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Full Length Research Paper

The effect of glutamine supplement on small intestinal morphology and xylose absorptive ability of weaned piglets

C. B. Hsu[1, 2], H. J. Huang[1], C. H. Wang[1] H. T. Yen[3] and B. Yu[2]*

[1]Kaohsiung Animal Propagation Station, Livestock Research Institute, COA, 372 Tungang Road, Laupi Village, Neipu, Pingtung 912, Taiwan, R.O.C
[2]Department of Animal Science, National Chung Hsing University, 250 Kuo Kuang Road, Taichung 402, Taiwan, R.O.C
[3]Animal Technology Institute Taiwan, P.O. box 23, Chunan, Miaoli 350, Taiwan, R.O.C

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The purpose of this study is to demonstrate the effects of glutamine (Gln) supplement on small intestinal morphology, xylose absorptive and growth performance of weaned piglets. Forty eight piglets weaned at 28 ± 2 days of age were randomly allotted to three treatment groups. A basal corn-soybean diet was formulated to contain 20.3% protein and 3450 kcal DE/kg diet. Glutamine was supplemented to the basal diet at 0% (control), 1% (Gln 1%) and 2% (Gln 2%). Pigs were fed experimental diets for three weeks. The results showed that the villous height of the Gln groups tended higher than the control group in duodenum and jejunum (P < 0.1). Glutamine supplementation increased plasma net xylose absorptive concentration from 0.78 to 1.20 and 0.95 to 1.23 in Gln 1% and Gln 2% group, respectively, which were better than the control group (0.86 to 0.97) in day 7 to 14 after weaning. Growth performance was not significantly affected by Gln supplement; however, average daily gain was approximately improved from 21 to 28% by Gln supplement compared to the control group during 21 days of experimental period. In summary, the results suggested that dietary supplementation of Gln could be beneficial in small intestinal villous morphology and xylose absorptive capacity, and could have a slight contribution to the average daily gain of weaned piglets.

Key words: Glutamine, growth performance, intestinal morphology, weaned piglets.

INTRODUCTION

Inadequate nutrient intake after weaning often causes damage to the intestinal villi resulting in poor growth of weanling pigs (van Beers-Schreurs et al., 1998). Amino mucosa. One of them, glutamine (Gln) is an essential acids provide the major energy source for the intestinal precursor for the synthesis of proteins as well as purine/pyrimidine nucleotides. Moreover, it can be used as an energy source to support rapidly, the differentiation and proliferation of intestinal epithelial cells (Newsholme et al., 2003; Wu et al., 1996) and activated lymphocytes cells (Wu et al., 1995). Therefore, Gln is the most abundant free amino acid found in the blood of animals and in the milk of sows (Wu and Knabe, 1994).

At normal intakes, dietary Gln is metabolized by the small intestine and essentially, all Gln within the body is synthesized *de novo* through the action of glutamine synthetase. The major sites of net Gln synthesis are lung, adipose tissue, and skeletal muscle and under some conditions, the liver. The intestine expresses proline 5-carboxylate (P5C) synthase, which means that proline is

*Corresponding author. E-mail: byu@dragon.nchu.edu.tw. Tel: +886-4-22860799. Fax: +886-4-22860265.

Abbreviations: Gln, Glutamine; **P5C,** proline 5-carboxylate; **DE,** digestible energy; **CP,** crude protein; **SI,** small intestine; **PBS,** phosphate buffered saline;; **EDTA,** ethylenediaminetetraacetic acid;; **TPN,** total parenteral nutrition; **IgA,** immunoglobulin A; **VH,** villous height; **CD,** crypt depth; **BW,** body weight; **ADG,** average daily gain; **ADFI,** average daily feed intake.

Table 1. The composition of basal diet.

Ingredient (%)	Basal diet
Maize, dent yellow	49.05
Soybean meal, 44 of CP	23.70
Dried skim milk	16.0
Whey	5.0
Soybean oil	1.0
Dicalcium phosphate	1.60
Limestone, pulverized	0.80
Salt	0.50
Vitamin premix [1]	0.10
Mineral premix[2]	0.15
Choline chloride, 50	0.10
Maize starch	2.0
Glutamine	0
Calculated values	
Crude protein	20.3
Calcium	1.01
Total phosphorus	0.77
Lysine	1.17

