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Impacts of amino acid nutrition on pregnancy outcome in pigs: Mechanisms and implications for swine production[1,2]

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ABSTRACT: Pigs suffer up to 50% embryonic and fetal loss during gestation and exhibit the most severe naturally occurring intrauterine growth retardation among livestock species. Placental insufficiency is a major factor contributing to suboptimal reproductive performance and reduced birth weights of pigs. Enhancement of placental growth and function through nutritional management offers an effective solution to improving embryonic and fetal survival and growth. We discovered an unusual abundance of the arginine family of AA in porcine allantoic fluid (a reservoir of nutrients) during early gestation, when placental growth is most rapid. Arginine is metabolized to ornithine, proline, and nitric oxide, and these compounds possess a plethora of physiological functions. Nitric oxide is a vasodilator and angiogenic factor, whereas both ornithine and proline are substrates for placental synthesis of polyamines, which are key regulators of protein synthesis and angiogenesis. Additionally, arginine, leucine, glutamine, and proline activate the mammalian target of rapamycin cell-signaling pathway to enhance protein synthesis and cell proliferation in placentae. To translate basic research on AA biochemistry and nutrition into application, dietary supplementation with 0.83% L-arginine to gilts on d 14 to 28 or d 30 to 114 of gestation increased the number and litter birth weight of live-born piglets. In addition, supplementing the gestation diet with 0.4% L-arginine plus 0.6% L-glutamine enhanced the efficiency of nutrient utilization, reduced variation in piglet birth weight, and increased litter birth weight. By regulating syntheses of nitric oxide, polyamines, and proteins, functional AA stimulate placental growth and the transfer of nutrients from mother to embryo or fetus to promote conceptus survival, growth, and development.

Key words: arginine, fetal growth, glutamine, litter size, reproduction, swine

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INTRODUCTION

Amino acids are not only the building blocks of proteins in cells, but are also precursors for syntheses of nitrogenous substances [e.g., nitric oxide (**NO**), polyamines, creatine, dopamine, and catecholamines] essential for whole-body homeostasis (Wu and Meininger, 2002; Odenlund et al., 2009; Suryawan et al., 2009). However, the NRC (1998) takes into consideration only those AA that are not synthesized by pigs [the nutritionally essential AA (**EAA**)]. Despite a marked increase in conceptus (i.e., embryo-fetus and associated membranes) growth with advancing pregnancy (Knight et al., 1977), the NRC (1998) recommends the same dietary intake of EAA by gilts or sows during the entire period of gestation. Given the current suboptimal feeding program for gestating swine (Kim et al., 2009), as well as increased prenatal mortality and severe intrauterine growth retardation (**IUGR**; Bazer et al., 2009), there is an impetus to develop novel and effec-

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tive strategies to improve pregnancy outcomes in pigs. The major objectives of this article are 1) to highlight recent advances in the roles for functional AA (**FAA**) in improving maternal and embryonic-fetal nutrition as well as embryonic-fetal survival and growth, and 2) to discuss the underlying cellular and whole-body mechanisms responsible for the beneficial effects of FAA.

LIMITATIONS TO LITTER SIZE IN PIGS

Litter size in mammals is a maternal trait and is affected by complex factors, including ovulation rate, uterine capacity, and embryonic and fetal survival (Wu et al., 2006; Distl, 2007). Among livestock species, pigs suffer the greatest prenatal loss (up to 50%) because of a suboptimal intrauterine environment (Bazer et al., 2009), which may include inadequate uterine secretions and suboptimal nutrition. This problem is even more severe in modern, very prolific pigs than in breeds used in the swine industry 25 or 30 yr ago because of selection for increased ovulation rate. For example, conceptus survival is only 60, 50, and 45% on d 25, 36, and 44 of gestation, respectively, in a commercial swine herd [i.e., Camborough Line; Pig Improvement Company, Hendersonville, TN (Vonnahme et al., 2002)]. Although prolific gilts or sows ovulate 20 to 30 oocytes, they deliver only 9 to 15 piglets at term (Town et al., 2005). The first peak of embryonic death occurs between d 12 and 15 of gestation (i.e., the peri-implantation period), with most of the prenatal losses (>75%) occurring during the first 25 or 30 d of gestation (Ford et al., 2002). A subsequent period of fetal mortality is d 30 to 40 of gestation, followed by losses on d 55 to 75 and during the period immediately before farrowing (Ford et al., 2002). Fetal losses after d 30 of gestation result from inadequate uterine capacity (Webel and Dziuk, 1974). Thus, sows with large litters (>10 fetuses) have the greatest rate of fetal mortality (Town et al., 2005).

Pigs develop a noninvasive, diffuse type of epitheliochorial placentae, whose weights vary greatly among conceptuses within the same uterus (Bazer et al., 2008). The greatest restraint on litter size in pigs is placental development and function in early gestation and inadequate uterine capacity at all periods of gestation, rather than simply the number of ovulations or embryos (Bazer et al., 1988). The problem of reduced prenatal survival is further exacerbated by the low heritability of litter size in pigs [i.e., 0.09 to 0.11 (Urban et al., 1966; Lund et al., 2002)]. Heritability for the number of liveborn piglets (i.e., 0.06 to 0.09) is even less than that for the total number of piglets born (Haley and Lee, 1992; Lund et al., 2002). Thus, improvement in litter size through animal breeding has been slow over the past decades (Distl, 2007). In fact, litter size in US swine increased at the rate of only 0.052 pigs/yr between 1980 and 2000 (Johnson, 2000).

