Arginine Nutrition in Neonatal Pigs¹,²

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ABSTRACT  
The concentration of arginine (an essential amino acid for neonates) in sow’s milk is remarkably low, and thus endogenous synthesis of arginine plays a crucial role in maintaining arginine homeostasis in milk-fed piglets. Paradoxically, intestinal synthesis of citrulline from glutamate/glutaminate and proline (the endogenous source of arginine) declines markedly in 7- to 21-d-old suckling pigs, compared with 1- to 3-d-old pigs. Therefore, plasma concentrations of arginine and its immediate precursors (ornithine and citrulline) decrease progressively by 20–41%, whereas plasma ammonia levels increase progressively by 18–46%, between d 3 and 14 of life. Dietary supplementation of 0.2 and 0.4% arginine to 7- to 21-d-old pigs (artificially reared on a milk feeding system) dose dependently enhances the plasma arginine concentration (30 and 61%), reduces the plasma ammonia level (20 and 35%), and increases weight gain (28 and 66%). These compelling metabolic and growth data demonstrate unequivocally that arginine is insufficient for supporting the maximal growth in milk-fed young pigs and that this arginine deficiency represents a major obstacle to realizing the growth potential in piglets. A low concentration of mitochondrial N-acetylglutamate (an activator of both pyrroline-5-carboxylate synthase and carbamoylphosphate synthase-I) is responsible for the striking decline in the intestinal synthesis of citrulline and arginine during the suckling period. Accordingly, oral administration of N-carbamoylglutamate [a metabolically stable analogue of N-acetylglutamate; 2 × 50 mg/(kg body wt·d)] enhances plasma arginine level (68%) and weight gain (61%) of 4- to 14-d-old sow-reared pigs. Thus, the metabolic activation of intestinal citrulline and arginine synthesis provides a novel, effective means to increase endogenous arginine provision and therefore piglet growth (a major goal of animal agriculture). Our findings not only generate new fundamental knowledge about amino acid utilization by neonatal pigs, but they also have important practical implications for improving the efficiency of pork production.


KEY WORDS:  
• amino acids  
• intestine  
• metabolism  
• milk  
• swine

Arginine is an essential amino acid for the maximal growth of young mammals (1–3). It is the most abundant nitrogen carrier in tissue proteins (4) and is used by multiple pathways, including arginase, nitric oxide (NO)⁴ synthase, argininosuccinate (5). Serving as a precursor for the synthesis of creatine, glutamine/glutamate, polyamines, and NO, arginine displays remarkable metabolic and regulatory versatility in cells. Notably, young mammals (including piglets) have a particularly high requirement of arginine for growth and metabolic function (1,2). An arginine deficiency (defined as insufficient arginine for supporting maximal growth or metabolic function in animals) may occur under various nutritional and clinical conditions. These conditions include a low supply of dietary arginine, reduced intestinal synthesis of citrulline, inherited deficiencies of arginine-synthetic enzymes, impaired intestinal transport of arginine, overexpression of intestinal arginase gene, and/or impaired conversion of citrulline into arginine in kidneys (1,6). Arginine deficiency causes growth retardation, intestinal and reproductive dysfunction, impaired immune and neurological development, cardiovascular and pulmonary abnormalities, impaired wound healing, hyperammonemia, and even death in animals (7–10). Because of the crucial metabolic roles of arginine, there was growing interest in its biochemistry, nutrition, and physiology in recent years (6,8). The major objective of this article is to review current knowledge about arginine nutrition in the neonatal pig, which has enormous agricultural importance worldwide (11) and is also an established animal model for studying human infant metabolism (9).

Submaximal growth of sow-reared piglets

Although sow’s milk was traditionally thought to provide adequate amino acids for supporting piglets’ growth, recent

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3 To whom correspondence should be addressed. E-mail: g-wu@tamu.edu.
4 Abbreviations used: CPS-I, carbamoylphosphate synthase-I; NAG, N-acetylglutamate; NCG, N-carbamoylglutamate; NO, nitric oxide; PSC, pyrroline-5-carboxylate.
studies identified submaximal growth of sow-reared piglets (12,13). For example, artificial rearing data show that the biological potential for neonatal pig growth is ≈400 g/d (average from birth to 21 d of age), or ≈74% greater than that for sow-reared piglets (230 g/d) (13). Interestingly, suckling piglets exhibit submaximal growth from d 8 after birth (13). The metabolic basis for the submaximal growth of sow-reared piglets is unknown, but it may be due to inadequate intake of protein (or an essential amino acid) and/or energy. Because preweaning growth is a major determinant of neonatal survival and postweaning growth performance in pigs (11), improving the growth of suckling piglets will greatly enhance the efficiency of pork production.