[1]Supplied per kg of diet: Vitamin A, 6,000 IU; vitamin D_3, 800 IU; vitamin E, 20 mg; vitamin K_3, 4 mg; vitamin B_2, 4 mg; vitamin B_6, 1 mg; vitamin B_{12}, 0.02 mg; niacin, 30 mg; calcium pantothenate, 16 mg; folic acid, 0.6 mg; biotin, 0.01 mg; choline chloride, 50 mg. [2]Supplied per kg of diet: Fe ($FeSO_4.H_2O$), 140 mg; Cu ($CuSO_4.5H_2O$), 7 mg; Mn ($MnSO_4.H_2O$), 20 mg; Zn (ZnO), 120 mg; I (KIO_3), 0.45 mg.

an end product of intestinal Gln catabolism (Wu et al., 1995).

Previous studies have shown that Gln is a conditionally-essential amino acid under weaning period as well as stressful conditions such as injury and infection (Newsholme, 2001). Yi et al. (2005) showed that Gln is beneficial for maintaining muscular Gln concentrations and normalizes lymphocyte function of *Escherichia coli*-challenged weaned pigs. Furthermore, due to the effect of Gln on regulation of systemic inflammation, it had been thought that it could be applied potentially to inflammatory diseases (Singleton and Wischmeyer, 2008). However, the endogenous Gln is insufficient and consequently, animals need to increase their requirement (Hall et al., 1996). Therefore, the aim of this study is to investigate the effects of dietary glutamine supplementation on small intestinal villous morphology/structure, xylose absorptive ability and growth performance of weaned piglets.

MATERIALS AND METHODS

Animals and diets

The animal feeding protocol of this research was approved by the Animal Care and Use Committee of Kaohsiung propagation station, Livestock Research Institute, Council of Agriculture. Forty-eight crossbred pigs (Landrace × Yorkshire × Duroc) weaned at 28 ± 2 days of age were obtained from 10 litters, and littermates were randomly allotted to 12 pens for three dietary treatments according to weight and sex. Same sex was allotted to each pen. The treatments consisted of a control group (C), control diet supplemented with glutamine in replace of maize starch for 1% (Gln 1%) and 2% (Gln 2%). The control diet (Table 1) was based on maize-soybean meal with digestible energy (DE) 3450 kcal/kg, crude protein (CP) 20.3%, and lysine 1.17% according to the standards of NRC (1998). During the 21 days of experimental period, pigs were housed in a traditional nursery room with wire-floored pens. Meal feed and nipple water were provided *ad libitum*. The individual pig weight, pen feed consumption, and pen feed efficiency (gain/feed) were recorded weekly.

Small intestinal morphology observation

Four pigs from each treatment (one pig per pen as duplicate) were sacrificed on day 14 post-weaning. Pigs were anaesthetized by halothane inhalation. Following intestinalectomy, the small intestine (SI) was removed and the length was determined; the positions at 10, 50, and 90% of the length of the SI were located at duodenum, jejunum and ileum, respectively. A 4 cm segments was taken from each portion for histological measurement. These samples were first rinsed with 0.1 M phosphate buffered saline (PBS) at pH 7.2, and then fixed with 10% neutral formaldehyde. After 24 h, the samples were removed from the fixative, cut into 1 cm^2 sections (two per location) and stored in fresh fixative. Then, they were embedded in paraffin, sectioned at 6 µm thickness and stained with hematoxylin as well as eosin for a light microscopy examination. The villous height (VH) and crypt depth (CD) were measured based on 15 apparently intact villi from each section according to Yu and Chiou (1997). Another 4 cm samples were taken from jejunum and ileum for morphological observation using a scanning electronic microscope according to the method of Yu and Chiou (1997). The gut samples were fixed in 10% buffered neutral formaldehyde, then rinsed in PBS 3 times and placed in 1% osmium tetraoxide overnight. They were rinsed again in PBS for 4 times. The samples were gradually dehydrated by increasing alcohol concentrations from 50 to 100%. They were then dehydrated, mounted on aluminum stubs, coated with gold for 30 min, and subsequently placed in the scanning electronic microscope (HITACHI S-300) for scanning.

