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Effects of Dietary Glutamine Supplementation on Nutrient Absorption and Activity of Enzymes Involved in Glutamine Metabolism and Energy Production in the Jejunum of Weaned Piglets

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Abstract: Glutamine has an essential role with a beneficial function in improving the nutrition status of young mammals. The influence of L-Glutamine (Gln) on the Coefficient of Total Tract Apparent Digestibility (CTTAD) and Apparent Ileal Digestibility (AID), the jejunal enzyme activity associated with nutrient absorption and the energy production in weaned piglets has not been sufficiently studied. The aim of the present study is to provide a profile of the effects of Gln on CTTAD, AID, the activities of jejunal enzymes in connection with nutrient digestion and absorption and energy production. The piglets were weaned at 21 days of age. There were two groups in Experiment 1 and 2 representing supplementation with 0 or 1% Gln to the basal diet. In Experiment 1, the CTTAD of the dietary components and energy was assessed at 3, 5, 10, 15 and 30 days after weaning. In Experiment 2, productive performance, AID, jejunal enzyme activities and expression of Peroxisome Proliferator-Activated Receptor gamma (PPARγ) were measured at 10 and 30 days post-weaning. Results showed that dietary Gln supplementation significantly improved the CTTAD of DM, OM, GE and AA and the CTTAD increased significantly with the extension of days after weaning. For the entire experiment, the average daily gain increased by 12.40% ($p = 0.049$) in the Gln group. Dietary Gln supplementation increased the AID of GE, Leu, Lys, Cys and Pro by 12.50 ($p = 0.047$), 7.03% ($p = 0.041$), 5.95% ($p = 0.036$), 9.30 ($p = 0.025$), 11.17% ($p = 0.009$), respectively at 10 days post-weaning; Pro by 6.11% ($p = 0.044$) at 30 days post-weaning. Jejunal brush border membrane-bound alkaline phosphatase activity increased in the Gln-supplemented pigs by 30.36% ($p = 0.048$) and 6.21% ($p = 0.30$) at 10 and 30 days post-weaning, respectively. Compared with the control pigs, the mRNA level of PPARγ decreased by 10.85% ($p = 0.14$) and 41.88% ($p = 0.023$) after the administration of 1% Gln for 10 and 30 days, respectively. The activity of glutamine synthetase decreased by 48.89% ($p = 0.044$) at 10 days post-weaning and pyruvate kinase by 13.13% ($p = 0.036$) at 30 days post-weaning in the Gln-supplemented pigs. In conclusion, 1% Gln supplementation to the post-weaned piglet diet enhanced the CTTAD and AID of diet, improved intestinal absorption and modified jejunal enzyme activities related to Gln metabolism and energy production.

Key words: Energy production, enzyme activity, L-glutamine, nutrient absorption, weaned piglets, China

INTRODUCTION

Weaning is marked by social, environmental and nutritional changes and is a critical stage of postnatal growth and metabolism in mammals (Henning, 1981; Wang *et al*., 2008). After weaning, digestive enzyme activity and paracellular barrier function decrease (Wijtten *et al*., 2011) and absorptive secretory electrolyte and fluid balances are disturbed in the intestines (Montagne *et al*., 2007). Weaned piglets often experience intestinal villus atrophy, crypts hyperplasia (Barszcz and Skomial, 2011) stimulation of pro-inflammatory cytokine expression (Pie *et al*., 2004) reduction of transcellular transport of macromolecules and passive transcellular absorption in the small intestine (Wijtten *et al*., 2011). Therefore, weaning is commonly associated with a period of low food consumption, decreased digestive capability and increased occurrence of enteric diseases and diarrhea (Bruininx *et al*., 2001).

Recent studies have confirmed that dietary L-Glutamine (Gln) supplementation can improve the intestinal physiology of young mammals (Wang *et al*., 2008; Rhoads and Wu, 2009). Gln is the most abundant free amino acid in the body and is now regarded as a conditionally essential nutrient under stress conditions such as weaning, injury and infection (Li *et al*., 2007;

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Abdeen *et al*., 2011). It is a primary energy source for enterocytes and immune cells (Reeds and Burrin, 2000). Gln as a metabolizable enteral solute also helps maintain cytoskeleton integrity, elevates filamentous-actin: globular-actin actin ratio and decreases mucosal permeability (Kozar *et al*., 2004). Additionally, Gln is required for the synthesis of bioactive molecules essential for gastrointestinal integrity (Wu, 1998; Li *et al*., 2007). Furthermore, Gln performs as a signal in enterocytes and plays a regulatory role in physiology and nutrition at gene and protein levels (Curi *et al*., 2005; Rhoads and Wu, 2009).

