

Effect of dietary soy oil, glucose, and glutamine on growth performance, amino acid profile, blood profile, immunity, and antioxidant capacity in weaned piglets

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Weaning stress results in gastrointestinal dysfunction and depressed performance in pigs. This study aimed to investigate the effect of soy oil, glucose, and glutamine on the growth and health of weaned piglets. Compared with those in the glutamine group, piglets in the glucose and soy oil groups had greater average daily gain, average daily feed intake, and gain: feed ratio from day 0 to 14, and gain: feed ratio for the overall period. There were no differences with regard to serum amino acids among the three groups on day 14, except glycine and threonine. The serum concentration of histidine, serine, threonine, proline, and cysteine was the highest in the glutamine group, while the content of glycine and lysine in the soy oil group on day 28 was the highest among all groups. Piglets fed with glutamine had greater serum glucose and creatinine on day 14, high-density lipoprotein on day 28, and serum IgG and IgM on day 28. Piglets in the glutamine group demonstrated lower serum total superoxide dismutase on day 14 and 28; however, they demonstrated higher total superoxide dismutase and total antioxidant capacity in the duodenum and ileum on day 14. Weaned pigs supplemented with glucose or soy oil demonstrate better growth performance possibly due to their enhanced feed intake, whereas those supplemented with glutamine may have improved immunity and intestinal oxidative capacity.

glucose, glutamine, soy oil

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INTRODUCTION

Despite gastrointestinal tissues representing only 4%–6% of body mass, these tissues disproportionately account for

20%–35% of the energy expenditure and protein turnover of the entire body (Lobley et al., 1980; Burke et al., 1989). Previous studies indicated that glutamate, aspartate, and glutamine were the major oxidative substrates utilized by the small intestinal epithelial cell and that glucose and fatty acid were the minor oxidative substrates (Windmueller and Spaeth, 1978; Stoll et al., 1998). In addition, glutamine

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specifically is the key link between the carbon metabolism of carbohydrate and protein (Reeds et al., 2000). Reportedly, in weaned pigs, glutamine can inhibit pro-inflammatory cytokines and plays an important role in improving the feed efficiency and gut health (Yi et al., 2005; Wang et al., 2008; Johnson and Lay, 2017).

It is well known that weaning stress decreases the feed intake, induces diarrhea, and damages the gut structure (Pluske et al., 1997; Pié et al., 2004; Wang et al., 2006). Low intake of feed immediately after weaning is attributed to insufficient energy supply in weaned piglets and morphological changes in their small intestine, which requires a high rate of energy expenditure to maintain gut integrity (van der Schoor et al., 2002). Previous studies have shown that different sources of fiber, lipid, or protein in the diet have different effects on the growth performance of weaned piglets (Makkink et al., 1994; Pluske et al., 2003a; Mendoza and van Heugten, 2014) and the intestinal environment and meat quality of growing pigs (Hanczakowska et al., 2014). However, glutamine, as a conditionally essential amino acid, is preferentially utilized by the intestine of the neonatal pig (Burrin and Stoll, 2002). Growing evidence indicates improvement in the growth performance and intestinal morphology of early-weaned piglets and intestinal immune response following dietary glutamine supplementation (Wu et al., 1996; Qian et al., 2005; Jiang et al., 2009). Based on previously published researches, we hypothesize that glutamine, as the major oxidative substrate of the small intestine, has stronger effects on the growth and health of weaned piglets than that with other energy sources. Therefore, the present study aimed to determine the effects of supplementation of different energy sources on the growth performance, serum biochemical parameters, immunity, antioxidant capacity, and intestinal morphology in weaned piglets.

RESULTS

Growth performance and visceral organ weights

Piglets fed with soy oil had greater ($P<0.05$) body weight (BW) on day 14 than those fed with glutamine, but no difference was observed in the BW on day 28 among dietary treatments (Table 1). Pigs fed with glucose or soy oil diet had greater ($P<0.05$) ADG and ADFI from day 0 to 14 and greater ($P<0.05$) G:F for the entire experiment than those fed with glutamine. However, there were no significant differences between the pigs in the glucose and soy oil groups with regard to the growth performance. The liver weight on day 28 was higher ($0.05<P<0.1$) in pigs fed with glucose compared with those fed with glutamine, but did not differ from those fed with soy oil. There were no differences in the heart, kidney, and spleen weight among the three treatment groups on both day 14 and 28.