Xylose absorption ability

On day 7 and 14 post weaning, eight pigs of each treatment group (2 pigs per pen) were carried to measure the ability of active absorption of small intestine. Before the procedure, the pig fasted for 16 h, and then fed 10% D-xylose (Sigma Chemical Inc, USA) solution at a dose of 1 ml/kg body weight by gavages. Blood samples (6 ml) were withdrawn through anterior vena cava with tubes containing ethylenediaminetetraacetic acid (EDTA) pre and post 1 h gavages. Plasma was obtained by centrifuging at 1500 g for 15 min and was stored at -20°C until analyzed for D-xylose concentration according to the procedures of Trinder (1975). The ability of absorption was determined by measuring the difference of xylose concentration in plasma between pre and post feeding of xylose. Another twelve pigs were randomly selected from the same herd at 28 day of age and their xylose absorption was measured as a reference data.

Statistical analysis

Data was analyzed by analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of the Statistical Analysis

Table 2. Effect of supplementing glutamine on the intestinal morphology of weaned pigs[1].

Item	Control	Gln 1%	Gln 2%	SE	Contrast (C vs. Gln)
Duodenum					
Villus hight,μm	282	345	332	23	0.069
Crypt depth,μm	266	282	260	32	0.914
VH/CD	1.06	1.21	1.27	0.20	0.941
Jejunum					
Villus hight,μm	306	377	397	33	0.068
Crypt depth,μm	202	242	211	16	0.236
VH/CD	1.52	1.56	1.84	0.19	0.634
Ileum					
Villus hight,μm	260	349	302	33	0.121
Crypt depth,μm	193	192	196	21	0.964
VH/CD	1.34	1.64	1.54	0.17	0.601

[1]Each value represents the mean of 4 pigs. VH, Villous height; CD, crypt depth.

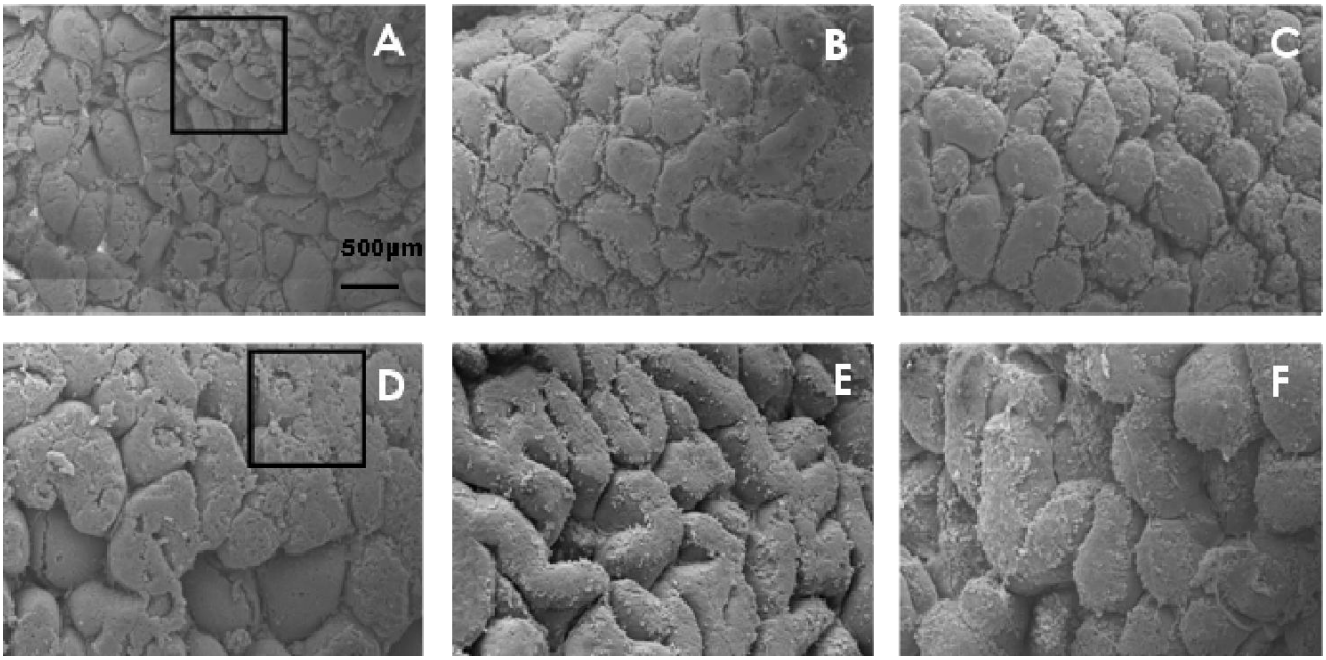

Figure 1. The scanning electron micrographs of the jejunal (A, B, C) and ileal (D, E, F) villi from control and glutamine supplement groups of pigs at 14 days postweaning. Control group (A and D); Gln 1% group (B and E); and Gln 2% group (C and F). The boxed areas showed erosion of surface epithelium at the apex of the villi in control group.