PROBLEMS OF IUGR IN PIG PRODUCTION

Pigs exhibit the most severe naturally occurring IUGR among livestock species (Wu et al., 2006). Before d 35 of gestation, porcine embryos are uniformly distributed within each uterine horn, and their weights do not differ appreciably within each litter. However, after d 35, uterine capacity becomes a limiting factor for fetal growth, even though fetuses are distributed relatively uniformly (Bazer et al., 1988). Rates of blood flow, and thus the supply of nutrients to conceptuses after d 30 of pregnancy, vary greatly along the length of the uterus of gestating swine (Père and Etienne, 2000) because of differences in the structure and density of its vasculature (Ford et al., 2002). Reduced growth of porcine fetuses is exacerbated by the widespread practice of restricted feeding programs (e.g., 2 kg of diet/d) in the swine industry during the entire gestation period to prevent excessive maternal BW gains (Kim et al., 2009). At birth, runt piglets may weigh only one-half or even one-third as much as the heaviest littermates (Widdowson, 1971). In some litters, most or nearly all of the piglets have reduced birth weights (<1.1 kg), particularly when a part or majority of the pregnancy period is subjected to environmental stress (e.g., hot or cold temperatures or disease). Notably, key organs involved in nutrient digestion and utilization in runt pigs (e.g., small intestine and skeletal muscle) suffer oxidative stress and are disproportionately smaller than those of the larger littermates (Wang et al., 2008).

Fetal growth restriction has permanent negative effects on neonatal adjustment to extrauterine life, preweaning survival, postnatal growth, efficiency of feed utilization, lifetime health, tissue composition (including protein, fat, and minerals), meat quality, reproductive function, and athletic performance (Wu et al., 2006). Most IUGR piglets die before weaning, and those that survive suffer permanent growth retardation. In a breeding study carried out between 2003 and 2009, we observed that IUGR piglets (<1.10-kg birth weights) represented 76% of preweaning deaths in pigs (Table 1). At present, IUGR piglets are culled on farms, and there is no nutritional support to increase their growth or survival during the suckling and postweaning periods. Because increased prenatal mortality and IUGR remain significant problems, increasing embryonic and fetal growth and development is important for optimizing the efficiency of pork production.

ROLE OF THE PLACENTA IN EMBRYONIC AND FETAL DEVELOPMENT

Immediately after implantation of the conceptus, various genes are expressed in the trophectoderm to initiate placental formation (Vonnahme and Ford, 2004). Implantation, followed by placentation, begins

on approximately d 15 of gestation in pigs (Geisert and Yelich, 1997). The placenta undergoes a rapid formation of new blood vessels (i.e., angiogenesis) and marked growth during pregnancy (Reynolds and Redmer, 2001). Thus, blood vessels are clearly visible in porcine placentae and allantoic membranes on d 25 of pregnancy. Notably, the porcine placenta grows rapidly between d 20 and 60 of gestation, and its development is maximal by d 70 (Knight et al., 1977; Wu et al., 2005), a period preceding rapid fetal growth. Placental angiogenesis is necessary to increase the utero-placental blood flow that supplies nutrients from mother to fetus (Wu et al., 2004; Reynolds et al., 2006). Indeed, the prolific Meishan pig exhibits more vascularization in the placenta and has 3 to 5 more piglets per litter than US or European pig breeds (Vonnahme and Ford, 2004). A well-developed placental vasculature enables the Meishan fetus to obtain sufficient nutrients from a relatively small placenta (Bazer et al., 1988), resulting in an increased rate of prenatal survival. Insufficient placental vascularization may lead to a progressive deterioration in placental function and a decrease in placental transfer of oxygen and nutrients to fetuses (Wu et al., 2006). Although no relationship exists between placental weight and embryonic survival before d 25 or 30 of pregnancy, the functional capacity of placentae for provision of nutrients and the exchange of gases is vital to fetal survival, growth, and development (Reynolds et al., 2006; Bazer et al., 2008). Therefore, understanding the mechanisms that regulate placental growth, including vascular growth and placental function, is crucial for improving litter size and fetal growth in pigs.