Serum amino acids

On day 14, supplementation of glucose and soy oil increased ($P<0.05$) the serum glycine level but reduced ($P<0.05$) serum threonine level compared with supplementation of glutamine (Table 2). Compared with pigs in the glutamine group, pigs in the glucose and soy oil groups had lower ($P<0.05$) serum histidine, threonine, and cysteine but greater ($P<0.05$) lysine on day 28. Supplementation of glutamine increased ($P<0.05$) serum serine and proline compared with that of glucose but not with supplementation soy oil. Soy oil supplementation also increased ($P<0.05$) serum glycine compared with glucose supplementation but not with glutamine supplementation.

Serum biochemical parameters and immunoglobulins

Pigs fed with soy oil tended ($P=0.087$) to have lower total protein compared with pigs fed with glucose and glutamine on day 14 (Table 3), whereas no significant differences were observed in the total protein among all the dietary treatments on day 28. Pigs fed with glutamine had greater ($P<0.05$) glucose and creatinine levels on day 14 and HDL-C on day 28 than that with those fed with soy oil or glucose. In addition, glutamine supplementation increased serum BUN on day 28 compared with soy oil supplementation ($P<0.05$) but not with glucose supplementation and tended to increase serum ALT and total cholesterol ($0.05<P<0.1$). There were no differences in the serum concentrations of ALP, triglycerides, and LCL-C among the three treatments on day 14 and 28. The glutamine group showed increased ($P<0.05$) serum IgM compared with the soy oil group on day 14. Pigs in the soy oil and glutamine groups had greater ($P<0.05$) IgA than those in the glucose group on day 28. The concentrations of IgG and IgM were markedly increased ($P<0.01$) in the glutamine group compared to those in the other two groups on day 28.

Serum antioxidative index

On day 14, pigs fed with soy oil and glucose had greater ($P<0.05$) serum total superoxide dismutase (T-SOD) than those fed with glutamine, whereas pigs fed with soy oil and glutamine tended to have greater ($P=0.064$) serum total antioxidant capacity (T-AOC) than those fed with glucose (Table 4). On day 28, supplementation of glutamine significantly increased ($P<0.05$) serum MDA compared with that of glucose, but did not differ with that of soy oil. Supplementation of glucose increased ($P<0.05$) T-SOD compared with that of soy oil or glutamine, and addition of soy oil significantly increased ($P<0.05$) serum T-SOD compared with that of glutamine.

Table 1 Effects of different sources of energy dietary supplementation on growth performance and organ weight in weaned piglets^{a)}

Item	Diet			P-value
	Glucose	Soy oil	Glutamine	
BW (kg)				
Day 0	7.36±0.23	7.39±0.19	7.43±0.17	0.968
Day 14	9.84±0.30 ^{ab}	10.03±0.30 ^a	9.08±0.22 ^b	0.046
Day 28	14.77±0.53	14.73±0.62	13.50±0.49	0.208
ADG (g d ⁻¹)				
Day 0–14	177.55±12.50 ^a	188.27±11.78 ^a	117.86±7.99 ^b	<0.0001
Day 0–28	266.07±17.07	260.71±16.99	216.84±13.96	0.083
ADFI (g d ⁻¹)				
Day 0–14	320.89±19.62 ^a	330.89±18.62 ^a	269.18±14.35 ^b	0.040
Day 0–28	498.76±27.60	503.73±20.45	481.22±30.52	0.815
G:F (g g ⁻¹)				
Day 0–14	0.55±0.02 ^a	0.57±0.02 ^a	0.44±0.02 ^b	<0.0001
Day 0–28	0.53±0.02 ^a	0.53±0.03 ^a	0.45±0.01 ^b	0.012
Organ weight/BW (g kg ⁻¹)				
Day 14				
Heart	4.93±0.15	5.13±0.15	5.02±0.14	0.545
Liver	20.66±0.66	20.71±0.48	19.72±1.04	0.596
Spleen	2.03±0.15	1.66±0.08	1.96±0.21	0.226
Kidney	3.11±0.09	3.14±0.09	3.04±0.23	0.904
Day 28				
Heart	4.94±0.15	5.26±0.11	5.15±0.10	0.205
Liver	23.90±0.72	22.85±0.64	21.60±0.63	0.078
Spleen	1.86±0.25	1.90±0.16	2.25±0.24	0.405
Kidney	3.04±0.13	3.17±0.15	2.77±0.21	0.244

a) Seven piglets per treatment. ADFI, average daily feed intake; ADG, average daily gain; G:F, gain:feed ratio. a, b, Values within a row with different superscripts differ significantly at $P<0.05$.

