Effect of Dietary Glutamine Supplement on Performance and Intestinal Morphology of Weaned Pigs*

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ABSTRACT : Two experiments were conducted to investigate the effect of dietary glutamine (Gln) supplement on the performance and villus morphology of weaned pigs. In Exp. 1, 48 pigs were fed diets supplemented with 0, 0.5, 1.0, or 1.5% Gln for 28 days. Dietary Gln supplemented levels did not influence performance and plasma Gln concentration of weaned pigs. In Exp. 2, 48 weaned pigs were fed the same treatment diets of Exp. 1 for 7 or 14 days. Dietary Gln supplement reduced the ratio of small intestine weight to empty carcass weight at d 14 postweaning. However, the villus height and villus height/crypt depth ratio at the duodenum were increased. IgA and protein in the bile from d 7 and d 14 postweaning were higher in the pigs fed the diet supplemented with 0.5% Gln. Plasma IgA concentration was not influenced by dietary Gln levels. In conclusion, dietary Gln supplement might benefit the development of the small intestine and bile IgA production in weaned pigs. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 12 : 1770-1776)***

Key Words : Glutamine, Weaned Pigs, Intestinal Morphology, Performance

INTRODUCTION

Glutamine (Gln) is one of the most abundant free amino acids in sow's milk (Wu and Knabe, 1994). Ingested Gln can further be degraded to α-ketoglutarate and amide group in the enterocytes; α-ketoglutarate is incorporated into Krebs cycle to supply energy, while amide group is used to synthesize purine, pyrimidines or the precursor of glucosamine in the intestinal cells (Kreb, 1980; Wu et al., 1995). Recent research has shown Gln is a major nutrient for intestinal epithelial cells (Wu et al., 1995). Although Gln makes up 3 to 10% in feedstuffs, it must be freed from the containing protein to be used by intestinal cells. However, the intestine enzymes responsible for the digestion of Gln are not well developed in pigs at 21 d of age (Madej et al., 1999). Therefore, it is suggested that the demand for Gln increases at weaning period of pigs (Wu et al., 1996).

Weaning at 21 d of age, often causes abnormalities in intestinal morphology. These alterations include decrease in villus height and increase in crypt depth. Hampson (1986) found that the small intestine villus height decreased to 75% within d 1 postweaning and decreased further to 50% by d 5 postweaning. As a result, pigs weaned at an early age often suffer from diarrhea and reduced growth performance during d 7 to 14 postweaning. The reasons for villus atrophy after weaning are complex (Pluske et al., 1996; Spreeuwenberg et al., 2001). One of the reasons proposed by Pluske et al. (1997) is that weaning stops the supply of Gln from maternal milk, affecting intestinal villus growth.

Although Gln is labile in acid conditions, Gln supplemented to the diet remains stable until it reaches the small intestine of weaned pigs (Krebs, 1980; Wu et al., 1996). Dietary Gln supplement can increase the absorption of sodium in 21 d old pigs suffering from *Rotavirus* infection (Rhoads et al., 1990), and maintain muscle Gln level under *E. coli* infection (Yoo et al., 1997). These results indicate that Gln is an important nutrient for weaned pigs. However, the effects of dietary Gln on the performance and intestinal physiology of weaned pigs are inconsistent (Wu et al., 1996; Kitt et al., 2002; Lee et al., 2002). This study was designed to clarify the effect of dietary Gln supplementation on performance and villus morphology in small intestine of weaned pigs.

MATERIALS AND METHODS

Animals and treatment

All pigs were the offspring of either Yorkshire or Landrace sows crossed with Duroc boars. Pigs were weaned at 21 d of age, and fed a basal diet containing corn, soybean meal, isolated soybean protein and whey, supplemented with vitamins and minerals. Pigs were fed 0, 0.5, 1.0 or 1.5% glutamine supplement diets (Ajinomoto Inc., Tokyo, Japan). All nutrients met the standards of the National Research Council (NRC, 1998). Table 1 lists the diet

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Table 1. Formula and nutrient composition of basal diet

Ingredients (%)	
Corn	50.09
Soybean meal	22.51
Soy protein (CP 65%)	12.00
Whey	10.00
DL-methionine	0.03
Soybean oil	1.12
Glutamine-corn starch[1]	1.50
Dicalcium phosphate	1.27
Limestone, pulverized	0.88
Salt	0.30
Vitamin premix[2]	0.10
Trace mineral premix[3]	0.10
Antibiotic premix[4]	0.10
Calculated nutrient composition	
ME (kcal/kg)	3,265
CP (%)	23.70
Ca (%)	0.80
P (%)	0.69
Analyzed nutrient composition	
Lys (%)	1.46
Met+Cys (%)	0.76
Glu+Gln (%)	3.70

