Effects of Dietary Supplementation with Combined Arginine and Glutamine on Growth Performance and Small Intestinal Development in Neonatal Piglets

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Abstract: This study aimed to determine the effects of dietary supplementation with combined arginine and glutamine on growth performance and intestinal development in neonatal piglets. The piglets (n = 144, 4 days old) were assigned randomly to receive one of four diets for 21 days: basal diet (control diet) or the basal diet supplemented with either 0.6% Arginine (Arg diet), 1.0% Glutamine (Gln diet) or both 0.6% arginine and 1.0% Glutamine (Arg/Gln diet). The results showed that Arg/Gln diet increased average daily gain and average daily feed intake compared with both control and Gln diets (p<0.05). Concentrations of serum T3 and insulin were greater for the Arg/Gln diet than the control diet (p<0.05). The Arg/Gln diet increased the duodenal villus height compared with the other three diets (p<0.05). In the jejunal mucosa, the Arg/Gln diet increased the glutathione peroxidase and catalase activities compared with the control diet (p<0.05). The Arg/Gln diet also increased the sucrase activity compared with both control and Arg diets (p<0.05). These results suggested that dietary supplementation with combined arginine and glutamine could improve small intestinal morphology and anti-oxidants and digestive capabilities and could be beneficial in growth performance and gut maturation of neonatal piglets.

Key words: Arginine, glutamine, neonatal piglets, growth performance, intestine, China

INTRODUCTION

The small intestine displays various functions including absorption of nutrients, secretion of mucin and immunoglobulins and selective barrier protection against harmful antigens and pathogens (Lalles et al., 2004). The marked changes that occur in intestine structure and function such as villous atrophy and crypt hyperplasia are generally associated with diarrhea and growth retardation (Pluske et al., 1997; Boudry et al., 2004). This is particularly important for neonates whose small intestine grows very rapidly and is more susceptible to external stimuli including dietary and environmental changes during the neonatal period (Lalles et al., 2007). For ameliorating the effects of adverse stimuli on the small intestine in neonatal piglets, numerous nutritional approaches such as ensuring the presence of adequate amino acids (arginine or glutamine) in the diet have been tested during the neonatal period.

Arginine (Arg) as an essential amino acid for the maximal growth of neonatal piglets, plays important roles in alleviating intestinal mucosa disruption and enhancing growth performance in neonatal piglets (Kim and Wu, 2004; Tan et al., 2009). Wu et al. (2004) proposed that the effects of Arg in neonatal piglets mainly modulated by stimulating the Arg-dependent production NO (a key regulator of immune response and a versatile signaling molecule) and polyamine. Glutamine (Gln) another essential amino acid for young suckling piglets, serves as an important fuel for intestinal cellular division and is also necessary to support the maximum growth and intestinal development in neonatal piglets (Wu et al., 1995, 2011).

Interestingly, an earlier report indicated that metabolic relation exists between Gln and Arg (Wu, 1998). Intestinal conversion of Gln leads to a release of citrulline from the gut which is the precursor for Arg synthesis in enterocytes (Wu et al., 1994). Subsequently, Arg can be hydrolyzed by arginase to ornithine which can be

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converted into Gln by ornithine aminotransferase or to polyamines by ornithine decarboxylase (Wu and Morris, 1998).

The intriguing link between Gln and Arg raises the important question of whether the benefit of simultaneous supplementation with Arg and Gln on the growth and intestinal development is great in neonatal piglets. However, to the best of the knowledge, there are no data available to address this question in neonatal piglets. Thus, this study was conducted to evaluate the effects of dietary supplementation with Arg and Gln in combination on the growth performance and intestinal development in neonatal piglets.

**MATERIALS AND METHODS**

**Animals and diets:** A total 144, 4 days old male neonatal piglets (Duroc x Large White x Landrace, average body weight 2.17±0.03 kg) were assigned randomly to one of four treatments on the basis of body weight. Each treatment was replicated using six pens with six piglets per pen. Piglets were housed in nursery pens equipped with a plastic slatted floor. The housing temperature was maintained at 30±1°C by heat lamps. The diets were made as a powder form and mixed with water at a 1:3 (wt/ wt) ratio freshly before feeding. All piglets were fed every 2 h from 08:00-24:00 h and had free access to warm water.