Antioxidative index in the intestinal mucosa

On day 14, pigs fed with glutamine had greater ($P<0.05$) duodenal T-SOD than those fed with glucose or soy oil; pigs fed with glutamine and glucose had greater ($P<0.05$) ileal T-SOD than those fed with soy oil (Table 5). Pigs fed with soy oil and glucose had greater ($P<0.05$) jejunal T-AOC than those fed with glutamine, whereas pigs fed with glutamine had greater ($P<0.05$) ileal T-AOC than pigs fed with glucose. On day 28, supplementation of soy oil increased ($P<0.05$) ileal glutathione compared with that of glucose but did not differ with that of glutamine. On day 28, supplementation of soy oil tended to increase ($P=0.063$) ileal T-SOD and increased jejunal T-AOC compared with that of glutamine ($P=0.05$) but did not differ with that of glucose.

DISCUSSION

Carbohydrates, fats and oils, and proteins in animal feed are essential as they are a source of energy to animals (Yang et

al., 2016). However, glucose, many fatty acids, and glutamine appear to affect different biological processes and physiological functions (Makkink et al., 1994; Pluske et al., 2003a; Mendoza and van Heugten, 2014). To understand how pigs utilize dietary energy, a simultaneous and detailed evaluation of the impacts of dietary energy sources on pig production is required.

Weaning piglets generally shift from high-fat and low-carbohydrate milk to low-fat and high-carbohydrate solid feed following weaning; however, their digestive and absorptive capacity of the gastrointestinal tract has limited ability to digest solid feed (Pluske et al., 1997). In addition, newly-weaned piglets must cope with a large increase in dry matter intake in order to maintain their growth (Pluske et al., 2003b). Owing to this, newly-weaned piglets generally have decreased feed intake and poor growth rate for 7–14 days after weaning. Enhanced feed intake in the first seven days post-weaning may improve the 28-day, post-weaning BW (Pluske et al., 2003b). In the present study, we observed that pigs supplemented with soy oil or glucose performed better compared with those supplemented with glutamine and de-

Table 2 Effects of different sources of energy dietary supplementation on serum amino acids of weaned piglets^{a)}

Item ($\mu\text{g mL}^{-1}$)	Diet			P-value
	Glucose	Soy oil	Glutamine	
Day 14				
His	3.56±0.24	3.03±0.27	3.70±0.36	0.291
Ser	13.58±1.06	14.01±1.31	13.45±1.11	0.939
Arg	10.94±1.09	8.86±0.76	8.61±1.12	0.270
Gly	105.57±8.37 ^a	104.92±11.12 ^a	63.26±6.49 ^b	0.003
Asp	4.05±0.36	3.95±0.09	3.72±0.26	0.684
Glu	40.38±1.40	40.08±2.33	39.83±3.14	0.988
Thr	8.13±0.58 ^b	9.29±1.99 ^b	179.00±24.48 ^a	<0.0001
Ala	29.79±2.06	32.45±2.94	30.71±2.36	0.757
Pro	16.5±0.83	16.61±1.22	15.68±1.47	0.841
Cys	1.76±0.24	2.32±0.27	2.89±0.46	0.106
Tyr	5.78±0.36	5.46±0.63	5.58±0.28	0.879
Met	3.87±0.21	3.62±0.36	3.42±0.26	0.414
Ile	4.92±0.28	4.42±0.15	4.77±0.16	0.243
Leu	8.74±0.60	7.34±0.64	7.82±0.51	0.267
Phe	5.26±0.24	4.57±0.44	4.78±0.23	0.309
Lys	14.64±1.23	15.45±2.13	14.24±0.82	0.835
Day 28				
His	1.56±0.22 ^b	1.46±0.14 ^b	3.84±0.27 ^a	<0.0001
Ser	7.38±0.62 ^b	10.90±0.96 ^a	13.36±1.07 ^a	0.001
Arg	9.15±1.14	9.51±0.96	10.31±0.70	0.675
Gly	33.25±6.22 ^b	75.51±13.46 ^a	52.81±4.02 ^{ab}	0.017
Asp	1.80±0.22	1.68±0.15	2.39±0.48	0.265
Glu	16.58±1.92	18.84±2.12	22.18±3.36	0.343
Thr	5.44±0.68 ^b	7.36±0.96 ^b	278.11±26.89 ^a	<0.0001
Ala	16.66±2.18	17.30±1.51	19.75±1.50	0.420
Pro	7.43±0.95 ^b	9.51±1.06 ^{ab}	11.77±1.01 ^a	0.028
Cys	1.44±0.26 ^b	1.54±0.35 ^b	3.95±0.69 ^a	0.003
Tys	4.17±0.56	3.99±0.42	5.20±0.40	0.149
Met	2.67±0.49	2.93±0.43	3.86±0.39	0.154
Ile	7.43±1.21	6.71±0.64	5.64±0.29	0.276
Leu	8.80±1.34	7.23±0.78	6.85±0.51	0.302
Phe	4.77±0.74	4.10±0.50	5.63±0.50	0.182
Lys	13.51±1.68 ^a	15.15±2.12 ^a	7.23±2.14 ^b	0.004

