

Effects of Dietary Glutamine and Glutamate Supplementation on Small Intestinal Structure, Active Absorption and DNA, RNA Concentrations in Skeletal Muscle Tissue of Weaned Piglets during d 28 to 42 of Age^a

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ABSTRACT : Seventy-four piglets were used to investigate the effects of dietary glutamine (Gln) and glutamate (Glu) on the mucosal structure and active absorption of small intestinal, DNA and RNA concentrations of skeletal muscle tissue in piglets during d 28 to 42 of age. Postweaning piglets were fed for 14 d corn- and soybean meal-based diets supplemented with 0.0 or 1.0% L-Gln or L-Glu. On d 7 and 14 postweaning, pigs' small intestinal sections and longissimus dorsi were collected, at the same time, the D-xylose absorption test was conducted. The results suggested that in comparison to control piglets, jejunal atrophy during the first week postweaning was prevented by the glutamine and glutamate supplementation (1%) and the capability of small intestine to absorb D-xylose was improved. Furthermore the RNA concentration in skeletal muscle tissue was increased. These results provide an experimental basis for use of glutamine and glutamate on alleviating the weaning stresses and improving piglets' growth performance. (*Asian-Aust. J. Anim. Sci.* 2002, Vol 15, No. 2 : 238-242)

Key Words : Glutamine, Glutamate, Small Intestine, Piglets

INTRODUCTION

Glutamine is the most abundant amino acid in the plasma of animals (Hamilton, 1945; Smith and Panico, 1985) and in sow's milk on d 22 and 29 of lactation (Wu and Knabe, 1994) and serves the primary metabolic fuel for rapidly dividing cells including intestinal epithelial cells (Windmueller and Spaeth, 1980; Windmueller, 1982) and activated lymphocytes (Alverdy, 1990; Ardawi, 1988; Brand et al., 1989). It plays an important role in promoting gut rescue, maintaining the gut barrier, and normalizing local immune function (Souba et al., 1990; Yoo et al., 1997). Glutamate is also a major fuel of gut mucosal cells (Reeds et al., 1997; Reeds et al., 1996). It is the obligatory precursor for the synthesis of glutamine (Neame et al., 1957), and glutathione synthesis in intestinal mucosa (Reeds et al., 1997) that plays an important role in maintaining the defenses of the mucosa against toxic and peroxidative damage. Because milk was removed from the piglet's diet after weaning and feed intake is minimal during a short period postweaning, inadequate supply of glutamine and glutamate might be the critical reason for the disordered gut mucosal structure and function, even the poor growth performance (Wu et al., 1996; Liu and Peng, 1999). The

previous results of our study have shown that glutamine and glutamate supplementation could improve the growth performance of weaning piglets (Liu and Peng, 1999). The present study was conducted to investigate the effects of dietary glutamine and glutamate supplementation on gut mucosal structure, active absorption of small intestine and DNA and RNA concentrations in skeletal muscle tissue.

MATERIALS AND METHODS

Animals and experimental design

Pigs were offspring of Hubei White × Large White, and were obtained from the Experimental Swine Center of Huazhong Agricultural University. They were nursed by their mothers until 28 d of age. At the time of weaning, littermates were randomly assigned to dietary treatments to ensure that genetic effects would be balanced across treatments. In all experiments, pigs had free access to water and corn- and soybean meal-based diets supplemented with 0.0 or 1.0% free L-Gln or L-Glu (table 1) for 14 days.

Chemicals

L-Glutamine was provided by Shanghai Boao Biotechnical Co., Ltd. imported from Ajinomoto Inc. (Tokyo, Japan). Sino-America Bioengineering Co. (Wuhan, China) provided L-glutamate. All other chemicals used in this study were purchased from Yuanpinghao Biotech. Co. Ltd. (Wuhan, P. R. China).

Experiment 1: Effect of dietary glutamine and glutamate supplementation on gut mucosal structure and DNA, RNA concentrations in skeletal muscle tissue.

