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Original Research Article

Glutamic acid supplementation recovers the reduced performance of weanling pigs fed reduced crude protein diets

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ABSTRACT

This study was conducted to evaluate the supplementation of glutamic acid (Glu) to reduced protein diets on the performance of weanling pigs. One hundred and eighty crossbred weanling pigs ([Yorkshire × Landrace] × Duroc, 21 d old) having similar body weight (BW) of 6.45 kg were randomly allotted to 1 of 6 dietary treatments (5 pigs per pen [2 barrows and 3 gilts]; 6 pens per treatment) based on BW and sex during a 6-week trial. Dietary treatments consisted of positive control (PC) diet formulated to have 226.9, 205.6, and 188.8 g crude protein (CP) during phases 1, 2, and 3, respectively, and negative control (NC) diets with 20 g CP reduction from PC diets and addition of Glu with increasing levels, resulting in the calculated Lys-to-Glu ratios of 1:2.25, 1:2.30.1:2.35, 1:2.40, and 1:2.45, designated as NC, NC1, NC2, NC3, and NC4, respectively. The BW of pigs receiving PC diet was higher ($P < 0.05$) than those receiving NC diet at d 7, 21 and 42. A higher ($P < 0.05$) average daily gain (ADG) from d 1 to 7, 8 to 21, 22 to 42 and during the overall experiment period was observed in pigs fed PC than NC diet. Pigs fed NC diets including the graded level of Glu linearly increased ($P < 0.05$) BW at d 42, ADG and gain-to-feed ratio (G:F) during the overall experimental period. In addition, trends in linear increase in BW ($P = 0.056$) at d 7 and ADG from d 1 to 7 and d 22 to 42 (linear effect, $P = 0.081$, $P = 0.058$ respectively) were observed. A tendency in the linear increment of NH_3 ($P = 0.082$) at d 21 and linear reduction in methyl mercaptans ($P = 0.054$) emission at d 42 was observed in pigs fed NC diets supplemented with graded level of Glu. In conclusion, supplementing the reduced protein diet with Glu enhanced the growth performance in weanling pigs suggesting that supplementation of Glu can compensate the reduction of 2% CP in the basal diets.

1. Introduction

High crude protein (CP) diets are fed to weaned piglets because they have a low capacity for feed intake and a high potential for protein accretion. Since protein is a relatively expensive nutrient, there is increased interest in finding alternatives to partially replace dietary protein for sustainable animal farming. With the increasing availability of crystalline amino acids (AA), the formulation of low-protein diets with a well-balanced AA content has become possible. Previous reports suggest that reducing the dietary CP by 2% to 4% and supplementing the diet with the most limiting synthetic AA (Lys, Met, Thr, Trp) can not only reduce feed costs, but also increase nitrogen utilization, reduce nitrogen excretion, and promote gut health thereby improving animal performance (Kerr et al., 2003; Houmard et al., 2007; Gloaguen et al., 2014). However, reducing the protein content in the diet whilst maintaining optimal animal performance is possible only if the knowledge on the requirements for the optimal quantity of AA is known.

In recent years there is a paradigm shift in the understanding of protein nutrition in animal feed (Wu et al., 2014). It has been

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suggested that animals have dietary requirements not only for essential AA but also for all of the synthesizable AA to achieve maximum growth and production performance (Wu et al., 2013). Some non-essential amino acids (NEAA) have been classified as conditionally essential because their utilization rates are higher than synthesis rates under certain conditions (e.g., early weaning, lactation, pregnancy, burns, injury, infection, heat stress, and cold stress) (Wu, 2009). Some examples of conditionally essential AA for weanling piglets include glutamic acid (Glu), Gln, Arg, Pro, Gly, and taurine. It has been reported that depression in growth performance in pigs receiving reduced CP diets by 4 percentage units can be ameliorated by supplementation with crystalline essential AA, including Lys, Trp, and Thr, indicating a critical role of sufficient CP in the diet for optimal growth performance of animals (Kerr et al., 2003). In a study using weaned piglets, the animals were fed a diet containing 20.7% (normal CP level), or a diet having reduced CP (12.7%) and supplemented with essential AA to achieve similar levels as the standard CP diet. Unfortunately, they found that pigs fed the low CP diet with supplemental essential AA had reduced protein synthesis in multiple tissues, such as liver, pancreas, and longissimus muscle (Deng et al., 2009) indicating the need for the supplementation of conditionally essential AA. Previous studies suggest that NEAA (e.g., Gln and Glu) may be required for weanling pigs under certain conditions such as weaning transition as well as to maintain the activation of translation initiation factors and optimal protein synthesis in neonates (Wu et al., 2014; Brasse-Lagnel et al., 2009). The dietary supply of NEAA is reduced in low CP diets resulting in the increased need for N for endogenous synthesis of NEAA to support protein synthesis (Gloaguen et al., 2014). In another study, Mansilla et al. (2017) demonstrated that the inclusion of non-protein nitrogen in diets improved the performance of pigs by compensating the deficient supply of NEAA in a very low CP levels diet.