Despite extensive research on the regulation of Gln in intestinal morphology, physiology and health, there is insufficient information on the effects of Gln supplementation on the Coefficient of Total Tract Apparent Digestibility (CTTAD) and Apparent Ileal Digestibility (AID) of diet and the activities of enzymes related to Gln metabolism and energy production in the small intestine of pigs. The aim of the present research was to study the effects of dietary Gln supplementation on the CTTAD and AID of nutrients and energy, the factors involved in nutrient absorption and the activities of enzymes associated with Gln metabolism in connection with energy production in the jejunum of weaned piglets.

MATERIALS AND METHODS

Animals and housing: All the experimental procedures described in these experiments were approved by the Animal Care and Use Committee of Zhejiang University in accordance with the Chinese guidelines for animal welfare and experimental protocol. The piglets (Duroc x Landrace x Large Yorkshire strain) were weaned at 21 days of age. Housing conditions were controlled during the whole experimental period as follows: a 12 h light/dark cycle and environment temperature maintained between 20 and 25°C.

Experimental diets: The basal diet without antibiotics was formulated to meet the recommendations of the National Research Council. There were two treatments, one with the Gln group and the other with the control group. The Gln and control groups represent 1% Gln (wt/wt) and 1.22% L-alanine (wt/wt; isonitrogenous control) in the basal diet, respectively. The amount of supplemental glutamine and the reason for selecting alanine as the isonitrogenous control were based on the studies of Wang *et al*. (2008) and Wu *et al*. (1996). The ingredient compositions of the experimental diets are shown in Table 1. The analyzed chemical compositions of the diets are shown in Table 2.

Table 1: Ingredient composition of the experimental diets (g kg^{-1} as fed basis)

Items	Control	Gln
Corn	565.0	567.2
Soybean meal	219.3	219.3
Extruded soybean	50.0	50.0
Fishmeal	40.0	40.0
Soybean oil	25.0	25.0
Whey powder	25.0	25.0
Glucose	40.0	40.0
Calcium hydrogen phosphate	9.0	9.0
Limestone	4.5	4.5
Vitamin/mineral premix[a]	10.0	10.0
Alanine	12.2	-
Glutamine	-	10.0

[a]Mineral and vitamin composition (mg kg^{-1} of feed): Cu, 200 as copper sulfate; Fe, 240 as ferrous sulfate monohydrate; Mn, 40 as manganese sulfate; Zn, 1848 as zinc oxide; Co, 0.5 as cobalt sulfate; I, 0.4 as potassium iodine; Se, 0.35 as sodium selenite anhydrous; Vitamin A, 17, 500 IU; Vitamin D3, 385 IU; Vitamin E, 70 IU; Vitamin K, 3.36; Vitamin B1, 3.43; Vitamin B2, 8.75; Vitamin B6, 5.15; Vitamin B12, 0.04; Ca-D-pantothenate, 17.15; niacin, 36; folic acid, 1.70; biotin, 0.26

Table 2: Analyzed chemical composition of the experimental diets (g kg^{-1} as fed basis)

Items	Control	Gln
DM	884.9	887.4
CP	216.1	214.8
GE (MJ kg^{-1})	14.1	14.1
Ash	51.9	52.2
Essential AA		
Arg	11.4	11.6
His	4.8	4.9
Iso	7.9	7.9
Leu	16.8	17.0
Lys	12.2	12.1
Met	3.0	3.2
Phe	8.8	9.0
Thr	8.4	8.5
Val	9.0	8.9
Nonessential AA		
Ala	20.0	9.9
Asp	17.9	17.9
Cys	4.0	4.0
Glu	38.3	49.3
Gly	8.6	8.4
Pro	9.1	9.0
Ser	8.9	9.0
Tyr	5.2	5.2

Analyzed in 3 replicates

Sampling

Experiment 1: Twelve 21 days old female piglets with 5.67±0.48 kg (mean±SEM) Body Weight (BW) were used. The piglets were individually caged and had *ad libitum* access to the feed during the experimental period. There were two treatments and six replicates in each treatment with one pig per replicate. At 3, 5, 10, 15 and 30 days after weaning, fresh fecal sample was collected from each pig. All samples were frozen at -20°C until chemical analysis to determine the CTTAD of Dry Matter (DM), Crude Protein (CP), Organic Matter (OM), Gross Energy (GE) and Amino Acid (AA).

