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Review article

Important roles for the arginine family of amino acids in swine nutrition and production

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Abstract

Arginine, glutamine, glutamate, proline, aspartate, asparagine, ornithine, and citrulline are interconvertible via complex interorgan metabolism in most mammals (including the pig). The major sites for their metabolism are the small intestine, kidneys, and liver, with cortisol being a key regulatory hormone. Because these amino acids (except for ornithine and citrulline) are usually abundant in plant and animal tissue proteins, pig producers have traditionally paid little attention to supplementing the arginine family of amino acids to swine diets. However, results of recent studies indicate that these amino acids serve important regulatory functions in nutrient metabolism and immune response, thereby affecting the efficiency of feed utilization by pigs. Arginine and glutamine are the prototypes with well-defined functions and expanded applications to pork production. Arginine deficiency, induced by a reduction in intestinal synthesis of citrulline, is a major factor limiting maximal growth of milk-fed piglets. Both enzymological and metabolic studies discovered that low availability of *N*-acetylglutamate in enterocyte mitochondria is responsible for limited synthesis of citrulline from both glutamine and proline in 7- to 21-day-old suckling piglets. Thus, either dietary supplementation with arginine or oral administration of *N*-carbamylglutamate (a metabolically stable analogue of *N*-acetylglutamate) increased muscle protein synthesis and body-weight gain in milk-fed piglets. Moreover, dietary supplementation with glutamine to early-weaned piglets prevented intestinal atrophy and improved growth performance. Remarkably, supplementing arginine to the diet of pregnant gilts between days 30 and 114 of gestation markedly increased the number of live-born piglets and litter birth-weight. Large-scale availability of feed-grade arginine and glutamine holds great promise for improving animal health and nutrient utilization in pig production worldwide.

Keywords: Amino acids; Biological functions; Production performance; Pigs

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1. Introduction

The arginine family of amino acids (AFAA), glutamine, glutamate, proline, aspartate, asparagine, ornithine, citrulline, and arginine, are interconvertible via complex interorgan metabolism in most mammals, including pigs (Fig. 1). Except for ornithine and citrulline, which are not substrates for protein synthesis, these amino acids are usually abundant in plant (e.g., corn and soybean meal) and animal tissue (e.g., fish meal and blood meal) proteins (Wu and Morris, 1998), and their provision from conventional diets is generally considered to be sufficient for growing, finishing, and pregnant pigs (NRC, 1998). Therefore, pig producers have traditionally paid little attention to supplementing AFAA to swine diets. However, recent biochemical studies revealed that these amino acids serve key regulatory functions in nutrient metabolism and immune response (Fu et al., 2005; Rhoads et al., 2006; Morris, 2006; Li et al., 2007), thereby affecting the efficiency of feed utilization by animals, including pigs (Table 1). In addition, there is evidence that arginine is deficient in the milk of most studied mammals, including pigs (Wu and Knabe, 1994; Davis et al., 1994; Kim et al., 2004) and that arginine transport from maternal to fetal blood in gilts is insufficient for maximal fetal growth (Wu et al., 1999; Mateo et al., 2007). Thus, there is a paradigm shift in our understanding of the roles for AFAA in swine nutrition and production. This new knowledge is highlighted in the present review, with a particular emphasis on the three most critical phases (fetal, neonatal, and postweaning) of pig growth and development.

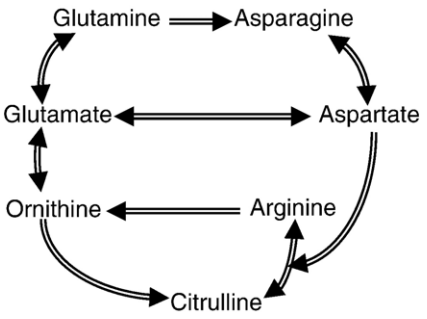

Fig. 1. Interconversion of the arginine family of amino acids in neonatal and postweaning pigs. Aspartate is required for both the synthesis of arginine from citrulline and the recycling of citrulline into arginine. The mitochondria-generated ornithine is readily converted into citrulline in the small intestine, whereas the ornithine provided from the diet is a poor substrate for arginine synthesis in the body because of the complex compartmentation of its intestinal metabolism.

2. Concentrations of AFAA in sow's milk and their metabolism in lactating porcine mammary tissue

2.1. Concentrations of AFAA in sow's milk

Our research on AFAA nutrition in pigs began with the measurement of amino acid concentrations in sow's milk. Based on careful review of the literature, it was clear that investigators failed to identify several physiologically important amino acids (ornithine, citrulline, glutamine and taurine) in porcine milk; therefore, we initiated studies to quantify free and protein-bound amino acids in sow's colostrum and milk between Days 1 and 28 of lactation (Wu and Knabe, 1994). Strikingly, concentrations of free glutamine in milk increased progressively with advancing lactation, and reach the highest mean value of 3.5 mM at Day 28 (Fig. 2), in comparison with 0.3 to 0.4 mM glutamine in plasma of lactating sows. In contrast, concentrations of arginine in sow's milk (free plus protein-bound) were much lower than those of glutamine plus glutamate, proline, lysine, and branched-chain amino acids (BCAA) at all days of lactation (Table 2). Concentrations of free arginine in sow's colostrum or milk are extremely low and account for less than 0.7% of its total arginine content (Wu and Knabe, 1994). On a gram basis, arginine/lysine ratios were 0.35 and 0.97, respectively, in sow's milk on Day 7 of lactation and in tissue proteins of 7-day-old piglets (Wu et al., 2004d).

We note that our values of both free and protein-bound arginine concentrations in sow's colostrum and milk (Wu and Knabe, 1994; Kim et al., 2004) are substantially lower than those reported by some investigators (Csapó et al., 1996; King et al., 1993) probably due to major differences in the analytical techniques used. For example, the analysis of amino acids in our studies involved precolumn derivatization with *o*-phthaldialdehyde, the separation of amino acid derivatives by high performance liquid chromatography, and fluorescence detection (Wu and Knabe, 1994; Kim et al., 2004). In contrast, the postcolumn derivatization of amino acids with ninhydrin after their separation on ion-exchange chromatography was employed for AFAA determination by other researchers (King et al., 1993; Csapó et al., 1996). It is not clear whether one or more of nitrogenous compounds in porcine milk could be co-eluted with

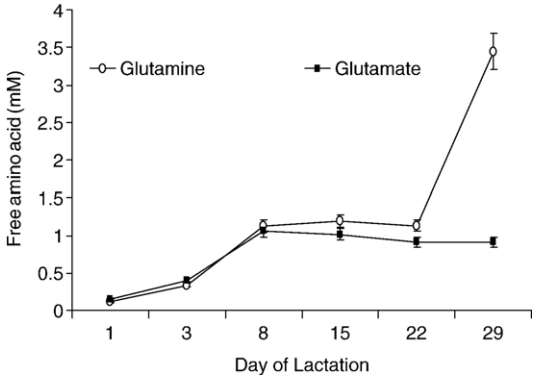

Fig. 2. Concentrations of free glutamine and glutamate in sows' milk. Adapted from Wu and Knabe (1994). Data are the mean ± SEM, $n = 10$.

