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Effects of Diets Supplemented with Recombinant Epidermal Growth Factor and Glutamine on Gastrointestinal Tract Development of Early-weaned Piglets*

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ABSTRACT : This study attempted to determine effects of recombinant porcine epidermal growth factor (pEGF) and glutamine (Gln) supplement on the growth performance and intestinal development of piglets weaned at 14 days of age. A total of ninety-six piglets were allotted to one of four dietary treatments which comprised inclusion of 1.0 mg pEGF supernatant/kg diet or 0.5% Gln both alone and in combination. Each treatment consisted of four replicates with six pigs per pen for a 28 days experimental period. Two pigs per replicate were sacrificed and gastrointestinal tract samples were collected on day 14. Data showed that dietary treatment failed to promote growth performance. On day 14, diets supplemented with pEGF elevated pancreatic chymotrypsin, jejunal alkaline phosphatase, sucrase, lactase and maltase activities ($p<0.05$), but failed to alter the small intestinal villus morphology, DNA, or protein content of gastrointestinal mucosa. Diets supplemented with Gln increased pancreatic chymotrypsin activity, tended to enhance the protein contents of gastric ($p = 0.08$) and jejunal mucosa ($p = 0.09$) but did not influence the serum IgA level or the enzyme activity in the gastrointestinal tract. On day 28, the diets supplemented with Gln increasedt ($p<0.05$) serum IgA and the proliferation of peripheral blood mononuclear cells by PHA stimulation. However, a combination of pEGF and Gln did not have a synergistic effect on these biomarkers in early-weaned piglets. The results demonstrate that diets supplemented with recombinant pEGF supernatant indeed improve intestinal digestive enzyme activity and diets supplemented with Gln increases the immune response in early-weaned piglets. (**Key Words** : Epidermal Growth Factor, Glutamine, Piglets, Gastrointestinal Tract)

INTRODUCTION

Piglets at weaning are subjected to dietary, environmental and physiological stress, which may raise the risk of diarrhea or growth retardation. These problems occur owing to the changes of morphology and enzyme activities in the porcine small intestine after weaning (Fan et al., 2002; Hampson, 1986; Kenworthy, 1976). The intestinal villus height reduced to 75% of pre-weaning values within 24 h post-weaning (Hampson, 1986) while crypt depths lifted after weaning (Pluske et al., 1996). The increase in crypt depth is ascribed to the mature enterocytes loss as the villi shorten and the failure of the immature enterocytes renewal to differentiate fully to express maximal enzyme activity (Hampson and Kidder, 1986). Approaches to improve weaning piglets through feeding management and particular nutrients supplement are becoming more critical and interesting issue.

Epidermal growth factor (EGF), a polypeptide identified in the milk of different mammals including rats, humans and swine (Cohen and Elliott, 1963; Jaeger et al., 1987; Moran et al., 1983) is highly varies in animal species and lactation period. Sow's milk typically contains 124 to 200 ng EGF/ml (Jaeger et al., 1987). EGF has many trophic effects on gastrointestinal tract including improved proliferation and differentiation of epithelial cells (Carpenter and Cohen, 1990; Wong and Wright, 1999). EGF has been demonstrated to increase stomach and small

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Table 1. Composition of the basal diets

Item	Day 0 to 14	Day 15 to 28
Ingredients (%)		
Corn	45.19	56.85
Soybean meal	25.00	23.83
Soybean protein (CP 65%)	12.08	8.00
Whey	10.00	5.00
Soybean oil	3.34	2.35
Yeast fermentive supernatant	1.25	1.25
Lysine·HCl	0.16	0.10
DL-methionine	0.13	0.03
Threoine	0.03	-
Limestone, purverized	1.12	1.07
Dicalcium phosphate	1.10	0.92
Salt	0.30	0.30
Vitamin premix[a]	0.10	0.10
Trace mineral premix[b]	0.10	0.10
Antibiotic premix[c]	0.10	0.10
Calculated nutrient composition		
ME (kcal/kg)	3,265	3,265
Crude protein (%)	23.70	20.70
Lysine (%)	1.35	1.15
Met+cys (%)	0.76	0.65

[a] 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.

[b] 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.