Experiment 2: One hundred twenty eight, 21±1 days old (mean±SEM) piglets with 5.57±0.51 kg BW were selected on the day of weaning and the same proportion of males and females was used. The piglets were weighed and designated to the experimental units according to sex and BW. There were two treatments and four replicates in each treatment with sixteen piglets per replicate. The piglets had free access to the feed and drinking water throughout the trial. At 10 and 30 days post-weaning, the piglets were weighed individually. Average Daily Gain (ADG), Average Daily Feed Intake (ADFI) and Feed Conversion Rate (FCR) were calculated by period and for the entire experiment.

After being weighed at 10 and 30 days post-weaning, eight female piglets (4 per treatment) were then killed after anesthesia. The jejunal segment was isolated and immediately flushed with ice-cold saline. A portion of the mid-jejunum was collected and frozen in liquid nitrogen for RNA isolation (Wang *et al*., 2008). Another set of samples were obtained for mucosa collection (Fan *et al*., 2002). The mucosa was scraped with a glass spatula. The mucosal scrapings of each pig were frozen in liquid nitrogen and stored at -80°C until further processing. To determine the AID of the DM, CP, OM, GE and AA, the ileal contents from the last 20-40 cm of the ileum were collected and frozen at -20°C until chemical analysis (Valencia *et al*., 2008).

Chemical analysis: Samples of faeces were heat-dried (65°C, 48 h) and samples of the ileal content were freeze-dried. Procedures set by the Association of Official Analytical Chemists (AOAC, 2000) were used to determine the concentrations of DM (934.01), total ash (942.05) and nitrogen (990.03). Amino acids were determined following acid hydrolysis using a Hitachi L-8900 amino acid analyzer (Hitachi, Tokyo, Japan) (994.12) as described by AOAC (2000). GE was measured by adiabatic bomb calorimetry (Model 1356, Parr Instrument Company, Moline, IL, USA). The Acid Insoluble Ash (AIA) of the diets, faeces and ileal content was determined employing the technique described by Vogtmann *et al*. (1975).

Tissue preparation and enzyme assays: The mucosal scrapings were rapidly thawed and manually homogenized using a glass homogenizer with a glass pestle. All procedures were performed on ice. The homogenates were centrifuged and the supernatant fraction diluted to an appropriate concentration. The resultant supernatants were collected for the assay of enzyme activities (Madej *et al*., 1999).

The jejunal brush border membrane-bound alkaline phosphatase activity (AKP, EC 3.1.3.1), glutamine synthetase (GS, EC 6.3.1.2), Hexokinase (HK, EC 2.7.1.1), Pyruvate Kinase (PK, EC 2.7.1.40), Alanine Aminotransferase (ALT, EC 2.6.1.2) and Aspartate aminotransferase (AST, EC 2.6.1.1) were evaluated using the detection kits following the manufacturer's instructions. Kits were provided by Jiancheng Bioengineering Ltd. Nanjing, P.R. China.

RNA preparation and real time RT-PCR: Total RNA was extracted from jejunum using Trizol Reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instruction. First-strand cDNA was synthesized using the SuperScript™ II RTase with random primers and an Rnase inhibitor (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. The relative abundance of Peroxisome Proliferator-Activated Receptor gamma (PPARγ) mRNA was determined using iQ5 Multicolor Real-Time PCR Detection System (Bio-Rad, California, USA) and SYBR Premix Ex Taq kit (Takara Biotechnology Co., Ltd. Japan).

The primers for pig PPARγ (GenBank Accession No.: NM214379) and the reference gene Glyceraldehyde-3-phosphate (GADPH) (Genbank Accession No.: NM017008) were designed using Primer Premier 6.0 (Premier, Canada). The sequences were as follows: PPARγ, forward 5'GTGGAGACCGCCCAGGTTTG 3', reverse 5'GGGAGGACTCTGGGTGGTTCA 3'; GADPH, forward 5'GGCAAATTCCACGGCACAGTCA 3', reverse 5'CTCGCTCCTGGAAGATGGT GAT 3'. All experiments were repeated in triplicate. Relative quantification of the target gene expression was evaluated by normalizing its signal to that of GAPDH using the $2^{-\Delta\Delta CT}$ Method (Livak and Schmittgen, 2001). Fold difference in the relative gene expression of the target was calculated as the $2^{-\Delta\Delta CT}$ value.