Arginine deficiency in sow’s milk and the crucial role of endogenous arginine synthesis in maintaining its homeostasis

We and others demonstrated that arginine is deficient in sow’s milk on the basis of 1) the amino acid patterns in the milk and piglets; and 2) the arginine supply from sow’s milk vs. the estimated arginine requirement of piglets for growth and metabolic function (14–16). For example, our analysis of amino acids, using established HPLC methods (4,15), showed that the ratio of arginine/lysine on a gram basis was 0.35 ± 0.02 and 0.97 ± 0.05 (means ± SEM, n = 10) in sow’s whole milk (d 7 of lactation) and 7-d-old pigs, respectively. This data suggests that a substantial amount of arginine is synthesized by the neonate to compensate for low levels of arginine in milk. Quantitatively, the relative contribution of milk vs. endogenous synthesis to meet arginine requirements by the suckling neonate can be estimated on the basis of arginine intake plus arginine accretion and catabolism in the body (17–20). Our calculations indicate that sow’s milk provides ≈40% of arginine requirements by the 1-wk-old pig (Table 1). Thus, endogenous synthesis of arginine must play a crucial role in maintaining arginine homeostasis in sow-reared piglets (21), as reported for human infants (22) and neonatal mice (23). This conclusion is further supported by our finding that an inhibition of intestinal conversion of ornithine into pyrroline-5-carboxylate (P5C) for 12 h reduces plasma levels of ornithine, citrulline, and arginine by 59, 52, and 76%, respectively, in 4-d-old sow-reared pigs (21).

**Table 1**

<table>
<thead>
<tr>
<th>Amounts of arginine</th>
<th>g/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine provision from sow’s milk</td>
<td>≤1.01</td>
</tr>
<tr>
<td>Milk intake (0.78 L/d; 1.43 g/L of whole milk)¹</td>
<td>1.12</td>
</tr>
<tr>
<td>Undigestible arginine in sow’s milk (9.6%)²</td>
<td>0.11</td>
</tr>
<tr>
<td>Arginine requirements for growth and metabolic function</td>
<td>≥2.7</td>
</tr>
<tr>
<td>Body weight gain (200 g/d; 27.2 g protein)³</td>
<td>1.88</td>
</tr>
<tr>
<td>Arginine catabolism via arginase and NO synthase⁴</td>
<td>0.65</td>
</tr>
<tr>
<td>Arginine utilization for creatine synthesis⁵</td>
<td>0.17</td>
</tr>
<tr>
<td>Arginine provision from endogenous synthesis</td>
<td>≥1.69</td>
</tr>
</tbody>
</table>

¹ Wu and Knabe (15).  
² Mavromichalis et al. (17).  
³ Wu et al. (18).  
⁴ Murphy et al. (19).  
⁵ Calculated on the basis of urinary excretion of creatinine [0.38 mmol/(kg body wt · d)] (20).

### Progressive decline in intestinal synthesis of citrulline and arginine in sow-reared piglets

We were puzzled by one of our previous findings that intestinal synthesis of citrulline and arginine from glutamine and glutamate decreases by 70–73% in 7-d-old suckling pigs in comparison with newborn pigs and declines further in 14- to 21-d-old pigs (16,27) (Fig. 2). Similarly, rates of citrulline and arginine synthesis from proline in enterocytes are 75–88% lower in 7-d-old pigs, when compared with newborn piglets and remain at reduced levels in 14- to 21-d-old pigs (27). Because the ratios of small-intestinal weight (or mucosal protein weight) to body weight do not change substantially in newborn and suckling piglets (28–32 g small intestine per kg body wt from 1 to 21 d of age), intestinal synthesis of citrulline and arginine per kg body wt is also strikingly low in 7- to 21-d-old piglets compared with 1- to 3-d-old piglets (10). The metabolic basis for the progressive decrease in citrulline and arginine synthesis by enterocytes of 7- to 21-d-old pigs was not...
Arginine deficiency as a growth-limiting factor in young pigs