System (SAS) programs (1999). Duncan's new multiple range test was applied for comparing the differences among treatments. The orthogonal contrasts were performed to compare the glutamine treatment effect. The difference was considered to be significant at $P < 0.05$, and $P < 0.10$ was considered as a trend.

RESULTS

Intestinal morphology

The morphology of the duodenum, jejunum and ileum at day 14 of the experiment is shown in Table 2. The results showed that glutamine supplement groups numerically improved the villus height of duodenum and jejunum when compared to the control ($P < 0.1$). Whereas, there were no difference between 1 and 2% Gln supplement groups. Figure 1 shows the scanning electron micrographs of the jejunal (Figures 1A, B and C) and ileal (Figures 1D, E and F) villi from the control and glutamine supplement groups, respectively. In general, blunted and folded villi were observed in most pigs (Figures 1B, C, E and F). Damaged villi with erosion of surface epithelium

Table 3. Effect of supplementing glutamine on the plasma xylose concentration (mmol/L) of weaned pigs[1].

Item	Control	Gln 1%	Gln 2%	SE
Day 7				
Preoral conc.	0.15	0.15	0.12	0.01
Postoral conc.	1.01	0.93	1.06	0.22
Net absorptive conc.	0.86	0.78	0.95	0.22
Day 14				
Preoral conc.	0.16	0.09	0.14	0.02
Postoral conc.	1.13	1.29	1.37	0.23
Net absorptive conc.	0.97	1.20	1.23	0.22

[1] Each value represents the mean of 8 pigs.

Table 4. Effect of supplementing glutamine on the growth performance of weaned pigs[1]

Item	Control	Gln 1%	Gln 2%	SE
Average BW (kg)				
Initial	6.66	6.69	6.67	0.22
7 day (12)[1]	7.26	7.28	7.37	0.24
14 day (12)	8.28	8.50	8.35	0.35
21 day (8)	9.78	10.35	10.36	0.58
ADG (kg/head)				
0 to 7 day	0.09	0.09	0.10	0.02
8 to 14 day	0.13	0.17	0.14	0.03
15 to 21 day	0.20	0.24	0.26	0.03
0 to 21 day	0.14	0.17	0.18	0.02
ADFI (kg/head)				
0 to 7 day	0.23	0.19	0.22	0.04
8 to 14 day	0.24	0.28	0.31	0.05
15 to 21 day	0.33	0.30	0.34	0.05
0 to 21 day	0.26	0.24	0.28	0.03
Gain/Feed				
0 to 7 day	0.41	0.45	0.44	0.08
8 to 14 day	0.53	0.60	0.46	0.12
15 to 21 day	0.67	0.80	0.76	0.09
0 to 21 day	0.54	0.63	0.64	0.05

[1]Values are presented as means with the numbers of piglets given in parentheses. BW, Body weight; ADG, average daily gain; ADFI, average daily feed intake.

at the apex of the villi were detected in some pigs from the control group (Figures 1A and D). The integrity of intestinal morphology was better in the glutamine supplementation groups than in the control group.

Absorption of xylose

Table 3 shows plasma xylose concentration of the three treatments. The average plasma xylose concentration at weaning was 0.17 mmol/L (data not shown). Glutamine supplementation increased plasma net xylose absorptive concentration from 0.78 to 1.20 and 0.95 to 1.23 in 1 and 2% Gln group, respectively, which were better than the control group (0.86 to 0.97) in day 7 to 14 after weaning.

Growth performance

The growth performance is presented in Table 4. No significant differences in average daily gain (ADG), daily feed intake and gain/feed were observed regardless of the treatment group. However, ADG was numerically improved ($P > 0.05$) 30 and 8% by Gln 1% and Gln 2%

compared to the control from d 8 to 14 of the experimental period ($P > 0.05$) and 20 and 30%, respectively, from day 15 to 21. Overall, ADG was improved 21 to 28 % approximately by glutamine supplement compared to the control group during 21 days of experimental period.

DISCUSSION

Weaning of piglets is known to be associated with gross changes in small intestinal morphology and structure such as villous atrophy, decreased villous height and increased crypt depth that will decrease the intestinal active absorption (van Beers-Schreurs et al., 1998). If the intestinal villous atrophy could be prevented, it would improve nutrient digestion and absorption and growth gap of weaned piglets (Pluske et al., 1997).