Table 1
Functions of the arginine family of amino acids in swine nutrition and production

Substrates for the synthesis of tissue protein
Cellular signaling via mTOR, cAMP, and cGMP pathways
Hormone synthesis and secretion (e.g., insulin, glucagon, growth hormone, prolactin, and placental lactogen)
Endothelial function, vasodilation, and blood blow
Nutrient metabolism (e.g., nutrient transport, protein turnover, fat synthesis and oxidation, glycolysis, glucose synthesis and oxidation, amino acid synthesis and oxidation, and urea synthesis for ammonia detoxification)
Acid-base balance and whole-body homeostasis
Intestinal integrity and function
Immune function and health (e.g., T-cell proliferation and B-cell maturation, antibody production by B-cells, and killing of pathogens)
Reproduction (e.g., spermatogenesis, male fertility, ovulation, ovarian steroidogenesis, embryo implantation, placental angiogenesis, placental growth, and fetal growth and development)
Postnatal production performance (e.g., preweaning and postweaning growth, feed efficiency, protein deposition, and lactation)

Adapted from Wu and Meininger (2000, 2002) and Flynn et al. (2002).

Table 2
Concentrations of amino acids in sow's whole milk on days 7–21 of lactation

Amino acids[a]	g/L	g/kg DM
Alanine	1.97	10.6
Arginine	1.43	7.69
Asn + Asp	5.12	27.5
Cysteine	0.72	3.87
Glu + Gln	9.44	50.8
Gly	1.12	6.02
Histidine	0.92	4.95
Isoleucine	2.28	12.3
Leucine	4.46	24.0
Lysine	4.08	21.9
Methionine	1.04	5.59
Phenylalanine	2.03	10.9
Proline	5.59	30.1
Serine	2.35	12.6
Threonine	2.29	12.3
Tryptophan	0.66	3.55
Tyrosine	1.94	10.4
Valine	2.54	13.7
Total	50.0	268.8

Adapted from Kim et al. (2004). DM, dry matter.
[a] The sum of free plus peptide-bound.

arginine in the ion-exchange chromatography with postcolumn derivatization, thereby resulting in an overestimation of arginine in milk samples. In this regard, it is noteworthy that concentrations of free arginine in sow's colostrum and milk reported by Csapó et al. (1996) were 11- and 4-times, respectively, those found from our published work (Wu and Knabe, 1994), despite only a modest difference in dietary CP content (16 vs. 14%) between the two studies. Furthermore, in our laboratory assays, standards of basic amino acids were prepared freshly from powder forms on the day of analysis to ensure their accurate quantification in samples. This is important because arginine, lysine and histidine from a commercial source of amino acid standard solution may adhere to the surface of its glass container during shipping and storage, therefore leading to overestimation of their concentrations in samples. Additionally, purified bovine insulin (Sigma, St. Louis, MO) with the known composition of arginine and other amino acids was used to verify our procedures of protein hydrolysis and their analysis using high-performance liquid chromatography (Wu and Knabe, 1994; Kim et al., 2004).

The high abundance of arginine in tissue proteins of piglets (Wu et al., 1999) and the remarkable deficiency of arginine in sow's milk (Wu and Knabe, 1994; Davis et al., 1994) led us to ask the following important questions: 1) what is the nutritional significance of the abundance of glutamine and proline in sow's milk? 2) why is arginine deficient in sow's milk? and 3) what are the nutritional and developmental implications for arginine deficiency in milk? Our subsequent research to answer these questions yielded important discoveries that significantly advance our knowledge of swine nutrition and production as well as amino acid biochemistry and physiology in mammals.

2.2. Metabolism of AFAA in the lactating porcine mammary tissue

The lactating porcine mammary gland takes up large amounts of arginine and BCAA (Spicer et al., 1969; Trottier et al., 1997). However, the output of these amino acids in sow's milk is much less than their uptake by the lactating gland (Spicer et al., 1969; Trottier et al., 1997). To understand this observation, we used mammary tissue slices to conduct metabolic studies, which revealed that lactating porcine mammary tissue actively degrades arginine and BCAA to form proline and glutamine, respectively (O'Quinn et al., 2002). Two isoforms of arginase (types I and II) are responsible for arginine hydrolysis to produce ornithine, which is subsequently converted into proline via ornithine aminotransferase and pyrroline-5-carboxylate (P5C) reductase. Increasing extracellular concentrations of arginine from 0.5 to 2 mM dose-dependently increases proline synthesis in mammary tissue. Similarly, BCAA transaminase and glutamine synthetase are active in lactating mammary tissue of sows (Wu et al., 2005b), as reported for rats (Watford et al., 1986; Conway and Hutson, 2000). Interestingly, catabolism of proline and glutamine is virtually undetectable in porcine mammary tissue, because it lacks proline oxidase and glutaminase (O'Quinn et al., 2002; Wu et al., 2005b). This metabolic coordination maximizes the output of both proline and glutamine in milk of the lactating mammary gland of pigs. Collectively, our findings provide a metabolic explanation for a low concentration of arginine and the abundance of glutamine and proline in milk from sows (Wu and Knabe, 1994; Davis et al., 1994).

3. Roles of glutamine and proline in piglet nutrition

3.1. Synthesis of citrulline and arginine from glutamine in enterocytes

The small intestine of pigs utilizes glutamine and releases citrulline and arginine (Wu et al., 1994a; Le Floc'h and Seve, 2000). Importantly, glutamine is the only amino acid in arterial blood that is taken up by the small intestine of pigs in the postabsorptive state (Wu et al., 1994a). Using the non-collagenase cell preparation technique (Watford et al., 1979), we successfully isolated biochemically viable enterocytes from the pig small intestine for incubation with glutamine (Wu et al., 1994b). Increasing extracellular concentrations of glutamine from 0.5 to 5 mM dose-dependently increased the synthesis of citrulline and arginine in porcine enterocytes (Wu et al., 1994b). The synthesis of arginine is consistent with the conversion of [U–^{14}C]glutamine into [^{14}C]arginine in enterocytes of 0 to 7-day-old piglets (Blachier et al., 1993). All substrates required for this synthetic pathway, including ammonia, HCO_3^-, glutamate, aspartate, and ATP, are produced from glutamine catabolism (Wu et al., 1995b). P5C synthase and *N*-acetylglutamate (NAG) synthase are the two key regulatory enzymes in the conversion of glutamine into citrulline (Fig. 3). Other major tissues of neonatal and postweaning pigs, including liver, kidney, and skeletal muscle, lack P5C synthase for synthesizing citrulline from glutamine or glutamate (Wu et al., 1997). The reactions for the formation of citrulline from glutamine occur in mitochondria of enterocytes, and the subsequent conversion of citrulline into arginine takes place in the cytosol (Dillon et al., 1999). These results indicate that glutamine plays an important role in