L-Glu is the most abundant free amino acid and a major energy substrate for the small intestine and major excitatory neurotransmitter of the vertebrate central nervous system (Wu, 1998). Gln via Glu is converted to α-ketoglutarate, an integral component of the citric acid cycle. In addition to serving as substrate for protein synthesis and adenosine tri-phosphate (ATP) produced from the Krebs cycle (Berres et al., 2010), it also participates in the biosynthesis of other AA and nitrogen-containing molecules, such as Gln, glutathione, and polyamines in porcine and human enterocytes, which are important compounds that modulate immune response and eliminates oxidants (Li et al., 2007; Vermeulen et al., 2011; Jiao et al., 2015). A study by Yin et al. (2015) demonstrated that Glu exhibited beneficial effects on diquat-induced oxidative stress in weanling pigs. Studies on supplementing reduced protein diets with Glu in weanling pig performance is limited and more studies are needed to confirm the effects of inclusion of Glu in weanling pig diets.

It was hypothesized that the supplementation of synthetic Glu to a 2% CP reduced diet for weanling pigs may enhance the growth performance of piglets by compensating for the reduction of CP in their basal diet as well as help meet the need for N for endogenous synthesis of NEAA. Therefore, the objective of the current study was to evaluate the graded levels of supplemental Glu to reduced protein diets in 3-phase feeding on the performance, digestibility, and fecal gases emission in weanling pigs.

2. Materials and methods

2.1. Animal ethics

The experimental protocol (DK-2-2006) for conducting this research study was reviewed and approved by the Animal Care and Use Committee of Dankook University, South Korea.

2.2. Glutamic acid

L-Glu used in the present study was purchased from NL FARM, 318, RIT center, Gyeonggi Technopark, Ansan-si, Gyeonggi-do, Republic of Korea.

2.3. Animals and diets

One hundred and eighty crossbred weanling pigs ([Yorkshire × Landrace] × Duroc, 21 d old) with an initial body weight (BW) of 6.45 kg were randomly allotted to 1 of 6 dietary treatments (5 pigs per pen [2 barrows and 3 gilts]; 6 pens per treatment) based on BW and sex. The experiment was divided into 3 phases: d 1 to 7 (phase 1), d 8 to 21 (phase 2), and d 22 to 42 (phase 3) in a 6-week trial. Dietary treatments consisted of positive control diets (PC) formulated to have 226.9, 205.6 and 188.8 g CP during phases 1, 2, and 3 respectively; and negative control diets (NC, NC1, NC2, NC3, and NC4) with 20 g CP reduction from PC diets and addition of Glu at increasing levels in the feed during each phase. Diets for phases 1, 2, and 3 were formulated to meet or exceed NRC (2012) recommendations for all nutrients except protein which was reduced by 2% for NC, NC1, NC2, NC3, and NC4 diets (Tables 1–3, respectively) and fed in mash form. The calculated Lys-to-Glu ratio for PC diet was 1:2.35, and those for NC, NC1, NC2, NC3, and NC4 diets were 1:2.25, 1:2.30.1:2.35, 1:2.40, and 1:2.45, respectively. All pigs were housed in an environmentally controlled room having a mechanical ventilation system. Each pen was equipped with stainless steel feeder and a nipple drinker and pigs were offered ad libitum feed and water throughout the experiment. Automatic regulation of lighting was done to provide 12 h of artificial light per day. The initial ambient temperature of the room was approximately 30 °C and was gradually reduced by 1 °C each week during the experiment.

2.4. Sampling and measurement

The individual BW and feed consumption on a pen basis were determined at the start of the experiment and at the end of each phase to calculate the average daily gain (ADG), average daily feed intake (ADFI), and gain-to-feed ratio (G:F). To determine dry matter (DM), nitrogen (N), and energy digestibility, chromium oxide was added to the diet as an indigestible marker at 2 g/kg of the diet for 7 d prior to fecal collection. Fecal samples were collected from 8 pigs randomly selected per treatment via rectal massage, and the sample was stored in a freezer at −20 °C until analyzed. All feed and fecal samples were freeze-dried and finely ground to pass through a 1-mm screen. Fecal samples were analyzed for DM (method 930.15), N (method 988.05) following the procedures established by AOAC International (2000). Chromium levels were determined via UV absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan). Gross energy was determined by measuring the heat of combustion in the samples using a Parr 6100 oxygen bomb calorimeter (Parr Instrument Co., Moline, IL, USA). Apparent total tract digestibility of DM, N, fat, and energy were calculated using indirect methods described by Williams et al. (1962). Feed samples were analyzed for CP (N × 6.25; method 988.05), crude fat (method 954.02, AOAC, 2000), crude fiber (method 962.09, AOAC, 2000), crude ash (method 942.05, AOAC, 2000) calcium (method 984.01, AOAC, 2000), phosphorous (method 965.17, AOAC, 2000), and AA (method 982.30E, AOAC, 2000).