Statistical analysis: The CTTAD or AID were calculated as indicated by Stein *et al*. (2001):

$$\text{Digestibility (\%)} = 100\times[1-(C_s\times AIA_d)\times(C_d\times AIA_s)]$$

Where:

C_d and C_s = The concentrations of dietary components and faeces or ileal components

AIA_d and AIA_s = The maker AIA concentrations of diet and faeces or ileum (all values in g kg^{-1} DM)

The experimental units were individual animals. In Experiment 1, data on CTTAD, the treatment effects were analyzed by repeated measures of ANOVA for 3, 5, 10, 15 and 30 days after weaning. All interactions were assessed at the 5% significant level. In Experiment 2, data on productive performance, *AID* gene expression and enzyme activities were analyzed by one-way ANOVA.

Results are presented as mean±SEM. Statistical significance was established at $p<0.05$ and p-value between 0.05 and 0.1 were considered a trend. All statistical analysis were made by Statistical Product and Service Solutions (Version 16.0; Chicago, IL, USA) as appropriate.

RESULTS AND DISCUSSION

Coefficient of total tract apparent digestibility (Experiment 1): The effects of Gln supplementation and days after weaning on the CTTAD are shown in Table 3. Dietary Gln supplementation significantly improved the CTTAD of DM, OM, GE and AA and tended to elevate the CTTAD of CP (p = 0.051). With the extension of days after weaning, the CTTAD of diets increased significantly. The interactions between Gln supplementation and days after weaning were significant in Arg (p = 0.016), Glu (p = 0.049) and Ser (p = 0.042).

Productive performance (Experiment 2): The pigs with dietary Gln treatment had better ADG (p = 0.041) and FCR (p = 0.047) than the control pigs at 10 days post-weaning (Table 4). From 11-30 days, Gln treatment increased ADG by 11.03% (p = 0.056) and decreased FCR by 8.96% (p = 0.12). As a consequence, for the entire experiment (1-30 days post-weaning), Gln supplementation increased ADG by 12.40% (p = 0.049) and decreased FCR by 10.45% (p = 0.086) but had no effect on ADFI.

Apparent ileal digestibility (Experiment 2): Dietary supplementation with 1% Gln increased AID of GE by 12.50% (p = 0.047) did not affect CP and OM at 10 days post-weaning (Table 5). The AID of DM, CP, OM and GE were not affected by Gln supplementation at 30 days post-weaning. The effect of Gln supplementation on the AID of AA showed the same trend as increase was observed for Leu, Lys, Cys, Pro by 7.03% (p = 0.041), 5.95% (p = 0.036), 9.30 (p = 0.025) and 11.17% (p = 0.009), respectively at 10 days post-weaning; Pro by 6.11% (p = 0.044) at 30 days post-weaning.

The supplementation with Gln tended to increase the AID of Val, Asp, Tyr by 6.14% (p = 0.074), 7.62% (p = 0.084) and 8.47 (p = 0.063), respectively at 10 days post-weaning; Lys, Cys, by 2.63% (p = 0.051), 5.81% (p = 0.085) and 4.28% Glu (p = 0.091), respectively at 30 days post-weaning.

Table 3: Influence of Gln on the total tract apparent digestibility (%) of the experimental diets