In the present study, glutamine supplementation showed a trend of improving villous heights of the duodenum and jejunum at day 14 of the weaning period ($P < 0.1$) and activation absorptive ability of xylose. The results agreed with the findings of Wu et al. (1996) as well as the previous research that glutamine or glutamine-dipeptide supplementation to total parenteral nutrition (TPN) solution prevented gut atrophy in humans and rats (Schroder et al., 1995). Liu et al. (2002) suggested that the jejunal atrophy was prevented by 1.0% glutamine supplementation during the first week post-weaning piglets. Yu et al. (2002) also suggested that a combination of 1.0% of glutamine and 1000 ppm of nucleotide in diet could improve feed intake and intestinal villus height. The reasons being that glutamine facilitated the survival and proliferation of intestinal mucosal cells and that glutathione synthesis from glutamine maintains the mucosal integrity and defenses. Another explanation could be the glutamine-dependent protein expression of intestinal epithelial tight junction barrier and cellular localization in Caco-2 cell monolayers (Liu et al., 2002; Wu et al., 1996). This mechanism may similarly relate to glutamine-mediated modulation of intestinal barrier function in stressed animals and humans (Li et al., 2004; DeMarco et al., 2003). Furthermore, enteral glutamine can stimulate the mucosal protein synthesis and preserve the paracellular permeability (Coeffier et al., 2003; Le Bacquer et al., 2003) that will be helpful for maintaining the epithelial barrier function. Our study indicated that the villi of the control pigs were damaged to some extent possibly due to inadequate nutrient intake and it may result in increase rate of cell turnover as well as decreasing villi height. The present study showed that the intestine xylose absorptive ability improved from day 7 to 14 after weanling in both Gln supplement groups when compared to the control group. Therefore, glutamine supplementation provided a beneficial environment for the proliferation of enterocytes, preventing intestinal atrophy and activation absorptive function.

Currently, glutamine supplementation had no significant improvement on the performance of piglets; however, it numerically improved 21 to 28% of ADG compared to the control group during 21 days of experimental period. Zou et al. (2006) found that pigs supplemented with 1% glutamine had a 12% lower feed/gain ratio during the first ten days after weaning and had a 27.8% higher ADG during day 11 to 20 post-weaning. Wu et al. (1996) had reported that 0.2 to 1.0% glutamine supplementation did not have any significant effects on the daily feed intake, ADG, and gain/feed during the first week post weaning, but the gain/feed of pigs supplemented with 1% glutamine was 25% higher than the control pigs during the second week of post-weaning. Lee et al. (2003) also found that 1.5% glutamine supplementation did not affect the feed intake, ADG, and gain/feed of pigs weaned on 21 days of age, but the small intestinal development and bile immunoglobulin A (IgA) production were improved. Similar results were also reported by Bartell and Batal (2007) in chicken, their results showed 1% Gln could improve growth performance, facilitate the health of GI tract, and increase the concentrations of sera IgG and IgA. In the study of Yi et al. (2005), they indicated that 2.0% glutamine supplementation on pigs weaned at 17 days of age did not affect growth performance during 11 days of feeding, but glutamine supplementation had beneficial effects on alleviating growth depression of *E. coli* K88+-challenged weaned pigs, through maintaining intestinal morphology and function. Focusing on glutamine supplementation for growth performance in weaned piglets, Zou et al. (2006) explained the importance of glutamine on energy source for enterocytes, the necessary precursor for DNA and protein synthesis, and the biological regulating function of glutamine metabolite. Recently, research on molecular mechanisms had revealed the findings that dietary glutamine supplement would increase intestinal expression (120 to 140%) of genes that is necessary for cell growth and removal of oxidants (Wang et al., 2008). However, the reason growth performance could not be consistently improved by glutamine might result from the fact that it may not be affected by glutamine alone, but with other uncertain environmental factors. Therefore, from the results obtained, it is suggested that glutamine supplementation for weaned piglets seemed to lack the significant improving effects on growth performance, but posses positive and improving efficacy for the maintenance of the intestinal villous morphology and function.

In conclusion, dietary supplementation of glutamine could be beneficial to small intestinal villous morphology, xylose absorptive capacity and slightly contribute to the average daily gain of weaned piglets.

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