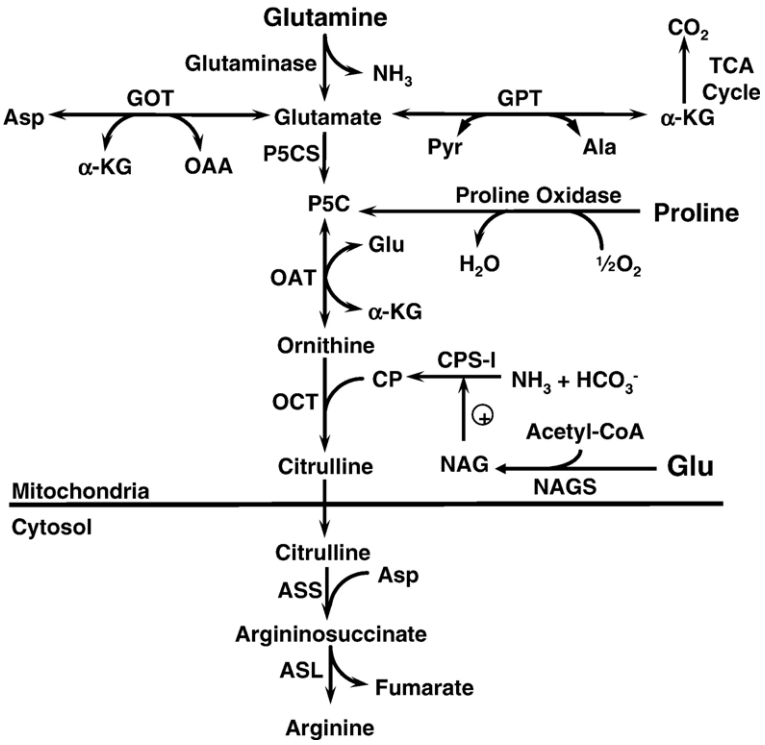

Fig. 3. Pathways of intestinal citrulline and arginine synthesis from glutamine and proline in pigs. Abbreviations: ASL, argininosuccinate lyase; Asp, aspartate; ASS, argininosuccinate synthase; CP, carbamoylphosphate; CPS-I, carbamoylphosphate synthase-I; Glu, glutamate; α-KG, α-ketoglutarate; NAGS, *N*-acetylglutamate synthase; OAT, ornithine aminotransferase; OCT, ornithine carbamoyltransferase; PDG, phosphate-dependent glutaminase; P5CS, pyrroline-5-carboxylate synthase; TCA, tricarboxylic acid. Arginine and *N*-acetylglutamate are essential allosteric activators of NAGS and CPS-I, respectively. Adapted from Wu and Morris (1998) and Wu et al. (2004d).

intestinal synthesis of citrulline (Fig. 4) and that the abundance of glutamine in sow's milk is of nutritional and physiological significance for compensating for low concentrations of arginine in this neonatal diet.

3.2. Synthesis of citrulline and arginine from proline in enterocytes

A puzzling observation arising from our studies was that the rates of citrulline synthesis from glutamine decreased markedly in 14- to 21-day-old piglets, as compared with 0- to 7-day-old piglets (Wu et al., 1995b). Thus, we hypothesized that an alternative precursor for intestinal synthesis of citrulline provided additional arginine in suckling piglets. Intriguingly, when sow-reared piglets were treated with gabaculine (an inhibitor of ornithine aminotransferase, an enzyme for converting P5C into ornithine), concentrations of both glutamine and proline in plasma increased two-fold (Flynn and Wu, 1996), suggesting that proline may be a substrate for citrulline synthesis via ornithine aminotransferase. This hypothesis is supported by evidence for the presence of proline oxidase in the small intestine of pigs (Samuels et al., 1989), although the cell types that expressed this enzymatic activity were not identified. Using radiochemical methodology, we discovered that proline is extensively catabolized by pig enterocytes to yield P5C, citrulline, and arginine via the mitochondrial proline oxidase pathway (Wu, 1997). The conversion of proline into citrulline requires ammonia and glutamate, both of which can be provided from glutamine degradation (Wu et al., 1995b). Because there is little uptake of arterial proline by the pig small intestine (Wu et al., 1994a,b), an enteral provision of large amounts of proline from sow's milk is crucial for compensating for its deficiency in arginine (Fig. 4). In support of this view, Ball and coworkers (Brunton et al., 1999) reported that there was little synthesis of arginine from arterial proline in piglets and intragastric administration of proline was effective in ameliorating a deficiency of arginine in neonatal pigs.

3.3. Role of dietary glutamine supplementation in preventing intestinal atrophy in early-weaned piglets

Glutamine is a major energy substrate for pig enterocytes (Wu et al., 1995b) and is an essential precursor for

