Postprint: A New Hypothesis on the Thrifty Mechanism of Porcine Amino Acid Metabolism

Authors: Sun Zhihong, Li Mao, Xu Qingqing, Yin Yulong, Zhu Weiyun, Jiang Qingyan, Feiruo Huang

Date: 2017-10-11T00:00:00+00:00

Abstract

Urinary nitrogen emissions from pigs constitute 60%~70% of total nitrogen emissions, and urea is the principal nitrogenous constituent in urine, with its synthesis rate largely determining the magnitude of both urinary nitrogen and total nitrogen excretion. Consequently, reducing the rate of hepatic urea synthesis in pigs represents the fundamental strategy for mitigating nitrogen emissions. This paper first reviews the nutritional modulation techniques currently employed for nitrogen reduction in swine, then respectively examines the direct precursor (ammonia) and indirect precursors (such as glycine and alanine) of hepatic urea synthesis, along with the amino acid metabolic fuel substitution mechanism. Building upon these discussions, a novel hypothesis concerning a thrifty mechanism of amino acid metabolism in pigs is proposed, which entails enhancing the energy provision efficiency of pyruvate/glucose and related metabolites to decrease the catabolic rate of amino acids like glutamate, thereby accomplishing the objective of reducing the net portal flux of urea precursors, hepatic urea synthesis, and urinary nitrogen emissions.

Full Text

A New Hypothesis for the Mechanism of Metabolic Saving of Amino Acids in Pigs

SUN Zhihong[1]*, LI Mao[1], XU Qingqing[1], YIN Yulong[2], ZHU Weiyun[3], JIANG Qingyan[4], HUANG Feiru[5]

[1]Laboratory for Bio-Feed and Animal Nutrition, Southwest University, Chongqing 400715, China
[2]Institute of Subtropical Agriculture, the Chinese Academy of Sciences, Changsha 410125, China
[3]College of Animal Science and Technology, Nanjing Agricultural University,

Nanjing 210095, China
[4]College of Animal Science and Technology, South China Agricultural University, Guangzhou 510642, China
[5]College of Animal Science and Technology, Huazhong Agricultural University, Wuhan 430070, China

Abstract

Urinary nitrogen excretion accounts for 60% to 70% of total nitrogen excretion in pigs, with urea being the primary nitrogenous compound in urine. The rate of urea synthesis largely determines urinary and total nitrogen emissions, making the reduction of hepatic urea synthesis rate a fundamental strategy for decreasing nitrogen excretion. This paper first reviews current nutritional regulation techniques for nitrogen reduction in swine, then discusses both direct (ammonia) and indirect (such as glycine and alanine) precursors of hepatic urea synthesis, along with the mechanism of amino acid metabolic fuel substitution. Based on these considerations, we propose a novel hypothesis for the regulatory mechanism of amino acid metabolic saving: promoting the energy supply efficiency of substances like pyruvate and glucose to reduce the metabolic rate of amino acids such as glutamate, thereby decreasing the net flux of urea precursors in the portal vein, hepatic urea synthesis, and ultimately urinary nitrogen excretion.

Keywords: pigs; nitrogen excretion; amino acids; metabolic saving; pyruvate dehydrogenase

---

Environmental pollution caused by nitrogen emissions has become increasingly severe with the continuous expansion and intensification of livestock production. Current global estimates place livestock nitrogen emissions at 89–164 million tons annually. In China alone, livestock nitrogen emissions reach approximately 30 million tons, with monogastric animals (primarily pigs) contributing about 60% of this total. Concurrently, protein resource scarcity represents a worldwide challenge; in 2014, China imported approximately 40 million tons of protein feed ingredients, with import dependence for fish meal and soybeans reaching 70%. Therefore, improving protein utilization efficiency and reducing nitrogen emissions has become an urgent scientific challenge for China's livestock industry, particularly in pig production.

