Maternal and fetal amino acid metabolism in gestating sows

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Among livestock species, swine exhibit the most severe naturally-occurring intra-uterine growth restriction (IUGR) primarily due to a reduction in net protein synthesis. Thus, new knowledge about fetal metabolism of amino acids (AA), which are building blocks for proteins and regulators of intracellular protein turnover, can provide a solution to this problem. Among all AA, requirements of glutamate and glutamine by fetal pigs are quantitatively the highest, but cannot be met through uterine uptake alone. Nearly all glutamate and ~70% glutamine in diets for gestating swine are degraded in the maternal small intestine and, therefore, do not enter the portal circulation. This necessitates interorgan AA metabolism involving maternal skeletal muscle, placenta, and fetal skeletal muscle to synthesize glutamate and glutamine from branched-chain AA, as well as storage of glutamate and glutamine in allantoic and amniotic fluids. The porcine placenta does not degrade arginine or glutamine, leading to their maximal transfer from maternal to fetal blood. Therefore, maternal sources of ornithine and proline play a major role in the placental synthesis of polyamines needed for placental growth including placental vascular growth. Likewise, during late gestation, uterine uptake of arginine, proline and aspartate/asparagine cannot meet requirements for optimal fetal growth. To provide sufficient arginine, the fetal small intestine synthesizes citrulline and arginine from glutamate and glutamine, and fetal kidneys convert citrulline into arginine. Collectively, glutamine and arginine are major sources of AA nitrogen transferred between mother and fetus. Results of recent studies indicate that dietary supplementation with these two AA can ameliorate IUGR in swine. These findings greatly advance the field of maternal-fetal AA metabolism in pigs, but also have important implications for improving reproductive efficiency in swine production worldwide.

Introduction

Domestic pigs have no brown adipose tissue (Satterfield & Wu 2011), but possess a high capacity for synthesizing triacylglycerides in white adipose tissue after birth (Smith et al. 1999). Excess white fat in the body causes insulin resistance, metabolic disorders, and impaired lactation performance in animals, including swine (Jobgen et al. 2006; Wu 2010a). Thus, during gestation, gilts or multiparous sows are usually fed a substantially reduced amount of

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diet (e.g., 2 to 2.2 kg/day that is approximately 50% of their ad libitum feed consumption) to reduce energy intake and prevent females from becoming overweight (Ashworth 1991; Kim et al. 2009; Pond et al. 1969, 1981). This strategy, however, results in inadequate provision of dietary amino acids (AA) to both mother and fetus (Wu et al. 2010). Amino acids are the building blocks for proteins in cells and, importantly, they are also precursors for synthesis of glucose and nitrogenous substances [e.g., nitric oxide (NO), polyamines, creatine, dopamine, thyroid hormones, and catecholamines] essential for whole body homeostasis (Wu 2009). Thus, AA have nutritional, physiological, and regulatory roles in animals (Fig. 1).

Current feeding programs for gestating swine remain suboptimal due, in part, to inadequate knowledge about maternal and fetal AA nutrition (Kim et al. 2009). Thus, among livestock species, swine suffer the greatest prenatal loss (up to 50%) and exhibit the most severe naturally-occurring intra-uterine growth restriction (IUGR) primarily because of a reduction in net protein synthesis (Vallet et al. 2002; Wu et al. 2006). This provides an impetus to develop novel and effective strategies to improve pregnancy outcome in pigs. The major objectives of this article are to highlight maternal-fetal AA metabolism and nutrition in gestating swine. Readers are referred to the work of Bazer et al. (2013) for recent advances in the roles of AA in regulating embryonic growth, survival and development during early pregnancy.

Sources of AA for the fetus

The porcine fetus receives AA from its mother via placental transport and can synthesize some AA, including alanine, arginine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, proline, serine, and tyrosine (Wu et al. 2010). Fetal blood, which contains relatively high concentrations of glutamine and glycine, is the parenteral source of all AA for the fetus. In addition, allantoic fluid, which is derived from both maternal and fetal sources, is a reservoir of AA for the fetus and contains unusually high concentrations of arginine (e.g., 4 to 6 mM)

![Fig. 1 Nutritional, physiological and regulatory roles of AA in animals. Reprinted from Wu (2010) with permission from American Society of Nutrition.](image-url)
and glycine (4 to 5 mM) during early and late gestation, respectively (Table 1). Nutrients in the allantoic fluid are transported through the allantoic epithelium, enter the fetal-placental circulation, and are utilized by fetal-placental tissues (Figure 2). During early pregnancy, marked increases in concentrations of each AA in allantoic fluid are not accounted for by changes in allantoic fluid volume (Table 1) or by changes in concentrations of AA in maternal and fetal plasma (Wu et al. 1995). AA may play an important role in regulating osmolality in allantoic fluid.