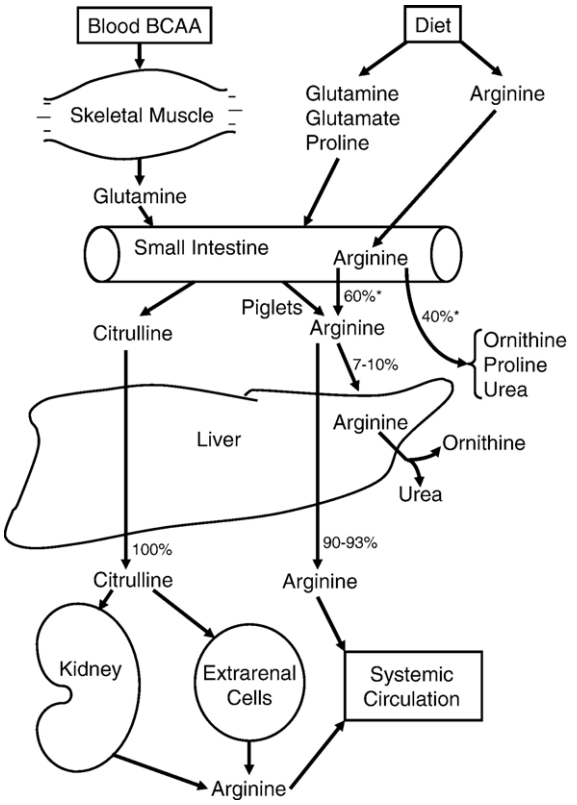

Fig. 4. Interorgan metabolism of citrulline and arginine in pigs. There is no net synthesis of arginine in the liver via the urea cycle because of its rapid hydrolysis by arginase. Therefore, the intestinal–renal axis plays an important role in arginine provision in neonatal and postnatal pigs. Branched-chain amino acids (BCAA) in arterial blood are taken up by skeletal muscle for the synthesis of glutamine, which is released into the circulation. Large amounts of arterial glutamine as well as dietary glutamine, glutamate and proline are utilized by enterocytes of the small intestine for the production of citrulline. Most citrulline is converted locally into arginine in the gut of neonatal pigs or released from the intestine of postweaning pigs. Because the small intestine of neonatal pigs lacks arginase activity for arginine catabolism, nearly all of the enterally delivered arginine that is not utilized for intestinal protein synthesis enters the portal circulation. In contrast, the presence of a high arginase activity in the small intestine of postweaning pigs is responsible for the catabolism of approximately 40% of dietary arginine in first pass. Virtually all of the intestine-derived citrulline bypasses the liver and is converted into arginine in kidneys and extrarenal cells (including endothelial cells and macrophages), whereas 7–10% of arginine in the portal vein is extracted by the liver in first pass. The symbol * denotes the flux in postweaning pigs.

the synthesis of purine and pyrimidine nucleotides that are essential for proliferation of cells (Curi et al., 2005), including porcine intraepithelial lymphocytes (Wu, 1996; Yoo et al., 1997). In addition, this amino acid increases expression of ornithine decarboxylase (ODC; Kandil et al., 1995), a key enzyme for the synthesis of polyamines that function to stimulate DNA and protein synthesis (Flynn et al., 2002). Further, glutamine is required for the synthesis of *N*-acetylglucosamine-6-phosphate, a common substrate for the synthesis of glycoproteins that are particularly rich in intestinal mucosal cells (Wu et al., 2001; Wang et al., 2006, 2007). As a substrate of glutamate, glutamine plays a role in the synthesis of glutathione, the most abundant small-molecular antioxidant in the small intestine (Wu et al., 2004b). Moreover, glutamine may increase the activity of the mammalian target of rapamycin (mTOR or FRAP1), a protein kinase that regulates protein synthesis in animal tissues and cells, including skeletal muscle (Suryawan et al., 2006) and small intestine (Fumarola et al., 2005). The discovery of the mTOR signaling pathway and its activation by glutamine and other amino acids (Fig. 5) is an exciting new development in nutrition research. Finally, glutamine regulates the synthesis of nitric oxide (NO), a signaling molecule that regulates mTOR activation (Pervin et al., 2007) and nutrient metabolism (Jobgen et al., 2006) at physiological concentrations in mammalian cells.

Because of a crucial role for glutamine in mucosal metabolism, this conditionally essential amino acid is expected to maintain intestinal barrier integrity and function in pigs (Wu, 1998). We used the early-weaned pig, which naturally develops intestinal epithelial damage within one-week postweaning (Dunsford et al., 1989), to test this hypothesis. By inserting a T-cannula into the mid-duodenum of postweaning pigs, we found that dietary glutamine was not subject to measurable acid hydrolysis in the stomach and upper part of the duodenum and, therefore, was effectively available to the small intestine for absorption and metabolic utilization (Wu et al., 1996c). In addition, dietary supplementation with 1% glutamine prevented jejunal atrophy (a major problem in swine production) during the first week postweaning and increased the gain/feed ratio by 25% during the second week postweaning (Fig. 6). This novel finding has led to the commercial development and availability of feed-grade glutamine (AminoGut) by Ajinomoto Co., Inc. (Tokyo, Japan) for use in swine diets (http://www.ajinomoto.com). Fig. 7 illustrates the possible mechanisms responsible for the beneficial effect of glutamine on intestinal function and growth.

4. Arginine nutrition in neonatal pigs

4.1. A crucial role for arginine synthesis in maintaining its homeostasis in milk-fed piglets

Arginine is an essential amino acid for young pigs (Southern and Baker, 1983; Roth et al., 1995; Urschel et al., 2006). Although arginine is formed in the liver via the urea cycle, there is no net synthesis of arginine by

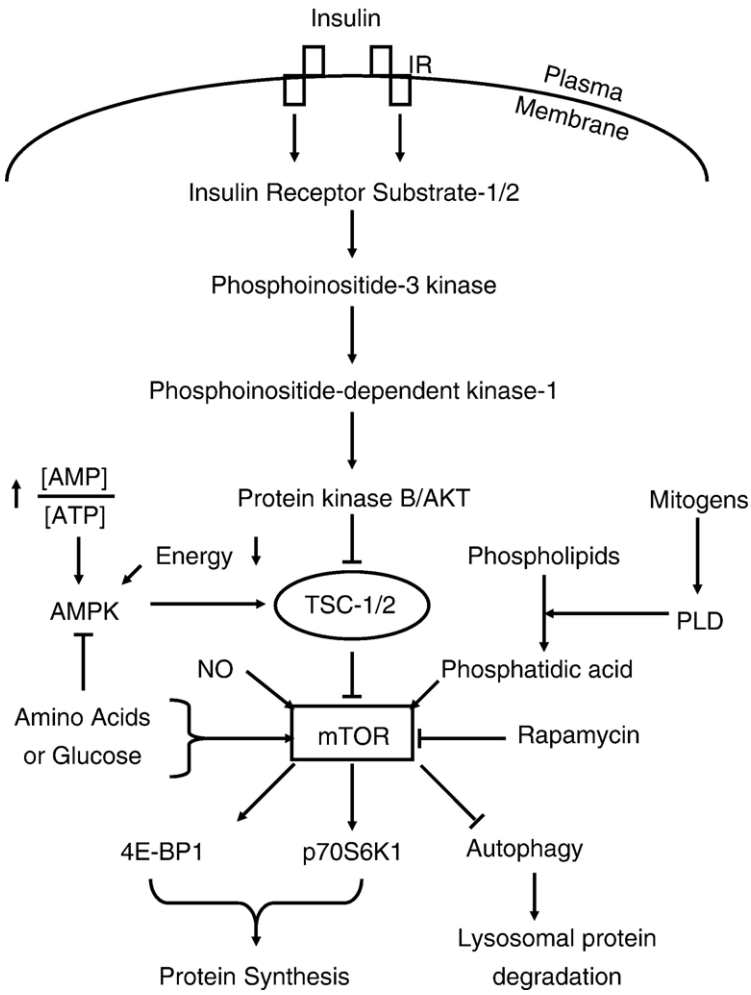

Fig. 5. Activation of protein synthesis by amino acids and growth factors through the mTOR signaling pathway. The mammalian target of rapamycin (mTOR) is a kinase, which phosphorylates 4E-BP1 (eIF4E-binding protein-1) and S6K1 (ribosomal protein S6 kinase-1), thereby initiating protein synthesis. In addition, mTOR may inhibit autophagy, a key step in lysosomal proteolysis. This kinase is inhibited by TSC-1/2 (tuberous sclerosis complex-1/2) whose activity is enhanced by AMPK (AMP-activated protein kinase) but suppressed by protein kinase B. Phosphorylation of protein kinase B in response to insulin and other growth factors relieves an inhibitory effect of TSC-1/2 on mTOR. Also, certain nutrients (e.g., glutamine, arginine, leucine, and glucose) and phosphatidic acid produced by phospholipase D (PLD) stimulate the phosphorylation of mTOR and thus increase its activity. Further, oxidation of amino acids and glucose increases cellular ratios of ATP:AMP and therefore reduces AMPK activity. Adapted from Meijer and Dubbelhuis (2004) and Suryawan et al. (2006).