During the 21 day experiment, piglets were fed with one of the four diets: basal diet (control diet); (2) 0.6% Arginine diet (Arg diet); (3) 1.0% Glutamine diet (Gln diet); (4) 0.6% Arginine + 1.0% Glutamine diet (Arg/Gln diet). All nutrients met NRC (1998) requirements for 3-5 kg piglets and appropriate amounts of L-alanine were added to make the diets isonitrogenous. Table 1 shows the basal diet formulation and its nutrient composition. The dosage of supplemental arginine (0.6%) was chosen because it was shown to further increase the weight gain in neonatal piglets compared with piglets supplemented with 0.4% arginine (Tan et al., 2009). Meanwhile, the dosage of supplemental glutamine (1.0%) was chosen because it was the effective dose for increasing the growth performance in neonatal piglets (Wu et al., 1996).

**Sample collection:** At 25 day of age, one piglet per replicate was selected at a random for blood collection. About 10 mL blood samples were obtained from the anterior vena cava. Serum was collected after centrifugation at 1,000×g for 10 min at 4°C and kept at -20°C until analyzed. Then, piglets were killed by jugular puncture after intramuscular injection of sodium pentobarbital (50 mg kg⁻¹ BW) and the small intestine collected. The small intestine in neonatal piglets is the portion of digestive tract between the pylorus of the stomach and the ileocecal valve with the first 10 cm segment being duodenum, the middle of the intestine being jejunum and the distal segment about 5 cm proximal to the ileocecal junction being ileum. The contents of whole small intestine were rapidly removed by flushing with ice-cold Phosphate Buffered Saline (PBS). Mucosa from the jejunal segment was removed by gentle scraping with a glass slide then rapidly placed in liquid nitrogen and stored at -80°C for analysis of the activities of selected enzymes. This study was carried out in accordance with the Chinese guidelines for animal welfare and was approved by the Animal Care and Use Committee of the Guangdong Academy of Agricultural Sciences.

**Preparation of intestinal mucosal samples:** The mucosa was weighed and suspended in ice-cold PBS (1:9, wt/vol). The mixture was homogenized for 45 sec using a Ultra-Turrax T8 homogenizer (IKA Labortechnik, Staufen, Germany) and followed by centrifuging at 1,000×g for 10 min at 4°C. The supernatant fluid was used for the determination of activities of antioxidant enzymes and disaccharidases.

| **Table 1: Nutrient composition of the basal diet (as-fed basis)** |
|------------------|------------------|
| **Items**        | **Content (%)**  |
| Whole milk powder (23% CP) | 50.42 |
| Whey protein concentrate (54% CP) | 17.00 |
| Plasma protein powder (78% CP) | 8.56 |
| Whey powder (48% CP) | 8.56 |
| Coconut oil | 5.00 |
| Lactose | 11.00 |
| L-lysine | 0.30 |
| DL-methionine | 0.42 |
| L-tryptophan | 0.08 |
| L-threonine | 0.29 |
| L-alanine | 2.70 |
| Vitamin-mineral premix | 1.00 |
| Dicalcium phosphate | 0.29 |
| Liquid chloroform (70%) | 0.12 |

**Nutrient composition**
- Digestible energy (Meal kg⁻¹): 4.72
- Fat (%): 15.56
- Crude protein (%): 24.53
- Calcium (%): 1.08
- Total phosphorus (%): 0.62
- Lactose (%): 37.01
- Lysine (%): 2.13
- Methionine (%): 1.26
- Tryptophan (%): 0.40
- Glutamic acid (%): 3.07
- Arginine: 0.81