Two pigs (1 barrow and 1 gilt) each pen from randomly selected 4 pens ($n = 8$) per treatment were bled via jugular venipuncture using a sterile needle. Blood samples from the same pigs per treatment on d 7, 21, and 42 were collected into 5-mL vacuum tubes containing no additive (Becton Dickinson Vacutainer Systems,

Table 1
Ingredients and chemical composition of experimental diets of phase 1 (%, as-fed basis).[1]

Item	PC	NC	NC1	NC2	NC3	NC4
Ingredients						
Corn	57.08	61.44	61.28	61.08	60.92	60.72
Soybean meal	36.66	31.34	31.36	31.4	31.42	31.46
Animal fat	2.31	2.6	2.66	2.73	2.79	2.86
Di-calcium phosphate	1.65	1.77	1.77	1.77	1.77	1.77
Limestone	0.84	0.81	0.81	0.81	0.81	0.81
Salt	0.2	0.2	0.2	0.2	0.2	0.2
Met (99%)	0.18	0.21	0.21	0.21	0.21	0.21
Lys (78%)	0.62	0.81	0.81	0.81	0.81	0.81
Thr (98%)	0.21	0.29	0.29	0.29	0.29	0.29
Trp (98%)	0.02	0.05	0.05	0.05	0.05	0.05
L-Glu (98%)	–	0.25	0.33	0.42	0.50	0.59
Mineral mix[2]	0.1	0.1	0.1	0.1	0.1	0.1
Vitamin mix[3]	0.1	0.1	0.1	0.1	0.1	0.1
Choline (25%)	0.03	0.03	0.03	0.03	0.03	0.03
Total	100	100	100	100	100	100
Calculated value						
ME, kcal/kg	3,300	3,300	3,300	3,300	3,300	3,300
CP	22.69	20.69	20.69	20.69	20.69	20.69
Ca	0.85	0.85	0.85	0.85	0.85	0.85
P	0.7	0.7	0.7	0.7	0.7	0.7
Lys	1.7	1.7	1.7	1.7	1.7	1.7
Met	0.51	0.51	0.51	0.51	0.51	0.51
Thr	1.06	1.05	1.05	1.05	1.05	1.05
Trp	0.3	0.3	0.3	0.3	0.3	0.3
Glu	4	3.83	3.91	4	4.08	4.17
Crude fat	4.81	5.16	5.22	5.28	5.33	5.4
Crude fiber	2.72	2.61	2.61	2.61	2.61	2.6
Ash	4.57	4.57	4.57	4.57	4.57	4.57
Analyzed value						
CP	22.7	20.67	20.72	20.7	20.65	20.73
Ca	0.83	0.84	0.84	0.85	0.85	0.85
P	0.69	0.69	0.7	0.68	0.71	0.71
Lys	1.68	1.68	1.68	1.69	1.71	1.71
Met	0.5	0.5	0.49	0.5	0.49	0.51
Thr	1.07	1.05	1.05	1.03	1.03	1.04
Trp	0.32	0.31	0.28	0.28	0.28	0.29
Glu	4.00	3.85	3.90	3.99	4.10	4.17
Crude fat	4.8	5.15	5.22	5.27	5.32	5.4
Crude fiber	2.73	2.63	2.61	2.61	2.59	2.6
Ash	4.57	4.55	4.55	4.58	4.59	4.56

[1] PC (positive control), the basal standard protein diet with the Lys-to-Glu ratio at 1:2.35; NC (negative control), NC1, NC2, NC3, and NC4, the basal diet with 2% CP reduction and Lys-to-Glu ratios at 1:2.25, 1:2.30, 1:2.35, 1:2.40, and 1:2.45, respectively.
[2] Provided per kilogram diet: Fe, 180 mg as ferrous sulfate; Cu, 17 mg as copper sulfate; Mn, 54 mg as manganese oxide; Zn, 90 mg as zinc oxide; I, 0.78 mg as potassium iodide; and Se, 0.36 mg as sodium selenite.
[3] Provided per kilogram diet: vitamin A, 10,800 IU; vitamin D_3, 4,000 IU; vitamin E, 40 IU; vitamin K_3, 4 mg; vitamin B_1, 6 mg; vitamin B_2, 21.6 mg; vitamin B_6, 9.6 mg; vitamin B_{12}, 0.084 mg; biotin, 0.48 mg; folic acid, 3 mg; niacin, 84 mg; D-calcium pantothenate, 54 mg.