this organ (Urschel et al., 2005) due to an exceedingly high activity of cytosolic arginase that rapidly hydrolyzes arginine (Wu and Morris, 1998). On the basis of concentrations of arginine in milk, daily milk intake, and weight gain as well as the synthesis of ornithine, NO, and creatine from arginine, we estimated that sow's milk provides at most 40% of arginine requirements of the 7-day-old pig (Wu and Knabe, 1995). This estimation is consistent with the previous finding that the endogenous synthesis of arginine provided ~ 45% of the arginine deposited in the body protein of the young pig fed a milk-based diet (Leibholz, 1982; Wilson and Leibholz, 1981). Thus, arginine synthesis via the intestinal–renal axis plays a crucial role in maintaining arginine homeostasis in sow-reared piglets. In support of this view, we found that inhibition of intestinal conversion of ornithine into P5C for 12 h reduced concentrations of ornithine, citrulline, and arginine in plasma by 59%, 52%, and 76%, respectively, in 4-day-old sow-reared piglets (Flynn and Wu, 1996). In enterocytes from 0- and 7-day-old piglets, most of the glutamine-derived citrulline is converted locally into arginine, whereas in older pigs, citrulline is largely released into the extracellular space (Wu and Knabe, 1995). The virtual

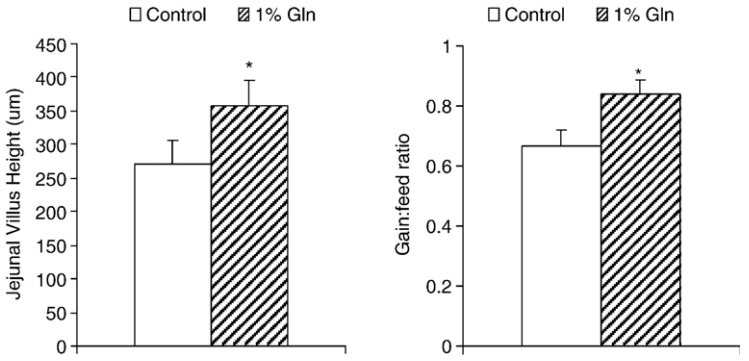

Fig. 6. Dietary glutamine supplementation enhances jejunal villus height and growth performance of early-weaned piglets. Data are the mean ± SEM, $n = 10$. Piglets were weaned at 21 days of age and fed a corn-soybean meal-based diet supplemented with 0 or 1% L-glutamine for 14 days. Jejunal villus height was measured at day 7 after the initiation of glutamine supplementation. Growth performance was measured at days 8 to 14 after the initiation of glutamine supplementation. * $P < 0.05$ vs. the control group. Adapted from Wu et al. (1996c). Gln, glutamine.

absence of arginase in enterocytes of preweaning pigs results in maximal output of arginine from the small intestine into the portal circulation (Wu et al., 1996b, 2007). Interestingly, endogenous synthesis of arginine from intragastrically administered glutamate and proline is regulated by dietary arginine intake in enterally-fed piglets (Wilkinson et al., 2004).

Using the isolated liver perfusion technique (Wu et al., 1991) and HPLC analysis of amino acids (Wu et al., 1994b), we found no evidence for citrulline uptake from the portal vein by the liver of 14-day-old suckling pigs ($n = 8$) when the organ was perfused with Krebs-bicarbonate buffer (30 ml/min per kg body wt) containing 5 mM glucose, 0.1, 0.2 or 0.5 mM L-citrulline, and plasma concentrations of other amino acids (Wu et al., 1997). When the liver from 14-day-old suckling pigs was similarly perfused with Krebs-bicarbonate buffer containing 5 mM glucose, 0.1, 0.2 or 0.5 mM L-arginine, and plasma concentrations of other amino acids (Wu et al., 1997), uptake of arginine from the portal vein was 6.8 ± 0.50%, 8.4 ± 0.63%, and 10.2 ± 0.77% (mean ± SEM, $n = 8$), respectively. Thus, as reported for adult rats (Windmueller, 1982; Dhanakoti et al., 1990), citrulline released by the small intestine of pigs is not extracted by the liver but is utilized for arginine synthesis by extrahepatic tissues (primarily kidneys) and cells (Wu and Brosnan, 1992; Wu and Knabe, 1995). Likewise, the uptake of physiological concentrations of arginine by pig and rat livers is limited (O'Sullivan et al., 1998) due to a low activity of the amino acid transport system y^+ in hepatocytes (Closs et al., 2004). Therefore, the small intestine is essential for the interconversion of AFAA in pigs (Wu, 1998; Bertolo et al., 2003), and citrulline or arginine derived from glutamine, glutamate and proline in the gut are equally effective as a source of arginine for the whole body (Fig. 4).

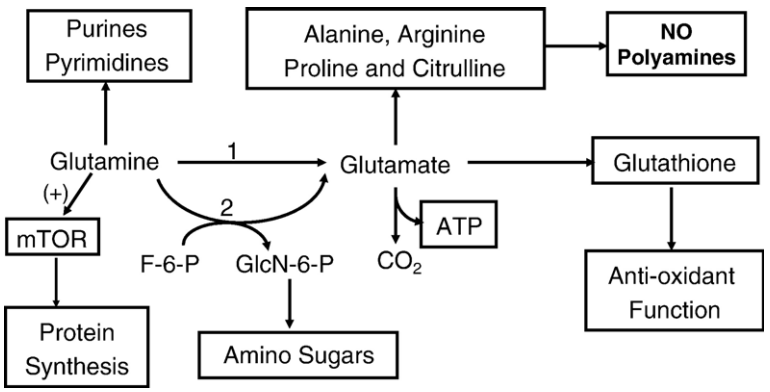

Fig. 7. Possible mechanisms responsible for the beneficial effect of glutamine on intestinal barrier function and growth. Abbreviations: F-6-P, fructose-6-phosphate; GlcN-6-P, *N*-acetylglucosamine-6-phosphate. The sign (+) denotes activation. Adapted from Wu (1998), Wu et al. (2001), and Curi et al. (2005).