[1] Glutamine substituted for corn starch. Glutamine levels were 1. 0.5, 1.0 or 1.5%. [2] Supplied per kg diet: vitamin A, 6,000 IU; vitamin D, 900 IU; vitamin E, 30 IU; vitamin K_3, 3 mg; vitamin B_2, 6 mg; pantothenic acid, 18 mg; niacin, 60 mg; vitamin B_{12}, 30 μg, and choline-HCl, 525 mg. [3] Supplied per kg diet: Cu, 20 mg; Zn, 100 mg; Fe, 140 mg; Mn, 4 mg; Se, 0.1 mg and I, 0.2 mg. [4] Supplied per kg diet: lincomycin-HCl 44 mg and spectinomycin sulfuate 44 mg.

formulation and nutrient composition. Feed and water were fed *ad libitum*. The total content of Gln and glutamate (Glu) in the basal diet was 3.70%, which was determined using the hydrolysis technique by amino acid automatic analyzer (System Gold, Beckman Inc., USA).

Effect of dietary glutamine levels on performance

Forth eight pigs from 6 litters were randomly allotted to dietary treatments based on sex and litter origin. Pigs were housed in a nursery room with four pigs per pen. Temperature in the nursery room was maintained around 26°C with heating supply during the first 2 weeks. Body weight and feed intake were recorded weekly during experiments. On d 14 and d 28, 10 ml blood was taken from two pigs in each pen and centrifuged at 2,000×g for 15 min. The plasma was mixed with an equal volume of 1.5 M perchloric acid and mixed well with a vortex. After 10 min the mixture was centrifuged using the method of Wu et al. (1996). The supernatant was used for determination of free amino acid composition with an automatic analyzer (System Gold, Beckman Inc.) and for Gln and Glu concentrations using an enzyme assay (Lund, 1983). Plasma IgA was measured using an ELISA kit (Bethyl Lab., Inc., USA).

Effect of dietary glutamine levels on intestinal morphology

In this Exp., 48 pigs from 6 litters were randomly allotted to dietary treatments based on sex and litter origin. The pigs were fed Gln supplement diet for 7 or 14 days. The basal diet and feeding condition were the same as in Exp. 1. Four and 8 pigs from each dietary group were killed on d 7 and d 14 postweaning, respectively. After citsol (1 mg/kg body weight, i.v.) injection pre-anesthesia, a mixture of 4% halothane and 95% oxygen for surgical anesthesia was administered by facemask. The abdomen was opened after anesthesia. The empty carcass was weighed after removing internal organs. Intestinal tissue samples at 5 cm

Table 2. Effects of dietary glutamine supplement on performance of weaned pigs (Exp. 1)

Item	Glutamine (%)				SEM
	0	0.5	1.0	1.5	
Initial weight (kg)	6.03	5.83	5.98	5.97	0.34
Finial weight (kg)	15.28	14.97	14.99	15.40	0.69
d 0 to 14					
ADG (g)	181.7	201.8	181.5	205.6	15.93
ADFI (g)	268.7	276.5	258.2	296.8	22.69
Feed efficiency (F/G)	1.50	1.40	1.46	1.51	0.09
Diarrhea[1] (%)	0.71	0.00	0.48	0.00	0.43
d 15 to 28					
ADG (g)	488.3	464.1	475.0	478.8	25.47
ADFI (g)	739.2	684.2	677.0	717.0	60.60
Feed efficiency (F/G)	1.53	1.49	1.43	1.53	0.05
Diarrhea[1] (%)	2.14	0.00	0.48	0.71	1.15
d 0 to 28					
ADG (g)	332.5	330.8	326.1	340.6	16.56
ADFI (g)	498.9	477.3	464.5	504.8	34.58
Feed efficiency (F/G)	1.53	1.46	1.44	1.51	0.06
Diarrhea[1] (%)	1.43	0.00	0.48	0.36	0.77

[1] Diarrhea (%)=Incidence of diarrhea daily × no. of pigs accumulated/period (day) × no. of pigs × 100%.