Amniotic fluid is derived from both the fetus (kidneys, lungs, epidermis, and fetal blood vessels in the placenta and umbilical cord) and the mother (blood vessels via amniotic membranes) (Schmidt 1992). This fluid is removed by both the fetus and the mother through the same

Table 1. Amino acid composition in the body and allantoic fluid of fetal pigs

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>AA composition in fetal pigs on different days of gestation (mg AA/g wet weight)</th>
<th>AA composition in allantoic fluid of fetal pigs on different days of gestation (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40  60  90  110  114</td>
<td>30  40  60  90  110</td>
</tr>
<tr>
<td>Alanine</td>
<td>3.63 3.01 4.26 5.87 6.46</td>
<td>233 281 92 396 669</td>
</tr>
<tr>
<td>Arginine</td>
<td>3.77 3.24 4.58 5.85 6.47</td>
<td>185 4103 642 128 465</td>
</tr>
<tr>
<td>Aspartate</td>
<td>2.90 2.34 3.01 3.92 4.25</td>
<td>16 164 85 170 195</td>
</tr>
<tr>
<td>Asparagine</td>
<td>2.41 1.94 2.50 3.25 3.53</td>
<td>61 280 56 83 139</td>
</tr>
<tr>
<td>Citrulline</td>
<td>0.023 0.024 0.032 0.046 0.054</td>
<td>10 72 46 96 30</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.78 0.67 0.92 1.19 1.28</td>
<td>70 220 308 230 604</td>
</tr>
<tr>
<td>Glutamate</td>
<td>4.94 4.21 5.34 6.81 7.72</td>
<td>112 2383 609 1182 1056</td>
</tr>
<tr>
<td>Glutamine</td>
<td>3.19 2.72 3.44 4.39 4.98</td>
<td>638 3442 273 652 701</td>
</tr>
<tr>
<td>Glycine</td>
<td>3.69 3.60 6.53 9.23 10.8</td>
<td>803 360 195 4227 3054</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.54 1.05 1.42 1.85 2.07</td>
<td>76 687 61 73 78</td>
</tr>
<tr>
<td>Hydroxyproline</td>
<td>0.50 0.91 2.08 3.01 3.48</td>
<td>61 49 28 41 82</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>2.18 1.71 2.08 2.61 2.89</td>
<td>28 80 31 10 13</td>
</tr>
<tr>
<td>Leucine</td>
<td>5.04 3.83 4.82 6.13 6.75</td>
<td>63 153 38 14 33</td>
</tr>
<tr>
<td>Lysine</td>
<td>5.15 3.42 4.14 5.19 5.77</td>
<td>362 1950 390 214 617</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.39 1.05 1.34 1.71 1.86</td>
<td>17 31 16 15 273</td>
</tr>
<tr>
<td>Ornithine</td>
<td>0.08 0.075 0.09 0.12 0.13</td>
<td>124 2524 645 76 85</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>2.78 2.03 2.52 3.14 3.44</td>
<td>36 40 34 195 51</td>
</tr>
<tr>
<td>Proline</td>
<td>3.68 4.07 5.42 7.06 7.82</td>
<td>261 144 46 117 336</td>
</tr>
<tr>
<td>Serine</td>
<td>3.05 2.35 2.96 3.98 4.33</td>
<td>553 902 79 603 1218</td>
</tr>
<tr>
<td>Taurine</td>
<td>0.28 0.23 0.28 0.36 0.38</td>
<td>457 641 298 450 658</td>
</tr>
<tr>
<td>Threonine</td>
<td>2.52 1.90 2.42 2.95 3.24</td>
<td>218 1780 89 343 545</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.72 0.61 0.82 1.03 1.11</td>
<td>13 53 69 164 574</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>2.13 1.58 1.85 2.27 2.43</td>
<td>45 70 22 42 39</td>
</tr>
<tr>
<td>Valine</td>
<td>3.40 2.41 3.13 3.88 4.21</td>
<td>80 163 66 57 144</td>
</tr>
</tbody>
</table>