1. Common Nutritional Regulation Techniques for Nitrogen Reduction in Pigs

Extensive research has been conducted on nitrogen reduction in pigs, encompassing diet formulation based on ideal amino acid patterns [1], reduction of dietary protein levels with supplementation of limiting amino acids [2–7], increased proportions of fermentable carbohydrates [2,8–9], and addition of enzymes, probiotics, and organic acids [10–11]. Although numerous studies have

confirmed that low-protein diets significantly reduce nitrogen excretion [2,6,12-13], this nutritional strategy has not become a standard practice in commercial pig production, particularly in intensive systems focused on rapid growth. Other nutritional regulation techniques can only reduce nitrogen emissions to a limited extent. Consequently, it is necessary to investigate the mechanisms of nitrogen excretion to identify key regulatory targets. Urinary nitrogen accounts for 60%-70% of total nitrogen excretion [9,14-15], and since urea is the main nitrogenous component in urine, its synthesis rate largely determines urinary and total nitrogen emissions. Thus, reducing the hepatic urea synthesis rate represents an important strategy for nitrogen reduction, and identifying the types and sources of urea precursors is the primary prerequisite for such research.

2.1. Ammonia—The Direct Precursor of Urea

Ammonia serves as the direct precursor for urea synthesis and originates primarily from amino acid catabolism. The portal-drained viscera (PDV) constitute a crucial site for amino acid metabolism, where 97% of dietary glutamate and aspartate, 70% of glutamine, 40%-50% of serine and glycine, 40% of arginine and proline, 20%-40% of branched-chain amino acids, and 30%-60% of other essential amino acids are catabolized [4,16-20]. Following deamination, amino acids are converted into acetyl-CoA, pyruvate, oxaloacetate, succinyl-CoA, fumarate, and α-ketoglutarate, which enter the tricarboxylic acid (TCA) cycle for oxidative energy production [21] (as shown in [Figure 1: see original paper]). This extensive amino acid metabolism in the PDV results in portal venous ammonia concentrations significantly higher than those in other body regions, with most blood ammonia being utilized for urea synthesis upon reaching the liver [22].

Figure 1. The oxidative metabolism pathways of amino acids [21]

2.2. Glycine and Alanine—Indirect Precursors of Urea

Previous studies have shown that in piglets fed diets containing 20%, 17%, and 14% crude protein, the net portal absorption rates of glutamate were -4.43, -5.65, and -6.64 mg/min, respectively, while net portal ammonia absorption rates were 2.86, 2.68, and 2.38 mg/min [23]. These results are consistent with other reports demonstrating extensive metabolism of glutamate and other amino acids in the porcine PDV, accompanied by substantial ammonia production [4,17,20]. Additionally, the net portal absorption of glycine and alanine accounted for 38.2%, 37.3%, and 37.0% of total amino acid net absorption in piglets fed the three protein levels, while hepatic consumption of glycine and alanine represented 52.0%, 49.5%, and 43.8% of total amino acid metabolism. This pattern of amino acid metabolism prompted deeper investigation into the origins and metabolic fates of glycine and alanine.

Traditional views hold that serine is the primary precursor of glycine, but Wu [21] proposed that only about 10% of glycine originates from serine. Alanine precursors include pyruvate, serine, and aspartate [24]. Based on amino acid

metabolic transformation pathways [21,24] (as shown in [Figure 2: see original paper]), we hypothesized that extensively metabolized amino acids in the PDV (such as glutamate, glutamine, and aspartate) are likely important precursors for glycine and alanine. Using catheterization and 15N stable isotope tracing techniques, we found that approximately 30% of glutamate metabolized in the PDV is converted to glycine and alanine. This metabolic pattern essentially reflects an important self-protective mechanism: if all ammonia produced from amino acid metabolism in the PDV entered the liver directly, it would create excessively high ammonia concentrations and potentially cause liver damage. Converting a portion of this ammonia into smaller molecules like glycine and alanine (with molecular masses of 75 and 89 u, respectively, far below the average amino acid molecular mass) effectively reduces ammonia concentration and liver burden while maintaining the metabolic fuel function of amino acids such as glutamate in the PDV.