Table 2
Ingredients and chemical composition of experimental diets of phase 2 (%, as-fed basis).[1]

Item	PC	NC	NC1	NC2	NC3	NC4
Ingredients						
Corn	62.77	67.12	66.92	66.77	66.62	66.45
Soybean meal	31.32	26	26.04	26.06	26.08	26.11
Animal fat	2.18	2.48	2.55	2.6	2.66	2.72
Di-calcium phosphate	1.5	1.65	1.66	1.66	1.66	1.66
Limestone	0.85	0.8	0.8	0.8	0.8	0.8
Salt	0.2	0.2	0.2	0.2	0.2	0.2
Met (99%)	0.15	0.18	0.18	0.18	0.18	0.18
Lys (78%)	0.59	0.78	0.78	0.78	0.78	0.78
Thr (98%)	0.19	0.28	0.28	0.28	0.28	0.28
Trp (98%)	0.02	0.05	0.05	0.05	0.05	0.05
L-Glu (98%)	–	0.23	0.31	0.39	0.46	0.54
Mineral mix[2]	0.1	0.1	0.1	0.1	0.1	0.1
Vitamin mix[3]	0.1	0.1	0.1	0.1	0.1	0.1
Choline (25%)	0.03	0.03	0.03	0.03	0.03	0.03
Total	100	100	100	100	100	100
Calculated value						
ME, kcal/kg	3,300	3,300	3,300	3,300	3,300	3,300
CP	20.56	18.56	18.56	18.56	18.56	18.56
Ca	0.8	0.8	0.8	0.8	0.8	0.8
P	0.65	0.65	0.65	0.65	0.65	0.65
Lys	1.53	1.53	1.53	1.53	1.53	1.53
Met	0.44	0.44	0.44	0.44	0.44	0.44
Thr	0.95	0.95	0.95	0.95	0.95	0.95
Trp	0.25	0.25	0.25	0.25	0.25	0.25
Glu	3.6	3.44	3.52	3.6	3.67	3.75
Crude fat	4.8	5.16	5.22	5.27	5.32	5.38
Crude fiber	2.64	2.54	2.53	2.53	2.53	2.52
Ash	4.57	4.57	4.57	4.57	4.57	4.57
Analyzed value						
CP	20.59	18.6	18.55	18.54	18.52	18.58
Ca	0.78	0.82	0.82	0.82	0.8	0.82
P	0.64	0.66	0.63	0.63	0.63	0.66
Lys	1.55	1.53	1.53	1.51	1.51	1.52
Met	0.42	0.44	0.42	0.43	0.43	0.44
Thr	0.94	0.95	0.97	0.97	0.96	0.96
Trp	0.23	0.27	0.24	0.24	0.27	0.25
Glu	3.62	3.42	3.54	3.61	3.69	3.73
Crude fat	4.79	5.16	5.23	5.27	5.33	5.39
Crude fiber	2.65	2.53	2.55	2.51	2.53	2.54
Ash	4.56	4.59	4.59	4.56	4.56	4.55

[1] PC (positive control), the basal standard protein diet with the Lys-to-Glu ratio at 1:2.35; NC (negative control), NC1, NC2, NC3, and NC4, the basal diet with 2% CP reduction and Lys-to-Glu ratios at 1:2.25, 1:2.30, 1:2.35, 1:2.40, and 1:2.45, respectively.
[2] Provided per kilogram diet: Fe, 180 mg as ferrous sulfate; Cu, 17 mg as copper sulfate; Mn, 54 mg as manganese oxide; Zn, 90 mg as zinc oxide; I, 0.78 mg as potassium iodide; and Se, 0.36 mg as sodium selenite.
[3] Provided per kilogram diet: vitamin A, 10,800 IU; vitamin D_3, 4,000 IU; vitamin E, 40 IU; vitamin K_3, 4 mg; vitamin B_1, 6 mg; vitamin B_2, 21.6 mg; vitamin B_6, 9.6 mg; vitamin B_{12}, 0.084 mg; biotin, 0.48 mg; folic acid, 3 mg; niacin, 84 mg; D-calcium pantothenate, 54 mg.

Franklin Lakes, NJ, USA) to obtain serum. Serum was separated by centrifugation at 3,000 × *g* for 15 min at 4 °C. The concentration of blood urea nitrogen (BUN) in the serum was measured with an automatic biochemical analyzer (Model 7,020, Hitachi, Tokyo, Japan). The serum creatinine concentration was determined using an Astra-8 analyzer (Beckman Instruments, Inc., Brea, CA, US).

For the evaluation of noxious gases in pig feces, fresh fecal samples were collected at d 21 and 42 of the experiment from 2 pigs per pen from 4 pens randomly selected per treatment ($n = 8$). The feces sample was collected via massaging the rectum and pooled on a pen basis. Thereafter, 300 g of fecal sample were mixed well and stored in 2.6-L plastic boxes having a small hole in the middle of one side wall and sealed with adhesive plaster. The samples were then allowed to ferment for 24 h at room temperature and the concentration of gases was determined after 24 h of fermentation. For the detection of gases, a gas sampling pump (Model GV-100; Gastec Corp., Ayase, Japan) and Gastec detector tubes (No. 3La for NH_3, No. 4LK for H_2S, and No. 70 for mercaptans; Gastec Corp.) were used. The samples were shaken manually for approximately 30 s to disrupt any crust formation on the surface and to homogenize them before the measurement. The adhesive plasters were punctured, and 100 mL of headspace air was sampled in duplicate approximately 2.0 cm above the slurry surface and then the average was calculated.