Table 1. Litter size, birth weights, and preweaning mortality of pigs[1]

Variable	Value[2]
Litter size, No. of gilts	132
Total piglets born, No.	1,468
Piglets born alive, No.	1,340
Piglets born dead, No.	128
Piglets born per litter, No.	11.12
Piglets born alive per litter, No.	10.15
Piglets born dead per litter, No.	0.97
Average birth wt of all piglets born, kg	1.35
Average birth wt of piglets born alive, kg	1.37
Piglets born dead, %	8.7
Piglets born alive, %	91.3
Proportion (%) of piglets in ranges of birth wt, kg	
0.50 to 0.69	3.0
0.70 to 0.89	7.4
0.90 to 1.09	13.2
1.10 to 1.29	31.4
1.30 to 1.49	27.8
1.50 to 1.69	14.3
1.70 to 2.09	2.9
Preweaning death, % of all piglets born alive	11.5

[1]Gilts (Yorkshire × Landrace dams and Duroc × Hampshire sire) were bred at 8 mo of age and fed 2 kg daily of a corn- and soybean meal-based diet. The diet ingredients (%) were as follows: corn grain, 80.4; soybean meal (48.5% CP), 10.0; alfalfa meal, 5.00; dicalcium phosphate, 2.20; potassium chloride, 0.75; limestone, 0.50; soybean oil, 0.50; salt, 0.35; mineral premix, 0.10; vitamin premix, 0.20. This diet provided 3,175 kcal of ME/kg and the following nutrients (% of diet; as-fed basis): DM, 89.4; CP, 12.4; alanine, 0.76; arginine, 0.70; asparagine, 0.59; aspartate, 0.71; cysteine, 0.21; glutamate, 1.02; glutamine, 1.19; glycine, 0.55; histidine, 0.31; isoleucine, 0.52; leucine, 1.13; lysine, 0.57; methionine, 0.22; phenylalanine, 0.57; proline, 1.08; serine, 0.52; threonine, 0.48; tryptophan, 0.14; tyrosine, 0.43; valine, 0.63. The content of minerals and vitamins per kilogram of complete diet (as-fed basis) was as follows: calcium, 8.1 g; phosphorus, 7.1 g; manganese, 46.7 mg; iron, 75 mg; zinc, 103.8 mg; copper, 9.5 mg; iodine, 0.72 mg; selenium, 0.23 mg; vitamin A, 7,556 IU; cholecalciferol, 825 IU; vitamin E, 61.9 IU; vitamin K, 4.4 IU; vitamin B_{12}, 54.9 μg; riboflavin, 13.7 mg; niacin, 54.9 mg; choline, 1,650 mg.

[2]Data are derived from analysis of litters born at the Texas A&M University Swine Center between 2003 and 2009. Intrauterine growth retardation piglets (<1.10 kg at birth) represented 76% of preweaning deaths.

ROLES OF AA IN PLACENTAL GROWTH

Among nutrients, AA play the most important role in placental growth because they are absolutely required for, and activate the machinery of, protein synthesis in cells (Li et al., 2009a; Palii et al., 2009; Rhoads and Wu, 2009). Based on dietary needs for N balance or growth, AA were traditionally classified as nutritionally essential (i.e., indispensable) or nonessential (i.e., dispensable; Elango et al., 2009). Essential AA are defined as either those AA whose carbon skeletons cannot be synthesized or those that are inadequately synthesized de novo by the body relative to needs and which must be provided from the diet to meet requirements (Chen et al., 2009; Wu, 2009). Nonessential AA are those AA that can be synthesized de novo in adequate amounts by the body to meet requirements (Baker, 2009). Conditionally essential AA are those that normally can be synthesized in adequate amounts by the organism, but which must be provided from the diet to meet needs under conditions in which rates of utilization are greater than rates of synthesis. Recent research has led to the development of FAA, which are defined as AA that can regulate key metabolic pathways to benefit the survival, growth, development, reproduction, and health of animals and humans (Wu, 2009). Functional AA (e.g., arginine, cysteine, glutamine, leucine, proline, and tryptophan) can be either EAA or nonessential AA (Kim and Wu, 2009; Stipanuk et al., 2009; Tan et al., 2009a,b).

A growing body of evidence supports the idea that NO and polyamines (i.e., putrescine, spermidine, and spermine), which are products of arginine catabolism, play important roles in placental growth (Wu et al., 2009). Arginine stimulates placental NO production by enhancing expression of guanosine triphosphate cyclohydrolase I, the first and rate-controlling enzyme in the synthesis of tetrahydrobiopterin (an essential cofactor for NO synthase; Figure 1). Additionally, glutathione, synthesized from glutamate, glycine, and cysteine, is the major antioxidative peptide in the conceptus and is abundant in uterine fluid (Gao et al., 2009e). Transport of AA requires multiple specific transporters (Grillo et al., 2008), whose expression in conceptuses increases

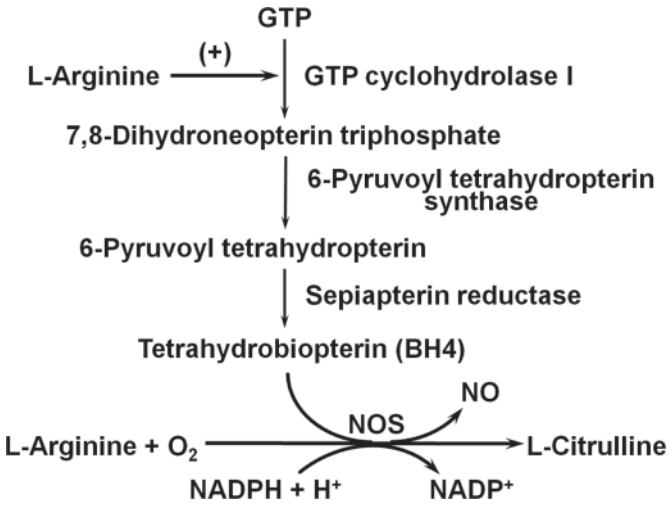

Figure 1. Tetrahydrobiopterin biosynthesis in porcine placentae via the de novo pathway. The plus sign (+) denotes enhancement of guanosine triphosphate (GTP) cyclohydrolase-I protein expression by arginine. Guanosine triphosphate cyclohydrolase I is the first and rate-controlling enzyme in the conversion of GTP into tetrahydrobiopterin [an essential cofactor for nitric oxide (NO) synthesis from L-arginine]. NOS = nitric oxide synthase; NADP = NAD phosphate; NADPH = reduced NAD phosphate.