Twenty-one pigs weaned at 28d of age were used in this

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Table 1. The basal diet composition

Ingredients (%)	
Com	60.55
Soybean meal	22.10
Fish meal	5.00
Dried whey	10.00
Limestone	0.45
Monohydrogen phosphate	0.60
L-Lysine-HCl	0.20
Salt	0.30
Premix ^a	0.80
Calculated nutrient composition	
Digestible energy (Mcal/kg)	3.46
Crude protein (%)	18.30
Methionine (%)	0.33
Lysine (%)	1.35
Calcium (%)	0.80
Phosphorus (%)	0.66

^a Supplied per kg diet: Cu, 250 mg; Zn, 200 mg; Fe, 100 mg; Terramycin, 50 mg; VA, 4,100 IU; VD3, 1,080 IU; VE, 36 IU; VK3, 10 mg; VB2, 10 mg; VB12, 30 µg; Folicin, 500 µg; Pantothenic acid, 25 mg.

experiment. Three were killed initially at 28 d of age to determine baseline values for intestinal villous height (VH) and lamina propria depth (LPD) after weighted individually. The remaining pigs were randomly assigned to dietary treatments (0.0 or 1.0% glutamine or glutamate supplementation) (table 1). Three pigs from each dietary treatment were killed on d 7 and d 14 postweaning. Briefly, pigs were anesthetized by intramuscular injections of pentobarbital (4%) at dose of 40 mg/kg BW. After five minutes, the anesthesia proceeding was completed normally. After the gut was removed, the small intestine was immediately placed in frozen physiological saline solution and the mesenteric web was cut, allowing the intestine to be laid out straight. Samples were taken at: 1) 5 cm from the pylorus (duodenum), 2) 5 cm from head of jejunum, 3) 50% of the small intestine's length (mid-jejunum), 4) 4 cm before the ileocecal junction. Briefly, all the samples were immersed in flaxative, 10% buffered formalin solution.

Table 2. Effect of glutamine and glutamate on VH and LPD

VH or LPD	Days after weaning	Duodenum			Head of jejunum			Mid-jejunum			End of jejunum						
		0%	+1%		0%	1%		0%	1%		0%	1%					
			Gln	Glu		SE ¹	Gln		Glu	SE ¹		Gln	Glu	SE ¹	Gln	Glu	SE ¹
VH	0	478 ^a			445 ^a			471 ^a			487 ^{ac}						
	7	400 ^b	387 ^c	409 ^{bc}	35	347 ^b	435 ^a	432 ^a	33	354 ^b	448 ^a	441 ^a	36	334 ^b	443 ^a	437 ^a	36
	14	519 ^{ad}	494 ^a	562 ^d	32	480 ^a	484 ^a	502 ^a	25	497 ^a	484 ^a	506 ^d	30	447 ^a	435 ^a	508 ^c	35
LPD	0	218 ^b			183 ^c			153 ^c			160 ^b						
	7	263 ^{ab}	258 ^{ab}	280 ^b	19	234 ^b	260 ^b	286 ^b	20	223 ^b	208 ^b	200 ^b	13	200 ^{ab}	199 ^{ab}	236 ^a	19
	14	342 ^a	351 ^a	326 ^a	17	297 ^a	252 ^b	281 ^{ab}	16	293 ^a	209 ^b	209 ^b	16	238 ^a	189 ^b	240 ^a	23

^{a,b,c,d} Means with difference in a row or column differ ($p < 0.05$). ¹ pooled standard error.

Abbreviations: VH, villous height; LPD, lamina propria depth.

They were then cleared in xylene before being embedded in paraffin and then sectioned. Six cross sections (15 µm thick) per sample were stained with hematoxylin and eosin. 10 longest VH and 10 LPD were measured with a 10 times microscope with an ocular micrometer. The LPD measurement extended vertically from the base of the villas to the muscular mucosa. The VH was the vertical height also.

Muscle biopsies (~5-6 g) of longissimus dorsi (LD) were taken and snap-frozen in liquid-nitrogen, and stored at -70°C until analyzed for DNA and RNA concentration as described previously (Zhujiang et al., 1981).

Experiment 2: Effect of glutamine and glutamate on active absorption of small intestine

Thirty-five pigs weaned at age of 28 d were used in this experiment. Five pigs were used at 28 d of age to determine baseline active absorption of small intestine. The remaining pigs were randomly assigned to dietary treatment as described in Exp.1. Before the procedure was carried, the pig's access to feed and water was prohibited for 24 h and 4 h respectively. On d 7 and 14 postweaning, pigs were fed 10% D-xylose solution at dose of 1 ml/kg BW. 1 h later, blood samples (6 ml) was withdrawn from anterior vena cava into unheparinized tubes, serum was obtained by centrifuging blood at 1,240 g × 10 min. Serum was stored at -20°C until analyzed for D-xylose concentration as described previously (Li et al., 1995).

Statistical analysis

All data were analyzed by using the GLM procedure of SAS (1990). Data was subjected to repeated-measures ANOVA. If a given ANOVA was significant at $p < 0.05$, comparison of means was performed using Duncan multiple range test.