	Control (days)					Gln (days)						p-value		
Items	3	5	10	15	30	3	5	10	15	30	SEM	1	2	3
DM	74.88	74.94	77.23	78.13	83.24	79.56	80.45	81.65	82.56	86.32	0.99	0.001	0.690	0.003
CP	55.08	61.52	70.71	73.96	82.71	65.94	68.58	75.61	77.40	84.98	2.28	***	0.170	0.051
OM	75.11	75.29	77.41	78.34	83.17	79.60	80.50	81.68	82.39	86.05	0.94	0.001	0.680	0.003
GE	74.32	74.38	76.71	77.67	83.86	78.15	79.03	80.31	81.19	85.19	1.77	***	0.480	0.004
Essential AA														
Arg	73.91	81.54	89.28	88.44	91.28	82.74	86.01	90.60	90.04	92.42	1.00	***	0.016	0.020
His	65.05	73.58	82.60	80.07	84.00	73.84	77.87	83.83	83.83	86.94	1.19	***	0.110	0.008
Iso	55.70	63.34	70.85	75.59	79.60	67.29	69.63	75.98	78.57	82.76	1.72	***	0.110	0.025
Leu	58.08	66.49	76.58	80.01	82.64	69.41	72.17	80.01	82.34	85.94	1.77	***	0.120	0.036
Lys	71.66	76.69	78.03	82.90	85.68	76.79	78.79	81.01	82.98	86.46	0.98	***	0.160	0.060
Met	69.28	69.93	82.67	84.73	89.13	79.86	81.62	86.57	85.68	91.55	2.04	0.001	0.120	0.012
Phe	60.04	67.98	75.56	79.63	82.36	71.50	74.19	80.35	82.28	85.48	1.59	***	0.060	0.027
Thr	59.53	65.77	75.91	75.70	81.87	69.73	71.80	79.73	79.14	83.89	1.28	***	0.073	0.013
Val	54.00	62.16	68.33	72.53	77.61	65.21	68.03	74.97	76.31	80.95	1.63	***	0.160	0.013
Nonessential AA														
Ala	76.99	80.39	83.98	86.02	88.95	63.67	65.75	73.56	74.73	80.02	1.34	0.001	0.240	***
Asp	62.02	67.70	75.53	77.94	83.27	71.05	73.08	80.06	80.41	85.26	1.51	***	0.190	0.028
Cys	59.06	66.59	68.06	70.33	78.86	69.75	72.12	77.28	76.97	80.03	1.67	***	0.140	0.004
Glu	62.22	70.04	79.96	79.44	85.95	78.40	80.53	87.82	86.63	90.89	1.53	***	0.049	0.002
Gly	56.84	63.15	68.63	70.82	77.62	66.87	69.56	75.38	75.79	81.16	1.42	***	0.230	0.005
Pro	52.99	58.91	63.89	68.13	75.41	70.24	73.81	81.59	82.94	84.91	1.67	***	0.100	***
Ser	58.02	66.12	78.57	76.51	83.57	69.84	72.50	81.61	80.45	86.33	1.37	***	0.042	0.013
Tyr	52.99	60.37	70.35	72.63	78.89	66.84	69.53	77.52	79.49	82.57	1.92	***	0.220	0.003

1: days after weaning effect; 2: the interaction between Gln supplementation effect and days after weaning; 3: Gln supplementation effect. ***$p<0.001$; Each value represents mean from six pigs

Table 4: Influence of Gln on the productive performance of pigs weaned at 21 days of age

Items	Control	Gln	SEM	p-value
First period (1-10 days post-weaning)				
ADG (g)	147.00	175.00	12.63	0.041
ADFI (g)	200.68	199.89	11.44	0.950
FCR	1.37	1.14	0.09	0.047
Second period (11-30 days post-weaning)				
ADG (g)	358.00	397.50	20.75	0.056
ADFI (g)	478.53	484.03	16.91	0.860
FCR	1.34	1.22	0.10	0.120
Whole trial (1-30 days post-weaning)				
ADG (g)	287.67	323.33	18.84	0.049
ADFI (g)	385.92	389.32	14.49	0.790
FCR	1.34	1.20	0.09	0.086

Each value represents mean from sixty four pigs

Table 5: Influence of Gln on the apparent ileal digestibility (%) of the experimental diets at 10 and 30 days post-weaning