Table 3. Effects of dietary glutamine supplement on concentration of plasma free amino acids of weaned pigs (Exp. 1)

Days postweaning	Glutamine (%)				SEM
	0	0.5	1.0	1.5	
d 14 (μmol/l)					
Gln[1]	224.0	180.7	175.4	18.07	20.20
Glu[1]	510.6	528.8	398.0	620.9	141.0
Pro	338.8[a]	262.1[ab]	158.2[b]	231.6[b]	34.64
Thr	259.4[a]	218.6[ab]	188.4[b]	185.3[b]	19.57
Gly	1,034.7	1,083.5	854.8	1,047.3	67.37
Ala	560.7[a]	459.9[ab]	393.9[b]	461.6[ab]	40.72
Val	255.4	220.62	228.05	249.78	19.85
Met	37.75[b]	45.62[a]	32.98[b]	38.92[b]	2.28
Cys	47.24	44.82	42.64	53.40	3.33
Ile	159.0	145.8	131.5	135.1	12.12
Leu	207.9	184.9	158.7	168.0	13.38
Tyr	121.5	112.0	89.38	99.19	10.14
Phe	88.04	96.93	86.17	72.69	6.48
His	87.18	94.33	72.38	78.48	6.74
Lys	108.3	84.86	84.06	111.1	14.40
Arg	191.0	164.1	132.3	164.6	14.54
d 28 (μmol/l)					
Gln[1]	181.9	172.4	183.6	199.2	17.34
Glu[1]	493.0	435.2	490.3	530.0	36.28
Pro	253.8	320.0	333.8	314.4	45.81
Thr	266.1	211.3	232.4	199.3	28.57
Gly	1,211.2[a]	1,223.6[a]	1,111.2[ab]	938.4[b]	66.41
Ala	472.7[a]	484.2[a]	346.4[b]	374.3[b]	36.22
Val	256.8	230.6	223.0	204.3	13.33
Met	42.90	46.80	43.28	38.72	3.77
Cys	53.53	55.25	47.63	47.19	2.65
Ile	132.5	137.2	129.3	112.1	8.81
Leu	196.7	196.9	179.0	174.9	10.30
Tyr	138.8	135.6	137.7	124.8	10.33
Phe	88.42[b]	109.8[a]	87.36[b]	94.47[b]	5.54
His	80.98[b]	95.08[ab]	70.85[b]	142.2[a]	17.72
Lys	93.22	104.72	93.77	95.38	8.55
Arg	164.4	177.6	153.6	157.9	16.53

[1] Determined by enzymatic analysis. [a, b] Means within the same row without common superscripts differ significantly ($p<0.05$).

from the pylorus, middle, and end were dissected, washed with 0.1 M phosphate buffer solution and fixed with 2.5% glutaraldehyde. The intestinal samples were embedded in paraffin according to the method of Spurr (1969), sectioned at 6 μm thickness and stained with hematoxylin and eosin for light microscopy examination (Olympus BX50, Japan). Villus height and crypt depth were measured based on 15 apparently intact villi. Bile IgA was measured with an ELISA kit (Bethyl Lab., Inc.), and the concentration of protein was measured using bicinchoninic acid protein assay kit (Pierce Inc., USA).

Statistical analysis

Experimental data were analyzed using the SAS (1999) statistical program. The general linear model was used to analyze variance, and Duncan's multiple range test was applied for comparing the differences between treatments.

RESULTS

Effect of dietary glutamine levels on performance of weaned pigs

Table 2 displays the performance of weaned pigs fed Gln at different levels (0, 0.5, 1.0 or 1.5%) in the diets. The level of Gln supplementation did not significantly influence performance or occurrence of diarrhea during d 0 to 28 postweaning. Table 3 lists the plasma free amino acid concentrations of weaned pigs at d 14 and d 28 postweaning. The concentration of Met increased, and the concentrations of Pro, Thr and Ala decreased in plasma from pigs with increasing dietary Gln levels at d 14 postweaning. Plasma Phe and His were higher, but Gly and Ala were lower in pigs fed diets supplemented with Gln than in the control group at d 28 postweaning. Plasma Gln and Glu concentrations remained constant throughout the experimental period.

The effect of dietary supplementation with Gln on plasma IgA concentration remained similar at d 14 and d 28 postweaning. The values were between 0.27 to 0.36 mg/ml (data not shown).

Effect of dietary glutamine levels on intestinal morphology of weaned pigs

Figure 1 illustrates the effect of dietary levels of Gln on the digestive tract weight of weaned pigs. Dietary treatments did not influence the digestive tract weight on d 7, but the small intestine/carcass weight ratio was decreased with increasing dietary Gln levels on d 14. The weight of stomach grew rapidly within 7 d postweaning, while pancreas and small intestine continued to grow thereafter till 14 d postweaning. Table 4 illustrates the effects of dietary Gln levels on the morphology of small intestinal villi. On d 7 postweaning, the villus height at the duodenum at 0.5% Gln supplementation and the villus height/crypt depth ratio at the ileum at 1.0% Gln supplementation were increased. On d 14, the villus height and villus height/crypt depth ratio at the duodenum increased when more than 0.5% Gln was supplemented. Villus height increased compared to the control group in all Gln supplemented groups, but the difference was non significant ($p>0.05$). The performance and occurrence of diarrhea in pigs were not affected by dietary Gln levels during 0 to 7 d or 0 to 14 d postweaning (data not shown).