Berthiaume et al. [25] and Doepel et al. [26] reported that the liver metabolizes substantial amounts of glycine and alanine, with glycine being an important ammonia-producing amino acid [27]. Alanine increases urea synthesis in hepatocytes from starved rats [28] and participates importantly in glycine metabolism [29]. These studies demonstrate that glycine and alanine are closely related to hepatic urea synthesis [27–29], though no reports have confirmed them as important nitrogen sources for urea synthesis. Based on these findings, we hypothesized that excess glycine and alanine in the liver are utilized for urea synthesis. Using catheterization and 15N stable isotope tracing, we confirmed that glycine and alanine serve as important indirect precursors for urea [30].

Figure 2. The pathways of metabolic transformation between amino acids [21,24]

3. The Mechanism of Metabolic Fuel Function Substitution of Amino Acids

In summary, reducing the generation of urea precursors (primarily ammonia, glycine, and alanine) in the PDV is crucial for decreasing urea synthesis and urinary nitrogen excretion. Providing alternative metabolic fuels to reduce amino acid oxidation rates represents an important strategy for achieving this goal. Exploration of amino acid metabolic fuel substitutes began in the 1990s, but limited research has prevented breakthrough progress. Besides glutamate/glutamine, glucose serves as an important fuel for various tissues; however, glucose typically cannot suppress glutamate/glutamine oxidation [17], while glutamate/glutamine can significantly reduce glucose oxidation rates [31–33]. Therefore, improving the oxidative energy supply efficiency of glucose in the PDV has become an urgent scientific challenge for nitrogen reduction research in pigs.

Although amino acids, fats, and glucose have different oxidation pathways, they all converge at the TCA cycle [34] (as shown in [Figure 3: see original

paper]). Acetyl-CoA, pyruvate, oxaloacetate, succinyl-CoA, fumarate, and α-ketoglutarate are the intermediate products through which amino acids enter the TCA cycle [21]. Pyruvate plays a pivotal role in connecting the metabolism of these three major nutrient classes, and abnormal pyruvate metabolism can lead to numerous diseases including diabetes, obesity [35], mitochondrial dysfunction [36], heart failure [37], neurodegenerative diseases [38], and cancer [39]. Studies have shown that pyruvate is an important regulator of amino acid oxidation [40–42]. Given its crucial role in the metabolism of the three major nutrients, we hypothesize that pyruvate may be a common regulatory target for amino acid and glucose metabolism, and that promoting pyruvate oxidation in the PDV could increase glucose oxidation rates, inhibit the metabolic fuel function of amino acids, and thereby reduce the generation of urea precursors (ammonia, glycine, and alanine) and urea synthesis.

Figure 3. The oxidative metabolism pathways of three major nutrients [34]

In mammalian cells, the pyruvate dehydrogenase complex (PDC) catalyzes the conversion of pyruvate to acetyl-CoA. PDC consists of three enzymes [pyruvate dehydrogenase (PDH), dihydrolipoyl transacetylase, and dihydrolipoyl dehydrogenase] and six cofactors [thiamine pyrophosphate, lipoic acid, flavin adenine dinucleotide (FAD), nicotinamide adenine dinucleotide (NAD), coenzyme A (CoA), and Mg^{2+}]. The upstream regulatory factors of PDH primarily include pyruvate dehydrogenase kinase (PDK) and pyruvate dehydrogenase phosphatase (PDP), with the regulatory mechanism illustrated in [Figure 4: see original paper] [43]. PDK1 inhibits PDH activity by phosphorylating serine residues (including Ser-293, Ser-300, and Ser-232), while PDP restores PDH and PDC activity through dephosphorylation [44]. Tyrosine phosphorylation activates PDK and inhibits PDP [45]. Collectively, the PDK/PDP/PDH axis likely represents a regulatory target for glucose and amino acid metabolism.

Figure 4. The regulatory mechanisms of pyruvate dehydrogenase complex [43]

The rate of pyruvate oxidation increases with elevated PDC activity [46]. The small molecule dichloroacetate (DCA) induces autophagy and reduces cell proliferation. Additionally, studies have shown that DCA activates PDH by inhibiting PDK, thereby decreasing glycolytic proportion and increasing glucose oxidation rates [47–48]. Glutamine oxidation rates decrease as glucose oxidation rates increase [49]. Research indicates that promoting pyruvate oxidation reduces glutamate dehydrogenase activity, thereby decreasing acetyl-CoA production from glutamine [49]. These findings demonstrate that regulating pyruvate/glucose oxidation rates to inhibit amino acid metabolic fuel function is feasible.