1Adapted from Wu et al. (1999).
3Traditionally classified as “nutritionally essential AA” (EAA).
4Traditionally classified as “nutritionally nonessential AA” (NEAA).
5Currently classified as “conditionally essential AA” (CEAA; Wu 2013).
channels, along with the participation of the fetal intestine following swallowing. Thus, with the development of intestinal amino acid transport systems during gestation, the drinking of amniotic fluid provides the enteral source of AA for fetal utilization. The nutritional significance of amniotic fluid is graphically illustrated by the finding that esophageal ligation, which prevents the entry of this fluid into the small intestine, results in IUGR in the fetus (Trahair & Harding 1995). The amniotic fluid provides a large amount of glutamine for supporting growth and development of the fetal small intestine (Dekaney et al. 2003). Likewise, in gestating gilts fed a low-protein (0.5% crude protein) diet, reduced concentrations of glutamine in amniotic fluid are associated with IUGR between d 0 and 60 of gestation (Wu et al. 1998a,b).

Based on growth or nitrogen balance, AA were traditionally classified as nutritionally essential or nonessential (Wu 2010b). Nutritionally essential AA (EAA) were defined as those AA whose carbon skeletons are not synthesized by animals, whereas nutritionally nonessential AA (NEAA) as those AA which are synthesized de novo in adequate amounts by animals to meet requirements for maintenance, growth, development and health and, therefore, need not be provided in the diet. However, EAA and NEAA are only categorizations according to definitions, and physiological functions of AA should be taken into consideration in formulating diets for animals. In fetal pigs, concentrations of free NEAA, the content of NEAA in proteins, and, therefore, requirements of NEAA for tissue protein synthesis are greater than those of EAA at all gestational stages (Table 3). To date, there is no compelling experimental evidence for sufficient synthesis of NEAA by swine or other animals to meet requirements for maximum growth or optimal health (Wu et al. 2013). Thus, in recent years, some NEAA have been reclassified as conditionally essential AA (CEAA), which are defined as those AA that normally can be synthesized in adequate amounts by animals, but which must be provided in the diet to meet optimal needs under certain conditions (e.g., gestation, lactation, and weaning) wherein rates of utilization are greater than rates of synthesis (Wu 2009).

**Limitations to uterine capacity in pigs**

Fetal growth and survival in mammals is affected by complex factors, including uterine capacity and environment, including AA nutrition (Ashworth et al. 2001; Wu et al. 2006).
pigs, inadequate uterine capacity is a major factor contributing to suboptimal fetal growth and survival after d 25 of gestation (Bazer et al., 2009). This problem is even more severe in modern highly prolific breeds than in breeds used in the swine industry 30 yr ago due to selection for increased litter size (Vallet et al. 2009). For example, IUGR piglets (< 1.10 kg birth weights) can account for up to 25% of total piglets born and represent 76% of preweaning deaths in pigs (Wu et al. 2010). At present, there is no nutritional support to increase growth and survival of IUGR piglets during postnatal periods to compensate for their reduced birth weight and, therefore, they are often culled on farms (Vallet & Freking 2006).

The gestating pig develops a noninvasive, diffuse type of epitheliochorial placentae, whose weights, lengths, and surface area vary greatly among conceptuses within the same uterus (Bazer et al. 2008). Immediately after implantation of the conceptus, various genes are expressed in the trophectoderm to initiate placental formation (Bazer et al. 2009; Vonnahme & Ford 2004). Implantation begins around d 14 of gestation and is completed around d 18 in pigs, followed by placentation (Geisert & Yelich 1997). The placenta undergoes rapid formation of new blood vessels (i.e., angiogenesis) and marked growth during pregnancy (Reynolds & Redmer 2001). Thus, blood vessels are clearly visible in porcine placentae and allantoic membranes on d 25 of pregnancy (Li et al. 2010). Notably, the porcine placenta grows rapidly between d 20 and 60 of gestation and its development is nearly maximal by d 70 (Knight et al. 1977; Wu et al. 2005), a period preceding rapid fetal growth (Table 2). Placental angiogenesis is necessary to increase blood flow from the placenta to the fetus that supplies nutrients from mother to fetus (Bazer et al. 1988; Ford et al. 2002; Reynolds et al. 2006). The functional capacity of the uterus and the placenta for provision of nutrients and oxygen from mother to fetus is vital to fetal survival, growth and development (Wu et al. 2004; Bazer et al. 2008).