RESULTS

Effect of dietary glutamine and glutamate supplementation on gut mucosal feature and structure

Table 2 summarizes the effects of glutamine and glutamate on gut mucosal structure. In pigs supplemented

with 0.0 or 1.0% glutamine and glutamate. VH in duodenum decreased ($p < 0.05$) on d 7 postweaning, but increased ($p < 0.05$) on d 14 postweaning heights. On d 7 postweaning, the VH in 3 sites of jejunum all decreased ($p < 0.05$) in control pigs, but maintained at preweaning value in pigs supplemented with 1.0% glutamine and glutamate. On d 14 postweaning, the VH in the end of jejunum of pigs supplemented with 1.0% glutamate was greater ($p < 0.05$) than in pigs supplemented with 1.0% glutamine and control group. The LPD of duodenum in all three groups progressively increased with increasing age. In control pigs, the LPD of head of jejunum and mid-jejunum also progressively increased with increasing age. However in glutamine and glutamate supplemented pigs, there was no difference ($p > 0.05$) in jejunum between d 7 and 14 postweaning.

Effect of dietary glutamine and glutamate supplementation on active absorption of small intestine

Table 3 showed the D-xylose concentration in serum of three treatments. In control pigs, D-xylose concentration on 7 d postweaning was lower significantly ($p < 0.05$) than preweaning value and on 14 d postweaning. However on 7 d postweaning D-xylose concentration in glutamine and glutamate supplementation pigs were greater ($p < 0.05$) than in control pigs. There was no difference in xylose concentration between glutamine and glutamate supplementation pigs on 7 and 14 d postweaning.

Effect of dietary glutamine and glutamate supplementation on DNA, RNA concentrations in skeletal muscle tissue

Table 3 also summarized the RNA, DNA concentrations in skeletal muscle tissue of pigs fed diets supplemented with 0.0 or 1.0% glutamine and glutamate. In pigs of three treatments, the RNA concentration in skeletal muscle tissue decreased ($p < 0.05$) on d 7 postweaning, and even on d 14 postweaning, the RNA concentration could not recover to the preweaning value. On 7 d postweaning, the RNA

concentration in glutamine and glutamate supplementation pigs were both greater than in control pigs, especially the glutamate supplementation pigs have significant value ($p < 0.05$). On 14 d postweaning the RNA concentration in glutamine and glutamate supplementation pigs were mathematically greater than control pigs, though there was no difference. Meanwhile there was no difference in the DNA concentration in muscle tissue between three groups in whole experimental period.

DISCUSSION

Glutamine has been shown to reduce both the mucosal atrophy and the bacterial translocation that occur during long periods of parenteral nutrition (Grant et al., 1988; O'Dwyer et al., 1989; Li et al., 1994). Experimental animals or human subjects deprived of enteral glutamine also demonstrated a reduction in intestinal villous height, relative depletion of enterocyte population or density, and impaired absorption of foodstuffs or inert markers (Helton, 1994; Lewis et al., 1995). Wu et al. (1996) demonstrated that supplementation of glutamine could prevent jejunal atrophy during the first week postweaning. Our present studies also showed that glutamine supplementation (1%) maintained the normal intestinal mucosal structure on d 7 postweaning. This result agreed with the finding from Wu et al. (1996). Windmueller et al. (1982) demonstrated that existence of glutamine in gut could decrease significantly the enterocytes uptake of circulating glutamine. Additional studies with glutamine-enriched TPN demonstrated activation of intestinal glutaminase acceleration of intestinal glutamine uptake (Salloum et al., 1990). Another observation from the study was that dietary glutamine prevented the increase in jejunal LPD on d 14 postweaning because of the effects of glutamine supplementation on maturation and migration of crypt cells which exists in lamina propria. All the studies indicated that the dietary supplementation of glutamine is feasible and reasonable.

Weaning of piglets is already known to be associated

Table 3. D-xylose concentration in serum and RNA, DNA concentrations in skeletal muscle tissue

	Days after weaning	Control	Glutamine (1%)	Glutamate (1%)	SE ¹
D-xylose (mmol/L)	0	0.8210 ^{ac}			
	7	0.6562 ^b	0.8383 ^c	0.8594 ^c	0.0350
	14	0.7914 ^a	0.8832 ^c	0.8670 ^c	0.0156
RNA (mg/g)	0	2.7573 ^a			
	7	2.3240 ^b	2.4060 ^b	2.6333 ^a	0.0556
	14	2.4880	2.5316	2.6300	0.0523
DNA (mg/g)	0	1.7500			
	7	1.6556	1.6536	1.6773	0.0307
	14	1.8096	1.6333	1.7515	0.0603