Provided per kg of diet: 11,000 IU Vitamin A, 660 IU Vitamin D3, 80 IU Vitamin E, 1.50 mg Vitamin K, 12.0 mg Vitamin B₂, 4.5 mg Vitamin B₁, 6.0 mg Vitamin B₆, 60.0 ug Vitamin B₁₂, 200 mg Vitamin C, 0.24 mg biotin, 0.90 mg folic acid, 90.0 mg nicotinic acid, 56 mg pantothenate, 20 mg Fe (FeSO₄·7H₂O), 10 mg Cu (CuSO₄·5H₂O), 2000 mg Zn Glu, 30 mg Mn (MnSO₄·H₂O), 0.6 mg Co (CoSO₄·7H₂O), 0.43 mg Se (Na₂SeO₃), 0.56 mg I (CaI₂).
Growth performance: Feed intake of piglets in each pen was recorded every day and Body Weights (BW) of piglets were measured individually before feeding on day 0, 10 and 21 of the experiment. Average Daily Gain (ADG) Average Daily Feed Intake (ADFI) and Gain:Feed ratio (G:F) were calculated for the four treatments.

Small intestinal morphology: A 3 cm segment was taken from each portion of the small intestine for histological measurement. These samples were washed with ice-cold PBS and then fixed in 10% neutral formaldehyde. After 24 h they were embedded in paraffin, sectioned at 5 μm thickness and stained with hematoxylin-eosin for examination using light microscopy. The Villous Height (VH) and Crypt Depth (CD) were measured according to the procedure described by Goodlad et al. (1991). The villus area was calculated according to the method published by Frankel et al. (1993).

Serum hormones, jejunal antioxidant capacity and activities of disaccharidases: Serum hormones including Insulin (INS), Triiodothyronine (T3) and Thyroxine (T4) were determined using commercially available ELISA kits (R and D Systems China Co. Ltd., Shanghai, China) and a multifunctional microplate reader (SpectraMax M5, MDC, America). Antioxidant capacity index, including Glutathione Peroxidase (GSH-Px), Superoxide Dismutase (SOD), Catalase (CAT), Malondialdehyde (MDA) and Total Anti-Oxycapacity Capability (T-AOC) were determined by using commercially available reagent kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Disaccharidases (maltase, lactase and sucrase) were also determined using commercially available reagent kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Statistical analysis: Data were analyzed statistically using the GLM procedures of the SAS statistical package (Statistical Analysis System, SAS Institute Inc., Cary, NC, USA, Version 9.1.3). Values were Table 1-6 shown as mean±SD.

The individual piglet was the experimental unit and all means±SD in Table 3-6 are based on analysis of samples from six piglets in each treatment except that the growth performance data in Table 2 was based on a per pen basis which was the experimental unit. Differences among means were determined using Tukey honestly significant difference. An α-level of p<0.05 was used for determination of statistical significance and p<0.10 was taken to indicate a statistical tendency.

Table 2: Effect of dietary supplementation with arginine and glutamine on growth performance of piglets