2.5. Statistical analysis

All data were subjected to the GLM procedures of SAS 9.0 (SAS Inst. Inc.; Cary, NC, USA) as a randomized complete block design with the pen being considered as the experimental unit for growth

Table 3
Ingredients and chemical composition of experimental diets of phase 3 (%, as-fed basis).[1]

Item	PC	NC	NC1	NC2	NC3	NC4
Ingredients						
Corn	67.68	72.03	71.89	71.73	71.58	71.45
Soybean meal	26.98	21.66	21.68	21.72	21.74	21.76
Animal fat	1.94	2.24	2.29	2.34	2.4	2.44
Di-calcium phosphate	1.33	1.45	1.45	1.45	1.45	1.45
Limestone	0.73	0.68	0.68	0.68	0.68	0.68
Salt	0.2	0.2	0.2	0.2	0.2	0.2
Met (99%)	0.13	0.16	0.16	0.16	0.16	0.16
Lys (78%)	0.59	0.78	0.78	0.78	0.78	0.78
Thr (98%)	0.17	0.26	0.26	0.26	0.26	0.26
Trp (98%)	0.02	0.05	0.05	0.05	0.05	0.05
L-Glu (98%)	–	0.26	0.33	0.40	0.47	0.54
Mineral mix[2]	0.1	0.1	0.1	0.1	0.1	0.1
Vitamin mix[3]	0.1	0.1	0.1	0.1	0.1	0.1
Choline (25%)	0.03	0.03	0.03	0.03	0.03	0.03
Total	100	100	100	100	100	100
Calculated value						
ME, kcal/kg	3,300	3,300	3,300	3,300	3,300	3,300
CP	18.88	16.88	16.88	16.88	16.88	16.88
Ca	0.7	0.7	0.7	0.7	0.7	0.7
P	0.6	0.6	0.6	0.6	0.6	0.6
Lys	1.41	1.41	1.41	1.41	1.41	1.41
Met	0.4	0.4	0.4	0.4	0.4	0.4
Thr	0.87	0.87	0.87	0.87	0.87	0.87
Trp	0.23	0.23	0.23	0.23	0.23	0.23
Glu	3.31	3.17	3.24	3.31	3.38	3.45
Crude fat	4.67	5.03	5.08	5.12	5.17	5.21
Crude fiber	2.59	2.48	2.48	2.47	2.47	2.47
Ash	4.57	4.57	4.57	4.57	4.57	4.57
Analyzed value						
CP	18.91	16.85	16.86	16.84	16.86	16.84
Ca	0.71	0.69	0.69	0.71	0.71	0.7
P	0.61	0.59	0.6	0.6	0.58	0.62
Lys	1.4	1.42	1.42	1.41	1.39	1.42
Met	0.38	0.39	0.38	0.38	0.39	0.4
Thr	0.86	0.86	0.89	0.87	0.87	0.85
Trp	0.22	0.21	0.21	0.25	0.24	0.22
Glu	3.3	3.19	3.23	3.29	3.36	3.45
Crude fat	4.66	5.01	5.08	5.11	5.18	5.2
Crude fiber	2.59	2.46	2.5	2.49	2.46	2.49
Ash	4.57	4.58	4.55	4.56	4.59	4.56

[1] PC (positive control), the basal standard protein diet with the Lys-to-Glu ratio at 1:2.35; NC (negative control), NC1, NC2, NC3, and NC4, the basal diet with 2% CP reduction and Lys-to-Glu ratios at 1:2.25, 1:2.30, 1:2.35, 1:2.40, and 1:2.45, respectively.
[2] Provided per kilogram diet: Fe, 180 mg as ferrous sulfate; Cu, 17 mg as copper sulfate; Mn, 54 mg as manganese oxide; Zn, 90 mg as zinc oxide; I, 0.78 mg as potassium iodide; and Se, 0.36 mg as sodium selenite.
[3] Provided per kilogram diet: vitamin A, 10,800 IU; vitamin D_3, 4,000 IU; vitamin E, 40 IU; vitamin K_3, 4 mg; vitamin B_1, 6 mg; vitamin B_2, 21.6 mg; vitamin B_6, 9.6 mg; vitamin B_{12}, 0.084 mg; biotin, 0.48 mg; folic acid, 3 mg; niacin, 84 mg; D-calcium pantothenate, 54 mg.

performance, blood profile and noxious gases indices, and individual animals were used as the experimental unit for digestibility indices. Pre-planned contrasts were used to compare PC (positive control) and NC (negative control). Means were also separated using orthogonal polynomial contrasts to examine linear/quadratic effects of increasing level of Glu supplementation in reduced protein diets. Variability in the data was expressed as the standard error of means (SEM) and a probability level of $P < 0.05$ were considered to be statistically significant and $P < 0.10$ as trends.