with gestation (Gao et al., 2009a,b). Of particular note, IUGR is associated with impaired transport of basic, neutral, and acidic AA by placentae (Regnault et al., 2005; Wu et al., 2008). Thus, maternal protein nutrition, which reduces AA availability in the conceptus (Wu et al., 1998a,b), greatly affects embryonic and fetal survival in pigs (Pond et al., 1969; 1981). Along with IGF, vascular endothelial growth factors, and other growth factors (Bazer et al., 2009), NO and polyamines are crucial for angiogenesis, embryogenesis, placental trophoblast growth, uteroplacental blood flow, and transfer of nutrients from mother to fetus, as well as fetal growth and development (Wu et al., 2006; Wu and Meininger, 2009).

We discovered an unusually large abundance of arginine in porcine allantoic fluid during early gestation (Wu et al., 1996). Indeed, arginine and ornithine account for 50 and 55% of the total α-AA nitrogen in porcine allantoic fluid on d 40 and 45 of gestation, respectively. Similarly, the arginine family of AA is also very abundant in ovine allantoic fluid (e.g., 10 m*M* citrulline and 25 m*M* glutamine at d 60; Kwon et al., 2003). These novel and intriguing observations raised important questions regarding the biological role for arginine in the growth and development of mammalian conceptuses. In support of this view, we found that rates of NO and polyamine synthesis in both porcine and ovine placentae were greatest during early gestation, when placental growth is most rapid (Kwon et al., 2004; Wu et al., 2005; Gao et al., 2009c). We hypothesized, based on these findings, that impaired placental growth, including vascular growth, or placental function can result from reduced placental synthesis of NO and polyamines, thereby contributing to IUGR in both underfed and overfed dams (Wu et al., 2004).

ROLES OF AA IN FETAL MUSCLE AND ADIPOSE TISSUE GROWTH

There is evidence that maternal protein nutrition during gestation can affect postnatal muscle growth and intramuscular fat content in pigs (Rehfeldt et al., 2004). Myocytes and adipocytes are derived from a common mesenchymal precursor (Sordella et al., 2003); therefore, excessive amounts of adipose tissue are developed at the expense of skeletal muscle when embryonic myogenesis is impaired (Kablar et al., 2003). There are 2 developing muscle fibers in fetal pigs: 1) primary fibers, formed by the rapid fusion of primary myoblasts between d 25 and 50 of gestation, and 2) secondary fibers, formed on the surface of primary fibers between approximately d 50 and 90 of gestation (Handel and Stickland, 1987). The numbers of secondary muscle fibers, but not primary muscle fibers, are affected by the uterine environment (Dwyer et al., 1994). Because the total number of muscle fibers is fixed at birth, their prenatal development affects the postnatal growth of skeletal muscle (Nissen et al., 2003). The differences in prenatal and postnatal growth rates between IUGR piglets and normal litter mates correlate with a smaller ratio of secondary to primary muscle fibers and a smaller size of the fibers in IUGR pigs (Handel and Stickland, 1987). Abnormal metabolic regulation of intracellular protein turnover, adipogenesis, and mitochondrial biogenesis is likely a major factor responsible for reduced protein deposition in skeletal muscle and increased fat accretion in IUGR fetuses or offspring. In this regard, it is noteworthy that recent results of proteomics studies indicate that newborn IUGR piglets have a greater abundance of proteasome (i.e., the major protease for nonlysosomal protein degradation) in skeletal muscle and liver, but less eukaryotic translation initiation factor 3, a key requirement for protein synthesis, in skeletal muscle compared with piglets with normal birth weights (Wang et al., 2008).

Polyamines are necessary for both proliferation and differentiation of cells (Montanez et al. 2008; Flynn et al., 2009) and likely mediate growth and development of fetal muscle fibers and adipocytes (Figure 2). Consistent with this view, we noted that concentrations of arginine, ornithine, proline, glutamine, and polyamines were reduced substantially in skeletal muscle of IUGR fetal pigs compared with littermates of average BW (Table 2). Similarly, concentrations of proline were much less in the allantoic and amniotic fluids of IUGR fetal pigs than in their normal counterparts (Wu et al., 2008). Emerging evidence shows that physiological concentrations of NO inhibit the growth of white adipocytes (Fu et al., 2005; Jobgen et al., 2006) and stimulate the oxidation of fatty acids and glucose in muscle (Jobgen et al., 2009a; Tan et al., 2009b). In addition to NO and polyamines, arginine and other FAA (e.g., glutamine, leucine, and proline) may regulate embryonic and fetal muscle growth and development (Wu et al., 2008; Gao et al., 2009c,d) via cell signaling through