a) Seven piglets per treatment. His, histidine; Ser, serine; Arg, arginine; Gly, glycine; Asp, aspartic acid; Glu, glutamine; Thr, threonine; Ala, alanine; Pro, proline; Cys, cysteine; Tyr, tyrosine; Met, methionine; Ile, isoleucine; Leu, leucine; Phe, phenylalanine; Lys, lysine. a, b, Values within a row with different superscripts differ significantly at $P<0.05$.

monstrated increased feed intake. The difference in the feed intake among the three groups in our study was likely due to the following reasons: pigs prefer the sweet taste of glucose supplemented feed (Seabolt et al., 2010) and the specific taste of fats or oils supplemented feed (Albin et al., 2001; Gu and Li, 2003). Previous studies have shown that glutamine can improve the performance of weanling piglets and contribute to the intestinal epithelium structure (Hsu et al., 2010; Wu, 2010; Yi et al., 2005; Lescano et al., 2013). However,

some studies also showed that there were no improvements on growth performance of piglets for 28 days post-weaning (Domeneghini et al., 2004; Lee et al., 2003a). In addition, a 12% lower feed: gain ratio was reported in 1% glutamine group during the first 10 days after weaning (Qian et al., 2005). Different duration of experimental treatments or dosage of glutamine may be the contributing factors to differing growth performance with glutamine supplementation (Lee et al., 2003b; Kitt et al., 2002).

Table 3 Effects of different sources of energy dietary supplementation on serum biochemical parameters of weaned piglets^{a)}

Item	Diet			P-value
	Glucose	Soy oil	Glutamine	
Day 14				
ALP (U L ⁻¹)	254.86±46.72	231.33±39.06	231.43±21.02	0.873
ALT (U L ⁻¹)	28.14±3.11	27.50±3.24	35.57±3.57	0.189
BUN (mmol L ⁻¹)	1.76±0.24	2.04±0.20	1.88±0.16	0.638
Creatinine (μmol L ⁻¹)	71.29±6.13 ^b	65.17±2.87 ^b	86.14±4.43 ^a	0.006
Total cholesterol (mmol L ⁻¹)	1.25±0.09	1.07±0.13	1.24±0.08	0.360
Glucose (mmol L ⁻¹)	2.80±0.32 ^b	2.73±0.21 ^b	3.87±0.42 ^a	0.050
HDL-C (mmol L ⁻¹)	0.38±0.04	0.37±0.06	0.41±0.04	0.827
LDL-C (mmol L ⁻¹)	0.76±0.05	0.57±0.08	0.71±0.05	0.110
Triglyceride (mmol L ⁻¹)	0.20±0.01	0.17±0.02	0.21±0.02	0.154
Total protein (g L ⁻¹)	28.84±1.80	22.92±1.95	28.77±2.12	0.087
IgA (mg dL ⁻¹)	0.49±0.05	0.38±0.06	0.41±0.03	0.334
IgG (mg dL ⁻¹)	104.69±5.28	94.80±6.63	110.23±9.64	0.376
IgM (mg dL ⁻¹)	10.47±1.73 ^{ab}	5.52±0.23 ^b	12.94±2.00 ^a	0.003
Day 28				
ALP (U L ⁻¹)	213.00±12.41	167.29±13.23	182.43±21.65	0.187
ALT (U L ⁻¹)	37.50±1.65	35.57±4.19	47.71±4.06	0.061
BUN (mmol L ⁻¹)	4.04±0.46 ^a	2.55±0.19 ^b	3.79±0.24 ^a	0.006
Creatinine (μmol L ⁻¹)	76.00±5.24	68.57±4.23	73.86±4.63	0.527
Total cholesterol (mmol L ⁻¹)	1.02±0.04	0.93±0.07	1.21±0.10	0.055
Glucose (mmol L ⁻¹)	2.60±0.18	2.51±0.46	3.40±0.33	0.128
HDL-C (mmol L ⁻¹)	0.33±0.02 ^b	0.31±0.02 ^b	0.45±0.03 ^a	0.001
LDL-C (mmol L ⁻¹)	0.56±0.03	0.48±0.05	0.62±0.09	0.283
Triglyceride (mmol L ⁻¹)	0.31±0.04	0.26±0.03	0.27±0.02	0.399
Total protein (g L ⁻¹)	24.15±1.70	20.60±1.80	25.53±1.82	0.152
IgA (mg dL ⁻¹)	0.70±0.07 ^b	1.74±0.25 ^a	1.46±0.22 ^a	0.008
IgG (mg dL ⁻¹)	92.55±4.95 ^b	75.43±8.64 ^b	126.47±14.40 ^a	0.009
IgM (mg dL ⁻¹)	9.33±1.23 ^b	5.24±1.05 ^b	19.71±1.98 ^a	<0.0001