As noted above, arginine content is relatively low in sow’s milk (14, 15), as reported for the milk of humans and cows (14). There is experimental evidence supporting the notion that a reduced availability of arginine may limit the maximal growth of young pigs. For example, Leibholz (36) reported that in piglets weaned at 3 to 4 d 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) numerically increased weight gain by 43 and 93%, respectively, during d 7 to 14 of life (statistical analysis was not performed). Most recently, we found that dietary supplementation of 0.2 and 0.4% arginine (as 0.242 and 0.484% arginine-HCl) to 7- to 21-d-old pigs (artificially reared on a milk feeding system) dose-dependently enhanced plasma arginine concentration (30 and 61%), reduced plasma ammonia level (20 and 35%), and increased weight gain (28 and 66%) (37). Dietary supplementation of 0.2 and 0.4% arginine resulted in elevated plasma levels of ornithine and citrulline but had no effect on plasma levels of lysine and histidine (37), suggesting the absence of amino acid antagonism under the experimental conditions.

Food intake was similar between the control and the arginine-supplemented piglets reared on the milk feeding system (37). We propose that dietary arginine supplementation enhanced the growth performance of milk-fed piglets through the following mechanisms: 1) augmenting arginine availability for tissue protein synthesis; 2) stimulating the arginine-dependent production of NO (a major vasodilator, a key regulator of immune response, and a versatile signaling molecule) by endothelial and endocrine cells as well as other cell types; 3) promoting the NO-mediated secretion of insulin and somatotropin (anabolic hormones); 4) improving tissue insulin...
sensitivity; and 5) increasing polyamine synthesis in various tissues. Our finding that dietary supplementation of 0.2% arginine had no effect on plasma levels of insulin and somatotropin, and yet increased piglet weight gain (37) suggests that the growth-promoting effect of arginine did not result solely from an increase in the circulating levels of these 2 hormones. Collectively, our results indicate that 1) the supply of arginine from the milk-based diet and endogenous synthesis is inadequate for supporting the maximal growth of 7- to 21-d-old pigs, 2) increasing the arginine provision has a great potential to enhance neonatal pig growth, and 3) an ideal dietary ratio of arginine/lysine is ≥55:100 to maximize weight gain in piglets <21 d of age. Thus, both metabolic and growth data provide direct, compelling evidence to substantiate an arginine deficiency in milk-fed young pigs.

Needs for metabolic activation of arginine synthesis in piglets

Although dietary arginine supplementation to artificially reared piglets was shown to improve growth performance (36,37), it may not be cost effective or practical for family farms or corporate producers at present. This is because weaning at such an early age (d 4 or 7) is labor and resource intensive, and requires highly specialized and expensive facilities as well as costly diets and arginine. Thus, piglets are normally nursed by sows until 14 to 21 d of age in the current U.S. swine industry (11). In view of a reduced arginine supply from endogenous synthesis in suckling piglets and the great potential of arginine to enhance neonatal pig growth, it is of crucial importance to identify an effective means for improving arginine nutrition in preweaning piglets.