[c] Supplied per kg diet: 44 mg lincomycin·HCl and 44 mg spectinomycin sulfate.

intestine digestive enzyme secretion and modulate jejunal villus morphology both in neonatal (Zijlstra et al., 1994) and weaned pigs (Jaeger et al., 1990; Lee et al., 2006). In our previous trial, diets supplemented with 0, 0.5, 1.0, or 1.5 mg pEGF /kg diet have found that piglets supplemented with 1.0 to 1.5 mg pEGF /kg diet could stimulate jejunal alkaline phosphatase (ALP) and maltase mRNA expression and lactase activities (Lee et al., 2007).

On the other hand, glutamine (Gln) is the most abundant free amino acid in sow's milk (Wu and Knabe, 1994) and is a major nutrient for intestinal epithelial cells (Wu et al., 1995). The ingested Gln can be further metabolized to α-ketoglutarate and amide group, which is incorporated into the Krebs cycle to supply energy and synthesize purine, pyrimidines in the intestinal cells (Krebs, 1980; Wu et al., 1995). Diets supplemented with Gln might enhance the intestinal growth and immune responses in 21-day weaned pigs (Lee et al., 2003a; Lee et al., 2003b). Furthermore, EGF and Gln have been demonstrated to be a synergistic effect on small intestinal function of the septic rats (Ardawi, 1992; Ko et al., 1993). However, dietary supplementation with EGF and Gln on the growth performance and the digestive tract development of early-weaned piglets has not been explored yet. This study attempted to understand whether dietary pEGF and Gln supplementation enhances the growth and function of the gastrointestinal tract in early-weaned piglets.

MATERIALS AND METHODS

Animals and diets

All pigs used in this study were the offspring of either Yorkshire or Landrace sows crossed with Duroc boar. Piglets were weaned at 14±1 day of age, and fed a corn-soybean meal-whey basal diet supplemented with either inclusion of 1.0 mg pEGF supernatant /kg diet or 0.5% Gln (Ajinomoto, Tokyo, Japan) both alone and in combination. pEGF from the fermentative supernatant of transformed yeast coding porcine EGF (pEGF) gene. Briefly, the *Pichia pastoris* strain was transformed with pEGF gene, and was then grown in buffered methanol-complex medium with a glass bioreactor as previously described for the production and quantification of recombinant pEGF (Lee et al., 2006). The concentration of pEGF in the supernatant was about 800 μg/ml using ELISA assay. All nutrients met National Research Council standards (NRC, 1998). Table 1 presents the diet formulation and its nutrient composition. This experiment was performed at the National Ilan University and received prior approval from the institution's Animal Protocol Review Committee. The treatment, housing, husbandry, and slaughtering conditions adhered to current Republic of China guidelines.

Animal treatment

Ninety-six piglets from fourteen litters were assigned a dietary treatment based on sex and litter origin. Six piglets per pen with four replicates were housed in a nursery room for a 28 days experimental period. The nursery room temperature was maintained at 26°C. Feed and water were given *ad libitum*. Body weight and feed intake were recorded weekly to evaluate ADG, ADFI, and gain/feed. On day 14, two piglets per replicate were sacrificed and gastrointestinal tract samples were collected. After pre-anesthesia with thiamylal sodium 25 mg/kg body weight i.v., a mixture of 4% halothane and 96% oxygen for surgical anesthesia was administered by facemask. Stomach tissue from the antrum and pancreases were collected immediately after anesthesia. Next, the intestinal tract samples at jejunum and ileum were opened longitudinally, flushed with ice-cold phosphate buffer solution and blotted to eliminate excess fluid. Additionally, the dissected samples were fixed with 2.5% glutaraldehyde for the evaluations of villus-crypt morphology. The mucosal samples were obtained by scraping each intestinal tract tissue with a glass slide. Finally, these mucosa scrapings were placed into pre-weighed cryovials, weighed and then frozen in liquid nitrogen to assay DNA, protein and digestive enzyme activities. On day 28, all piglets were bled to analyze

peripheral blood mononuclear cells (PBMC) proliferation and serum IgA.

Preparation and chemical analysis of samples

All chemicals (unless otherwise notes) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The intestinal samples were embedded in paraffin using the method outlined by Spurr (1969), sectioned at 6 μm thickness and stained with hematoxylin and eosin following light microscopy examination (BX50, Olympus, Tokyo, Japan). The average villus height and crypt depth were determined from 15 well-oriented, intact villi in a blind fashion. Frozen mucosa samples were homogenized on ice in saline with 0.1% triton X-100 using a motor-driven homogenizer (Pro 200, PRO Sci., CT, USA) and a polytron homogenizer. The mucosa DNA concentration was measured according to the method as Labarca and Paigen (1980) described. The protein concentration in each sample was measured using a commercial bicinchoninic acid (BCA) protein assay kit (Pierce, IL, USA). Blood samples were drawn from the piglets and centrifuged at 2,000×g 15 min for serum collection. Serum IgA was determined using commercial ELISA kit (Bethyl, TX, USA), which serum was diluted 1,000 fold to meet the reagents supplied by the manufacturer.