a) Seven piglets per treatment. ALP, alkaline phosphatase; ALT, alanine transaminase; BUN, blood urea nitrogen; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; a, b, Values within a row with different superscripts differ significantly at $P<0.05$.

In the present study, serum concentration of histidine, threonine, serine, cysteine and proline increased in glutamine supplemented piglets compared with soy oil or glucose supplemented pigs, which confirmed the role of glutamine in modulating amino acid metabolism (Xiao et al., 2012). It is well known that the intestinal tract has a high metabolic rate and the fate of dietary excesses of glutamine, glutamate, and proline largely depends on splanchnic metabolism, especially first-pass metabolism by the gut (Bertolo and Burrin, 2008). Glutamine is metabolized to amino acids such as alanine, aspartate, citrulline, ornithine, and proline (Wu et al., 1995; Stoll et al., 1998). Approximately 7% of glutamine carbon metabolized in the rat small intestine was used for proline synthesis (Windmueller et al., 1974). Proline promotes animal growth and has a versatile role in cell metabolism and physiology, including cellular redox reactions

and antioxidative capacity (Wu et al., 2011). Cysteine is also known to be involved in a myriad of immune-modulatory and antioxidant pathways (Wu et al., 2007; Kim et al., 2009). Lysine and threonine are both indispensable amino acids and can be synthesized using aspartate (derived from glutamine) as a precursor. Threonine is a major component of plasma γ -globulin in poultry, rabbits, and human beings (Li et al., 1999); it is utilized at a high rate in the intestine of piglets mainly due to its incorporation into mucosal proteins (Schaart et al., 2005). However, dietary, rather than systemic threonine was preferentially utilized for mucosal protein synthesis (Schaart et al., 2005). In addition, intestinal lysine oxidation accounted for one third of the whole-body lysine oxidation (Wu et al., 2005), indicated that amino acid patterns in the systemic circulation of the glutamine group have been altered by extensive catabolism of enteral amino acids

Table 4 Effects of different sources of energy dietary supplementation on serum antioxidative index of weaned piglets^{a)}

Item	Diet			<i>P</i> -value
	Glucose	Soy oil	Glutamine	
Day 14				
MDA (nmol mL ⁻¹)	2.65±0.49	2.68±0.73	3.91±0.41	0.229
Catalase (U mL ⁻¹)	106.49±11.51	113.85±25.83	114.32±13.24	0.934
T-SOD (U mL ⁻¹)	136.97±4.09 ^a	140.67±6.08 ^a	110.67±9.11 ^b	0.015
T-AOC (U mL ⁻¹)	1.67±0.14	2.27±0.13	2.52±0.35	0.064
Day 28				
MDA (nmol mL ⁻¹)	4.13±0.51 ^b	5.10±0.34 ^{ab}	6.67±0.81 ^a	0.033
Catalase (U mL ⁻¹)	226.88±20.63	243.50±20.83	204.76±23.43	0.453
T-SOD (U mL ⁻¹)	47.62±1.39 ^a	41.36±2.85 ^b	31.30±1.14 ^c	0.002
T-AOC (U mL ⁻¹)	1.33±0.12	1.46±0.16	1.13±0.20	0.398

a) Seven piglets per treatment. MDA, malondialdehyde; a, b, Values within a row with different superscripts differ significantly at *P*<0.05.