After several years’ studies, we now recognize that there are practical difficulties in improving arginine provision in sow-reared piglets through either dietary arginine supplementation to sows or oral administration of arginine and citrulline to the neonates. This is primarily because of extensive arginine catabolism by the lactating mammary gland (38) and nutritional antagonism among basic and/or structurally related amino acids (31). For example, we found that supplementing 0.4% arginine (as 0.48% arginine-HCl) to the sow’s conventional diet, containing 0.81% arginine (15) (n = 6 sows), between d 1 and 21 of lactation had no effect (P > 0.05) on total arginine content in milk, compared with isonitrogenous supplementation of 0.82% alanine (e.g., 1.45 ± 0.17 vs. 1.53 ± 0.19 g/L of whole milk; means ± SEM, n = 6 sows). Oral administration of arginine to sow-reared piglets (145 mg/kg body wt) 2 times/d enhanced plasma concentrations of arginine but reduced those of lysine and histidine (essential amino acids) compared with alanine administration (Table 2). Plasma concentrations of arginine were higher in sow-reared piglets receiving oral administration of citrulline twice daily than in isonitrogenous-control pigs (Table 3). This result supports the view that the sow-reared piglet has a high capacity for synthesizing arginine from citrulline (16) and that arginine deficiency in 7- to 21-d-old sow-reared pigs results from a limited supply of citrulline from the small intestine. An adverse effect of the citrulline administration at the chosen dose (121 mg/kg body wt) was a significant reduction in plasma concentrations of tryptophan, histidine, and lysine (Table 3), suggesting impaired intestinal absorption of these amino acids. Consistent with this notion, we found that in vitro transport of 0.5 nmol/L tryptophan, histidine, and lysine by incubated porcine enterocytes was 35, 28, and 32% lower, respectively, in the presence of 5 nmol/L citrulline, compared with its absence (n = 6). Thus, oral administration of relatively large doses of arginine or citrulline twice daily is unlikely to be beneficial for improving amino acid nutrition in sow-reared piglets. Interestingly, oral arginine administration at the chosen daily dose 6 times/d had no effect on plasma concentrations of arginine and, instead, enhanced plasma levels of cortisol (an indicator of stress) (Table 2). Cortisol is a potent

### TABLE 2

Enterocyte arginase activity as well as plasma levels of cortisol and amino acids in 14-d-old sow-reared piglets receiving oral administration of water, alanine, or arginine

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Frequency of oral dosing</th>
<th>Enterocyte arginase activity</th>
<th>Plasma cortisol</th>
<th>Arginine</th>
<th>Lysine</th>
<th>Histidine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>times/d</td>
<td>nmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>0.22 ± 0.03a</td>
<td>75 ± 8.2a</td>
<td>147 ± 10b</td>
<td>241 ± 17a</td>
<td>113 ± 9.7a</td>
</tr>
<tr>
<td>Water</td>
<td>2</td>
<td>0.24 ± 0.04a</td>
<td>78 ± 9.1a</td>
<td>149 ± 12b</td>
<td>232 ± 14a</td>
<td>118 ± 13a</td>
</tr>
<tr>
<td>Arginine</td>
<td>2</td>
<td>0.28 ± 0.03a</td>
<td>86 ± 12a</td>
<td>145 ± 8.2b</td>
<td>237 ± 12a</td>
<td>108 ± 7.3a</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.30 ± 0.04a</td>
<td>83 ± 10a</td>
<td>265 ± 14a</td>
<td>170 ± 10b</td>
<td>74 ± 5.8b</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>0.26 ± 0.04b</td>
<td>72 ± 7.4b</td>
<td>152 ± 9.3a</td>
<td>239 ± 13a</td>
<td>106 ± 8.2a</td>
</tr>
<tr>
<td>Water</td>
<td>6</td>
<td>2.83 ± 0.35a</td>
<td>416 ± 46a</td>
<td>114 ± 7.6b</td>
<td>225 ± 12a</td>
<td>114 ± 9.5a</td>
</tr>
<tr>
<td>Alanine</td>
<td>6</td>
<td>2.57 ± 0.17a</td>
<td>421 ± 53a</td>
<td>116 ± 7.2b</td>
<td>234 ± 11a</td>
<td>101 ± 8.8a</td>
</tr>
<tr>
<td>Arginine</td>
<td>6</td>
<td>2.94 ± 0.21a</td>
<td>437 ± 58a</td>
<td>122 ± 7.6b</td>
<td>229 ± 13a</td>
<td>97 ± 8.2a</td>
</tr>
</tbody>
</table>

1 Data are means ± SEM, n = 10. At 4 d of age, pigs received oral administration of arginine-HCl (145 mg/kg body wt), alanine (245.5 mg/kg body wt; isonitrogenous control), or water every 12 h (2 times/d) (Experiment 1), or of arginine-HCl (48.3 mg/kg body wt), alanine (81.8 mg/kg body wt), or water every 4 h (6 times/d) (Experiment 2). The amount of arginine (provided as arginine-HCl) represents 53% of estimated daily arginine intake by the piglet from milk (18). Some pigs did not receive oral administration of any solution and were not handled (the None group). At 14 d of age, 2 h after oral administration of water, alanine or arginine, jugular venous blood samples (3 mL) were obtained for determining plasma amino acids and cortisol, as previously described (5,6). Arginase activity was measured as described by Wu et al. (16). Data were analyzed by two-way ANOVA and the Student-Newman-Keul multiple comparison test (SAS, Cary, NC). a–c: Means without a common superscript letter within a column of each experiment differ (P < 0.01).