**Uterine uptake of AA for fetal growth in gestating pigs**

To assess the availability of AA present in maternal blood to the placenta, we previously determined uterine uptake of AA in gestating gilts on d 110–114 of pregnancy based on uterine arteriovenous concentrations and the rate of uterine blood flow (243 mL/min/fetus). All AA, except for γ-aminobutyric acid, which is negligible (< 0.1 μM) in uterine arterial and venous plasma, are taken up by the uterus of pregnant gilts (Table 3). Uterine uptake of glutamine is greatest, followed by glycine, proline, leucine, alanine, lysine, and arginine, in decreasing order. However, uterine uptake of aspartate/asparagine and glutamate represents only 9 and 29% of fetal accretion, respectively. Note that glycine, which has versatile physiological functions (Wang et al. 2013), has the highest rate of accretion in fetal tissue proteins among all AA (Table 3). Because AA are oxidized by the fetus, most of the NEAA and CEAA (including glutamine, glutamate, aspartate, glycine, arginine, and proline) taken up by the uterus are likely to be insufficient for fetal protein synthesis. Because hydroxyproline is not utilized for protein

<table>
<thead>
<tr>
<th>Variable</th>
<th>18</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>60</th>
<th>90</th>
<th>110</th>
<th>114</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placental weight, g</td>
<td>---</td>
<td>0.22</td>
<td>33</td>
<td>59</td>
<td>182</td>
<td>208</td>
<td>237</td>
<td>225</td>
</tr>
<tr>
<td>Fetal weight, g</td>
<td>0.030</td>
<td>0.063</td>
<td>1.7</td>
<td>11</td>
<td>130</td>
<td>596</td>
<td>1176</td>
<td>1486</td>
</tr>
<tr>
<td>Allantoic fluid, ml</td>
<td>1.0</td>
<td>4.1</td>
<td>227</td>
<td>74</td>
<td>347</td>
<td>83</td>
<td>56</td>
<td>29</td>
</tr>
<tr>
<td>Amniotic fluid, ml</td>
<td>0.02</td>
<td>0.06</td>
<td>2.2</td>
<td>12.5</td>
<td>119</td>
<td>127</td>
<td>81</td>
<td>32</td>
</tr>
</tbody>
</table>

Adapted from Bazer 1988 and Wu et al. (2005).
synthesis, peptide-bound hydroxyproline in fetal pigs is derived from proline. Pathways for AA synthesis via inter-organ cooperation in mammals including pigs are now largely known (Wu 2013) and are illustrated in Figure 3. For example, the maternal and fetal skeletal muscle, as well as the placenta of gestating swine synthesize glutamine and glutamate from branched-chain AA and \( \alpha \)-ketoglutarate. In both the sow and her fetuses, the small intestine converts arterial glutamine as well as glutamine, glutamate, and proline within its lumen into citrulline and arginine. The citrulline and ornithine transported from the maternal blood is used by almost