Table 5 Effects of different sources of energy dietary supplementation on intestinal mucosal antioxidative index of weaned piglets^{a)}

Item	Diet			<i>P</i> -value
	Glucose	Soy oil	Glutamine	
Day 14				
Glutathione (mg g ⁻¹ protein)				
Duodenum	33.72±6.43	36.87±4.11	38.83±11.68	0.889
Jejunum	30.62±3.29	33.20±1.99	30.35±2.14	0.686
Ileum	26.92±2.67	31.44±3.11	34.82±3.72	0.241
T-SOD (U mg ⁻¹ protein)				
Duodenum	7.84±1.02 ^b	9.33±0.75 ^b	12.16±1.04 ^a	0.011
Jejunum	19.85±2.25	17.30±1.55	19.68±1.49	0.546
Ileum	18.08±1.69 ^a	13.43±0.66 ^b	17.78±1.25 ^a	0.033
T-AOC (U mg ⁻¹ protein)				
Duodenum	2.77±0.16	2.91±0.19	3.19±0.34	0.448
Jejunum	2.19±0.22 ^a	1.97±0.17 ^a	1.43±0.12 ^b	0.017
Ileum	0.87±0.09 ^b	1.57±0.28 ^b	1.82±0.27 ^a	0.040
Day 28				
Glutathione (mg g ⁻¹ protein)				
Duodenum	38.32±8.90	36.05±4.59	42.81±10.01	0.840
Jejunum	33.50±3.77	35.22±1.84	37.20±2.73	0.658
Ileum	34.65±3.99 ^b	59.80±5.23 ^a	45.86±5.82 ^{ab}	0.012
T-SOD (U mg ⁻¹ protein)				
Duodenum	6.91±0.62	5.33±0.62	6.41±0.48	0.175
Jejunum	12.35±1.28	12.01±1.04	14.98±2.12	0.356
Ileum	13.40±0.90	14.63±1.03	11.76±0.41	0.063
T-AOC (U mg ⁻¹ protein)				
Duodenum	3.46±0.14	3.72±0.19	3.77±0.12	0.362
Jejunum	1.54±0.25 ^{ab}	1.80±0.30 ^a	0.91±0.17 ^b	0.050
Ileum	1.61±0.13	2.02±0.18	1.90±0.44	0.564

a) Seven piglets per treatment. a, b, Values within a row with different superscripts differ significantly at *P*<0.05.

in the small intestine. Complex interactions are involved in the modified amino acid profiles of the intestinal amino acid

metabolism in glutamine supplemented pigs. Further exploration is warranted regarding this aspect.

In this study, supplementation of glutamine increased serum glucose compared with that of soy oil or glucose. This observation was consistent with lower ADG in glutamine supplemented pigs on day 14. Carbohydrates are broken down to glucose, which is released in the systemic circulation and can be oxidized to provide energy and channeled into pathways for the synthesis of fatty acids (Uyeda and Repa, 2006). The increased glucose concentration in the serum of glutamine supplemented pigs suggests a lower rate of glucose used in this group compared with glucose or soy oil group (He et al., 2012). The higher glucose concentration in serum is also probably due to the high corn content in the diet of glutamine supplemented group. Serum BUN concentration has been used as an indicator of amino acid utilization efficiency, and it is related to the status of protein metabolism and retained dietary nitrogen in animals (Jin et al., 2010). In the present study, supplementation of glutamine or glucose increased serum BUN compared with that of soy oil. This indicates the glutamine supplementation may cause an unbalanced amino acid composition in the diet, thereby decreasing protein synthesis, which was indicated by the reduced growth performance of glutamine supplemented pigs. A previous study also showed that BUN has a negative relationship with G:F (Whang and Easter, 2000). In the present study, piglets fed the diet containing glucose also had higher serum BUN values, which may indicate that these pigs have reduced protein synthesis or enhanced protein metabolism. These observations are consistent with the serum total protein concentration of piglets in the glutamine or glucose group. The high concentration of HDL-C in glutamine supplemented pigs also indicates that glutamine altered the carbohydrate, protein, and lipid metabolism unlike that with soy oil or glucose supplemented pigs.

Growth performance of animals is affected by their health status and immunity (Yan and Kim, 2011). Previous studies reported that glutamine supplementation could increase serum immunoglobulin and improve systemic immune function in pigs (Burke et al., 1989; Alverdy, 1990; O'Riordain et al., 1994; Bartell and Batal, 2007). Intestinal intraepithelial lymphocytes also utilize glutamine as an important energy source and for their proliferation (Dugan et al., 1994). The current study's findings showed that supplementation with glutamine increased the concentration of IgA, IgG, or IgM compared with that with glucose and soy oil. The increased serum immunoglobulin in glutamine supplemented pigs indicates that glutamine supplementation may enhance the innate immunity of weanling pigs.