Table 2. Concentrations of members of the arginine family of AA and activities of arginine metabolic enzymes in skeletal muscle of fetal pigs at d 60 of gestation[1]

Variable	NIUG	IUGR	SEM
Gastrocnemius muscle, mg	256	201*	7.2
Arginine, nmol/g of tissue	438	356*	9.5
Proline, nmol/g of tissue	520	423*	13
Ornithine, nmol/g of tissue	84	65*	4.1
Glutamine, μmol/g of tissue	4.37	3.69*	0.27
Putrescine, nmol/g of tissue	126	81*	5.8
Spermidine, nmol/g of tissue	191	128*	7.6
Spermine, nmol/g of tissue	225	174*	8.2
Tetrahydrobiopterin, nmol/g of tissue	1.20	0.77*	0.06
ODC[2] activity, nmol/min per gram of tissue	2.57	1.85*	0.14
cNOS[3] activity, nmol/min per gram of tissue	1.34	1.02*	0.08

[1]Data are means with pooled SEM (n = 6). Gilts (Yorkshire × Landrace dams and Duroc × Hampshire sire) were fed 2 kg daily of a corn- and soybean meal-based diet (Table 1) between d 0 and 60 of gestation. Fetal weights at d 60 of gestation were 126 and 94 g (SEM = 4.5 g), respectively, in the normal intrauterine growth (NIUG) and intrauterine growth retardation (IUGR) groups. Amino acids, polyamines, and tetrahydrobiopterin, as well as enzyme activities in skeletal muscle were determined using established methods (Meininger and Wu, 2002; Wu et al., 2005; Wu and Meininger, 2008).

[2]ODC = ornithine decarboxylase.

[3]cNOS = constitutive nitric oxide synthase.

*$P < 0.05$ vs. the NIUG group, as analyzed by *t*-test.

the mammalian target of rapamycin (now known as FKBP12-rapamycin complex-associated protein 1; Liao et al., 2008). In addition, arginine promotes the growth of skeletal muscle and reduces accretion of white adipose tissue (Jobgen et al., 2009b; Nall et al., 2009). Most important, dietary supplementation with arginine during early gestation increased the ratio of secondary to primary muscle fibers in fetal pigs at d 70 of pregnancy (Berard et al., 2009). Conversely, decreased availability of arginine and NO increases the proliferation of preadipocytes and adipocytes in IUGR fetuses (Figure 2). Thus, arginine regulates nutrient partitioning to promote skeletal muscle growth over white-fat accretion.

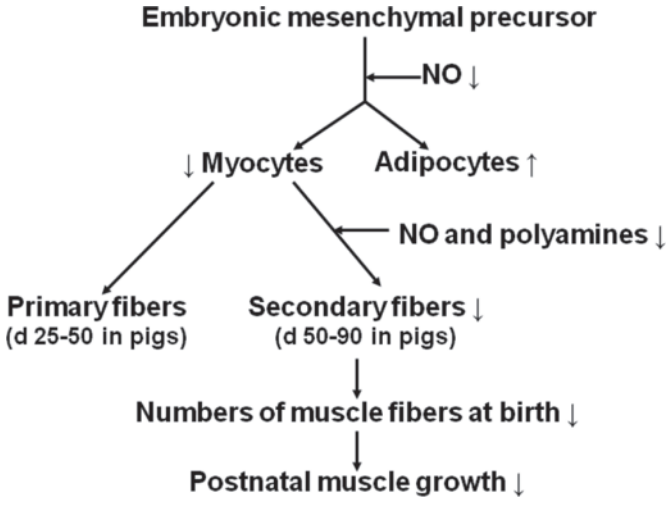

Figure 2. A possible role for nitric oxide (NO) and polyamines in the development of skeletal muscle cells and adipocytes. The embryonic mesenchymal precursor is differentiated into myocytes and adipocytes under the influence of NO. Development of myocytes into secondary fibers is affected by NO and polyamines (products of arginine). Secondary fibers further develop into skeletal muscle cells at birth. Deficiency of NO synthase during pregnancy favors formation of white adipocytes but impairs development of muscle cells.

IMPROVEMENT OF PREGNANCY OUTCOME BY FAA

As noted previously, the naturally occurring inability of placentae to supply an adequate amount of nutrients to fetuses in pigs is exacerbated further by the current widespread practice in the swine industry of restricted feeding programs to prevent excessive BW gain by gilts and sows during gestation (Kim et al., 2009). Although such a feeding regimen also can ameliorate farrowing difficulties and appetite reduction during lactation, gilts and sows cannot receive sufficient amounts of dietary AA to support optimal embryonic and fetal survival and growth during early to late gestation (i.e., d 14 to 114; Mateo et al., 2007; Berard et al., 2009; Kim et al., 2009). Of particular interest, the current feeding program for gestating swine results in inadequate provision of arginine from mother to fetuses (Wu et al., 1999). Unfortunately, the current version of NRC (1998) recommends little or no requirement for dietary arginine by gestating gilts or sows (Table 3).