<table>
<thead>
<tr>
<th>Items</th>
<th>Control</th>
<th>0.6% Arg</th>
<th>1% Gin</th>
<th>0.6% Arg + 1% Gin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0 BW (kg)</td>
<td>2.18±0.12a</td>
<td>2.17±0.10a</td>
<td>2.16±0.10a</td>
<td>2.16±0.09a</td>
</tr>
<tr>
<td>Day 21 BW (kg)</td>
<td>7.61±0.23a</td>
<td>7.95±0.17a</td>
<td>7.61±0.10a</td>
<td>8.13±0.18a</td>
</tr>
<tr>
<td>Days 0-10</td>
<td>ADG (g)</td>
<td>141.6±0.688b</td>
<td>156.5±3.32b</td>
<td>147.8±3.28b</td>
</tr>
<tr>
<td></td>
<td>ADFI (g)</td>
<td>122.7±4.100b</td>
<td>134.1±12.82b</td>
<td>124.5±1.42b</td>
</tr>
<tr>
<td></td>
<td>GF</td>
<td>1.15±0.030b</td>
<td>1.17±0.02b</td>
<td>1.18±0.02b</td>
</tr>
<tr>
<td>Days 10-21</td>
<td>ADG (g)</td>
<td>401.3±8.990b</td>
<td>421.6±8.35b</td>
<td>396.7±5.26b</td>
</tr>
<tr>
<td></td>
<td>ADFI (g)</td>
<td>327.9±3.780b</td>
<td>328.2±8.13b</td>
<td>321.1±1.44b</td>
</tr>
<tr>
<td></td>
<td>GF</td>
<td>1.22±0.010b</td>
<td>1.29±0.01b</td>
<td>1.24±0.01b</td>
</tr>
<tr>
<td>Days 0-21</td>
<td>ADG (g)</td>
<td>251.5±10.18b</td>
<td>275.3±8.01b</td>
<td>259.3±3.11b</td>
</tr>
<tr>
<td></td>
<td>ADFI (g)</td>
<td>225.3±5.240b</td>
<td>231.8±3.92b</td>
<td>223.0±0.83f</td>
</tr>
<tr>
<td></td>
<td>GF</td>
<td>1.15±0.040b</td>
<td>1.19±0.01b</td>
<td>1.16±0.01b</td>
</tr>
</tbody>
</table>

Table 3: Effect of dietary supplementation with arginine and glutamine on small intestinal morphology of piglets

<table>
<thead>
<tr>
<th>Items</th>
<th>Control</th>
<th>0.6% Arg</th>
<th>1% Gin</th>
<th>0.6% Arg + 1% Gin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td>Villus height (μm)</td>
<td>445.9±9.7900b</td>
<td>472.1±9.7900b</td>
<td>486.7±11.89b</td>
</tr>
<tr>
<td></td>
<td>Crypt depth (μm)</td>
<td>161.9±7.1250</td>
<td>123.8±9.1090</td>
<td>148.7±21.59</td>
</tr>
<tr>
<td></td>
<td>Villus area (mm²)</td>
<td>0.330±0.033b</td>
<td>0.287±0.034b</td>
<td>0.331±0.016b</td>
</tr>
<tr>
<td>Jejunum</td>
<td>Villus height (μm)</td>
<td>516.9±31.190b</td>
<td>356.4±26.650b</td>
<td>352.9±13.05</td>
</tr>
<tr>
<td></td>
<td>Crypt depth (μm)</td>
<td>148.1±7.2000</td>
<td>140.2±6.5000</td>
<td>148.66±11.35</td>
</tr>
<tr>
<td></td>
<td>Villus area (mm²)</td>
<td>0.372±0.027b</td>
<td>0.374±0.029b</td>
<td>0.363±0.0070</td>
</tr>
<tr>
<td>Ileum</td>
<td>Villus height (μm)</td>
<td>474.7±14.490b</td>
<td>575.6±26.710b</td>
<td>541.0±8.00b</td>
</tr>
<tr>
<td></td>
<td>Crypt depth (μm)</td>
<td>120.3±16.220</td>
<td>129.5±6.9100</td>
<td>123.9±8.71</td>
</tr>
<tr>
<td></td>
<td>Villus area (mm²)</td>
<td>0.315±0.020b</td>
<td>0.324±0.038b</td>
<td>0.359±0.018b</td>
</tr>
</tbody>
</table>

1Data are presented as means±SD (n = 6/treatment); 2Values in a row without a common letter differ (p<0.05).