Many factors including birth and weaning processes, housing, and transportation can disrupt oxidative balance and cause oxidative injury in weanling piglets (Yin et al., 2013). The present experiment observed that piglets supplemented with soy oil or glucose had greater systemic antioxidant capacity and, therefore, had better growth performance than

pigs supplemented with glutamine. However, piglets supplemented with glutamine had greater intestinal antioxidant capacity during the first 2 weeks of weaning. Glutathione, which is formed from cysteine, glutamate, and glycine, is the major antioxidant in cells that regulates the homeostasis of free radicals (Wu et al., 2005). However, there were no differences in the intestinal glutathione concentration among the three groups in the present study.

In conclusion, results of the present study indicate that piglets supplemented with glucose or soy oil have better growth performance, which is likely due to their enhanced feed intake and better systemic antioxidant capacity. Supplementation of glutamine increases innate immunity and intestinal oxidative capacity of weaned piglets. These new findings also present glucose, soy oil, and glutamine as different energy sources with differing impact on growth performance and antioxidant capacity of weanling pigs. Further research is warranted to explore the effects of the energy source on intestinal microbial metabolism.

MATERIALS AND METHODS

The experimental design and procedures used in this study were approved by the Animal Care and Use Committee of the Institute of Subtropical Agriculture, Chinese Academy of Sciences (IACUC # 201302).

Animals and experimental treatments

Weanling piglets used in the experiment were bred from Duroc boars and Landrace×Large Yorkshire sows. A total of 42 piglets weaned at the age of 21 days; these were blocked by body weight (BW) and sex and were randomly assigned to one of the three treatments. Fourteen replicates, including seven gilts and seven barrows, were performed for each treatment. All piglets were fed an isocaloric diet supplemented with one of the three energy sources, glucose, soy oil, and glutamine. The experiment was conducted over 28 days with two phases and two weeks in each phase. Glucose, soy oil, and glutamine accounted for 2.5% of the metabolic energy (ME) in each of the diet. Glucose and soy oil were edible grade refined and glutamine (G3126) was obtained from Sigma (USA). The diets were formulated to meet the nutritional requirements of the weanling piglets (NRC, 2012); the nutrient compositions are depicted in Table 6.

Animal management and sample collection

Piglets were individually housed in an environmentally controlled nursery barn with hard-plastic slatted flooring. All piglets had *ad libitum* access to drinking water. The BW of piglets was recorded at the initiation of the experiment and

Table 6 Composition of the diets^{a)}

Composition (%)	Phase I			Phase II		
	Glucose	Soy oil	Glutamine	Glucose	Soy oil	Glutamine
Corn (8.5% CP)	43.50	43.39	47.52	64.65	64.61	68.51
Puffed corn	20.00	20.00	20.00	–	–	–
Soybean meal, 43	6.52	7.00	5.25	14.31	14.44	14.17
Soybean protein concentrate powder	3.75	3.45	3.36	3.00	2.93	1.83
Whey powder	8.00	8.00	8.00	5.70	5.70	5.70
Fish meal	5.00	5.00	5.00	3.00	3.00	3.00
Plasma protein powder	5.00	5.00	5.00	–	–	–
Lys, 98%	0.49	0.49	0.54	0.64	0.64	0.69
DL-Met	0.10	0.10	0.11	0.17	0.17	0.18
L-Thr	0.11	0.11	0.13	0.19	0.19	0.22
L-Trp	0.04	0.04	0.05	0.06	0.06	0.07
Glucose	2.44	0.00	0.00	2.49	0.00	0.00
Ala	1.95	1.95	0.00	1.95	1.95	0.00
Soybean oil	0.00	0.94	0.00	0.00	0.96	0.00
Gln	0.00	0.00	1.95	0.00	0.00	1.98
Fill in the blanks content	0.00	1.45	0.00	0.34	1.86	0.00
Stone powder	1.04	1.04	1.05	1.03	1.03	0.14
Dicalcium phosphate	0.50	0.50	0.50	0.42	0.42	0.42
Choline chloride (50%)	0.10	0.10	0.10	0.10	0.10	0.10
Suckling pig premix	1.00	1.00	1.00	1.00	1.00	1.00
ZnO	0.30	0.30	0.30	0.30	0.30	0.30
Citric acid	0.16	0.14	0.14	0.30	0.30	0.30
Antioxidants	–	–	–	0.05	0.05	0.05
salt	–	–	–	0.30	0.30	0.30
Nutrient composition*						
GE, kcal kg ⁻¹	4,276	4,233	4,209	4,122	4,102	4,102
ME, kcal kg ⁻¹	3,400	3,400	3,400	3,350	3,350	3,350
CP, %	18.50	18.50	18.50	20.00	20.00	20.00
Ca, %	0.80	0.80	0.80	0.70	0.70	0.70
SID, Lys, %	1.35	1.35	1.35	1.23	1.23	1.23
SID, Met+Cys, %	0.74	0.74	0.74	0.68	0.68	0.68
SID, Thr, %	0.79	0.79	0.79	0.73	0.73	0.73
SID, Trp, %	0.22	0.22	0.22	0.20	0.20	0.20