Stomach mucosal pepsin activity (EC 3. 4. 23. 1) was determined by measuring the hydrolysis rate of haemoglobin (Rick and Fritsch, 1974). Before the pepsin activity assay, the pepsinogen in the homogenate was activated by incubating at 4°C for 10 min with 0.1 M HCl at pH 2.0. The pancreatic trypsin (EC 3. 4. 21. 4) and chymotrypsin (EC 3. 4. 21. 1) activities were measured following the methods outlined by Geiger (1984) and Geiger and Fritz (1984). Trypsinogen and chymotrypsinogen in the homogenates were activated with enterokinase at a concentration of 4 mg/ml and incubated at 4°C for 24 h. Trypsin and chymotrypsin activities were determined by measuring the hydrolysis rates of benzoyl-L-arginine-p-nitroanilide and of N-succinyl-L-phenyl-alanine-p-nitroanilide, respectively. The jejunal and ileal mucosa disaccharidase activities were determined as described by Petersen et al. (2002). Maltose (300 mM), sucrose (375 mM) and lactose (56 mM) dissolved in sodium malate buffer (62.5 mM, pH 6.0) were employed as substrates for maltase activity (EC 3. 2. 1. 20), sucrase (EC 3. 2. 1. 48) and lactase (EC 3. 2. 1. 23), respectively. Alkaline phosphatase activity (EC 3. 1. 3. 1) was determined using colorimetric assay with a commercial assay kit (Roche, Basal, Switzerland).

The PBMC proliferation was measured by incorporating ^{3}H-thymidine into cells as previously described in Lee et al. (2003a). Brief, the lymphocyte cells from blood were centrifugally isolated on a cushion of Ficoll-hypaques (GE Healthcare, Buckinghamshire, UK). The cells were then washed with Roswell Park Memorial Institute-1640 (RPMI-1640) medium and cultured at a concentration of 1×10^{5} cells/well triplicate in an RPMI-1640 with 10% fetal bovine serum and 100 U/ml penicillin culture medium (Gibco, Invitrogen, CA, USA) at 37°C for 72 h in 5% CO_2 and 95% air. The culture medium contained 5 μg/ml concanavalin A (Con A), 10 μg/ml phytohemagglutinin (PHA), or 5 μg/ml pokeweed mitogen (PWM) to determine the lymphocyte response to mitogen stimulation. Lymphocyte proliferation was pulsed with 0.5 μCi/well ^{3}H-thymidine (Specific activity 80Ci /mmol, GE Healthcare) for 18 h, and was terminated by harvesting cells onto glass fiber filters using an automatic cell harvester (Packard, CT, USA). Filters were immersed in a cocktail after drying, and radioactivity was measured using a microplate scintillation and luminescence counter (Packard). Stimulation index was calculated as counts per minute (cpm) of radioactivity in the presence of a mitogen/cpm of radioactivity compared with the absence of mitogen as a basal treatment.

Statistical analysis

Experimental data were analyzed as a factorial design using the GLM procedures of SAS (SAS, 1999). When a significant F-value for treatment means ($p<0.05$) was observed in the ANOVA, treatment means were compared with Duncan's multiple range test (Duncan, 1955). The individual pigs were the experimental units, except the feed and feed efficiency data of per pen were as the experiment units.

RESULTS

One piglet was dead and diarrhea occasionally occurred that had no difference among diet treatments throughout the experimental period. Table 2 illustrates the effects of dietary treatments on growth performance of early-weaned piglets. Diets supplemented with pEGF and Gln failed to influence the ADG, ADFI, and feed efficiency during the 14 or 28 days of testing. Dietary treatments also did not affect on the stomach, pancreas, liver, kidney, spleen or small intestine weights (data not shown). Table 3 illustrates the effects of dietary treatments on DNA and the protein contents of stomach mucosa, pancreas, and jejunal mucosa on day 14 of experiment. Diets supplemented with pEGF or Gln had no effect on DNA or protein contents in these tissues. By contrast diets supplemented with Gln was increased the gastric ($p = 0.08$) and jejunal mucosa ($p = 0.08$) protein levels and jejunal mucosa DNA content ($p = 0.09$) but did not reach a significant level. Moreover, diets supplemented with pEGF or Gln alone was increased the pancreas DNA ($p = 0.07$), but had a decreased tendency in pEGF+Gln treatment pigs. Diets supplemented with pEGF and Gln