<table>
<thead>
<tr>
<th>AA</th>
<th>AA content in maternal diet (% as-fed basis)</th>
<th>AA intake from 2 kg diet by gilt (g/day/gilt)</th>
<th>Entry of dietary AA into portal vein (g/day/gilt)</th>
<th>Uterine uptake of AA (mg/day/fetus)</th>
<th>AA accretion in fetal pig (mg/day/fetus)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>0.78</td>
<td>15.6</td>
<td>11.7</td>
<td>891</td>
<td>723</td>
</tr>
<tr>
<td>Arginine</td>
<td>0.70</td>
<td>14.0</td>
<td>7.22</td>
<td>749</td>
<td>732</td>
</tr>
<tr>
<td>Aspartate</td>
<td>0.76</td>
<td>15.2</td>
<td>0.65</td>
<td>76</td>
<td>461</td>
</tr>
<tr>
<td>Asparagine</td>
<td>0.58</td>
<td>11.6</td>
<td>8.68</td>
<td>264</td>
<td>382</td>
</tr>
<tr>
<td>Citrulline</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>388</td>
<td>7.0</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.23</td>
<td>4.6</td>
<td>3.28</td>
<td>202</td>
<td>136</td>
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<tr>
<td>Glutamate</td>
<td>1.07</td>
<td>21.4</td>
<td>0.74</td>
<td>420</td>
<td>922</td>
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<tr>
<td>Glutamine</td>
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<td>24.4</td>
<td>6.92</td>
<td>2171</td>
<td>595</td>
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<tr>
<td>Glycine</td>
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<td>11.0</td>
<td>7.76</td>
<td>1617</td>
<td>1375</td>
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<tr>
<td>Histidine</td>
<td>0.33</td>
<td>6.6</td>
<td>4.54</td>
<td>281</td>
<td>240</td>
</tr>
<tr>
<td>Hydroxyproline</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>119</td>
<td>434</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.51</td>
<td>10.2</td>
<td>5.70</td>
<td>416</td>
<td>328</td>
</tr>
<tr>
<td>Leucine</td>
<td>1.17</td>
<td>23.4</td>
<td>13.3</td>
<td>1031</td>
<td>756</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.58</td>
<td>11.6</td>
<td>7.48</td>
<td>762</td>
<td>661</td>
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<tr>
<td>Methionine</td>
<td>0.18</td>
<td>3.6</td>
<td>2.45</td>
<td>245</td>
<td>202</td>
</tr>
<tr>
<td>Ornithine</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>218</td>
<td>14.5</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.62</td>
<td>12.4</td>
<td>8.32</td>
<td>452</td>
<td>381</td>
</tr>
<tr>
<td>Proline</td>
<td>1.03</td>
<td>20.6</td>
<td>10.8</td>
<td>1179</td>
<td>887</td>
</tr>
<tr>
<td>Serine</td>
<td>0.50</td>
<td>10.0</td>
<td>7.14</td>
<td>575</td>
<td>471</td>
</tr>
<tr>
<td>Taurine</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>122</td>
<td>38.3</td>
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<tr>
<td>Threonine</td>
<td>0.49</td>
<td>9.8</td>
<td>6.07</td>
<td>482</td>
<td>361</td>
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<tr>
<td>Tryptophan</td>
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<td>2.6</td>
<td>1.81</td>
<td>204</td>
<td>137</td>
</tr>
<tr>
<td>Tyrosine</td>
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<td>9.0</td>
<td>6.19</td>
<td>340</td>
<td>254</td>
</tr>
<tr>
<td>Valine</td>
<td>0.65</td>
<td>13.0</td>
<td>7.27</td>
<td>585</td>
<td>455</td>
</tr>
</tbody>
</table>

1 The diet was based on corn and soybean meal. Feed intake by each gilt was 2.0 kg per day during the entire period of gestation (Wu et al. 2010). The average litter size at d 110-114 of gestation was 10.0 fetuses per gilt.
2 Assuming true ileal digestibility of 86% for each AA.
3 Days 110-114 of gestation (Wu et al. 1999).

Values for the bioavailability (%) AA in the lumen of the small intestine to the portal vein are based on those for growing pigs (Wu et al. 2010): Ala, 87; Arg, 60; Asn, 87; Asp, 5; Cys, 83; Glu, 4; Gln, 33; Gly, 82; His, 80; Ile, 65; Leu, 66; Lys, 75; Met, 79; Phe, 78; Pro, 61; Ser, 83; Thr, 72; Trp, 81; Tyr, 80; and Val, 65). Products of intestinal AA metabolism that enter the portal vein are not included.
all cell types in the fetus for the synthesis of arginine and proline, respectively. At present, quantitative aspects of placental uptake of AA in gestating swine are unknown due to the lack of data on placental blood flow. However, the parity between uterine uptake and accretion of AA in fetal pigs is particularly large for glutamate and aspartate, and to a lesser extent, for proline and arginine. Therefore, these AA must be synthesized from branched-chain AA and glucose, and possibly from other AA by the placenta and/or the fetus.

Fetal to maternal ratios of glutamine and branched-chain AA in plasma are much greater and less than 1, respectively (Wu et al. 1995). For example, in the plasma of pigs, fetal/maternal ratios for glutamine are 3.87 and 2.88, respectively, at d 45 and 110 of gestation. Such disparate glutamine levels between mother and fetus suggest that glutamine is actively synthesized by the placenta, which then releases glutamine into the fetal circulation. In contrast, fetal/maternal ratios for branched-chain AA are less than 0.70 between d 45 and 110 of gestation, suggesting extensive catabolism of uterus-derived BCAA in the placenta. Results of both enzymological and metabolic studies indicate that branched-chain amino acids and glucose donate the amino group and the carbon skeleton, respectively, for the synthesis of glutamine by the porcine placenta (Self et al. 2004). Interestingly, there is no detectable catabolism of glutamine in the porcine placenta throughout pregnancy (Self et al. 2004), which ensures maximum output of glutamine by this tissue (Figure 3). These findings provide valuable insight into the dynamic role of the placenta in fetal metabolism and nutrition.