	10 days			30 days			p-value	
Items	Control	Gln	SEM	Control	Gln	SEM	1	2
DM	70.11	74.44	0.74	76.28	78.56	0.91	0.069	0.610
CP	65.34	70.88	1.68	75.69	77.84	1.82	0.160	0.420
OM	71.46	75.48	1.44	76.82	79.51	1.04	0.190	0.180
GE	69.54	78.23	1.17	77.64	78.42	1.97	0.047	0.730
Essential AA								
Arg	80.48	82.25	1.34	82.42	83.11	1.01	0.850	0.410
His	71.54	71.99	1.01	72.63	72.91	1.93	0.920	0.990
Iso	63.78	64.19	2.22	71.80	74.22	2.37	0.910	0.720
Leu	69.34	76.37	1.32	75.79	79.25	1.57	0.041	0.170
Lys	73.56	79.51	0.99	76.69	79.32	0.82	0.036	0.051
Met	75.97	76.87	1.09	81.66	82.79	1.09	0.850	0.680
Phe	70.48	72.13	1.71	75.42	77.19	1.85	0.750	0.690
Thr	67.45	74.21	2.13	71.57	73.26	1.63	0.110	0.590
Val	62.57	68.71	1.75	71.58	73.62	1.25	0.074	0.430
Nonessential AA								
Ala	77.89	76.97	1.26	81.45	79.12	1.35	0.920	0.770
Asp	68.35	75.97	1.62	75.40	77.21	1.10	0.084	0.180
Cys	60.15	69.45	1.31	65.87	71.68	1.67	0.025	0.085
Glu	77.64	81.69	1.19	81.53	85.81	1.34	0.290	0.091
Gly	60.47	68.46	1.06	69.45	70.38	1.17	0.041	0.960
Pro	58.26	69.43	1.20	64.38	70.49	1.09	0.009	0.044
Ser	71.48	73.56	1.49	78.11	80.54	1.84	0.650	0.670
Tyr	63.22	71.69	1.83	67.57	70.16	2.02	0.063	0.210

1: Control vs. Gln 10 days post-weaning; 2: Control vs. Gln 30 days post-weaning. Each value represents mean from four pigs

The factors involved in nutrient absorption (Experiment 2): Jejunal brush border membrane-bound AKP activity increased by 30.36% (p = 0.048) 10 days after weaning and by 6.21% (p = 0.30) 30 days after weaning by Gln treatment (Fig. 1). Gene expression of PPARγ in the jejunum was downregulated by the Gln treatment (Fig. 2). When administrated for 10 and 30 days, the mRNA level of PPARγ decreased by 10.85% (p = 0.14) and 41.88% (p = 0.023), respectively.

The activities of the jejunal enzymes involved in Gln metabolism and energy production (Experiment 2): In the metabolic enzymes studied, the activities of GS and PK in the jejunum decreased by 48.89% (p = 0.044) and 3.70% (p = 0.35) 10 days after weaning and by 14.29%

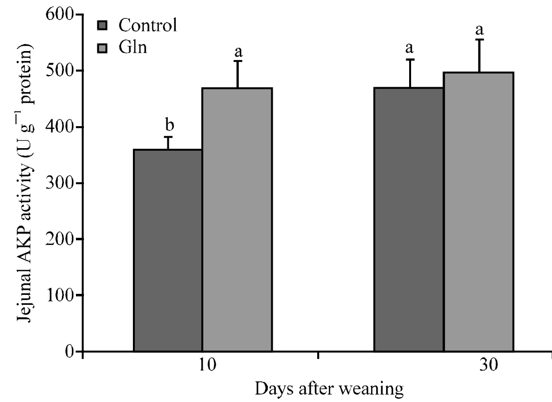

Fig. 1: Effect of Gln on the jejunal brush border membrane-bound alkaline phosphatase activity in weanling piglets. Each column represents the mean of four individual pigs ±SEM; Different uppercase letters indicate a significant difference at $p<0.05$

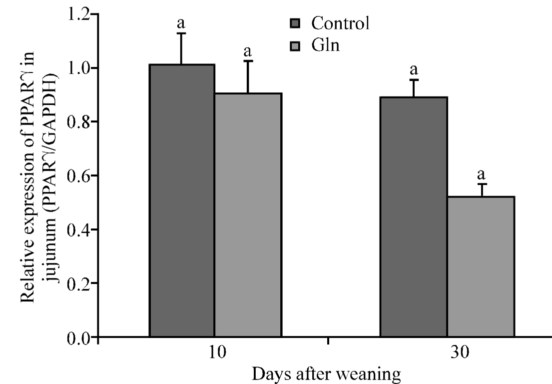

Fig. 2: Effects of Gln on the relative mRNA level of jejunal PPARγ in weanling piglets. Each column represents the mean of four individual pigs ±SEM; Different uppercase letters indicate a significant difference at $p<0.05$

(p = 0.62) and 13.13% (p = 0.036) 30 days after weaning. The activities of HK was numerically upregulated (p = 0.085) by Gln treatment 10 days after weaning and ALT numerically upregulated (p = 0.076) 30 days after weaning whereas the activities of AST was not affected (Table 6).