4.2. Arginine deficiency limiting maximal growth of sow-reared piglets

Data from artificial rearing systems indicate that the biological potential for neonatal pig growth is at least 400 g/day (average from birth to 21 days of age) or ≥ 74% greater than that for sow-reared piglets (230 g/d) and that suckling piglets start to exhibit submaximal growth from Day 8 after birth (Boyd et al., 1995). Interestingly, the submaximal growth of suckling piglets occurs around the time when intestinal synthesis of citrulline and arginine is markedly reduced (Wu and Knabe, 1995). We have reported that intestinal synthesis of citrulline and arginine from glutamine decreased by 70–73% in 7-day-old suckling pigs in comparison with newborn pigs, and declined further in 14- to 21-day-old pigs (Wu, 1997). Similarly, rates of citrulline and arginine synthesis from proline were 75–88% lower in enterocytes of 7-day-old pigs, compared with newborn pigs, and remained at reduced levels in 14- to 21-day-old pigs (Wu, 1997). Thus, the endogenous synthesis of arginine in piglets declines remarkably during the suckling period due to the reduced release of citrulline from the small intestine. Accordingly, concentrations of arginine and its immediate precursors (ornithine and citrulline) in plasma decreased progressively by 20 to 41% from days 3 to 14 of postnatal development (Flynn et al., 2000). In addition, plasma concentrations of ammonia increased progressively by 18–46%, whereas those of nitrite plus nitrate decreased by 16–29% in 7- to 14-day-old suckling pigs, compared with 1- to 3-day-old pigs (Flynn et al., 2000). These metabolic data showing impaired hepatic ureagenesis and reduced systemic NO synthesis revealed a previously unrecognized deficiency of arginine in 7- to 21-day-old sow-reared pigs. Experimentally, we found that dietary supplementation with 0.2 and 0.4% L-arginine to 7- to 21-d-old milk-fed pigs (artificially reared on a liquid-milk feeding system) dose-dependently enhanced plasma arginine concentrations (30 and 61%), reduced plasma ammonia levels (20 and 35%), and increased weight gain (28 and 66%) (Fig. 8). Similarly, Leibholz (1982) reported that, in piglets weaned at 3 to 4 days of age, supplementing 0.2 and 0.4% arginine to a dried milk diet containing 19.2% crude protein and 0.75% arginine (similar to the protein and arginine content in sow's milk) increased weight gain by 43 and 93%, respectively, during days 7 to 14 of life. Thus, both metabolic and growth data provide compelling evidence that arginine deficiency is a major factor limiting maximal growth of young pigs (Kim et al., 2004).

4.3. A low mitochondrial concentration of NAG as the unifying mechanism for reduced intestinal citrulline synthesis from both glutamine and proline in young pigs

NAG synthase, which catalyzes the synthesis of NAG from glutamate and acetyl-CoA, is restricted to mitochondria of the liver and small-intestinal mucosa (Wakabayashi et al., 1991; Bush et al., 2002). NAG is an essential allosteric activator of carbamoylphosphate synthase-I (Meijer et al., 1990), which synthesizes mitochondrial carbamoyl-phosphate necessary for the conversion of ornithine into citrulline (Fig. 2). In addition, we recently discovered that NAG is an activator of P5C synthase in enterocyte mitochondria, as NAG at 0.1 mM increased its enzymatic activity by 124% (Wu et al., 2004d). Thus, addition of *N*-carbamylglutamate (NCG, a metabolically stable

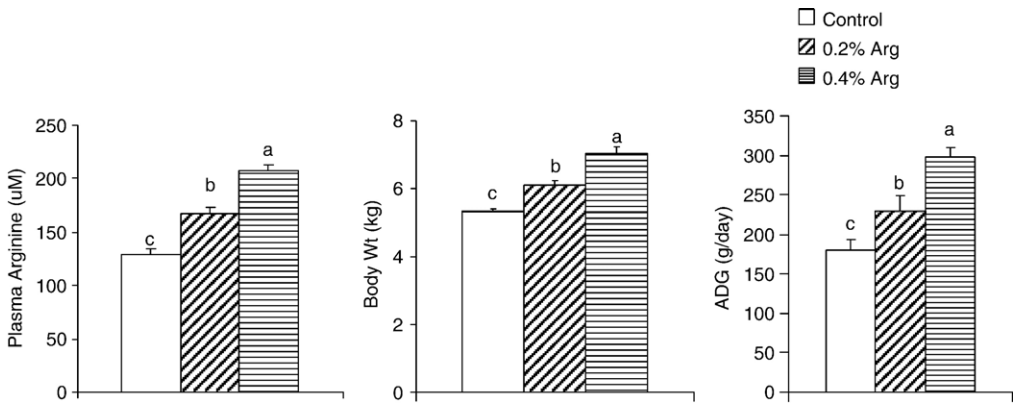

Fig. 8. Dietary arginine supplementation enhances the growth performance of milk-fed piglets. Data are the mean ± SEM, $n = 8$. Piglets were weaned at 7 days of age and then fed for 2 weeks a liquid milk replacer diet supplemented with 0, 0.2 or 0.4% L-arginine. Concentrations of arginine in plasma and body weight were measured at 21 days of age. Daily weight gain was measured between at 7 and 21 days of age. a–c: $P < 0.05$ within a measured parameter. Adapted from Kim et al. (2004). ADG, average daily gain. Arg, arginine.

analogue of NAG) to incubation medium greatly stimulated the conversion of glutamine and proline into citrulline in enterocytes of 14-day-old pigs (Wu et al., 2004d). Clearly, both enzymological and metabolic studies show that, in 14- to 21-day-old pigs, a low availability of NAG due to a reduced activity of mitochondrial NAG synthase is the unifying biochemical mechanism responsible for the limited intestinal synthesis of citrulline from both glutamine and proline and, therefore, for the primary cause of arginine deficiency in the neonates (Wu et al., 2004d).