3. Results

The effect of supplementation of increasing level of Glu to reduced protein diets is shown in Table 4. The pigs receiving PC diet had a higher BW ($P < 0.05$) than those receiving NC diet at d 7, 21 and 42. A higher ADG during d 1–7, 8–21, 22–42 as well as during overall experimental period was observed in pigs fed PC than NC diet ($P < 0.05$). An increase (linear effect, $P < 0.05$) in BW at d 42, ADG, and G:F during the overall experimental period was observed in pigs fed reduced protein diets supplemented with increasing levels of Glu. In addition, trends in linear increment in BW ($P = 0.056$) at d 7, ADG during d 1–7 ($P = 0.081$), and d 22–42 ($P = 0.058$) were observed in pigs fed reduced protein diets supplemented with increasing levels of Glu.

As shown in Tables 5 and 6, the nutrient digestibility as well as serum BUN and creatinine concentrations remained unaffected in PC compared with the NC group. The inclusion of increasing levels of Glu to reduced protein diets had no effects ($P > 0.05$) on DM, N, and energy digestibility as well as serum BUN and creatinine concentrations.

There were no differences between pigs fed PC and NC diets on fecal noxious gases (NH_3, H_2S, methyl mercaptans, and acetic acid) emissions (Table 7). The increase of the inclusion level of Glu to reduced protein diets showed a tendency in the linear increment of NH_3 ($P = 0.082$) at d 21 and a linear reduction in methyl mercaptans ($P = 0.054$) at d 42.

4. Discussion

In weanling pigs, a large proportion of undigested dietary nutrient is subjected to bacterial fermentation in the large intestine due to incomplete digestibility in the small intestine. To minimize the amount of fermentable protein that enters the lower gut and the associated enteric problems, the concept of reducing CP and addition of AA has been suggested in nursery pigs (Nyachoti et al., 2006; Heo et al., 2013). Thus, optimizing the recommendations of dietary AA requirements for animals is important for maximizing their growth and production performance while improving their health and reducing dietary protein levels. The requirements for AA are more often expressed relative to that of Lys on a standardized ileal digestible (SID) basis. Emerging evidence shows that non-ruminants are incapable of synthesizing NEAA or conditionally essential AA in adequate amounts to realize their full growth potential (Wu et al., 2014). In the present study, increasing level of Glu, the non-essential AA which is reported to be conditionally essential was supplemented into the protein-reduced corn-soybean meal-based diet with an objective to estimate the optimal Lys-to-Glu ratio for weanling pig growth enhancement.

Results from the present study showed that piglets fed standard diet formulated to meet NRC (2012) recommendation for weanling pigs had higher ADG and BW than those fed lower CP diets (reduced by 2%) during all 3 phases of the experiment. Although lower dietary protein levels have been shown to be a nutritional strategy to improve the intestinal structure and function of weaned piglets, it may result in impaired piglet growth performance (Williams et al., 2015). Thus, the adverse effect of reduced CP diets on the growth performance of weanling pigs may be due to an inadequate supply of AA in the diet. However, supplementing Glu with a increasing level showed a significant difference or trends in linearly increasing BW, ADG, and G:F but no effects on ADFI. Glu has received increasing attention from nutritionists owing to its role in metabolism and physiology. In agreement with the present findings, the supplementation of Glu in low protein diets has been reported to increase the daily gain of weaned piglets without affecting feed conversion ratio (Duan et al., 2016) suggesting that low protein diets do not provide enough Glu to weaned piglets. In particular, Glu has been recognized as a nutritionally essential amino acid for the intestinal and systemic homeostasis of piglets (Li et al., 2016). Moreover, the feed intake in weaned piglets during the first week after weaning usually decreases rapidly. Therefore, the weanling piglet diet can only provide half the amount of AA

Table 4
Effects of dietary supplementation of glutamic acid to reduced protein diets on growth performance in weanling pigs. [1, 2]

Item	PC	NC	NC1	NC2	NC3	NC4	SEM	*P*-value		
								PC vs. NC	Linear	Quadratic
Body weight, kg										
Initial	6.45	6.45	6.45	6.45	6.46	6.46	0.01	0.902	0.350	0.633
d 7	8.44	8.31	8.37	8.33	8.43	8.39	0.04	0.020	0.056	0.704
d 21	14.23	13.78	13.88	13.97	14.12	13.98	0.11	0.010	0.101	0.362
d 42	25.36	24.20	24.33	24.60	25.10	24.90	0.29	0.008	0.024	0.694
d 1 to 7										
ADG, g	284	265	274	269	282	276	5.19	0.014	0.081	0.599
ADFI, g	315	300	306	300	312	307	5.94	0.084	0.272	0.813
G:F	0.905	0.887	0.896	0.895	0.903	0.897	0.009	0.175	0.160	0.482
d 8 to 21										
ADG, g	414	392	394	403	406	399	7.10	0.034	0.210	0.362
ADFI, g	515	499	500	504	507	503	8.34	0.197	0.518	0.712
G:F	0.803	0.781	0.787	0.800	0.803	0.795	0.01	0.148	0.331	0.357
d 22 to 42										
ADG, g	530	496	497	506	523	520	11.21	0.041	0.058	0.997
ADFI, g	813	795	797	802	808	806	12.61	0.339	0.408	0.891
G:F	0.650	0.622	0.623	0.628	0.647	0.643	0.01	0.093	0.10	0.921
Overall (d 1 to 42)										
ADG, g	450	423	426	432	444	439	6.78	0.008	0.027	0.682
ADFI, g	630	614	616	619	625	622	7.63	0.140	0.266	0.789
G:F	0.714	0.688	0.692	0.697	0.710	0.705	0.008	0.020	0.045	0.773