2 Values are nmol/(mg protein · min).
Yamada and Wakabayashi (29) reported a profound decrease in the oral nature of neonatal pigs, which exhibit significant stress in response to frequent handling and gavaging. This resulted in a lower level of plasma arginine in the stressed (unstressed) pigs that were not handled or gavaged (Table 2). Our finding may be explained by the behavioral nature of neonatal pigs, which exhibit significant stress in response to frequent handling and gavaging.

In view of the foregoing results, metabolic activation of intestinal citrulline and arginine synthesis may be an attractive approach to augment arginine provision in sow-reared piglets. However, only by elucidating the mechanism responsible for the marked decline in intestinal synthesis of citrulline and arginine (the major source of endogenous arginine) in sow-reared piglets, can we design a scientifically sound means to enhance arginine availability in neonates. This consideration prompted us to address the question of why intestinal citrulline synthesis is low in 7- to 21-d-old pigs, compared with newborn pigs.

**A possible role for NAG in regulating intestinal synthesis of citrulline and arginine in neonatal pigs**

NAG synthase, which catalyzes the synthesis of NAG from glutamate and acetyl-CoA, is restricted to mitochondria of the liver and intestinal mucosa (30,40). NAG is an allosteric activator of carbamoylphosphate synthase-I (CPS-I) (5), which synthesizes mitochondrial carbamoyl-phosphate necessary for the conversion of ornithine into citrulline (Fig. 1).

Two lines of evidence from our work suggest that NAG may play an important role in regulating intestinal synthesis of citrulline and arginine in pigs. First, although ornithine aminotransferase and ornithine carbamoyltransferase are abundant in pig enterocytes, only ∼35% of proline-derived P5C is converted into citrulline in enterocytes of 14-d-old pigs (41). This suggests a low concentration of mitochondrial carbamoylphosphate in enterocytes of 2- to 3-wk-old pigs. Second, mitochondrial NAG concentration [measured as described by Bush et al. (32)] was decreased progressively in enterocytes of 7- to 14-d-old pigs, compared with newborn pigs, as was intestinal synthesis of citrulline and arginine (Fig. 2). Further, we found that a reduced availability of mitochondrial NAG resulted from a marked postnatal decline (P < 0.01) in enterocyte NAG synthase activity [measured as previously described (29)], which was 229 ± 36, 72 ± 8.5, 26 ± 3.9, and 28 ± 3.3 pmol/(mg protein·min) (means ± SEM, n = 5) for 0-, 7-, 14-, and 21-d-old pigs, respectively. Notably, Yamada and Wakabayashi (29) reported a profound decrease in NAG synthase activity in the small intestinal mucosa of 3-d-old rats, compared with newborn rats. Although the amounts of intestinal CPS-I protein are similar between 2- and 21-d-old pigs on the basis of the enzyme activity measured under in vitro optimal conditions, including an optimal NAG concentration (42), a low level of mitochondrial NAG may limit in vivo intestinal citrulline and arginine synthesis from both glutamine and proline in suckling piglets.

PSC synthase is another key regulatory enzyme in intestinal citrulline synthesis. To determine whether P5C synthase may be activated by NAG, we conducted a study to measure P5C synthase activity in enterocytes from 14-d-old suckling pigs in the presence of 0 or 0.1 mmol/L NAG, by using our established method (16). Our results showed that 0.1 mmol/L NAG increased P5C synthase activity by 124 ± 11% (means ± SEM, n = 5). Thus, NAG is a novel activator of P5C synthase in enterocytes. Through modulating P5C synthase and CPS-I activities, mitochondrial NAG levels may play a crucial role in regulating in vivo intestinal synthesis of citrulline and arginine. If this hypothesis is correct, increasing mitochondrial NAG availability may stimulate intestinal synthesis of citrulline and arginine, thereby enhancing endogenous arginine provision.