^{a,b,c} Means with difference in a row or column differ ($p < 0.05$). ¹ pooled standard error.

with gross changes in intestinal feature and structure that decline in active absorption (Miller et al., 1984; Hetty et al., 1998; Hampson et al., 1986; Tong et al., 1989). Such a conclusion is also supported by the fall in xylose absorption observed in experiment 2 (table 3). Rhoads et al. (1994) have shown that glutamine oxidation stimulated intestinal and enterocytes Na^+/H^- exchange leading to a high rate of NaCl absorption in health and diseased jejunal epithelium (Rhoads et al., 1991, 1992). A similar observation in this study also showed that dietary glutamine supplementation increased the serum xylose concentration compared with control pigs, and maintained the intestinal active absorption on 7 d postweaning. The possible explanations are following 1) Being the major fuels of intestinal mucosal cells, glutamine accelerated the cellular survival and proliferation (Reeds et al., 1996). 2) Glutathione synthesis from glutamine maintained the defenses of mucosa against toxicant peroxidative damage (Reeds et al., 1997).

The major tissue involved in glutamine synthesis in the human body and other animals is skeletal muscle (Vinnars et al., 1975; Newsholme et al., 1990; Souba et al., 1990). This has the enzymatic capacity to synthesize glutamine, and has been reported to store and release glutamine into the bloodstream at a high rate (Parry-Billings et al., 1990). However, when animals are in critical illness or stressful conditions, which results in a decrease in the rate of protein synthesis and increase in the rate of protein degradation (Klasing et al., 1984a,b; Klasing et al., 1987), glutamine utilization may exceed its production resulting in a decreased plasma glutamine level and a depletion of glutamine stores (Castell and Newsholme, 2001). We assumed that if dietary glutamine supplementation could support the intestinal fuel requirements during a short period postweaning, the protein degradation rate in skeletal muscle tissue may decrease and intramuscular glutamine concentration be maintained. Therefore the RNA concentration difference between several diet treatments was studied. As shown in table 3, on d 7 postweaning, dietary glutamine supplementation increased the RNA concentration in skeletal muscle tissue. The higher concentration of RNA in tissue indicated the increased tissue protein and/or the enzyme synthesis, which were essential to maintain the increasing protein-synthesis status. Enteral diets that contain glutamine may provide appropriate fuels to the gastrointestinal mucosa and therefore eliminates the need for excessive glucocorticoid secretion that would mobilize muscle glutamine store and accelerate gut glutamine uptake (Souba et al., 1985) in order to satisfy intestinal fuel requirements (Yoo et al., 1997). There are many other investigators who evaluate the direct effects of exogenous glutamine on muscle glutamine content, protein synthesis, and muscle mass. Kapadia and colleagues showed that a glutamine infusion during the postoperative period could diminish the efflux of glutamine

from hindlimb using a canine model (Kapadia et al., 1985). Other demonstrated that muscle glutamine concentration correlated directly with the rate of muscle protein synthesis and the RNA/protein ratio and was inversely correlated with the rate of protein degradation (Millward et al., 1989). But the direct relationship between dietary glutamine and intramuscular glutamine concentration, protein synthesis and degradation in weaned pigs merits further study.

Furthermore, it is very exciting that glutamate have the same function as glutamine in preventing jejunal atrophy, decreasing jejunal LPD, and improving active absorption of small intestine as well as increasing RNA concentration in skeletal muscle tissue. Reeds et al. (2000) indicated that under fed conditions, enteral (dietary) glutamate was a far more important oxidative substrate than arterial glutamine. Meanwhile, dietary glutamate was clearly playing a quantitatively significant role in the biosynthesis of two conditionally essential amino acids (proline and arginine) and was a key factor responsible for protection of the mucosa (glutathione). Though these two closely related amino acids have different functional roles in the multicellular system termed the intestinal mucosa, glutamate might perform the function to maintain a health gut as shown in our studies. Whether dietary glutamate is an essential factor for maintenance of mucosal health and the extent to which mucosal glutamate metabolism confers direct physiological benefit are at present unknown and merit further examination.

In conclusion, the results of this study demonstrated that dietary glutamine and glutamate supplementation maintained the intestinal feature and structure and improved the intestinal active absorption and the RNA concentration in skeletal muscle tissue in weaned pigs during the first week postweaning. These results provide an experimental basis for use of glutamine and glutamate on alleviating the weaning stresses and improving piglets' growth performance.

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