Utilization of AA for placental growth and development

The placenta uses both EAA and NEAA to synthesize proteins to support its growth and development. Protein synthesis is stimulated by polyamines (i.e., putrescine, spermidine, and spermine) (Wu et al. 2008). Unlike most other tissues which convert arginine to polyamines via arginase and ornithine decarboxylase, the porcine placenta lacks arginase and, therefore, cannot synthesize ornithine from arginine (Wu et al. 2005). This feature of AA catabolism helps maximize the transfer of arginine from maternal to fetal blood, leading to an unusual abundance of arginine in porcine allantoic fluid for supporting fetal growth (Wu et al. 1996). At present, it is unknown whether arginine can be metabolized in the placenta to generate putrescine via arginine decarboxylase and agmatinase (Bazer et al. 2013). Interestingly, the porcine placenta expresses a high activity of proline oxidase for oxidizing maternal blood-derived proline to form pyrroline-5-carboxylate, a precursor of ornithine and, therefore, polyamines (Figure 3). The synthesis of polyamines requires methionine as a donor of the methyl group via the formation of S-adenosylmethionine. In maternal tissues, proline is synthesized from arginine (Wu et al. 2011). Besides polyamines, NO (a product of arginine degradation by NO synthase) plays an important role in placental growth, including placental angiogenesis and vascular growth, as well as blood flow (Meininger & Wu 2002; Wu et al. 2009). Generation of NO from arginine is increased by some AA (e.g., taurine and citrulline) and is inhibited by others (e.g., lysine and homocysteine) (Wu & Meininger 2002). Thus, appropriate proportions and amounts of all AA, including EAA and NEAA, are important factors affecting NO synthesis and uterine capacity.

Besides serving as substrates for placental synthesis of protein and nitrogenous metabolites, AA (e.g., arginine, glutamine, and leucine) regulate these metabolic pathways via various cell signaling mechanisms, including phosphorylation of the mechanistic target of rapamycin (MTOR) protein (Jobgen et al. 2006; Li et al. 2009; Bazer et al. 2012). Using trophectoderm cells derived from conceptuses on day 12 of gestation, we found that addition of 100 and 350 \( \mu M \) arginine to culture medium increased the abundance of total and phosphorylated proteins for MTOR, ribosomal protein S6 kinase 1, and eukaryotic initiation factor 4E-binding protein-1.
Fig. 3 Amino acid metabolism in the porcine placenta. The enzymes catalyzing the indicated reactions are: 1, BCAA transaminase; 2, glutamate dehydrogenase; 3, pyrroline-5-carboxylate synthase; 4, pyrroline-5-carboxylate reductase; 5, aspartate transaminase; 6, alanine transaminase; 7, asparagine synthetase; 8, enzymes for synthesis of glycoproteins; 9, enzymes of glycolysis; 10, glutamine:fructose-6-phosphate transaminase; 11, phosphate-dependent glutaminase; 12, glutamine synthetase; 13, ornithine aminotransferase; 14, arginase; 15, proline oxidase; 16, enzymes of the pentose cycle; 17, fructose kinase; 18, enzymes converting glucose into fructose; 19, serine hydroxymethyl transferase; 20, enzymes for converting D-3-phosphoglycerate and glutamate into serine; 21, argininosuccinate synthase; 22, argininosuccinate lyase; 23, enzymes for GTP synthesis; 24, enzymes for converting GTP into tetrahydrobiopterin; 25, NO synthase; 26, branched-chain α-ketoacid dehydrogenase; 27, enzymes for converting glucose into α-KG; 28, enzymes for synthesis of nucleotides; 29, enzymes for DNA and RNA synthesis; 30, enzymes for protein synthesis; 31, polyamine synthesis via ornithine decarboxylase, spermidine synthase, and spermine synthase; 32, putrescine synthesis via arginine decarboxylase and agmatine synthase. BCAA = branched-chain amino acids; BCKA = branched-chain α-ketoacids; D3PG = D-3-phosphoglycerate; F6P = fructose-6-phosphate; GlcN-6-P = glucosamine-6-phosphate; HCy = homocysteine; α-KG = α-ketoglutarate; MTH = N<sup>5</sup>-N<sup>10</sup>-methylene tetrahydrofolate; OH-Pro = hydroxyproline; KGM = α-ketoglutaramate; OAA = oxaloacetate; P5C = pyrroline-5-carboxylate; THF = tetrahydrofolate. Adapted from Wu (2013). The porcine placenta contains no activity of phosphate-activated glutaminase (reaction 11) or arginase (reaction 14) (Self et al. 2004; Wu et al. 2005).