Table 2. Effects of diets supplemented with pEGF and glutamine on growth performance of early-weaned pigs

Item		Initial weight (kg)	ADG		ADFI		ADG:ADFI	
			Day 0 to 14* (g/d)	Day 15 to 28* (g/d)	Day 0 to 14 (g/d)	Day 15 to 28 (g/d)	Day 0 to 14	Day 15 to 28
pEGF	Gln							
-	-	4.66	214.8	476.8	243.3	732.3	0.88	0.61
-	+	4.68	197.4	434.0	231.4	684.3	0.85	0.60
+	-	4.72	220.9	454.4	245.8	707.0	0.89	0.59
+	+	4.55	207.0	466.6	235.3	720.2	0.86	0.60
SEM		0.15	14.12	17.37	17.20	42.87	0.03	0.02
pEGF effect								
-		4.67	206.1	455.4	237.3	708.3	0.86	0.60
+		4.63	213.9	460.5	240.6	713.6	0.88	0.60
SEM		0.11	10.24	12.38	12.68	30.58	0.02	0.01
Gln effect								
-		4.69	217.9	465.6	244.5	719.7	0.88	0.60
+		4.61	202.2	450.3	233.3	702.3	0.86	0.59
SEM		0.11	10.24	12.38	12.68	30.58	0.02	0.01
Sources of variance (p-value)								
pEGF		0.808	0.576	0.772	0.852	0.906	0.671	0.984
Gln		0.610	0.266	0.385	0.523	0.695	0.437	0.572
pEGF×Gln		0.541	0.902	0.122	0.978	0.494	0.885	0.617

* Values are means of six and four pigs of each pen during the day 0 to 14 and day 15 to 28 periods, respectively.

Table 3. Effects of diets supplemented with pEGF and glutamine on the DNA and protein levels of gastric mucosa, pancreas and jejunal mucosa in early-weaned piglets

Item		Stomach		Pancreas		Jejunum	
		DNA (mg/g mucosa)	Protein (mg/g mucosa)	DNA (mg/g tissue)	Protein (mg/g tissue)	DNA (mg/g tissue)	Protein (mg/g tissue)
pEGF	Gln						
-	-	0.56	71.20	0.51	139.5	0.89	95.97
-	+	0.59	74.79	0.61	129.0	0.95	98.34
-	+	0.54	70.83	0.62	135.4	0.89	95.40
+	+	0.68	81.02	0.58	140.4	0.96	107.4
SEM		0.06	3.74	0.04	7.67	0.03	3.92
pEGF effect							
-		0.58	72.99	0.56	134.3	0.92	97.16
+		0.61	75.92	0.60	137.9	0.92	101.4
SEM		0.04	2.62	0.03	5.37	0.02	2.80
Gln effect							
-		0.55	71.01	0.57	137.5	0.89	95.69
+		0.63	77.90	0.59	134.7	0.95	102.9
SEM		0.04	2.63	0.03	5.38	0.02	2.80
Sources of variance (p-value)							
pEGF		0.572	0.437	0.353	0.634	0.902	0.293
Gln		0.164	0.077	0.456	0.722	0.091	0.082
pEGF×Gln		0.389	0.382	0.067	0.319	0.826	0.236

failed to affect the villus height, crypt depth, or the villus height/crypt depth ratio in the jejunum and ileum (data not shown). Table 4 illustrates the effects of dietary treatments on the gastrointestinal tract enzyme activities. Diets supplemented with pEGF or Gln had no effect on gastric pepsin activity. The pepsin activity of control, pEGF, Gln, and pEGF+Gln was 311.1, 301.4, 313.8, and 339.0 μmol/mg protein/min, respectively. Diets supplemented with pEGF and Gln increased the pancreatic chymotrypsin activity, but did not affect the trypsin activity. Diets supplemented with pEGF also increased the jejunal ALP, sucrase, lactase, and maltase activities ($p<0.05$). However, dietary Gln supplementation or the interactive effect of pEGF and Gln did not affect these enzyme activities. Table 5 illustrates the effects of diets supplemented with pEGF and Gln on the immune responses of early-weaned piglets on day 28 of experiment. Pigs fed Gln diet had a higher level of serum IgA ($p<0.05$) and PBMC proliferation without mitogen stimulation ($p<0.05$) and with Con A ($p = 0.07$) and PHA ($p<0.05$) stimulation. Diets supplemented with pEGF failed to affect serum IgA or PBMC proliferation of early-weaned pigs.