4.4. Use of NCG to enhance piglet growth

Because of extensive catabolism of arginine by the lactating mammary gland (O'Quinn et al., 2002), supplementing arginine to the diet for lactating sows is not an effective approach to increase arginine concentration in milk (Kirchgessner et al., 1991; Wu et al., 2004d). However, our efforts to elucidate the mechanism responsible for arginine deficiency in 2- to 3-week-old suckling pigs led to development of enteral supplementation with NCG as a novel and effective means to enhance piglet growth (Wu et al., 2004d). Notably, oral administration of NCG to 4-day-old sow-reared pigs at 50 mg/kg body wt every 12 h until 14 days of age increased concentrations of arginine in plasma by 68%, prevented the marked postnatal decline in plasma levels of arginine, and enhanced piglet weight gain by 61% between Days 4 and 14 of age (Fig. 9). Most recently, we found that oral administration of NCG increased absolute protein synthesis in longissimus and gastrocnemius muscles of young pigs by 30 and 21%, respectively (Frank et al., 2007). Interestingly, the increase in skeletal muscle protein synthesis did not entirely account for the increase in body-weight gain of NCG-treated piglets (Frank et al., 2007). This result suggests that arginine not only increases protein synthesis, but also inhibits proteolysis in skeletal muscle.

As we noted previously (Wu et al., 2004d), the major advantages of NCG administration are: (1) a lack of interference of intestinal absorption of dietary tryptophan and basic amino acids; (2) promotion of a balanced provision of arginine to piglets relative to the supply of other basic amino acids from milk during the suckling period because of constant NCG activation of intestinal synthesis of citrulline; (3) a low effective dose of NCG as a metabolic activator of both P5C synthase and CPS-I; (4) a relatively high *in vivo* half-life of NCG in the intestinal mucosa; and (5) the ready chemical synthesis of NCG and its potentially low costs. These advantages are expected to benefit pork producers.

5. Hormonal regulation of intestinal metabolism of AFAA in weanling pigs

Weaning is characterized by a marked change in arginine and proline nutrition in swine (Ball et al., 1986; Chung and Baker, 1993; Kim et al., 2007). This is manifested by a shift in arginine and proline requirements from nutritionally essential to nonessential amino acids. To explain the underlying mechanisms, we conducted a series of biochemical and feeding experiments with weanling pigs and reported increases in the activities of P5C synthase and NAG synthase for citrulline synthesis in enterocytes of 29-day-old pigs weaned at 21 days of age (Wu et al., 1994b, 2004d). Further, our results indicated that intestinal expression of arginase and ODC was markedly enhanced to promote the synthesis of proline, urea, and polyamines in enterocytes of weaned pigs (Wu et al., 2000a,b). Importantly, we discovered a functional urea cycle for ammonia detoxification in the small intestine of postweaning pigs (Wu, 1995). Interestingly, expression of type-II arginase, but not type I arginase, was enhanced in enterocytes of weanling pigs (Flynn et al., 1999). The induction of type II arginase in the small intestine may be important for tissue remodeling, because proline (a product of arginine catabolism) is a major component of collagen proteins in the extracellular matrix (Davis and Wu, 1998). Notably, the rates of syntheses of citrulline and arginine are greater than their rates of degradation in enterocytes of weanling pigs (Wu et al., 1994b), resulting in an increase in the net release of these two amino acids from the small intestine after weaning (Wu et al., 1994a).

The weaning-induced increase in intestinal arginine metabolism is independent of a change in age or diet (Dugan et al., 1995) but is associated with an increase in concentration of cortisol in plasma (Worsae and Schmidt, 1980). Administration of RU486 (a glucocorticoid receptor antagonist) to weanling pigs markedly diminished the increased expression of the key enzymes of arginine metabolism, as well as arginine synthesis from glutamine, the conversion of ammonia into urea, and polyamine synthesis in enterocytes (Flynn and Wu, 1997a,b; Wu et al., 2000a,b). Also, administration of cortisol to 14- to 21-day-old suckling piglets increased intestinal arginine synthesis and degradation, which was abolished by co-administration of RU486 (Flynn and Wu, 1997a,b). These results indicate that a cortisol surge, acting via a glucocorticoid receptor-mediated mechanism, plays an important role in regulating intestinal arginine and proline metabolism during weaning. Our findings also provide a molecular

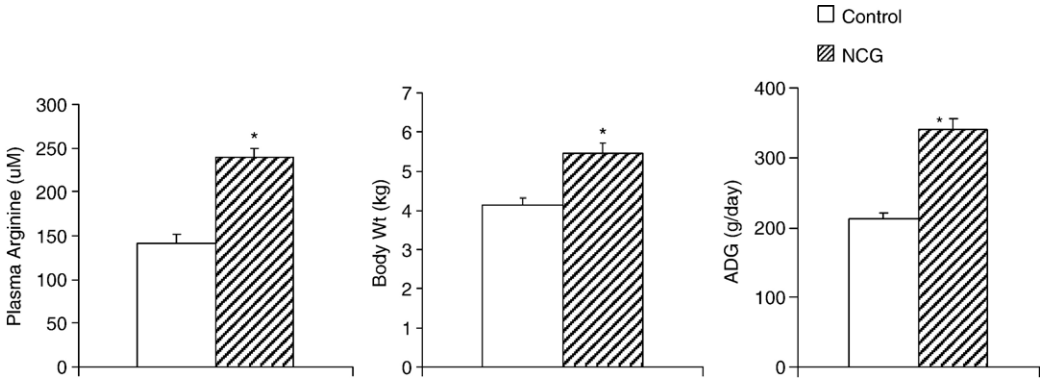

Fig. 9. *N*-Carbamoylglutamate (NCG) supplementation enhances plasma arginine concentration and the growth performance of sow-reared piglets. Data are the mean ± SEM, $n = 10$. Four-day-old sow-reared piglets received oral administration of 0 or 50 mg NCG per kg body weight every 12 h for 10 days. Concentrations of arginine in plasma and body weight were measured at 14 days of age. Daily weight gain was measured between 4 and 14 days of age. * $P < 0.01$ vs. the control. Adapted from Wu et al. (2004d). ADG, average daily gain.

mechanism to explain why arginine and proline are essential amino acids for suckling and young pigs, but not postweaning growing pigs. It should be borne in mind that this does not necessarily mean that endogenous synthesis of arginine is sufficient for maximal growth performance of growing-finishing pigs. Future research is needed to determine whether supplementing arginine to their diets may favorably alter the proteomes and cellular signaling pathways in major tissues, thereby increasing protein deposition and decreasing fat accretion in the body.