[1] Values represent the means of 6 pens with 5 pigs per pen.
[2] PC (positive control), the basal standard protein diet with the Lys-to-Glu ratio at 1:2.35; NC (negative control), NC1, NC2, NC3, and NC4, the basal diet with 2% CP reduction and Lys-to-Glu ratios at 1:2.25, 1:2.30, 1:2.35, 1:2.40, and 1:2.45, respectively.

Table 5
Effects of dietary supplementation of glutamic acid to reduced protein diets on apparent total tract nutrient digestibility in weanling pigs at d 42 (%). [1, 2]

Item	PC	NC	NC1	NC2	NC3	NC4	SEM	*P*-value		
								PC vs. NC	Linear	Quadratic
Dry matter	81.40	79.29	79.97	80.02	80.92	80.83	1.55	0.345	0.464	0.917
Nitrogen	79.89	77.89	78.96	79.32	79.70	79.61	1.23	0.260	0.323	0.646
Energy	80.16	78.27	78.47	78.82	79.87	79.49	1.28	0.305	0.373	0.925

[1] Values represent the means of randomly selected 8 pigs per treatment.
[2] PC (positive control), the basal standard protein diet with the Lys-to-Glu ratio at 1:2.35; NC (negative control), NC1, NC2, NC3, and NC4, the basal diet with 2% CP reduction and Lys-to-Glu ratios at 1:2.25, 1:2.30, 1:2.35, 1:2.40, and 1:2.45, respectively.

Table 6
Effects of dietary supplementation of glutamic acid to reduced protein diets on serum BUN and creatinine in weanling pigs at d 7, 21 and 42 (mg/dL). [1,2]

Item	PC	NC	NC1	NC2	NC3	NC4	SEM	*P*-value		
								PC vs. NC	Linear	Quadratic
d 7										
BUN	6.25	6.00	6.00	6.25	6.50	6.50	0.50	0.728	0.341	1.000
Creatinine	1.19	1.18	1.18	1.20	1.22	1.23	0.03	0.714	0.162	0.917
d 21										
BUN	6.50	6.00	6.25	6.75	6.75	7.00	0.54	0.526	0.117	0.780
Creatinine	1.18	1.16	1.17	1.19	1.23	1.24	0.04	0.702	0.100	0.958
d 42										
BUN	6.75	6.25	6.50	6.75	7.00	7.25	0.51	0.502	0.119	1.000
Creatinine	1.19	1.18	1.16	1.19	1.21	1.23	0.04	0.928	0.166	0.496

BUN = blood urea nitrogen.
[1] Values represent the means of 4 pens with 2 pigs per pen.
[2] PC (positive control), the basal standard protein diet with the Lys-to-Glu ratio at 1:2.35; NC (negative control), NC1, NC2, NC3, and NC4, the basal diet with 2% CP reduction and Lys-to-Glu ratios at 1:2.25, 1:2.30, 1:2.35, 1:2.40, and 1:2.45, respectively.

required for piglet intestinal development. Obviously, dietary supplementation with Glu is essential for the performance and health of weanling piglets. Among the different NC diets tested in the present study, feeding NC3 diets with calculated Lys-to-Glu ratio of 1:2.40 to weanling pigs had the closest mean values to that of PC diets.

Unlike growth performance, a moderate reduction in CP of weanling pigs' diet did not have any significant effects on total tract DM, N and energy digestibility compared with pigs fed PC diet. Tuitoek et al. (1997) stated that low protein diets improved the utilization efficiency of nitrogen without affecting the digestibility and retention of nitrogen in growing to finishing pigs. In addition, Gloaguen et al. (2014) noted that reduction of CP by 3% had no impact on N retention in weanling pigs. Moreover, the inclusion of Glu at an increasing level to CP reduced diet had comparable effects as that of PC diets.