In conclusion, the abnormally increased glycine and alanine in the PDV result from excessive metabolism of amino acids such as glutamate, and these amino acids serve as important precursors for hepatic urea synthesis. Reducing amino acid oxidation rates is key to decreasing urea precursor generation and hepatic urea synthesis. Promoting the energy supply efficiency of pyruvate/glucose in the porcine PDV is expected to increase glucose oxidation rates,

inhibit the metabolic fuel function of amino acids, and consequently reduce urea precursor generation and urinary nitrogen excretion, with the PDK/PDP/PDH axis potentially serving as the regulatory target. Although this approach has been validated in vitro, in rodent studies, and in human clinical trials, porcine metabolism differs substantially from cellular, rodent, and human systems, and the research objectives vary considerably. Therefore, this hypothesis requires extensive in vivo and in vitro validation.

References

[1] BOISEN S, HVELPLUND T, WEISBJERG M R. Ideal amino acid profiles as a basis for feed protein evaluation[J]. Livestock Production Science, 2000, 64(2/3): 239–251.

[2] SHRIVER J A, CARTER S D, SUTTON A L, et al. Effects of adding fiber sources to reduced-crude protein, amino acid-supplemented diets nitrogen excretion, growth performance, and carcass traits of finishing pigs[J]. Journal of Animal Science, 2003, 81(2): 492–502.

[3] LORDELO M M, GASPAR A M, LE BELLEGO L, et al. Isoleucine and valine supplementation of a low-protein corn-wheat-soybean meal-based diet for piglets: growth performance and nitrogen balance[J]. Journal of Animal Science, 2008, 86(11): 2936–2941.

[4] YIN Y L, HUANG R L, LI T J, et al. Amino acid metabolism in the portal-drained viscera of young pigs: effects of dietary supplementation with chitosan and pea hull[J]. Amino Acids, 2010, 39(5): 1581–1587.

[5] ZHANG G J, SONG Q L, XIE C Y, et al. Estimation of the ideal standardized ileal digestible tryptophan to lysine ratio for growing pigs fed low crude protein diets supplemented with crystalline amino acids[J]. Livestock Science, 2012, 149(3): 260–266.

[6] GALLO L, DALLA MONTÀ G, CARRARO L, et al. Growth performance of heavy pigs fed restrictively diets with decreasing crude protein indispensable amino acids content[J]. Livestock Science, 2014, 161: 130–138.

[7] GLOAGUEN M, LE FLOC'H N, CORRENT E, et al. The use of free amino acids allows formulating crude protein diets piglets[J]. Journal of Animal Science, 2014, 92(3): 637–644.

[8] GALASSI G, COLOMBINI S, MALAGUTTI L, et al. Effects of high fibre and low protein diets on performance, digestibility, nitrogen excretion and ammonia emission in the heavy pig[J]. Animal Feed Science and Technology, 2010, 161(3/4): 140–148.

[9] PATRÁŠ P, NITRAYOVÁ S, BRESTENSKÝ M, et al. Effect of dietary fiber and crude protein content in feed on nitrogen retention in pigs[J]. Journal of Animal Science, 2015, 90(S4): 158–160.

[10] ROTZ C A. Management to reduce nitrogen losses in animal production[J]. Journal of Animal Science, 2004, 82(E-Suppl): E119-E137.

[11] PUIMAN P, STOLL B, MØLBAK L, et al. Modulation of the gut microbiota with antibiotic treatment suppresses whole body urea production in neonatal pigs[J]. American Journal of Physiology-Gastrointestinal and Liver Physiology, 2013, 304(3): G300-G310.

[12] HITOSUGI T, FAN J, CHUNG T W, et al. Tyrosine phosphorylation of mitochondrial pyruvate dehydrogenase kinase 1 important for cancer metabolism[J]. Molecular Cell, 2011, 44(6): 864-877.

[13] NYACHOTI C M, OMOGBENIGUN F O, RADEMACHER M, et al. Performance responses indicators of gastrointestinal health in early-weaned pigs fed low-protein amino acid-supplemented diets[J]. Journal of Animal Science, 2006, 84(1): 125-134.