The early post-weaning phase consists of an acute deterioration of intestinal structure and function (Montagne *et al.*, 2007), accompanied by smaller activity of the brush-border enzymes, decrease in the villous length and reduction in the transepithelial electrical resistance (Pacha, 2000; Wijtten *et al.*, 2011). These are the critical factors related to the compromised absorption

Table 6: Influence of Gln on the activities of enzymes associated with Gln metabolism and energy production in the jejunum at 10 and 30 days post-weaning

	10 days			30 days			p-value	
Items	Control	Gln	SEM	Control	Gln	SEM	1	2
GS (U mg^{-1} protein)	3.60	1.84	0.33	0.63	0.54	0.11	0.044	0.620
ALT (U mg^{-1} protein)	27.34	29.50	1.99	27.69	30.47	0.92	0.190	0.076
AST (U mg^{-1} protein)	30.65	34.41	0.75	28.63	28.42	1.45	0.260	0.670
PK (U mg^{-1} protein)	29.71	28.61	1.01	32.68	28.39	0.38	0.350	0.036
HK (U g^{-1} protein)	2.77	3.28	0.15	4.52	5.03	0.72	0.085	0.200

1: Control vs. Gln 10 days post-weaning; 2: Control vs. Gln 30 days post-weaning. Each value represents mean from four pigs; GS: Glutamine Synthetase; ALT: Alanine Aminotransferase; AST: Aspartate aminotransferase; PK: Pyruvate Kinase; HK: Hexokinase

of nutrients. In the present study, the CTTAD of CP and AA were found to be low from 3-10 days and increased with the extension of the days post-weaning. The late post-weaning phase corresponds to an adaptation of the gut to the weaning diet (Montagne *et al*., 2007). The enzyme activities are restored to the level before weaning 2 weeks later (Barszcz and Skomial, 2011). The secretagogue-induced secretion is also linked with the factor representing time (Montagne *et al*., 2007). Additionally, the gastrointestinal tract development of young pigs was quick after weaning. At 3-7 days post-weaning, the relative weight of the small intestine increases by 25% and at 10-14 days, it increases by up to 52% (Dividich and Seve, 2000). All these changes result in a significant increase of the TTAD of nutrients and energy.

Recent studies have proven that Gln supplementation prevents jejunal atrophy, elevates intestinal oxidative-defense capacity and provides an energy source for enterocytes in weaned piglets (Wu *et al*., 1996; Wang *et al*., 2008). Gln serves as a specific survival factor in enterocytes, thus preventing the apoptosis in intestinal epithelial cells (Rhoads and Wu, 2009). Maintaining the structural integrity of the jejunal morphology in Gln-supplemented pigs may elevate the digestion and absorption of nutrients during the days post-weaning (Wu *et al*., 1996). Gln modulates intestinal barrier function and increases transcellular transport (Kozar *et al*., 2004; Wijtten *et al*., 2011). The results of the study indicated that dietary Gln supplementation significantly increased the CTTAD and AID of nutrients and gross energy in early weaned pigs.

The intestinal brush border membrane-bound AKP is regarded as a key maker enzyme associated with changes in the primary digestive and absorptive functions of the small intestine (Hodin *et al*., 1995). Recently, the gut mucosal defense role of intestinal AKP as an endogenous detoxification factor against luminal pathogenic bacterial lipopolysaccharide endotoxin has been recognized to be very beneficial (Geddes and Philpott, 2008). Early weaning commonly reduces small intestinal AKP expression in pigs (Lackeyram *et al*., 2010). In the current study, jejunal brush border membrane-bound AKP activity was increased by Gln supplementation. The expression of PPARγ, a maker of pro-inflammatory cytokine gene expression (Chamorro *et al*., 2010) was reduced in the jejunum by Gln supplementation. A previous study has reported that the expression of PPARγ is reduced by medium supplementation with Gln in human colorectal Caco-2 cells (Fiatte *et al*., 2008). Rhoads and Wu (2009) reported that Gln possesses anti-inflammatory effects in the intestine. The findings confirm that Gln can relieve intestinal inflammation caused by early weaning in piglets. These results help explain the improvement of CTTAD and AID of nutrients and gross energy and the productive performance in Gln-supplemented piglets.

Gln starvation leads to an increase in GS activity (Feng *et al*., 1990). Early weaning is commonly related to the reduction of Gln intake (Wu *et al*., 1996) which causes Gln starvation for enterocytes. In the present study, the jejunal GS activity was relatively high in the weanling pigs but was decreased significantly by Gln treatment. A previous report has indicated that the response of GS activity to Gln depends on physiologically meaningful Gln concentration (Labow *et al*., 2001). If the extra Gln is not consumed, a feedback loop increases the degradation rate of the GS protein (Labow *et al*., 2001).