in a dose-dependent manner (Kong et al. 2012). Therefore, arginine at these concentrations stimulated protein synthesis and inhibited proteolysis, leading to enhanced proliferation of the cells (Kong et al. 2012). Inactivation of autophagy appears to be a major mechanism for AA to inhibit protein degradation in cells (Wu 2013). Interestingly, an inhibition of NO synthesis by 76% had only a modest effect on intracellular protein turnover in trophectoderm cells (e.g., a 13% decrease in protein synthesis and a 20% increase in proteolysis) (Kong et al. 2012). Thus,
arginine promotes growth of porcine placental cells largely via an NO-independent pathway. Results of recent studies indicate that glutamine and leucine also activate the MTOR pathway to stimulate protein synthesis in porcine and ovine trophectoderm cells (Kim et al. 2011a,b,c; 2012). Similar results have been reported for pregnant rats (Zheng et al. 2008; 2012).

Mechanisms for amino acids to regulate fetal muscle growth and development

Enhancing the availability of AA in the fetus can increase fetal growth through providing the building blocks and activating the MTOR signaling pathway to enhance muscle protein synthesis (Wu et al. 2010). Because myocytes and adipocytes are derived from a common mesenchymal precursor (Sordella et al. 2003), fetal skeletal muscle is formed maximally while differentiation of stem cells into preadipocytes is impaired in response to appropriate signals (e.g., transcription factors Myf5 and MyoD) that favor development of myocytes (Kablar et al. 2003). Thus, at birth, white adipose tissue in newborn pigs is only approximately 1%, in striking contrast to 40% muscle mass (Wu et al. 1999). There are two 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 & Stickland 1987; Bee 2004). The numbers of secondary muscle fibers, but not primary muscle fibers, are affected by the uterine environment (Dwyer et al. 1994) and the number of fetuses in utero (Town et al. 2004). Because the total number of muscle fibers is fixed at birth, their prenatal development impacts 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 lower ratio of secondary to primary muscle fibers and a smaller size of the fibers in IUGR pigs (Handel & Stickland 1987). Polyamines are necessary for both proliferation and differentiation of cells (Flynn et al. 2009) and likely mediate growth and development of fetal muscle fibers (Wu et al. 2010). In support of this view, concentrations of arginine, ornithine, proline, glutamine, and polyamines were reduced substantially in skeletal muscle of IUGR fetal pigs, compared with normal-body-weight (NBW) littermates (Wu et al. 2010). Similarly, concentrations of polyamines were much lower in allantoic and amniotic fluids of IUGR than in NBW fetal pigs (Wu et al. 2008). Whether dietary supplementation with polyamines can improve growth and development of fetal skeletal muscle has yet to be determined.

Intracellular protein turnover, adipogenesis, and mitochondrial biogenesis play major roles in regulating protein deposition in skeletal muscle and fat accretion in fetuses (Wu et al. 2010; Wang et al. 2012). In this regard, it is noteworthy that results of proteomics studies indicate that newborn IUGR piglets have a greater abundance of proteasomes (i.e., the major protease complex 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, when compared with NBW piglets (Wang et al. 2008). Recently, Wang et al. (2013) reported that muscle fiber diameters were smaller in IUGR fetal pigs than in NBW fetal pigs on all days of gestation. Although the number of primary fibers did not differ between these two groups of fetal pigs on d 60 of gestation, the total number of muscle fibers in IUGR fetal pigs was lower on d 90 and 110 of gestation (amounting to a 25% difference on d 110), when compared with NBW fetal pigs. Proteomic analysis revealed 37 differentially expressed proteins, which are involved in energy supply, protein metabolism, structure and type of muscle fibers, proliferation and differentiation of muscle fibers, nutrient transport, intracellular environment, and tissue integrity (Wang et al. 2013). Twenty five of these proteins were less abundant in IUGR fetal pigs, including transferrin, glutathione S-transferase omega-1, mitochondrial ATP synthase,
myosin-7, citrate synthase, creatine kinase, and bifunctional purine biosynthesis protein. These findings provide some insight into mechanisms responsible for reduced growth and impaired development of skeletal muscle in IUGR piglets.

