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Effects of L-glutamine on growth performance, antioxidant ability, immunity and expression of genes related to intestinal health in weanling pigs

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ABSTRACT

Although there were many reports about the L-glutamine (Gln) on growth performance and intestine health, little studies were conducted to determine the effects of Gln on nutrient digestibility and expression of genes related to intestinal health. Besides, the glutamic acid with lower price was assumed to substitute the glutamine partly, which can partly be synthesized by the glutamic acid. Therefore, this study was conducted to determine the effects of Gln on growth performance, apparent total tract digestibility (ATTD), blood profiles, related enzyme activity, small intestinal mucosal morphology and expression of genes related to intestinal health in weanling pigs. A total of 250 [(Landrace × Yorkshire) × Duroc] pigs (9.22 ± 0.11 kg) were blocked on the basis of sex and body weight, and then randomly assigned to 1 of the following 5 treatments: 1) CON (basal diet); 2) 1/9 Gln/Glu (CON +0.1% Gln +0.9% Glu); 3) 2/8 Gln/Glu (CON +0.2% Gln +0.8% Glu); 4) 1% Gln (CON +1% Gln); 5) 1% Glu (CON +1% Glu). There were 5 replications (pens) per treatment and 10 pigs per pen in this 28-d experiment. Pigs fed the 1% Gln diet had a higher ADG and G: F ($P < 0.05$) than those fed CON diet during d 0–14 and the overall period. The ATTD of dry matter (DM) and nitrogen (N) was the greatest ($P < 0.05$) in response to the 1% Gln diet on d 28. Compared with CON, feeding the 1% Gln diet increased ($P < 0.05$) the content of superoxide dismutase (SOD), while it decreased ($P < 0.05$) the malondialdehyde (MDA) content on d 28 in the serum. The levels of immunoglobulin G (IgG) and immunoglobulin M (IgM) in the serum was increased in the 1% Gln group ($P < 0.05$), whereas the percentage of the cluster of differentiation 8 receptors (CD8+) was reduced ($P < 0.05$) in the 1% Gln group compared with CON. The activity of lactase was improved ($P < 0.05$) in pigs fed the 1% Gln diet compared with those fed CON diet. However, the activity of glutamine synthetase (GS) was reduced ($P < 0.05$) in pigs fed Gln1, Gln2, the 1% Gln and 1% Glu diets compared with those fed CON diet. Feeding of the 1% Gln diet declined ($P < 0.05$) the relative expression levels of peroxisome proliferator-activated receptor γ (PPARγ) and mammalian target of rapamycin (mTOR), while improving ($P < 0.05$) the relative expression level of pyruvate kinase (PK) in the duodenum, jejunum and ileum, respectively, compared with CON. Taken together, the 1% Gln supplementation to weanling pig diets could modify intestinal health and improve ATTD of nutrients so as to benefit the growth performance. Nevertheless, the Gln/Glu use could not achieve the effects of 1% Gln group in this study.

1. Introduction

Due to the sudden changes of physiology, nutrition and circumstance, early weaning of piglets with the immature digestive tract was associated with the intestinal villus atrophy, the crypt hyperplasia, the decrease in digestive enzyme activity, the reduction in digestion and absorption of biological macromolecules (Wijtten et al., 2011), the inhibition of intestinal immune cell growth and differentiation, which may stimulate the expression of inflammatory cytokines (Pies et al., 2004). These aforementioned influences may result in the weaned stress syndrome in the piglet: the reduction in feed intake, the inhibition of growth, and the increase in diarrhea incidence after weaning. The supplementation of several immunomodulators may rectify the intestinal

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impairment and modulate the immune function of animals so as to improve their performance and health status (Li et al., 2007).