[14] SHIRALI M, DOESCHL-WILSON A, KNAP P W, et al. Nitrogen excretion at different stages of growth and its association with production traits in growing pigs[J]. Journal of Animal Science, 2012, 90(6): 1756-1765.

[15] JØRGENSEN H, PRAPASPONGSA T, VAN THI K V, et al. Models to quantify excretion of dry matter, nitrogen, phosphorus and carbon in growing pigs fed regional diets[J]. Journal of Animal Science and Biotechnology, 2013, 4: 42.

[16] KIRCHGESSNER A L. Glutamate in the enteric nervous system[J]. Current Opinion in Pharmacology, 2001, 1(6): 591-596.

[17] STOLL B, BURRIN D G. Measuring splanchnic amino acid metabolism in vivo using stable isotopic tracers[J]. Journal of Animal Science, 2006, 84(Suppl): E60-E72.

[18] ROMERO-GÓMEZ M, JOVER M, GALÁN J J. Gut ammonia production and its modulation[J]. Metabolic Brain Disease, 2009, 24(1): 147-157.

[19] WU G. Amino acids: metabolism, functions, and nutrition[J]. Amino Acids, 2009, 37(1): 1-19.

[20] EL-SABAGH M, SUGINO T, OBITSU T, et al. Effects of forage intake level on nitrogen net flux by portal-drained viscera of mature sheep with abomasal infusion of an amino acid mixture[J]. Animal, 2013, 7(10): 1614-1621.

[21] WU G Y. Functional amino acids in growth, reproduction, and health[J]. Advances in Nutrition, 2010, 1: 31-37.

[22] DAM G, KEIDING S, MUNK O L, et al. Branched-chain amino acids increase arterial blood ammonia in spite of enhanced intrinsic muscle ammonia metabolism in patients with cirrhosis and healthy subjects[J]. American Journal Physiology-Gastrointestinal Liver Physiology, 2011, 301(2): G269-G277.

[23] CHEN Cheng. Effects of dietary protein level on hepatic amino acid metabolism and transformation in piglets[D]. Master' s thesis. Chongqing: Southwest University, 2015: 21-24.

[24] REZAEI R, WANG W W, WU Z L, et al. Biochemical and physiological bases for utilization of dietary amino acids by young pigs[J]. Journal of Animal Science and Biotechnology, 2013, 4: 7.

[25] BERTHIAUME R, THIVIERGE M C, PATTON R A, et al. Effect of ruminally protected methionine on splanchnic metabolism of amino acids in lactating dairy cows[J]. Journal of Dairy Science, 2006, 89(5): 1621-1634.

[26] DOEPEL L, LOBLEY G E, BERNIER J F, et al. Effect of glutamine supplementation on splanchnic metabolism in lactating dairy cows[J]. Journal of Dairy Science, 2007, 90(9): 4325-4333.

[27] ROSE C F. Ammonia-lowering strategies treatment of hepatic encephalopathy[J]. Clinical Pharmacology & Therapeutics, 2012, 92: 321-331.

[28] WIECHETEK M, SOUFFRANT W B, GARWACKI S. Utilization of nitrogen from 15NH4Cl and [15N]alanine for urea synthesis in hepatocytes from fed and starved rats[J]. International Journal of Biochemistry, 1986, 18(7): 653-658.

[29] KRISTIANSEN R G, ROSE C F, FUSKEVÅG O M, et al. L-Ornithine phenylacetate reduces ammonia in pigs with acute liver failure through phenylacetylglycine formation: a novel ammonia-lowering pathway[J]. American Journal of Physiology-Gastrointestinal and Liver Physiology, 2014, 307(10): G1024-G1031.

[30] YANG Jing. Metabolic fate of glycine and alanine in the liver[D]. Master' s thesis. Chongqing: Southwest University, 2016: 27-35.

[31] KIGHT C E, FLEMING S E. Oxidation of glucose carbon entering the TCA cycle is reduced glutamine small intestine epithelial cells[J]. The American Journal Physiology, 1995, 268(6): G879-G888.