Table 7
Effects of dietary supplementation of glutamic acid to reduced protein diets on fecal gases emission in weanling pigs at d 21 and 42 (μg/mL).[1, 2]

Item	PC	NC	NC1	NC2	NC3	NC4	SEM	*P*-value		
								PC vs. NC	Linear	Quadratic
d 21										
NH_3	1.25	0.88	1.00	1.13	1.38	1.50	0.29	0.370	0.082	0.904
H_2S	1.00	0.85	0.90	1.03	0.90	0.95	0.14	0.451	0.609	0.589
Methyl mercaptans	2.00	1.50	1.50	2.00	1.75	1.88	0.38	0.364	0.396	0.716
Acetic acid	6.88	6.13	6.25	7.13	6.38	6.75	0.51	0.311	0.438	0.589
d 42										
NH_3	1.50	1.38	1.38	1.50	1.63	1.75	0.20	0.657	0.154	0.754
H_2S	1.13	1.05	1.03	1.08	1.18	1.13	0.19	0.780	0.632	1.000
Methyl mercaptans	2.25	1.88	1.63	1.88	2.50	2.38	0.29	0.369	0.054	0.559
Acetic acid	6.88	6.75	6.63	6.75	7.38	7.00	0.45	0.848	0.381	1.000

[1] Values represent the means of 4 pens with 2 pigs per pen.
[2] PC (positive control), the basal standard protein diet with the Lys-to-Glu ratio at 1:2.35; NC (negative control), NC1, NC2, NC3, and NC4, the basal diet with 2% CP reduction and Lys-to-Glu ratios at 1:2.25, 1:2.30, 1:2.35, 1:2.40, and 1:2.45, respectively.

The BUN concentration and production are influenced by protein catabolism, and its concentration is negatively correlated with the utilization of proteins and AA (Coma et al., 1995). Creatinine is a natural waste product created by the muscles and is removed from the body by the kidney. In the present study, BUN and creatinine levels were unaffected in the low CP diet compared with normal protein diets. Consistently, Yue and Qiao (2008) also observed that serum urea nitrogen levels in piglets fed the 18.9% and 17.2% CP diets were similar throughout the study. In addition, supplementing the low CP diets in the present study with graded levels of Glu in the different phases in weanling pigs' diets did not have significant linear or quadratic responses.

The lack of significant effects on the digestibility of DM, N, and energy, BUN and creatinine levels in pigs fed NC diets compared with PC diet may be due to a slight reduction in CP in the NC diet. However, the closest mean values for digestibility and blood indices were observed by feeding NC3 diets compared with other NC diets, suggesting that the Lys-to-Glu ratio at 1:2.40 has comparable values as that of standard CP diet.

In a review, Wang et al. (2018) noted that Lys being the first limiting amino acid in the swine diet, the concentration of CP in the conventional corn-soybean meal-based diet is higher so as to satisfy Lys requirement. Therefore, excess nitrogen is excreted in the urine as urea. Because of the negative environmental impact due to ammonia emission from manure, it is necessary to reduce nitrogen as well as other noxious gases excretion in feces (Webb et al., 2014). One effective approach to reducing nitrogen emission is to reduce the CP content in the diet and supplementing with AA, so as to closely match the pig's ideal protein pattern. Most studies indicated that a reduction of dietary CP by more than 2% could effectively reduce ammonia and odor emissions (Hayes et al., 2004; Leek et al., 2007). In the present study, CP content reduced by 2% in the diet (NC) did not have a significant effect on the noxious gases (NH_3, H_2S, acetic acid, and mercaptan) emissions as compared with normal diet (PC). Supplementing graded levels of Glu to NC diets showed a slight increment in NH_3 and trends in a linear reduction in mercaptan at d 21 and 42, respectively. Among the treatment diets, the lowest level mean values of NH_3 was seen in NC diet and that of mercaptan was seen in NC1 diet. The reason for a slight increment in NH_3 emission in Glu supplemented diet is not clear. More studies are needed to confirm these findings.

5. Conclusions

Taken together, the reduction in dietary CP contents by 2% reduced the performance of weanling pigs but had no effects in the digestibility of DM, N, and energy, as well as on the BUN and creatinine levels, and odorous gases emission from feces. The effect of reduction of CP in growth performance was reversed by adding Glu in the low CP diet suggesting the role of Glu in promoting growth performance in weanling pigs. Thus, from the finding of this study, the inclusion of Lys-to-Glu ratio at 1:2.40 is suggested to be beneficial in reduced CP diets for piglets in achieving similar levels of performance as that of standard diets.

Author contributions

In Ho Kim, **Young Hwa Kim, Santi D. Upadhaya,** and **Zhenlong Wu** designed the study; **Sang Seon Lee** and other lab students contributed to the animal experiment; **In Ho Kim** provided supervision to the experiment; **Santi D. Upadhaya** and **Sang Seon Lee** performed data analysis; **Santi D. Upadhaya** wrote the original draft of manuscript. Manuscript review and editing was done by **Zhenlong Wu**, **Young Hwa Kim** and **In Ho Kim**. **In Ho Kim** had the primary responsibility for the final content. All authors have read and approved the final manuscript.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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