Because of extensive catabolism of arginine by arginase in the small intestine (Bergen and Wu, 2009), only 60% of dietary arginine enters the portal circulation of pregnant gilts (Wu et al., 2007). Therefore, increasing the dietary provision of arginine beyond that from a typical corn- and soybean meal-based diet may be an effective means to enhance circulating concentrations and improve pregnancy outcomes in pigs. Several lines of experimental evidence support this hypothesis. First, dietary supplementation with 1.0% arginine-HCl between d 30 and 114 of gestation increased the number of live-born piglets by 2 and the litter birth

weight by 24% (Mateo et al., 2007). The arginine-to-lysine ratio in the supplemental diet was 2.64 (Table 3), which did not affect intestinal absorption of lysine or histidine (Mateo et al., 2007). An arginine-to-lysine ratio of greater than 3:1 in the diet would likely result in antagonism among basic AA and should be avoided in dietary formulation (Mateo et al., 2008; Wu, 2009). Second, dietary supplementation with 1% arginine to gilts or sows between d 14 and 28 of gestation increased the number of live-born piglets by approximately 1 at birth (Ramaekers et al., 2006; Campbell, 2009). Third, supplementation with 1% arginine between d 14 and 28 of gestation increased the number of fetuses on d 70 by 3 per litter (Berard et al., 2009). Similarly, dietary supplementation with arginine during early or midgestation increased embryonic survival and litter size in rats (Zeng et al., 2008).

Arginine may cooperate with other FAA to further improve the reproductive performance of pigs. One of the FAA is glutamine, because its uptake by the uterus of gestating gilts is the greatest among all the AA (Wu et al., 1999) and because it is an abundant AA in both uterine (Gao et al., 2009e) and fetal (Wu et al., 1995, 1996) fluids. Thus, glutamine may play an important role in fetal nutrition and growth. This hypothesis led us to develop an arginine-glutamine mixture for feeding to gestating swine (Table 3). The rationale for supplementation with both AA is that arginine and glutamine regulate protein synthesis by activating 1) the production of polyamines, which are essential for gene expression and mRNA translation, and 2) the mammalian target of rapamycin signaling pathway (Figure 3). We found that adding 0.6% glutamine and 0.4% arginine to a corn- and soybean meal-based diet prevented the decline in glutamine concentrations in gilt plasma (Table 4) that occurred in response to dietary supplementation with only arginine between d 30 and 114 of gestation (Mateo et al., 2007). This modified diet markedly reduced 1) concentrations of ammonia (–29%) and urea (–27%) in maternal plasma (the reduction in both ammonia and urea is an indicator of improved efficiency of utilization of dietary protein and AA); 2) variation in birth weights among either all piglets born (–27%) or live-born piglets (–24%; Table 4); and 3) the proportion of piglets with birth weights of 0.6 to 1.29 kg (–23% for all piglets born and –22% for live-born piglets; Table 5). It is important to note that dietary supplementation with arginine plus glutamine increased 1) the number of live-born piglets by 1.4 per litter; 2) litter birth weight for either all piglets born (+10%) or live-born piglets (+15%), and 3) the proportion of piglets with birth weights of 1.3 to 1.49 kg (+37% for all piglets born and +30% for live-born piglets; Tables 4 and 5). The proportion of piglets with greater birth weights (1.5 to 1.69 kg or 1.7 to 2.09 kg) did not differ between control and arginine plus glutamine-supplemented gilts (Table 5). Taken together, these results support an important role for FAA in improving pregnancy outcomes in pigs.

Table 3. National Research Council-recommended requirements of DM, CP, energy, and AA for gestating gilts and use of an arginine-supplemented diet to improve litter size and fetal growth[1]

Nutrient	NRC value[2]	Arginine diet[3]
DM, % of diet	90.0	89.3
CP, % of diet	12.8	12.2
ME, kcal/kg	3,265	3,110
AA, % of diet		
EAA[4]	2.97	4.66
Histidine	0.18	0.33
Isoleucine	0.32	0.51
Leucine	0.49	1.17
Lysine	0.57	0.58
Methionine	0.15	0.18
Phenylalanine	0.32	0.62
Threonine	0.45	0.49
Tryptophan	0.11	0.13
Valine	0.38	0.65
NEAA[5]	0.48	8.70
Alanine	0.00	0.78
Arginine	0.03	1.53
Asparagine	0.00	0.58
Aspartate	0.00	0.76
Cysteine	0.23	0.23
Glutamate	0.00	1.07
Glutamine	0.00	1.22
Glycine	0.00	0.55
Proline	0.00	1.03
Serine	0.00	0.50
Tyrosine	0.22	0.45

[1]All values are expressed on an as-fed basis.

[2]NRC (1998) for gilts weighing 150 kg at breeding and gaining 45 kg during the entire period of gestation. NRC-recommended requirements of arginine were 0.06 and 0.00% of the diet, respectively, for sows with BW of 125 and 175 kg at breeding.