Table 4. Effects of diets supplemented with pEGF and glutamine on digestive enzyme activity of gastrointestinal tract in early-weaned piglets

Item		Pancreas		Jejunum			
		Trypsin	Chymotrypsin	ALP	Sucrase	Lactase	Maltase
pEGF	Gln						
-	-	94.37*	2.87	520.3	60.71	29.65	109.0
-	+	80.84	3.51	562.4	69.00	39.88	120.2
+	-	91.22	3.47	724.6	112.1	54.43	177.2
+	+	87.89	4.39	652.6	93.30	46.14	153.2
SEM		7.86	0.26	70.33	12.15	5.82	17.83
pEGF effect							
-		92.79	3.17[a]	541.4[a]	64.86[a]	34.76[a]	114.6[a]
+		84.36	3.95[b]	688.6[b]	102.7[b]	50.28[b]	165.2[b]
SEM		5.50	0.19	49.28	8.51	4.07	12.49
Gln effect							
-		87.60	3.19[a]	622.5	86.39	42.04	143.1
+		89.55	3.93[b]	607.5	81.15	43.01	136.7
SEM		5.51	0.18	49.35	8.52	4.08	12.51
Sources of variance (p-value)							
pEGF		0.288	0.006	0.045	0.004	0.013	0.008
Gln		0.805	0.009	0.833	0.668	0.869	0.723
pEGF×Gln		0.518	0.586	0.420	0.271	0.120	0.328

* Activity unit is expressed as μmol/mg protein/min.

[a, b] Means within the same row without common superscripts differ significantly.

Table 5. Effects of diets supplemented with pEGF and glutamine on serum IgA and peripheral blood mononuclear cells (PBMC) proliferation of early-weaned piglets

Item		IgA	PBMC proliferation			
		(μg/ml)	Basal	Con A	PHA	PWM
pEGF	Gln					
-	-	190.6	1.00*	381.5	369.6	84.0
-	+	259.6	1.93	709.5	759.4	220.1
+	-	220.2	1.07	427.9	568.5	194.8
+	+	236.2	1.71	501.2	793.6	144.9
SEM		16.17	0.31	108.9	125.1	55.3
pEGF effect						
-		225.1	1.46	545.5	564.5	152.1
+		228.2	1.39	464.6	681.1	169.9
SEM		11.53	0.22	77.01	88.47	39.1
Gln effect						
-		205.4[a]	1.03[a]	404.7	469.0[a]	139.4
+		247.9[b]	1.82[b]	605.4	776.5[b]	182.5
SEM		11.53	0.22	77.01	88.47	39.10
Sources of variance (p-value)						
pEGF		0.849	0.811	0.464	0.359	0.751
Gln		0.012	0.014	0.073	0.018	0.443
pEGF×Gln		0.109	0.643	0.251	0.517	0.101

* The cpm value of control treatment without mitogen stimulation was 277±124 (mean±SE) and was used to calculate the stimulation index to control ratio.

[a, b] Different letter represents the significant difference between treatments.

DISCUSSION

Previous reports have found that EGF can increase the absorption of GLN (Salloum et al., 1993; Hardin et al., 1996). This factorial design work attempted to investigate the effects of pEGF and Gln on the growth performance, intestine development and immune response of early weaning piglets particular in the interactive effect of pEGF and Gln. Therefore, the chose dosage of pEGF and Gln was low in this experiment and the results focused on the 14 days after weaning that is critical period for the intestinal development of piglets (Bianchi et al., 1992). However, dietary pEGF and Gln supplementation did not show significantly interactive effect. Interestingly, dietary pEGF

elevated intestinal enzyme activity and dietary Gln supplement increased serum IgA and PBMC proliferation, respectively, but did not alter the growth performance. The result is associated with the rat study that EGF and Gln show no synergistic effect on enzyme activities, and the increase of EGF administration (i.v.) in the intestinal sucrase and maltase activities is more efficient than that of Gln administration (Ardawi, 1992).

