek PJ, Löwenberg B, Valk PJ (2008) SNPExpress: integrated visualization of genome-wide genotypes, copy numbers and gene expression levels. BMC Genomics 9:41

Sinclair KD, Allegrucci C, Singh R, Gardner DS, Sebastian S, Bispham J, Thurston A, Huntley JF, Rees WD, Maloney CA, Lea RG, Craigon J, McEvoy TG, Young LE (2007) DNA methylation, insulin resistance, and blood pressure in offspring determined by maternal periconceptional B vitamin and methionine status. Proc Natl Acad Sci 104:19351–19356

Stoll B, Burrin DG (2006) Measuring splanchnic amino acid metabolism in vivo using stable isotopic tracers. J Anim Sci 84(E Suppl):E60–E72

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, Yin Y, Liu Z, Li X, Xu H, Kong X, Huang R, Tang W, Shinzato I, Smith SB, Wu G (2008) 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

Ule J, Ule A, Spencer J, Williams A, Hu JS, Cline M, Wang H, Clark T, Fraser C, Ruggiu M, Zeeberg BR, Kane D, Weinstein JN, Blume J, Darnell RB (2005) Nova regulates brain-specific splicing to shape the synapse. Nat Genet 37:844–852

Vercauteren FGG, Arckens L, Quirion R (2007) Applications and current challenges of proteomic approaches, focusing on two-dimensional electrophoresis. Amino Acids 33:405–414

Wang J, Li D, Dangott LJ, Wu G (2006) Proteomics and its role in nutrition research. J Nutr 136:1759–1762

Wang J, Chen L, Li P, Li X, Zhou H, Wang F, Li D, Yin Y, Wu G (2008a) Gene expression is altered in piglet small intestine by weaning and dietary glutamine supplementation. J Nutr 138:1025–1032

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

Wang JJ, Chen LX, Li DF, Yin YL, Wang XQ, Li P, Dangott LJ, Hu WX, Wu G (2008c) Intrauterine growth restriction affects the proteomes of the small intestine, liver and skeletal muscle in newborn pigs. J Nutr 138:60–66

Wang W, Shi CY, Zhang JS, Gu W, Li TJ, Gen MM, Chu WY, Huang RL, Liu YL, Hou YQ, Li P, Yin YL (2008d) Molecular cloning, distribution and ontogenetic expression of the oligopeptide transporter PepT1 mRNA in Tibetan suckling piglets. Amino Acids. doi:10.1007/s00726-008-0178-7

Wang Y, Xue Z, Shen G, Xu J (2008e) PRINTR: prediction of RNA binding sites in proteins using SVM and profiles. Amino Acids 35:295–302

Waterland RA (2006) Assessing the effects of high methionine intake on DNA methylation. J Nutr 136:1706S–1710S

Weaver IC, Champagne FA, Brown SE, Dymov S, Sharma S, Meaney MJ, Szyf M (2005) Reversal of maternal programming of stress responses in adult offspring through methyl supplementation: altering epigenetic marking later in life. J Neurosci 25:11045–11054

Wolff GL, Kodell RL, Moore SR, Cooney CA (1998) Maternal epigenetics and methyl supplements affect agouti gene expression in Avy/a mice. FASEB J 12:949–957

Wu G, Knabe DA (1994) Free and protein-bound amino acids in sow's colostrum and milk. J Nutr 124:415–424

Wu G, Bazer FW, Tuo W, Flynn SP (1996a) Unusual abundance of arginine and ornithine in porcine allantoic fluid. Biol Reprod 54:1261–1265

Wu G, Meier SA, Knabe DA (1996b) Dietary glutamine supplementation prevents jejunal atrophy in weaned pigs. J Nutr 126:2578–2584

Wu G, Bazer FW, Cudd TA, Meininger CJ, Spencer TE (2004a) Maternal nutrition and fetal development. J Nutr 134:2169–2172

Wu G, Bazer FW, Wallace JM, Spencer TE (2006) Intrauterine growth retardation: implications for the animal sciences. J Anim Sci 84:2316–2337

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

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

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

Wu G, Knabe DA, Kim SW (2004b) Arginine nutrition in neonatal pigs. J Nutr 134:2783S–2390S

Xin L, Cao WX, Fei XF, Wang Y, Liu WT, Liu BY, Zhu ZG (2007) Applying proteomic methodologies to analyze the effect of methionine restriction on proliferation of human gastric cancer SGC7901 cells. Clin Chim Acta 377:206–212

Yan GR, He QY (2008) Functional proteomics to identify critical proteins in signal transduction pathways. Amino Acids 35:267–274

Yin FG, Liu YL, Yin YL, Kong XF, Huang RL, Li TJ, Wu GY, Hou YQ (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

Zamore PD, Tuschl T, Sharp PA, Bartel DP (2000) RNAi: double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. Cell 101:25–33

Zeng X, Wang F, Fan X, Yang W, Zhou B, Li P, Yin Y, Wu G, Wang J (2008) Dietary arginine supplementation during early pregnancy enhances embryonic survival in rats. J Nutr 138:1421–1425

Zhan Z, Ou D, Piao X, Kim SW, Liu Y, Wang J (2008) Dietary arginine supplementation affects microvascular development in the small intestine of early-weaned pigs. J Nutr 138:1304–1309

Zu XL, Besant PG, Imhof A, Attwood PV (2007) Mass spectrometric analysis of protein-histidine phosphorylation. Amino Acids 32:347–357