a) *, Gross energy concentrations were analyzed values, while all other nutrient compositions were calculated. Gln, glutamine; Trp, Tryptophan; SID, standardized ileal digestible.

before feeding on day 14 and 28. Average daily gain (ADG), average daily feed intake (ADFI), and gain:feed (G:F) ratio were used to measure the growth performance in piglets (Yin et al., 2010). On day 14, seven piglets (four barrows and three gilts) were randomly selected from each treatment and were euthanized for blood and tissue sampling. The remaining pigs were euthanized after the completion of the experiment (day 28). Therefore, the growth performance from day 14 to 28 was measured using replicates of seven pigs per treatment.

Blood samples were collected in 10-mL tubes following jugular vein puncture and were centrifuged at 3,000×g for

10 min at 4°C to recover serum samples (Yang et al., 2013). Serum samples thus obtained were immediately stored at –80°C until they were further analyzed for biochemical profile, immunoglobulins, and antioxidant capacity. The piglets were euthanized using an overdose of sodium pentobarbital solution (40 mg kg⁻¹ BW) followed by exsanguinations (Ren et al., 2014). The heart, liver, spleen, and kidneys were collected and weighed; duodenum, mid-jejunum, and ileum were harvested and rinsed several times with ice-cold phosphate-buffered saline to subsequently scrape off the mucosal cell layers, which were rapidly frozen in liquid nitrogen. The duodenum was collected from the

junction of stomach and small intestine, the ileum from the anterior to the ileocecal junction, while the jejunum from the middle portion of the small intestine.

Analysis of serum samples

Amino acid contents were determined from the serum samples obtained on day 14 and 28. Briefly, 1 mL of the serum sample and 2.5 mL of 7.5% trichloroacetic acid solution were mixed thoroughly and centrifuged at $12,000\times g$ at 4°C for 15 min. The supernatant was analyzed for the amino acid content by an ion-exchange amino acid analyzer (Hitachi, Japan) (Wang et al., 2016). Ten biochemical parameters were measured in the serum samples, including alkaline phosphatase (ALP), alanine transaminase (ALT), total protein, total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), glucose, blood urea nitrogen (BUN), and creatinine using a biochemical analytical instrument (TBA-120FR, Toshiba Medical Systems Corporation, Japan). Serum immunoglobulins IgA (No. IGA7170), IgG (No. IGG7170), and IgM (No. IGM7170) on day 14 and 28 were measured with commercial kits (Weifang). The antioxidant capacity, including total antioxidant capacity (T-AOC; No. A015) and malondialdehyde (MDA; No. A003-1), superoxide dismutase (SOD; No. A001-1), glutathione (No. A006-1), and catalase (No. A007-1-1) activity in serum samples, was determined using assay kits according to the manufacturer's instructions (Nanjing Jiancheng).

Measurement of intestinal antioxidant capacity

Intestinal mucosa was homogenized in 0.9% of ice-cold saline solution with a Tissue Tearor (IKA products, T8 ultraturax, Germany) and then centrifuged at $3,000\times g$ at 4°C for 10 min. The supernatant was collected to determine the antioxidant capacity (T-AOC, SOD, and glutathione) using assay kits according to the manufacturer's instructions (Nanjing Jiancheng).

Statistical analysis

All statistical analyses were performed using SPSS software 19.0 (SPSS Inc., USA). The differences among the treatments were evaluated using one-way analysis of variance and Duncan's multiple range tests. The data are presented as means, and $P<0.05$ indicated statistical significance, whereas $0.05<P<0.1$ indicated a trend toward significance.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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