Several studies have already identified glutamine (Gln) has the function of alleviating early weaning stress (Newsholme, 2001; Wu, 2011). The Gln primary functions were as follows: 1) it was the important energy source for intestinal epithelial cells, lymphocytes and reticular cell proliferation (Feng, et al., 2009); 2) it was often the most abundant free AA in pig colostrum and milk, and it was considered as a conditional necessary AA under stress condition such as injury and disease infection (Li et al., 2007); 3) it could maintain the integrity of the structure and function of gastrointestinal tract in early weaning piglets (Wu et al., 1996; Kozar et al., 2004; Li et al., 2007); 4) it also can strengthen the immune function and improve the antioxidant capacity (Yoo et al., 1997; Johnson et al., 2006); 5) it was regarded as a key regulator of gene expression and cell signaling pathways (Rhoads and Wu, 2009). Several studies have already identified its primary functions including the improvement of animal production and gut health, increase in nutrient digestibility and boost in immune ability (Zou et al., 2006; Wang et al., 2008; Jiang et al., 2009; Haynes et al., 2009; Wu et al., 2011).

Although there were many reports about the Gln on growth performance and intestine health, little studies were conducted to determine the effects of Gln on nutrient digestibility and expression of genes related to intestinal health. Moreover, the Gln could be expensive, which may restrict the use in the feed industry. We assumed that the glutamic acid with lower price can substitute the glutamine partly because the glutamine can partly be synthesized by glutamic acid, which may reduce the cost. Therefore, the objective of this trial was to determine the effects of Gln and Glu on growth performance, ATTD of nutrients, blood profiles, related enzyme activity, small intestinal mucosal morphology and expression of genes related to intestinal health in weanling pigs.

2. Materials and methods

2.1. Animals, housing, and treatments

A total of 250 pigs at the age of d 35 ± 1 [(Landrace $\times$ Yorkshire) $\times$ Duroc, weaned at 28 days of age] with an average initial BW of 9.22 ± 0.11 kg were assigned to 1 of 5 dietary treatments. There were 5 replications (pens) per treatment and 10 pigs per pen (5 castrated males and 5 females). The males were castrated at the age of d 5. The dietary treatments included a maize-soybean meal-based control diet or control diet supplemented with different levels of Gln or Glu: 1) CON (basal diet); 2) 1/9 Gln/Glu (CON +0.1% Gln +0.9% Glu); 3) 2/8 Gln/Glu (CON +0.2% Gln +0.8% Glu); 4) 1% Gln (CON +1% Gln); 5) 1% Glu (CON +1% Glu). Gln or Glu was added at the expense of maize. The diets were formulated to provide all of the nutrients to meet or exceed NRC (1998) requirements (Table 1). The experiment lasted for 28 d. All the experimental protocols used in this study were approved by the Animal Welfare Committee of Southwest University of Science and Technology in China.

All of the pigs were housed in an environmentally controlled nursery facility with slatted plastic flooring and a mechanical ventilation system. The environmental temperature was maintained at 30 °C for the first week of the experiment, and was then reduced by 1 °C per week over the next three weeks. Each pen (2×2.5 m) was provided with a stainless steel feeder and one nipple waterer, which allowed ad libitum access to feed and water throughout the experiment.

Table 1
Diet composition (as-fed basis)[a].