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RESULTS

Growth performance: Effects of dietary supplementation with Arg or and Gln on performance of piglets were shown in Table 2. On day 21 of the experiment, final Body Weight (BW) was greater for piglets on the Arg/Gln diet compared with the control and Gln diets (p<0.05). Between days 0 and 10, ADG for piglets on the Arg diet was greater compared with the control diet (p<0.05) while ADFI was greater compared with the control diet and the Gln diet (p<0.05). Similarly, the Arg/Gln diet increased the ADO and ADFI for piglets compared with both control diet and Gln diets (p<0.05). Between days 10 and 21 as compared with the control and piglets diets, piglets fed the Arg diet and Arg/Gln diet had a greater G:F ratio (p<0.05) while piglets fed the Arg/Gln diet had a greater ADG (p<0.05). Between days 0 and 21, ADG and G:F ratio for piglets on the Arg diet and Arg/Gln diet were greater compared with the control diet (p<0.05). ADG and ADFI for piglets fed the Arg/Gln diet were greater compared with the Gln diet (p<0.05). However, the Arg/Gln diet did not improve the ADG, ADFI and G:F ratio for piglets when compared with the Arg diet throughout the experiment (p>0.05).

Small intestinal morphology: The Arg/Gln diet increased the duodenal villus height for piglets compared with the other three diets (p<0.05; Table 3) and increased the duodenal villus area for piglets compared with the Arg diet (p<0.05). Piglets fed with both Arg, Gln and Arg/Gln diets increased the ileal villus height compared with the control diet (p<0.05). Piglets fed the Arg diet increased the ileal villus area compared with the control diet (p<0.05). There was no significant difference on jejunal morphology among treatments.

Serum hormones: Concentrations of serum T3 and INS were greater for piglets fed the Arg/Gln diet compared with the control diet (p<0.05; Table 4) while piglets fed with the Arg diet tended to increase the serum T3 level compared with the control diet (p<0.10). There was no significant difference on concentration of serum T4 among treatments.

Jejunal antioxidant capacity: Jejunal mucosa T-AOC increased in response to the Arg diet compared with the control and Gln diets (p<0.05; Table 5) while MDA content decreased in response to the Arg diet compared with the control and Arg/Gln diets (p<0.05). Piglets receiving the Gln diet had a lower content of MDA in the jejunal mucosa compared with the control diet (p<0.05). Activities of GSH-Px and CAT in jejunal mucosa were greater for piglets on the Arg/Gln diet compared with the control diet (p<0.05). There was no significant difference on activity of SOD in jejunal mucosa among treatments.

Levels of jejunal disaccharidases: Lactase activity in the jejunal mucosa was greater for piglets fed the Arg, Gln and Arg/Gln diets compared with the control diet (p<0.05; Table 6). Sucrase activity in the jejunal mucosa was greater for piglets fed the Gln and Arg/Gln diets compared with the control diet (p<0.05). Additionally, the Arg/Gln diet increased the sucrase activity as compared with that for piglets fed the Arg diet (p<0.05). However, there was no significant difference on maltase activity in jejunal mucosa among treatments.

DISCUSSION

This study was conducted to quantify the effects of Arg and Gln in combination on growth performance, selected serum hormones, intestinal morphology, jejunal
mucosa redox status and disaccharidase activities in neonatal piglets. Arginine was reported to be the major factor limiting maximal growth of sow-reared piglets because the endogenous synthesis of arginine is impaired in sow-reared piglets (Wu, 2010; Geng et al., 2011). Results of the study demonstrated that dietary supplementation with Arg alone increased the ADG and G:F ratio throughout the 21 days experiment when compared to the control diet. The improvement of growth performance in response to Arg was similar to that reported previously (Wu et al., 2004; Tan et al., 2009). Whereas supplementation with Gln alone did not significantly affect growth performance of neonatal piglets in the present study, which is contradictory to earlier studies that showed supplementation with Gln in corn-soybean based diet was effective in enhancing BW gain in 10 days old neonatal piglets (Lackeyram et al., 2001). The discrepancy is probably due to the age of piglets, composition of the basal diet and environmental factors. Moreover, researchers did observe a marked increase in final BW, ADG and G:F ratio in piglets fed the Arg/Gln diet in the study, when compared to both the control and Gln diets. This is the first report, to the best of the knowledge, on the cooperative effects of Arg and Gln on the growth performance in neonatal piglets. These findings suggest that Arg alone or in combination with Gln have favorable effects on growth performance in neonatal piglets.