Table 5 lists the effects of Gln supplementation levels on bile composition and plasma IgA. Bile IgA and protein concentrations were increased at 0.5% of dietary Gln at d 7 and d 14 postweaning, respectively. Plasma IgA remained constant regardless of dietary Gln supplementation levels.

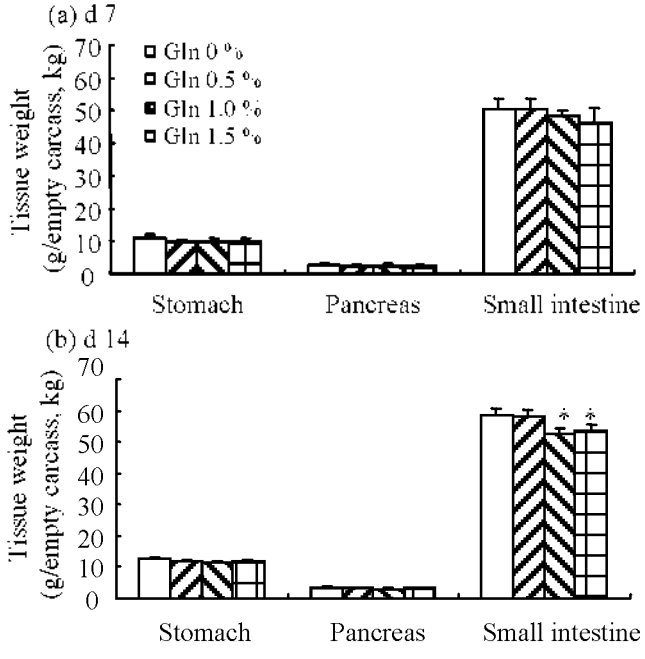

Figure 1. Effects of dietary glutamine supplement on digestive tract weight of weaned pigs at d 7 (a), and d 14 postweaning (b). * Means compare to the Gln 0% group differed significantly ($p<0.05$) (Exp. 2).

DISCUSSION

The reduced growth rate and intestinal atrophy at the early phase of weaning pigs (Hampson, 1986; Dunsford et al., 1989) was confirmed in this study. It is recognized that intestinal atrophy in early weaned pigs remains a major problem in this period. Digestion and absorption of nutrients are affected (Miller et al., 1986) and there is an increased demand for Gln to support their metabolism after weaning (Dugan et al., 1994). Wu et al. (1996) reported that supplementing to a simple basal diet with 1% Gln improved feed efficiency in pigs weaned at 21 d of age. However, no improvement in performance was observed in this study. This could be due to the 10% whey added to our basal diet. Kitt et al. (2002) also reported that supplementation of Gln to a simple diet resulted in greater feed efficiency in pigs, compared to Gln supplemented to a complex diet containing whey, plasma and fish meal.

Dietary Gln supplement did not influence the concentrations of Gln and Glu in plasma on d 14 or d 28 postweaning. The result is similar to those of Yoo et al. (1997). They reported that the plasma Gln concentration was unchanged by the addition of 4% Gln for pigs weaned at 21 d of age. These data indicating that dietary Gln supplement might be utilized by the cells in the digestive tract or metabolized by liver and other organs before entering plasma (Souba and Austgen, 1990; Stoll et al., 1998). Among the many rapidly dividing cells in gut tissue, activated lymphocyte and intestinal epithelial cells utilize Gln extensively (Szondy and Newsholme, 1989; Souba, 1990; Wu, 1998). Windmueller and Spaeth (1980) determined the amount of intestinal uptake of circulating Gln was above 25%. Changes in plasma concentrations of certain amino acids in weaned pigs supplemented with Gln (Table 3) were also noticed, but the mechanism involved is not known.