6. Arginine nutrition in pregnant pigs

6.1. Roles of arginine in placental angiogenesis and association between impaired placental arginine metabolism and intrauterine growth retardation (IUGR)

Arginine is a common substrate for NO and polyamine syntheses via NO synthase (NOS) and ODC, respectively (Wu and Morris, 1998). Arginine is hydrolyzed by arginase to form ornithine, which is decarboxylated by ODC to yield putrescine. NO is a major endothelium-derived relaxing factor (Wu and Meininger, 2000), and plays an important role in regulating placental-fetal blood flow and, thus, the transfer of nutrients and O_2 from mother to fetus (Bird et al., 2003). Likewise, polyamines regulate DNA and protein synthesis, and, therefore, cell proliferation and differentiation (Igarashi and Kashiwagi, 2000). Arginine is also a potent secretagogue of hormones (Newsholme et al., 2005). Growing evidence shows that NO and polyamines are key regulators of angiogenesis and embryogenesis, as well as placental and fetal growth (Reynolds and Redmer, 2001; Wu et al., 2004a). There are also data indicating that maternal undernutrition and hypercholesterolemia in pigs are associated with reduced concentrations of arginine and ornithine in the conceptus, reduced expression of NOS and ODC in the placenta and endometrium (Wu et al., 1998a,b), as well as impaired placental and fetal growth (Schoknecht et al., 1994). Therefore, we proposed a novel hypothesis that inadequate placental synthesis of NO and polyamines is a unifying mechanism for IUGR in response to both maternal undernutrition and overnutrition (Wu et al., 2004a).

6.2. Unusual abundance of AFAA in the porcine conceptus

On the basis of our findings on citrulline and arginine synthesis in enterocytes of neonatal pigs (Wu and Knabe, 1995), we asked whether this pathway might occur in fetal pigs (Dekaney et al., 2001, 2003). In analyzing fetal fluids from pigs at various days of gestation, we discovered an unusual abundance of arginine (4 to 6 mM) in porcine allantoic fluid on Day 40 of gestation (term = 114 days), when compared with maternal plasma levels (0.1 to 0.14 mM) (Wu et al., 1995a, 1996a, 1998a). In addition, we found particularly high concentrations of ornithine (1 to 3 mM) and glutamine (3 to 4 mM) in porcine allantoic fluid on Day 40 of gestation, when compared with maternal plasma levels (0.05 to 0.1 mM for ornithine and 0.3 to 0.45 mM for glutamine) (Wu et al., 1995a, 1996a). Remarkably, concentrations of arginine, ornithine, and glutamine in porcine allantoic fluid increase by 23-, 18-, and 4-fold, respectively, between Days 30 and 40 of gestation, with their nitrogen accounting for 67% of the total free α-amino acid nitrogen (Wu et al., 1996a). The unusual abundance of AFAA in fetal fluids is associated with maximal

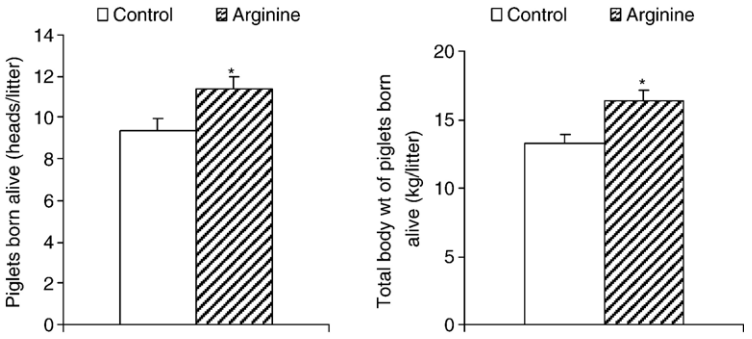

Fig. 10. Dietary arginine supplementation to pregnant gilts enhances litter size and total body-weight of live-born piglets. Data are the mean ± SEM. Pregnant gilts were fed a standard corn-soybean meal-based diet (McPherson et al., 2004) supplemented with 1.0% L-arginine-HCl ($n=25$) or 1.7% L-alanine (isonitrogenous control; $n=28$) between days 30 and 114 of gestation. *$P<0.05$ vs. the control. Adapted from Mateo et al. (2007).

placental NO and polyamine syntheses in the first half of pregnancy (Wu et al., 2003; Wu et al., 2005a) when placental growth is most rapid (Knight et al., 1977; Self et al., 2004). These novel findings suggest a crucial role for arginine-dependent metabolic pathways in conceptus growth and development.

6.3. Dietary supplementation with arginine improves pregnancy outcome in pigs

Modern high prolific sows ovulate 20 to 30 oocytes, but only deliver 9 to 15 piglets at term because of high prenatal losses (Town et al., 2005). There is a positive relationship between uterine capacity and fetal mortality (Wilson and Ford, 2001). Available evidence suggests that the greatest restraint on litter size in pigs is placental development and function in early gestation and uterine capacity at all periods of gestation (Bazer et al., 1988; Vallet et al., 2002; van der Lende and Rens, 2003). Interestingly, among domestic animals, pigs exhibit the most severe naturally occurring IUGR (Wu et al., 2006). The current restricted feeding program for pregnant gilts and sows (NRC, 1998) may not be most desirable for fetal growth and development because dietary provision of amino acids does not appear to meet requirements during pregnancy (McPherson et al., 2004; Ji et al., 2005). As noted previously, arginine plays a key role in placental angiogenesis and growth in mammals. Thus, we conducted a study to test the hypothesis that increasing arginine provision may enhance reproductive performance of gilts (Mateo et al., 2007). Our results indicate that dietary supplementation with 1.0% arginine–HCl between Days 30 and 114 of gestation increased concentrations of arginine, ornithine, and proline in plasma by 77, 53, and 30%, respectively (Mateo et al., 2007). The arginine treatment did not affect body weight or backfat thickness of gilts, but increased the number of live-born piglets by 2 and litter birth-weight by 24% (Fig. 10). Similarly, other investigators reported that supplementing 1% arginine to the diet of sows between days 14 and 28 of gestation increased the number of live-born piglets by 1 without affecting their average birth weight (Ramaekers et al., 2006). These exciting findings provide the first evidence for improved pregnancy outcomes in gilts through dietary arginine supplementation. It is noteworthy that our pioneering work on arginine nutrition in the porcine conceptus led to the commercial development and availability of feed-grade arginine (Progenos) by Nutreco for enhancing pig reproductive performance (http://www.trouwnutritionusa.com). Dietary supplementation to gestating pigs may have important implications for improving pregnancy outcomes, as well as postnatal growth performance, health and meat quality of the progeny (Wu et al., 2006).

7. Conclusion

The AFAA serves as essential precursors for the synthesis of a variety of molecules with enormous importance, and also regulates key metabolic pathways that are vital to health, growth, development, reproduction, and homeostasis of animals. Arginine and glutamine are currently the prototypes of AFAA with well-defined functions and expanded applications to swine production. Importantly, these findings exemplify the power of basic research to discover new knowledge and solve significant practical problems in animal agriculture. In view of the crucial regulatory roles for AFAA in nutrient metabolism, we anticipate that their supplementation to conventional diets will be highly beneficial for enhancing immune function, growth, and development of fetal, neonatal, and postweaning pigs. Achieving large-scale availability of

feed-grade arginine and glutamine holds great promise for improving animal health, the efficiency of nutrient utilization, and meat quality in pig production worldwide.

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