**Effects of N-carbamoylglutamate on citrulline and arginine synthesis in incubated enterocytes**

The cytosol of mammalian cells, including enterocytes, contains a high deacylase activity to catabolize NAG (43), thus limiting the use of extracellular NAG to increase mitochondrial NAG concentrations. NCG [an analogue of NAG

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**TABLE 3**

Plasma concentrations of amino acids in 14-d-old sow-reared piglets receiving oral administration of alanine or citrulline

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Citrulline</th>
<th>Arginine</th>
<th>Tryptophan</th>
<th>Histidine</th>
<th>Lysine</th>
<th>Glutamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>76 ± 5.2</td>
<td>138 ± 10</td>
<td>45 ± 2.1</td>
<td>103 ± 6.6</td>
<td>237 ± 14</td>
<td>516 ± 19</td>
</tr>
<tr>
<td>Citrulline</td>
<td>148 ± 10*</td>
<td>260 ± 18*</td>
<td>36 ± 1.6*</td>
<td>88 ± 5.0*</td>
<td>194 ± 10*</td>
<td>504 ± 23</td>
</tr>
</tbody>
</table>

1 Data are means ± SEM, n = 10. At 4 d of age, pigs received oral administration of citrulline (121 mg/kg body wt) or alanine (185 mg/kg body wt; isonitrogenous control) every 12 h (twice daily). At 14 d of age, 2 h after oral administration of alanine or citrulline, jugular venous blood samples (3 mL) were obtained for determining plasma amino acids, as previously described (5). Data were analyzed by unpaired t test (SAS Institute). * P < 0.01 vs. the alanine group.
FIGURE 4  Effect of N-carbamoylglutamate (NCG) on citrulline and arginine synthesis from glutamine (panel A) and proline (panel B) in enterocytes of 14-d-old sow-reared pigs. Data are means ± SEM, n = 5. Jejunal enterocytes were incubated at 37°C for 45 min in Krebs bicarbonate buffer (pH 7.4) containing 2 mmol/L [U-14C]glutamine or 2 mmol/L glutamine plus 2 mmol/L [U-14C]proline (27) and 0 or 2 mmol/L NCG. Rates of citrulline and arginine synthesis were measured as described by Wu (27). *P < 0.01 vs. control (no NCG), as analyzed by paired t test (SAS Institute).

NAG readily enters cells and mitochondria to exert its effect (43,44). Indeed, the activation of hepatic citrulline synthesis from both glutamine and proline by pig enterocytes (Fig. 4). These results are the first to demonstrate the feasibility of enhancing intestinal citrulline and arginine synthesis through the metabolic activation of P5C synthase and CPS-I.

**Effects of oral NCG administration on plasma arginine concentrations and growth of sow-reared piglets**

Results of our in vitro study are exciting and therefore prompted us to conduct an in vivo study to determine whether NCG can increase plasma concentration of arginine and thus the growth of sow-reared piglets. Four-d-old sow-reared piglets received oral administration of 0 or 50 mg NCG per kg body wt every 12 h (2 times/d) until 14 d of age. This dose of NCG was chosen on the basis of the previous studies with human infants (46), and 2) our preliminary finding that oral administration of NCG at 50 to 100 mg/kg body wt twice daily maximally enhanced plasma arginine concentration in 14-d-old sow-reared pigs, compared with 10 and 25 mg NCG/kg body wt.

Concentrations of ammonia or glutamine in the jejunal lumen did not differ (P > 0.05) between control and NCG-treated piglets (e.g., 0.21 ± 0.03 vs. 0.23 ± 0.02 mmol/L for ammonia; 2.21 ± 0.28 vs. 2.09 ± 0.34 mmol/L for glutamine; means ± SEM, n = 5). This result suggests that NCG did not affect the availability of intestinal ammonia or other nitrogenous precursors needed by gut microbes. However, oral administration of NCG enhanced plasma concentrations of arginine by 68% and prevented the marked postnatal decline in plasma levels of arginine in 14-d-old pigs (Table 4), indicating that NCG activates in vivo synthesis of citrulline and arginine by the small intestine. Importantly, NCG treatment had no effect on plasma levels of tryptophan, lysine, or histidine in piglets (data not shown), suggesting that NCG did not affect intestinal absorption of these amino acids. In support of this view, we found that rates of transport of 0.5 mmol/L tryptophan, histidine, or lysine by porcine enterocytes did not differ (P > 0.05) between the presence of 0 and 1 mmol/L NCG (n = 5).