In present study, the average amount of pEGF consumption of each piglet was 240 and 714 μg/d during day 0 to 14 and day 15 to 28, respectively. Dietary pEGF supplement increased ($p<0.05$) the pancreatic chymotrypsin and jejunal enzyme (ALP, sucrase, lactase and maltase) activities, but did not influence the gastric pepsin or pancreatic trypsin activity. The result of pepsin activity is similar to the previous study that early-weaned piglets fed 0.5 mg pEGF/kg diet could enhance the gastric pepsin activity and return to the control level in the adding amount exceed 1.0 mg pEGF/kg diet (Lee et al., 2007). Literatures has been well demonstrated that EGF enhances the intestinal brush border enzyme activity (Carpenter and Cohen, 1990; Jaeger et al., 1990; Zijlstra et al., 1994; Wong et al., 1999; Lee et al., 2007). Previous report has indicted that administration with high dose (372 μg/d) EGF in 21 days weaned piglets for three consecutive days, but not with low dose (124 μg/d), can elevate jejunal disaccharidases (sucrase and lactase) activities (Jaeger et al., 1990). Moreover, our previous study has found that dietary supplemented with 193 μg pEGF/d could enhance the mRNA expression and activity of these enzymes (Lee et al., 2007). In this work, oral administration 240 μg pEGF/d should be sufficient to augment the enzyme activity of piglets. And we also found that dietary pEGF enhancing effect on the jejunal enzyme activity was more effective than on ileal enzyme activity. This may be attributed to orally EGF treatment faced destruction by the digestive hydrolysis in the gastrointestinal tract with some loss before reaching to ileum (Shen and Xu, 1996). Recently, pEGF possesses the increase of nutrients absorption has been reported (Wolfgang et al., 2003). However, the elevation of intestinal enzyme activity with dietary pEGF supplement did not result in improvement of growth performance in this study. The improvement of digestibility and absorption may be insufficient to leading the growth of piglets. Dietary pEGF supplementation did not affect growth, mucosa DNA and protein contents, or intestinal villus morphology of early-weaned piglets in this trial. This finding is also agreed with other reports (James et al., 1987; Jaeger et al., 1990). Our previous work in feeding 14 days weaned piglets for 28 days has found that pEGF (541 μg/d) does not change mucosal DNA content but expand villus crypt depth in jejunum (Lee et al., 2006). Conversely, feeding pEGF (240 μg/d) to weaning piglet for 14 days did not observe any different morphological change in this experiment. This discrepancy is probably attributed to the dosage effect on morphological change.

Dietary Gln supplement did not promote growth performance during the initial 14 or 28 days post-weaning and this finding is consistent to the previous works with piglets weaned at 14 days (Lee et al., 2002), 21 days (Lee et al., 2003b), or 28 days (Zhou et al., 2006). Dietary Gln supplementation raised the jejunal crypt depth on day 14 of the trial, but did not change the ratio of villus height to crypt depth. The level of Gln (0.5%) used in this study was lower than that in previous trial. One percent of Gln could increase the villus height and the DNA and protein contents in the small intestinal mucosa (Lee et al., 2003b). Dietary Gln supplement did not affect on digestive enzyme activity is associated with the rat experiment (Li et al., 2004; Hou et al., 2006). Dietary Gln supplementation increased serum IgA levels on day 28 ($p<0.05$). This result was agreed with our previous works, dietary Gln supplement tended to increase the concentration of plasma and bile IgA of weaned pigs (Lee et al., 2002; Lee et al., 2003b). In this study, Gln supplementation also increased the PBMC proliferation in early-weaned piglets compared with the control group. Inclusion of Gln is also increased the proliferation of PBMC, mesenteric lymph node, and splenocytes in normal and infected weaned piglets (Yoo et al., 1997; Lee et al., 2003a). Therefore, Gln performs a specific and unique role in the immune response process.

IMPLICATIONS

Diet supplemented with recombinant yeast cultures containing enriched porcine epidermal growth factor improves digestive enzyme activity in weaned piglets. Dietary glutamine supplementation stimulates the immune responses in early-weaned piglets. However, combined supplementation of porcine epidermal growth factor and glutamine does not show a synergistic effect on the intestinal development in early-weaned piglets.

REFERENCES

Ardawi, M. S. M. 1992. Effects of epidermal growth factor and glutamine-supplemented parenteral nutrition on the small bowel of septic rats. Clin. Sci. 82:573-580.

Ayonrinde, A. I., I. H. Williams, R. McCauley and B. P. Mullan. 1995. Reduced plasma concentrations of glutamine and its metabolites in weaned pigs. In: Manipulating Pig Production, vol. V. (Ed. D. P. Hennessy and P. D. Cranwell). Aust. Pig Sci. Assoc. Werribee, Australia. p. 179.