In this study, we found that intestinal growth was increased significantly during the 14 d weaning period. Feeding Gln decreased the relative growth of the small intestine, a finding contrary to a previous study (Lackeyram et al., 2001). Although the composition or the activity of the microflora in the digestive tract was not determined in this study, other studies have indicated that Gln supplement reduced the attack from pathogens (Souba et al., 1990; Arndt et al., 1999). Thus, the weight of the small intestine might be reduced by decreasing inflammation. Dietary Gln supplement improved the development of intestine villi, particularly in the duodenum, which generally atrophied

Table 4. Effects of dietary glutamine supplement on villus morphology of weaned pigs (Exp. 2)

Days postweaning	Glutamine (%)				SEM
	0	0.5	1.0	1.5	
d 7					
No. of pigs	4	4	4	4	
Duodenum					
Villus height (μm)	249.61[a]	406.10[b]	348.86[ab]	316.24[ab]	31.12
Crypt depth (μm)	196.95	230.28	250.16	213.51	13.11
Villus height/crypt depth	1.32	1.76	1.41	1.48	0.16
Jejunum					
Villus height (μm)	221.22	266.17	286.37	241.05	21.61
Crypt depth (μm)	193.72	199.53	223.37	213.15	12.81
Villus height/crypt depth	1.14	1.34	1.29	1.16	0.13
Ileum					
Villus height (μm)	208.83	256.32	293.37	293.07	26.52
Crypt depth (μm)	209.87	202.59	186.29	208.80	13.58
Villus height/crypt depth	0.99[a]	1.27[ab]	1.65[b]	1.38[ab]	0.13
d 14					
No. of pigs	8	8	8	8	
Duodenum					
Villus height (μm)	281.78[a]	378.83b	429.96[b]	437.76[b]	24.85
Crypt depth (μm)	253.95	243.20	248.04	256.47	11.41
Villus height/crypt depth	1.12[a]	1.57[b]	1.75[b]	1.72[b]	0.11
Jejunum					
Villus height (μm)	320.94	401.44	375.71	385.54	22.45
Crypt depth (μm)	208.07	213.04	221.89	211.04	12.68
Villus height/crypt depth	1.56	1.92	1.72	1.92	0.15
Ileum					
Villus height (μm)	296.23	353.48	361.48	350.80	24.02
Crypt depth (μm)	223.27	220.86	207.18	215.11	10.74
Villus height/crypt depth	1.33	1.62	1.76	1.71	0.14

[a,b] Means within the same row without common superscripts differ significantly ($p<0.05$).

Table 5. Effects of dietary glutamine supplement on IgA concentrations of bile and plasma in weaned pigs (Exp. 2)

Days postweaning	Glutamine (%)				SEM
	0	0.5	1.0	1.5	
d 7					
No. of pigs	4	4	4	4	
Plasma IgA (μg/ml)	49.75	53.43	46.38	50.70	6.95
Bile IgA (μg/ml)	11.78[a]	30.22[b]	14.08[ab]	24.18[ab]	3.14
Bile protein (mg/ml)	2.22	3.46	2.54	4.42	0.77
Bile IgA/protein (μg/ml)	6.00	9.31	5.57	6.46	0.94
d 14					
No. of pigs	8	8	8	8	
Plasma IgA (μg/ml)	89.02	98.56	81.61	83.98	9.67
Bile IgA (μg/ml)	21.77	34.58	22.87	31.29	6.82
Bile protein (mg/ml)	1.56[a]	3.11[c]	2.02[ab]	2.63[bc]	0.28
Bile IgA/protein (μg/ml)	14.45	10.96	11.29	12.49	2.27

[a,b,c] Means within the same row without common superscripts differ significantly ($p<0.05$).

more seriously than in the jejunum and ileum after weaning. In the pigs fed with 0.5% Gln, the villi grew back to normal height by d 7 postweaning. This finding was in accordance with earlier findings that the addition of Gln increased the villus height of weaned pigs (Ayonride et al., 1995; Wu et al., 1996; Liu et al., 2002). Therefore, our results suggest that dietary Gln reduces intestinal mass and yet increases villus height.

IgA secreted from the digestive tract could be circulated through the lymph system into bile (Alverdy, 1990). Burke et al. (1989) noted that the IgA level dropped by 50% in weaning rats, however adding Gln to the diet brought the level back to normal. Research on the effect of dietary Glu supplement on bile IgA concentration in weaned pigs is rare.

Our previous study found that the dietary Gln supplement tended to increase the concentration of plasma IgA at d 12 postweaning of pigs weaning at 14 d of age (Lee et al., 2002). In a separate report of this experiment, we found that the addition of Gln increased the proliferation of peripheral blood mononuclear cells, mesenteric lymph node, and splenocytes (Lee et al., 2003). Therefore, increase of IgA concentration in bile by dietary Gln supplementation might benefit immune responses in the digestive tract.

In conclusion, this study has shown that the growth performance of weaned pigs was not affected by dietary Gln supplement in the basal diet containing 10% whey. However, the results provide an experimental basis that Gln supplement might benefit the development of the small intestine and bile IgA secretion in weaned pigs.

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