Using the weigh–suckle–weigh technique (48), we found that milk consumption was not different (P > 0.05) between 14-d-old control and NCG-treated piglets [51.6 ± 6.2 vs. 52.3 ± 6.4 g dry matter (kg body wt–d); means ± SEM, n = 5].

| Piglets | Plasma arginine | Body weight | Weight gain
<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmol/L</td>
<td>d 4</td>
<td>d 14</td>
</tr>
<tr>
<td>Control</td>
<td>206 ± 8.0</td>
<td>142 ± 9.2</td>
<td>2.01 ± 0.09</td>
</tr>
<tr>
<td>NCG</td>
<td>202 ± 9.1</td>
<td>239 ± 11*</td>
<td>2.05 ± 0.27</td>
</tr>
</tbody>
</table>

1 Data are means ± SEM, n = 10. Four-d-old sow-reared piglets received oral administration of 0 or 50 mg NCG (dissolved in water) per kg body wt every 12 h (2 times/d at 0730 and 1930). At d 4 and 14 of age, 2 h after suckling and oral NCG administration, respectively, jugular venous blood samples (3 mL) were obtained from pigs for determining plasma amino acids (16); body weights were measured. Data were analyzed by unpaired t test (SAS Institute). * P < 0.01 vs. the control group.
Remarkably, NCG treatment increased \((P < 0.01)\) piglet weight gain by 61% from d 4 to 14 after birth (Table 4). To determine the components of weight gain, we quantified body composition in 14-d-old control and NCG-treated piglets (10 d after initiating NCG treatment), using standard methods (4). Our results showed that the content (\%) of water (69.5 ± 0.85 vs. 69.7 ± 0.89), dry matter (30.5 ± 0.85 vs. 30.3 ± 0.89), protein (14.9 ± 0.53 vs. 15.3 ± 0.56), fat (12.1 ± 0.97 vs. 11.6 ± 1.04), and minerals (3.0 ± 0.12 vs. 2.9 ± 0.13) (means ± SEM, \(n = 5\)) did not differ \((P > 0.05)\) between control and NCG-treated pigs. Thus, the weight gain in NCG-treated piglets resulted in part from a greater absolute amount of tissue protein accretion, rather than from a disproportionate change in fat deposition. Our novel findings further support the conclusion that arginine is a major factor limiting the maximal growth of milk-fed young pigs.

NCG offers unique and important advantages over oral administration of arginine or citrulline to sow-reared piglets. First, oral administration of NCG does not affect intestinal absorption of dietary tryptophan, histidine, or lysine in pigs. Second, because of constant activation of intestinal synthesis of citrulline and arginine, oral administration of NCG ensures a balanced supply of arginine to piglets relative to the supply of other basic amino acids from sow’s milk during the suckling period. This is particularly important for sow-reared piglets, which suckle about every 1 to 1.5 h. Third, as a metabolic activator of P5C synthase and CPS-I, a low dose of NCG is required to metabolically activate arginine synthesis in sow-reared piglets. Thus, oral administration of NCG provides the major metabolic activator of P5C synthase and CPS-I, a low dose of NCG is required to metabolically activate arginine synthesis in sow-reared piglets.

In conclusion, our results indicate that arginine deficiency, owing to its reduced intestinal synthesis, is a major metabolic basis for the submaximal growth of sow-reared piglets and that increasing arginine provision has a great potential to promote the growth of the neonates. Because of a reduced availability of mitochondrial NAG in enterocytes of 7 to 21-d-old sow-reared pigs, the metabolic activation of intestinal citrulline and arginine synthesis with NCG provides a new, effective strategy to augment arginine provision in sow-reared piglets. Our findings not only generate new fundamental knowledge about amino acid utilization by neonatal pigs but also have important practical implications for improving the efficiency of pork production.