[3]From Mateo et al. (2007). The BW of gilts at breeding was 155 kg. A corn- and soybean meal-based diet (containing 0.70% arginine) was supplemented with 1.0% L-arginine-HCl (equivalent to 0.83% L-arginine) between d 30 and 114 of gestation. Asparagine plus aspartate and glutamine plus glutamate in the arginine diet were analyzed by HPLC after acid hydrolysis (Wu et al., 1999). The ratios of asparagine:aspartate and glutamine:glutamate in the diet were determined using a bioassay method, which involved incubation of 50 mg of a finely ground sample or 50 mg of water (blank) with 2.5 mL of porcine gastric fluid (2 h at 37°C) and, after neutralization, with 5 mL of porcine small-intestinal luminal fluid (4 h at 37°C). Porcine gastric and small-intestinal luminal fluids were obtained from 12-h food-deprived 35-d-old pigs weaned at 21 d of age. Molecular weights of intact AA were used for the calculation of AA content in the diet.

[4]EAA = nutritionally essential AA.

[5]NEAA = nutritionally nonessential AA.

At present, little is known about the effects of supplementation with arginine or other FAA on conception rates or early embryo survival in swine. There is evidence that interactions between stage of gestation and dose of arginine supplementation have critical effects on embryonic and fetal survival (De Blasio et al., 2009). The underlying mechanisms may involve alterations in 1) the development or function of corpora lutea; 2) the production of progesterone (X. L. Li, F. W. Bazer, G. A. Johnson, and G. Wu, unpublished data), a major hormone for maintaining pregnancy in mammals (Bazer et al., 2008); 3) NO signaling (Li et

Table 4. Concentrations of AA in maternal plasma and reproductive performance of gilts fed diets without or supplemented with functional AA (FAA)[1]

Variable	Control[2] (n = 32)	FAA[3] (n = 30)	Pooled SEM
Arginine concentrations in maternal plasma, μM	202	285*	10
Glutamine concentrations in maternal plasma, μM	380	392	18
Proline concentrations in maternal plasma, μM	283	334*	15
Ornithine concentrations in maternal plasma, μM	81	133*	6.7
Lysine concentrations in maternal plasma, μM	128	126	5.5
Ammonia concentrations in maternal plasma, μM	76	54*	4.2
Urea concentrations in maternal plasma, mM	2.09	1.52*	0.12
Total piglets born per litter, No.	11.03	11.90*	0.40
Total piglets born alive per litter, No.	9.91	11.33*	0.33
Average birth wt of all piglets born, kg	1.35	1.36	0.02
Average birth wt of all piglets born alive, kg	1.37	1.37	0.02
Total litter wt at birth for all piglets born, kg	14.6	16.0*	0.36
Total litter wt at birth for all live piglets, kg	13.4	15.4*	0.32
Piglets born dead per litter, No.	1.13	0.57*	0.12
Variation in birth wt among all piglets born,[4] %	17.3	12.7*	0.58
Variation in birth wt among all piglets born alive,[4] %	15.1	11.5*	0.53

[1]Data are means with pooled SEM. Supplemental AA were products of Ajinomoto Co. Inc. (Tokyo, Japan). All pregnant gilts (Yorkshire × Landrace dams and Duroc × Hampshire sire) were fed 2 kg daily of a corn- and soybean meal-based diet (Mateo et al., 2007) in 2 equal meals at 0700 and 1800 h. The composition of AA in the basal diet (containing 0.70% arginine and 1.22% glutamine on an as-fed basis) is given in Table 3. The BW of gilts at breeding was 114 ± 1.6 kg, n = 62). Blood samples (approximately 0.1 mL) were obtained from the ear vein of gilts at d 110 of gestation at 2 h after feeding for analysis of metabolites in plasma (Jobgen et al., 2008). Duration of gestation did not differ ($P > 0.05$) between control and FAA-supplemented gilts (114 ± 0.2 d, n = 62).

[2]L-Alanine (31 g; isonitrogenous control) was added to the 2-kg basal diet as top dressing between d 30 and 114 of gestation.

[3]A mixture of 8 g of L-arginine and 12 g of L-glutamine was added to the 2-kg basal diet as top dressing between d 30 and 114 of gestation. This FAA diet contained 1.1% arginine and 1.8% glutamine.

[4]CV (SD/mean × 100%).

*$P < 0.05$ vs. the control group, as analyzed by unpaired t-test.

al., 2009b); and 4) cellular redox state (Jobgen et al., 2009a). Without doubt, an increase in the number of live-born pigs will markedly reduce production costs associated with reproduction and lactation in dams. Additionally, a reduction in the number of IUGR piglets will greatly improve the management of neonatal pigs and maximize preweaning survival and growth. Our findings provide a compelling basis for revising the current NRC (1998)-recommended requirements for AA, including arginine, glutamine, and proline, of gestating gilts and sows.