PK is a key regulatory glycolytic enzyme modulated by diet (Stifel *et al*., 1969). PK activity in the jejunum decreased in the Gln-supplemented piglets in the present study. Gln and glucose are the major oxidative substrates utilized for energy production in the intestinal epithelial tissue in young mammals (Fleming *et al*., 1997; Blachier *et al*., 2009; Rhoads and Wu, 2009) and their metabolic pathways are across each other in the Tricarboxylic Acid (TCA) cycle (Fig. 3). The present data indicate that the glucolysis capacity for conversion of glucose to pyruvate is lower in dietary Gln supplemented piglets. This suggests glutamine influences the model of energy metabolism in the jejunum in support of the

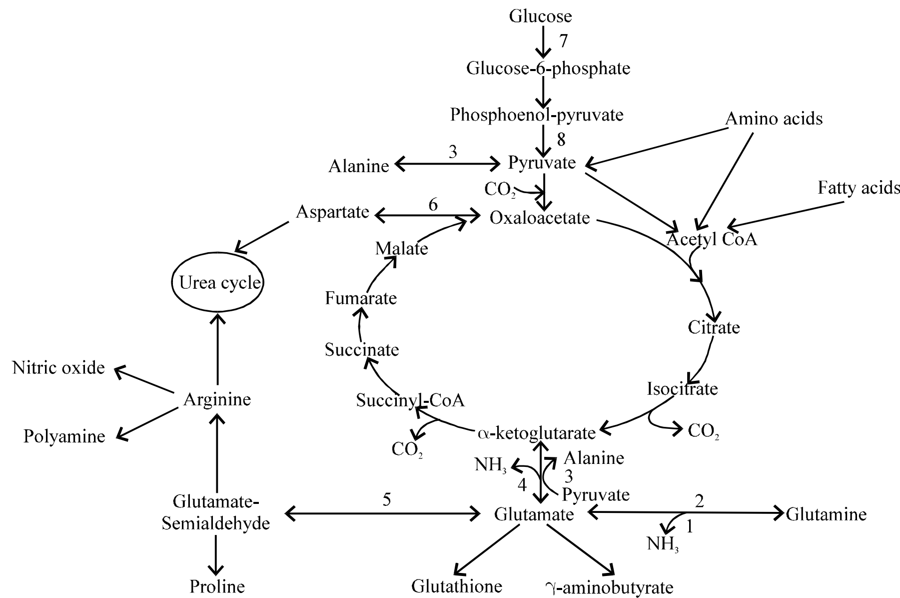

Fig. 3: Schematic drawing of the metabolic pathways for Gln and glucose: 1) glutaminase, 2) glutamine synthenase, 3) alanine aminotransferase, 4) glutamate dehydrogenase, 5) glutamate semi-aldehyde dehydrogenase, 6) aspartate aminotransferase, 7) pyruvate kinase and 8) hexokinase

findings of Madej *et al*. (2002). The capability of the TCA cycle to transform α-ketoglutarate from other sources aside from acetyl-CoA is strong such as glutamine (Quan *et al*., 1998; Madej *et al*., 2002). ALT is associated with the metabolism of glutamine, assisting its entrance into the TCA cycle as α-ketoglutarate (Board *et al*., 1990). The activities of ALT and AST increased numerically in the Gln treatment piglets. This result indicates that most of the α-ketoglutarate supplied in the TCA cycle is derived from glutamine conversions rather than from substrates entering the TCA cycle as acetyl-CoA, consistent with the results of Darcy-Vrillon *et al*. (1994).

CONCLUSION

The results of the present study demonstrate that 1% Gln supplementation to the post-weaned piglet diet enhanced the CTTAD and AID of nutrients and energy. The jejunal brush border membrane-bound AKP activity increased by Gln treatment but the expression of jejunal PPARγ decreased. This finding indicates that Gln supplementation can partially improve intestinal absorption and health status in weanling pigs. The observation of the comparisons between the activities of GS, PK, HK, ALT and AST in the Gln-supplemented piglets and the control piglets further supports the knowledge regarding the well-known role of these enzymes in the formation of pyruvate and intermediates for the TCA cycle (α-ketoglutarate and oxaloacetate) and the modification of the jejunal energy production in the small intestine.

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