Bianchi, A. T. J., R. J. Zwart, S. H. M. Jeurissen and H. W. M. Moonen-Leusen. 1992. Development of the B- and T-cell

compartments in porcine lymphoid organs from birth to adult life: an immunohistological approach. Vet. Immuno. Immunopath. 33:201-221.

Carpenter, G. and S. Cohen. 1990. Epidermal growth factor. J. Bio. Chem. 265:7709-7712.

Cohen, S. and G. A. Elliott. 1963. The stimulation of epidermal keratinization by a protein isolated from the submaxillary gland of the mouse. J. Invest. Dermatol. 40:1-5.

Duncan, D. B. 1955. Multiple range and multiple *F*-test. Biometrics 11:1-42.

Fan, M. Z., O. Adeola, E. K. Asem and D. King. 2002. Postnatal ontogeny of kinetics of porcine jejunal brush border membrane-bound alkaline phosphatase, aminopeptidase N and sucrase activities. Comp. Biochem. Physiol. 132:599-607.

Geiger, R. 1984. Chymotrypsin. In: Methods of Enzymatic Analysis. (Ed. H. U. Bergmeyer). Academic Press, New York. pp. 99-118.

Geiger, R. and H. Fritz. 1984. Trypsin. In: Methods of Enzymatic Analysis. (Ed. H. U. Bergmeyer). Academic Press, New York. pp. 119-129.

Hampson, D. J. 1986. Attempts to modify changes in the piglet small intestine after weaning. Res. Vet. Sci. 40:313-317.

Hampson, D. J. and D. E. Kidder. 1986. Influence of creep feeding and weaning on brush border enzyme activities in the piglet small intestine. Res. Vet. Sci. 40:24-31.

Harid, J. A., J. K. Wong, C. I. Cheeseman and D. G. Gall. 1996. Effect of luminal epidermal growth factor on enterocyte glucose and praline transport. Am. J. Physiol. 271:G509-515.

Hou, Y. Q., Y. L. Liu, J. Hu and W. H. Shen. 2006. Effects of lactitol and tributyrin on growth performance, small intestinal morphology and enzyme activity in weaned pigs. Asian-Aust. J. Anim. Sci. 19:1470-1477.

Jaeger, L. A., C. H. Lamar, G. D. Bottoms and T. R. Cline. 1987. Growth-stimulating substances in porcine milk. Am. J. Vet. Res. 48:1531-1533.

Jaeger, L. A., C. H. Lamar, T. R. Cline and C. J. Cardona. 1990. Effect of orally administered epidermal growth factor on the jejunal mucosa of weaned pigs. Am. J. Vet. Res. 51:471-474.

James, P. S., M. W. Smith, D. R. Tivey and T. J. G. Wilson. 1987. Epidermal growth factor selectively increases maltase and sucrase activities in neonatal piglet intestine. J. Physiol. 393:583-594.

Kenworthy, R. 1976. Observations on the effects of weaning in the young pig. Clinical and histopathological studies of intestinal function and morphology. Res. Vet. Sci. 21:69-75.

Ko, T. C., R. D. Beauchamp, C. M. Townsend, Jr. and J. C. Thompson. 1993. Glutamine is essential for epidermal growth factor-stimulated intestinal cell proliferation. Surgery 114:147-154.

Krebs, H. 1980. Glutamine metabolism in the animal body. In: Glutamine: Metabolism, Enzymology, and Regulation. (Ed. J. Mora and R. Palacios). Academic Press, New York. pp. 319-329.

Labarca, C. and K. Paigen. 1980. A simple, rapid, and sensitive DNA assay procedure. Anal. Biochem. 102:344-352.

Lee, D. N., Y. H. Cheng, B. J. Chen, I. T. Yu, C. W. Liao and H. T. Yen. 2002. Effect of dietary glutamine supplementation on the growth performance and immune response of early weaned piglets. J. Chin. Soc. Anim. Sci. 31:99-110.

Lee, D. N., C. F. Weng, Y. H. Cheng, T. Y. Kuo, J. F. Wu and H. T. Yen. 2003a. Dietary glutamine supplementation enhances weaned pigs mitogen-induced lymphocyte proliferation. Asian-Aust. J. Anim. Sci. 16:1182-1187.

Lee, D. N., Y. H. Cheng, F. Y. Wu, H. Sato, I. Shinzato, S. P. Chen and H. T. Yen. 2003b. Effect of dietary glutamine supplement on performance and intestinal morphology of weaned pigs. Asian-Aust. J. Anim. Sci. 16:1770-1776.