Arginine was reported to be effective in stimulating the secretion of insulin and growth hormone in neonatal piglets (Wu and Morris, 1998; Yao et al., 2008). In the present study, researchers observed that Arg alone tended to increase concentrations of serum T3 in neonatal piglets. Conversely, Gln alone did not affect concentrations of serum hormones which is similar to the results of Yi et al. (2005) in the Escherichia coli K88+-challenged weaned pigs. Moreover, the results showed that Arg combined with Gln increased the concentrations of serum T3 and INS throughout the experiment which indicated that the better growth performance of piglets on the Arg/Gln diet may relate to the hormone secretion.

The morphology of the small intestine has been used widely to assess intestinal health and function in neonatal piglets (Cera et al., 1998, Yi et al., 2005; Zhong et al., 2011). Alternations in intestinal morphology is usually associated with a reduced disaccharidase activity and growth retardation in neonatal piglets (Achkar et al., 1996). Zhan et al. (2008) reported that dietary supplementation with 0.7(%) Arg increased villus height throughout the small intestine in neonatal piglets. Consistent with those results, the study showed that Arg alone increased both the villus height and villus area in the ileum. Similarly, the increase of ileal villus height in piglets fed the Gln diet supports the results of Wu et al. (1996). In addition, researchers observed that the Arg/Gln diet increased the duodenal villus height compared with that for pigs fed the other three diets, increased the duodenal villus area compared with that for pigs fed the control and Arg diets and increased the ileal villus height compared with that for pigs fed the control diet. Several studies revealed that there is coordination of Gln and Arg metabolism in intestinal mucosa cells and intestinal bacteria (Wu, 1998; Dai et al., 2011, 2012). These findings suggest that the combination of Arg and Gln exert their beneficial effects on small intestinal morphology probably due to their cooperative regulation on Arg and Gln utilization and metabolism in the small intestinal cells and microbiota of neonatal piglets.

The balance of Reactive Oxygen Species (ROS) generation and antioxidant capacity within the enterocytes determines the oxidative environment. When the ROS exceed the antioxidant capacity of the cell, oxidative stress results. Oxidative stress can initiate and/or mediate a number of signaling cascades that can impair the integrity of the intestine (Turan and Mahmoud, 2007). The antioxidant enzymes, either present in the intracellular milieu or released into the extracellular milieu can directly scavenge these oxidants or prevent their conversion to toxic species (Prakash and Srinivasan, 2010). In the study, Arg alone increased the T-AOC activity and decreased the MDA content in jejunal mucosa. Arg is reported as a precursor of NO, a free radical which has antioxidant functions in mammalian cells (Lin et al., 2005; Dasgupta et al., 2006). The antioxidant capacity of Arg is probably due to the NO pathway. Similarly, the study also revealed a decrease in MDA content in response to the Gln diet which is consistent with the observation of Wang et al. (2008). Earlier research suggested that glutathione, a metabolic production of Gln is an important antioxidant for the small intestine (Gibson et al., 1993). This indicated that Gln might inhibit the lipid peroxidation in intestinal mucosa by the synthesis of glutathione. However, in the present study, the Arg/Gln diet did not decrease the content of MDA but increased activities of GSH-Px and CAT in jejunal mucosa. This is probably due to the opposite effects of Arg and Gln on NO production via iNOS in endothelial cells (Wu, 2009).

Increased expression of disaccharidase activities is associated with differentiation of enterocytes as they migrate from the crypt to the villus (Fan et al., 2001). Reductions in disaccharidase activities were generally associated with reductions in villus height and increases in crypt depth in the small intestine of neonatal piglets.

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CONCLUSION

The results demonstrate that dietary supplementation with Arg and Gln in combination increased the concentrations of selected serum hormones, up-regulated the anti-oxidants and digestive capabilities of the intestinal mucosa and modified the morphology of the small intestine which contributed to the intestinal development and resulted in better growth performance in neonatal piglets. These results could be the basis of prospective studies for evaluating the effects of dietary supplementation with combined Arg and Gln in neonatal piglets.

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