**Use of functional AA to improve fetal survival and growth in pigs**

The current swine industry adopts restricted feeding programs to prevent excessive weight gain by gilts and sows during gestation (NRC 2012). Such a strategy can ameliorate farrowing difficulties and appetite reduction during lactation; however, gilts and sows do not receive sufficient amounts of dietary AA to support optimal embryonic and fetal growth during early-to late-gestation (i.e., d 14 to 114) (Wu et al. 2010). This problem is exacerbated further by: 1) degradation of large amounts of dietary AA in the small intestine; and 2) the naturally occurring inability of placentae to supply an adequate amount of nutrients to fetuses in pigs. For example, due to extensive catabolism of arginine by arginase in the small intestine (Bergen & Wu 2009), 60% of dietary arginine enters the portal circulation of pregnant gilts (Wu et al. 2007a). Therefore, increasing dietary provision of arginine beyond that from a typical corn- and soybean meal-based diet may be an effective means to enhance circulating levels and improve fetal survival and growth in gestating swine. The following 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 litter birth-weight by 24% (Mateo et al. 2007). The contents of crude protein and arginine in the basal diet were 12.2% and 0.70% (on an as-fed basis), respectively. The arginine to lysine ratio in the supplemental diet was 2.64, 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 formulations (Wu et al. 2007b). Second, compared with control gilts, dietary supplementation with 0.4% or 0.8% L-arginine between d 14 and 25 of gestation increased placental growth by 21-34% and the number of viable fetuses per litter by approximately 2 (Li et al. 2011). Likewise, 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). Third, supplementation with 1% arginine between d 14 and 28 of gestation increased the number of fetuses on d 70 by 3 per litter and the ratio of secondary to primary muscle fibers in fetal pigs (Berard et al. 2009). Fourth, supplementing 1% arginine to gilts beginning on d 17 of gestation for 16 days increased placental weight by 16% and the number of live-born piglets per litter by 1.2 (Gao et al. 2012). Finally, dietary supplementation with 0.83% arginine between d 90 and 114 of gestation increased average birth weight of live-born piglets by 16% (Wu X. et al. 2012). Collectively, well-designed studies have demonstrated beneficial effects of arginine supplementation on reproductive performance in gestating swine managed under practical production conditions.

As a functional AA, arginine acts in concert with other functional AA to further improve reproductive performance of pigs (Wu et al. 2009). One of the functional AA is glutamine, whose uptake by the uterus of gestating gilts is the highest among all AA (Wu et al. 1999) and whose concentrations during late gestation are reduced (Wu et al. 2011). Supplementing a corn- and soybean meal-based diet (containing 0.70% arginine and 1.22% glutamine) with 0.4% arginine and 0.6% glutamine (on an as-fed basis) between d 30 and 114 of gestation prevented a decline in glutamine concentrations in the plasma of gilts (Wu et al. 2011) that occurred when only arginine was supplemented to the basal diet (Mateo et al. 2007). Supplemental arginine and glutamine markedly reduced: 1) concentrations of ammonia (−29%) and urea (−27%) in maternal plasma (the reduction in both ammonia and urea indicate improved efficiency of
utilization of dietary AA); 2) variation in birth weights among all piglets born (–27%) and live-
born piglets (–24%); 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). Additionally, 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). The proportion of piglets with heavier birth weights (1.5 to 1.69 or 1.7 to 2.09 kg) did 
not differ between control and arginine plus glutamine-supplemented gilts (Wu et al. 2011). 
Collectively, our results indicate an important role for functional AA in improving pregnancy 
outcome in swine. These findings led to the recent recognition of arginine and glutamine as 
conditionally essential AA for growing and gestating pigs (NRC 2012).

Concluding remarks

Placental insufficiency contributes to high rates of embryonic/fetal mortality and the incidence of 
IUGR in swine. As building blocks of protein and regulators of metabolic pathways, inadequate 
or disproportionate amounts of AA in maternal diets are major factors affecting pregnancy 
outcomes. Many of the traditionally classified “nutritionally nonessential AA” are not adequate 
in conventional sow rations to meet demands of the fetus, because rates of their utilization 
are much greater than those for “nutritionally essential AA” that are not synthesized by animal 
tissues. Growing evidence shows that dietary supplementation with arginine to gilts or sows 
increases placental growth (including vascular growth), litter size, and litter birth weight. Also, 
a combination of arginine with glutamine during late gestation further improves fetal growth, 
while reducing variation in birth weights of piglets. These new results not only greatly advance 
basic knowledge about reproductive biology, but also have important implications for enhancing 
fetal survival and growth in swine and other livestock species (e.g., sheep and cattle).

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing 
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