Lee, D. N., T. K. Kuo, M. C. Chen, T. Y. Tang, F. H. Liu and C. F. Weng. 2006. Expression of porcine epidermal growth factor in *Pichia pastoris* and its biology activity in early-weaned piglets. Life Sci. 78:649-654.

Lee, D. N., Y. S. Chuang, H. Y. Chiou, F. Y. Wu, H. T. Yen and C. F. Weng. 2007. Oral administration recombinant epidermal growth factor enhances the jejunal digestive enzyme genes expression and activity of early-weaned piglets. J. Anim. Physiol. Anim. Nutr. (In press)

Li, N., K. Liboni, M. Z. Fang, D. Samuelson, P. Lewis, R. Patel and J. Neu. 2004. Glutamine decreases lipopolysaccharide-induced intestinal inflammation in infant rats. Am. J. Physiol. Gastrointest. Liver Physiol. 286:G914-921.

Moran, J. R., M. E. Courtney, D. N. Orth, R. Vaughan, S. Coy, C. D. Mount, B. J. Sherrell and H. L. Greene. 1983. Epidermal growth factor in human milk: daily production and diurnal variation during early lactation in mothers delivering at term and at premature gestation. J. Pediatr. 103:402-425.

National Research Council. 1998. Nutrient Requirements of Swine. 10th Ed. National Academy Press, Washington, DC.

Petersen, Y. M., J. Elnif, M. Schmidt and P. T. Sangild. 2002. Glucagon-like peptide 2 enhances maltase-glucoamylase and sucrase-isomaltase gene expression and activity in parenterally fed premature neonatal piglets. Pediatr. Res. 52:498-503.

Pluske, J. R., I. H. Williams and F. X. Aherne. 1996. Maintenance of villous height and crypt depth in piglets by providing continuous nutrition after weaning. Anim. Sci. 62:131-144.

Rick, W. and W. P. Fritsch. 1974. Chymotrypsin, trypsin and pepsin. In: Methods of Enzymatic Analysis, vol. 2. (Ed. H. U. Bergmeyer). Academic Press, New York. pp. 1006-1057.

SAS. 1999. SAS/STAT User's Guide: Version 6.12th edn. SAS Institute Inc., Cary, North Carolina.

Salloum, R. M., B. R. Stevens, G. S. Schultz and W. W. Souba. 1993. Regulation of small intestinal glutamine transport by epidermal growth factor. Surgery 113:552-559.

Shen, W. H. and R. J. Xu. 1996. Stability of epidermal growth factor in the gastrointestinal lumen of suckling and weaned pigs. Life Sci. 59:197-208.

Spurr, A. R. 1969. A low viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26:31-43.

Wolfgang, C. L., C. M. Lin, Q. H. Meng, A. M. Karinch, T. C. Vary and M. Pan. 2003. Epidermal growth factor activation of intestinal glutamine transport is mediated by mitogen-activated protein kinases. J. Gastrointest. Surg. 7:149-156.

Wong, W. M. and N. A. Wright. 1999. Epidermal growth factor, epidermal growth factor receptors, intestinal growth, and adaptation. J. Parenteral. Enteral. Nutr. 23:S83-88.

Wu, G. and D. A. Knabe. 1994. Free and protein-bound amino acids in sow's colostrum and milk. J. Nutr. 124:415-424.

Wu, G., D. A. Knabe, W. Yan and N. E. Flynn. 1995. Glutamine and glucose metabolism in enterocytes of the neonatal pig. Am. J. Physiol. 268:R334-342.

Yoo, S. S., C. J. Field and M. I. McBurney. 1997. Glutamine supplementation maintains intramuscular glutamine concentrations and normalizes lymphocyte function in infected early weaned pigs. J. Nutr. 127:2253-2259.

Zhou, R. Y., J. Peng, Z. L. Liu and Z. F. Fang. 2006. Effects of Biocom as a replacement of glutamine on performance and blood biochemical indexes of early weaned piglets. Asian-Aust. J. Anim. Sci. 19:872-876.

Zijlstra, R. T., J. Odle, W. F. Hall, B. W. Petschow, H. B. Gelberg and R. E. Litov. 1994. Effect of orally administered epidermal growth factor on intestinal recovery of neonatal pigs infected with rotavirus. J. Pediatr. Gastroenterol. Nutr. 19:382-390.