Conclusion and Perspectives

Placental insufficiency is a major factor contributing to IUGR in pigs (Vallet et al., 2002). The underlying mechanisms are likely complex but may include inadequate or disproportionate amounts of AA available to the conceptus. Multiple and interacting signal transduction pathways in the conceptus are crucial for control of cell attachment and migration (key events in the initiation of implantation), as well as cellular growth and proliferation through regulation of intracellular protein turnover. Several of these pathways are regulated by AA, their metabolites (including NO, polyamines, and glutathione), or both. Thus, dietary supplementation with arginine to gilts or sows increases litter size and litter birth weight, and its combination with other FAA (e.g., glutamine, leucine, and proline) can reduce variation in birth weights of piglets. To date, the biochemical mechanisms responsible for the effects of AA are

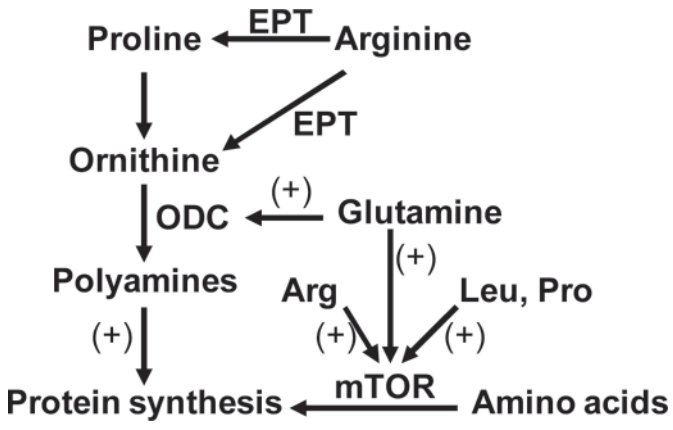

Figure 3. An important role for functional AA in regulating protein synthesis in porcine placentae. Polyamines are key regulators of DNA and protein synthesis in cells. Arginine is metabolized to ornithine and proline in extraplacental tissues (EPT). In placentae, proline is degraded to ornithine, which is utilized for polyamine synthesis via ornithine decarboxylase (ODC). Expression of ODC is stimulated by glutamine. In addition, protein synthesis is regulated by the mammalian target of rapamycin (mTOR) signaling pathway, which is activated by arginine, glutamine, leucine, and proline. Selective activation of the machinery for polyamine and protein syntheses by functional AA may provide a novel and effective mechanism to enhance placental growth and development. Arg = L-arginine; Leu = L-leucine; Pro = L-proline.

Table 5. Distribution of piglet birth weights in gilts fed diets without or supplemented with functional AA (FAA)[1]

	All piglets born			Piglets born alive		
Item	Control[2]	FAA[3]	SEM	Control	FAA	SEM
Birth wt, kg						
0.60 to 1.09 kg	0.98 (83)	1.01 (60)	0.01	1.01 (59)	1.01 (49)	0.01
1.10 to 1.29 kg	1.24 (106)	1.23 (87)	0.01	1.24 (98)	1.23 (83)	0.01
1.29 to 1.49 kg	1.46 (98)	1.39 (136)	0.01	1.46 (96)	1.39 (134)	0.01
1.50 to 1.69 kg	1.65 (54)	1.58 (58)	0.01	1.65 (52)	1.58 (58)	0.01
1.70 to 2.09 kg	1.89 (12)	1.80 (16)	0.02	1.89 (12)	1.80 (16)	0.02
Proportion of piglets in each birth wt range, %						
0.60 to 1.09 kg	23.5	16.8†		18.6	14.4†	
1.10 to 1.29 kg	30.0	24.4†		30.9	24.4†	
1.30 to 1.49 kg	27.8	38.1†		30.3	39.4†	
1.50 to 1.69 kg	15.3	16.2		16.4	17.1	
1.70 to 2.09 kg	3.4	4.5		3.8	4.7	

[1]Data are means with pooled SEM. The values in parentheses represent the numbers of piglets. Supplemental AA were products of Ajinomoto Co. Inc. (Tokyo, Japan). All pregnant gilts (Yorkshire × Landrace dams and Duroc × Hampshire sire) were fed 2 kg daily of a corn- and soybean meal-based diet (Mateo et al., 2007) in 2 equal meals at 0700 and 1800 h. The composition of AA in the basal diet (containing 0.70% arginine and 1.22% glutamine on an as-fed basis) is given in Table 3. Body weight of gilts at breeding was 114 ± 1.6 kg (n = 62). Duration of gestation did not differ ($P > 0.05$) between control and FAA-supplemented gilts (114 ± 0.2 d, n = 62).

[2]L-Alanine (31 g; isonitrogenous control) was added to the 2-kg basal diet as top dressing between d 30 and 114 of gestation.

[3]A mixture of 8 g L-arginine and 12 g L-glutamine was added to the 2-kg basal diet as top dressing between d 30 and 114 of gestation. This FAA diet contained 1.1% arginine and 1.8% glutamine.

†$P < 0.05$ vs. the control, as analyzed by χ^2 analysis.

largely unknown and are being studied primarily by standard low-output methods, such as analysis of metabolites, reverse transcription-PCR, and Western blot analysis (Hu et al., 2008a; Phang et al., 2008; Haynes et al., 2009; Yin et al., 2009). The recent development of high-output approaches (e.g., genomics, epigenomics, proteomics, and metabolomics) is transforming nutrition research (Hu et al., 2008b; Yan and He, 2008; He et al., 2009; Wang et al., 2009a,b). These powerful discovery tools can be used to rapidly advance our understanding of how dietary AA regulate gene and protein expression in the conceptus via cellular and molecular mechanisms, including epigenetics, to affect embryonic and fetal survival as well as placental and fetal growth. This new knowledge will greatly facilitate translation of basic research on AA biochemistry and physiology into feeding practice to further improve the reproductive performance of swine.

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