[32] DIENEL G A, CRUZ N F. Astrocyte activation in working brain: energy supplied by minor substrates[J]. Neurochemistry International, 2006, 48(6/7): 586-595.

[33] TORRES F V, HANSEN F, LOCKS-COELHO L D. Increase of extracellular glutamate concentration increases its oxidation and diminishes glucose oxidation in isolated mouse hippocampus: reversible TFB-TBOA[J]. Journal Neuroscience Research, 2013, 91(8): 1059-1065.

[34] EL BACHA T, LUZ M, DA POIAN A. Dynamic adaptation of nutrient utilization in humans[J]. Nature Education, 2010, 3(9): 8.

[35] DEFRONZO R A, TRIPATHY D. Skeletal muscle insulin resistance is the primary defect in type 2 diabetes[J]. Diabetes Care, 2009, 32(Suppl. 2): S157-S163.

[36] Review clinical trials mitochondrial disorders: 1997-2012[J]. Neurotherapeutics, 2013, 10(2): 307-319.

[37] FILLMORE N, LOPASCHUK G D. Targeting mitochondrial oxidative metabolism as an approach to treat heart failure[J]. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 2013, 1833(4): 857-865.

[38] YAO J, RETTBERG J R, KLOSINSKI L P, et al. Shift in brain metabolism in late onset Alzheimer' s disease: implications for biomarkers and therapeutic interventions[J]. Molecular Aspects of Medicine, 2011, 32(4/5/6): 247-257.

[39] TENNANT D A, DURÁN R V, GOTTLIEB E. Targeting metabolic transformation for cancer therapy[J]. Nature Reviews Cancer, 2010, 10(4): 267-277.

[40] BRICKER D K, TAYLOR E B, SCHELL J C, et al. A mitochondrial pyruvate carrier required for pyruvate uptake in yeast, Drosophila, and humans[J]. Science, 2012, 337(6090): 96-100.

[41] VACANTI N M, DIVAKARUNI A S, GREEN C R, et al. Regulation of substrate utilization by the mitochondrial pyruvate carrier[J]. Molecular Cell, 2014, 56(3): 425-435.

[42] GRAY L R, SULTANA M R, RAUCKHORST A J, et al. Hepatic mitochondrial pyruvate carrier 1 is required for efficient regulation of gluconeogenesis and whole-Body glucose homeostasis[J]. Cell Metabolism, 2015, 22(4): 669-681.

[43] PATEL M S, KOROTCHKINA L G. Regulation of mammalian pyruvate dehydrogenase complex phosphorylation: complexity of multiple phosphorylation sites kinases[J]. Experimental & Molecular Medicine, 2001, 33: 191-197.

[44] ROCHE T E, BAKER J C, YAN X, et al. Distinct regulatory properties of pyruvate dehydrogenase kinase and phosphatase isoforms[J]. Progress in Nucleic Acid Research and Molecular Biology, 2001, 70: 33-75.

[45] SHAN C L, KANG H B, ELF S, et al. Tyr-94 phosphorylation inhibits pyruvate dehydrogenase phosphatase 1 and promotes tumor growth[J]. Journal of Biological Chemistry, 2014, 289: 21413-21422.

[46] STACPOOLE P W, NAGARAJA N V, HUTSON A D. Efficacy of dichloroacetate as a lactate-lowering drug[J]. Journal of Clinical Pharmacology, 2003, 43(7): 683-691.

[47] BONNET S, ARCHER S L, ALLALUNIS-TURNER J, et al. A mitochondria-K+ channel axis is suppressed in cancer and its normalization promotes apoptosis and inhibits cancer growth[J]. Cancer Cell, 2007, 11(1): 37-51.

[48] SUN Y, LI T, XIE C, et al. Dichloroacetate treatment improves mitochondrial metabolism and reduces brain injury in neonatal mice[J]. Oncotarget, 2016, doi: 10.18632/oncotarget.9150.

[49] YANG C D, KO B, HENSLEY C T, et al. Glutamine oxidation maintains the TCA cycle and survival during impaired mitochondrial pyruvate transport[J]. Molecular Cell, 2014, 56(3): 414-424.

Note: Figure translations are in progress. See original paper for figures.