Items	CON	1/9 Gln/Glu	2/8 Gln/Glu	1% Gln	1% Glu
Ingredients, %					
Maize	54.61	53.61	53.61	53.61	53.61
Extruded maize	10.00	10.00	10.00	10.00	10.00
Soybean meal (CP 46%)	25.00	25.00	25.00	25.00	25.00
Fish meal (CP 65%)	3.00	3.00	3.00	3.00	3.00
Soybean oil	2.80	2.80	2.80	2.80	2.80
L-Glutamine (99%)	–	0.10	0.20	1.00	–
Glutamic acid (99%)	–	0.90	0.80	–	1.00
Limestone	0.65	0.65	0.65	0.65	0.65
Dicalcium phosphate	0.96	0.96	0.96	0.96	0.96
NaCl	0.35	0.35	0.35	0.35	0.35
L-Lys HCl (78.8%)	0.36	0.36	0.36	0.36	0.36
DL-Methionine (99%)	0.08	0.08	0.08	0.08	0.08
L-Threonine (98.5%)	0.10	0.10	0.10	0.10	0.10
L-Tryptophan (10%)	0.23	0.23	0.23	0.23	0.23
Choline chloride (50%)	0.10	0.10	0.10	0.10	0.10
Zinc Oxide (80%)	0.25	0.25	0.25	0.25	0.25
Phytase	0.01	0.01	0.01	0.01	0.01
Acidifier	0.50	0.50	0.50	0.50	0.50
Vitamin premix[b]	0.50	0.50	0.50	0.50	0.50
Trace mineral premix[c]	0.50	0.50	0.50	0.50	0.50
Analytical composition					
DE, Mcal/kg[d]	3400	3400	3400	3400	3400
Crude protein, %	19.4	19.8	20.3	20.4	20.0
Lysine, %	1.42	1.46	1.40	1.37	1.44
Ca, %	0.68	0.65	0.69	0.66	0.68
P, %	0.56	0.59	0.57	0.58	0.57
Aspartic acid	1.97	1.98	1.93	1.88	1.88
Threonine	0.86	0.85	0.85	0.82	0.81
Serine	0.90	0.89	0.87	0.85	0.85
Glutamic acid	3.57	4.48	4.55	4.37	4.42
Glycine	0.87	0.88	0.86	0.84	0.83
Alanine	1.06	1.06	1.05	1.02	1.03
Cysteine	0.30	0.33	0.35	0.35	0.37
Valine	0.94	0.95	0.93	0.91	0.92
Methionine	0.40	0.41	0.39	0.38	0.38
Isoleucine	0.85	0.85	0.84	0.81	0.82
Leucine	1.76	1.76	1.76	1.71	1.74
Tyrosine	0.74	0.83	0.82	0.79	0.83
Phenylalanine	1.02	1.05	1.02	1.00	1.02
Lysine	1.42	1.46	1.40	1.37	1.44
Histidine	0.55	0.57	0.55	0.54	0.55
Arginine	1.30	1.30	1.26	1.23	1.24
Proline	1.19	1.22	1.19	1.18	1.22
Total AA	19.7	20.86	20.62	20.06	20.33

[a] CON (basal diet); 1/9 Gln/Glu (CON + 0.1% Gln + 0.9% Glu); 2/8 Gln/Glu (CON + 0.2% Gln + 0.8% Glu); 1% Gln (CON + 1% Gln); 1% Glu (CON + 1% Glu).
[b] Provided per kilograms of diet: 20,000 IU of vitamin A; 4,000 IU of vitamin D3; 80 IU of vitamin E; 16 mg of vitamin K3; 4 mg of thiamine; 20 mg of riboflavin; 6 mg of pyridoxine; 0.08 mg of vitamin B12; 120 mg of niacin; 50 mg of Ca-pantothenate; 2 mg of folic acid and 0.08 mg of biotin.
[c] Provided per kg diet: 80 mg of Fe; 140 mg of Cu; 179 mg of Zn; 12.5 mg of Mn; 0.5 mg of I; 0.25 mg of Co and 0.4 mg of Se.
[d] Calculated values.

2.2. Experimental procedures, sampling, and analysis

Individual pig BW was measured initially and on d 14 and 28 of the experiment. Feed consumption per group (pen) was also assessed on d 14 and 28 of the experiment. The ADG, ADFI, and G:F were also calculated.

During d 22–28, chromic oxide (0.2%) was added to all the diets as an indigestible marker for the determination of apparent nutrient digestibility. On the last two days of the experiment, fecal samples (at least 0.5 kg) were collected from at least two pigs at random from each pen via rectal massage, then pooled within the pens. All the feed and fecal samples were stored at −20 °C until further analysis. Concentrations of DM and N in the feed and feces were analyzed in accordance with AOAC (2000) procedures. The