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Effects of varying levels of dietary protein and net energy on growth performance, nitrogen balance and faecal characteristics of growing-finishing pigs

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ABSTRACT - This experiment was conducted to evaluate the effects of dietary protein and net energy (NE) levels on growth performance, nutrient digestibility, nitrogen metabolism, and faecal microbiota of growing-finishing pigs. Eighteen crossed barrows were randomly allocated into one of three dietary treatments: high protein + high NE diet, low protein + high NE diet, and low protein + low NE diet. The whole experiment lasted 90 days and was divided into three phases (phase I: 25-50 kg; phase II: 50-75 kg; phase III: 75-105 kg). All pigs were individually housed in a metabolism cage and subjected to four-day total faeces and urine collection period at the end of each phase. There was no significant difference in growth performance, nutrient digestibility, serum total protein, and albumin concentrations of pigs among the dietary treatments. Compared with the high protein + high NE diet, pigs fed low protein + high NE and low protein + low NE diets had lower N intake, urine N, and total N excretion in each phase. At the end of the experiment, pigs fed the low protein + high NE and low protein + low NE diets had lower blood urea nitrogen, serum NH_3-N concentrations, faecal pH value, faecal NH_3-N concentration, and faecal *Escherichia coli* count than those fed the high protein + high NE diet. However, there was no significant difference in all of the above indexes between low protein + high NE and low protein + low NE diets. Decreasing the dietary protein content by 3.5 percentage units has no adverse effects on growth performance and nutrient digestibility of pigs while significantly reduces N excretion and faecal *Escherichia coli* count. Moreover, further decreasing dietary NE level in the low-protein diet by 0.35-0.5 MJ/kg does not affect growth performance, nutrient digestibility, N excretion, blood profiles, and faecal *Escherichia coli* count of pigs.

Keywords: faecal microbiota, nitrogen metabolism, nutrient digestibility, pigs

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Introduction

Maximization of pig performance has traditionally been the goal of swine producers and nutritionists. However, over-supplementation of diets with nutrients to ensure maximum pig performance can result in an excessive amount of nutrients being excreted in the faeces and urine, ultimately into the environment, especially nitrogen (NRC, 2012). It was reported that retention of dietary N was only ranging from 30 to 60% of the intake of pigs, the rest were excreted in faeces and urine (Jongbloed and Lenis, 1992; Otto et al., 2003). Some previous studies confirmed that properly reducing dietary crude protein (CP) content, while maintaining adequate supplies of essential amino acids (EAA), allowed a significant reduction of total N excretion without any adverse effect on feed intake, growth rate, or feed

efficiency (Dourmad et al., 1993; Kerr et al., 2003; Carpenter et al., 2004). Shriver et al. (2003) reported that decreased CP of growing pig diet from 18 to 14% supplemented with synthetic lysine, methionine, threonine, tryptophan, isoleucine, and valine had no significant effect on growth performance, while notably decreased urine N and total N emission by 40 and 50%, respectively, and also markedly reduced the content of ammonia nitrogen and total volatility fatty acids of excreta. A summary of 33 swine metabolism data indicated that the total N excretion could be reduced by approximately 8% for each percentage unit reduction in dietary CP (but balanced for amino acid limitations) (Kerr et al., 2003).

Previous studies have shown that intake of low-protein diets increase the deposition of carcass fat in pigs; the main reason was that the amount of deamination and transamination of amino acids decreased in low protein diets fed pigs; hence, the dietary energy used for protein and AA metabolism was decreased, and more energy was deposited as fat. Besides, intake of low-protein diets decrease the weight of the visceral organs of pigs, which results in a reduction in energy requirement for maintenance and total heat production of pigs (Kerr et al., 2003); thus, more energy is used for fat deposition. Furthermore, in the case of corn-soybean meal low-protein diets, the content of CP is reduced by replacing part of the soybean meal with corn, which results in the increase of dietary NE level. Although there is similar metabolisable energy (ME) content between corn and soybean meal, net energy (NE) density of corn is higher than that of soybean meal. It has been suggested that the NE system appears to be superior to digestible energy (DE) and ME systems, which takes into account the heat consumption and energy loss via faeces, urine, and gases (Le Bellego et al., 2001; Le Bellego et al., 2002). Net energy reflects the energy requirement for animal and the dietary energy content on the same basis. Early research showed that formulation of low-protein diets using the NE system and appropriately decreased dietary NE density could improve pig carcass characteristics (Saraiva et al., 2014; Main et al., 2008; Chen et al., 2011). Protein degradation and synthesis are the processes of energy consumption. However, little information is available on the effects of reducing dietary NE density on the metabolism of protein in pigs.

Furthermore, research on low-protein diets mainly focuses on the influence on pigs themselves, but there are few studies on the effects of intestinal microbes. Diet is a key factor affecting the composition of the gut microbiota, which can serve as substrates for the fermentation of intestinal microorganisms, thereby regulating the proliferation and reproduction of microbes (Edwards, 1993).

Therefore, this study was designed to evaluate the effects of different dietary protein and NE levels on growth performance, nutrient digestibility, N metabolism, and faecal characteristics of growing-finishing pigs.

Material and Methods

The experimental protocols used in the current study were reviewed and approved by the Animal Care and Use Committee of Sichuan Province (case no. SYXK (Sichuan) 2014-187). The experiment was conducted in Yaan, Sichuan, China (29°40' N and 102°51' W).

Eighteen crossbred barrows, (Landrace × Yorkshire) × Duroc, with initial body weight of 24.17±0.50 kg, were blocked based on initial body weight and randomly allotted to one of three dietary treatments: high protein + high NE diet, low protein + high NE diet, and low protein + low NE diet. There were six replicates per treatment and one pig per replicate. Only barrows were selected to facilitate the collection of urine and faeces. The whole experiment lasted 90 days and was divided into three phases: phase I, 25-50 kg and lasted 35 days; phase II, 50-75 kg and lasted 28 days; and phase III, 75-105 kg and lasted 35 days). In the high protein + high NE treatment, the contents of CP and NE were 17.00-15.50-13.50% and 10.36-10.36-10.36 MJ/kg for each phase, respectively; in the low protein + high NE treatment, the contents of CP and NE were 13.50-12.00-10.00% and 10.36-10.36-10.36 MJ/kg for each phase, respectively; in the low protein + low NE treatment, the contents of CP and NE were 13.50-12.00-10.00%, and 9.86-10.01-10.01 MJ/kg for each phase, respectively. The high NE level was set according to NRC (2012), and the low NE level was adjusted according to the results of Yi et al. (2010). Experimental diets were formulated to meet or exceed the nutrient requirement recommended

by NRC (2012), except dietary CP and NE contents (Table 1). All pigs were individually hosted in a stainless-steel metabolism cage (1.7 × 0.6 × 0.7 m) and subjected to a four-day total faeces and urine collection period at the end of each phase. Pigs were fed at 8.00, 14.00, and 20.00 h and had free access to water.

Pigs were individually weighed on days 0, 34, 63, and 90 of the experiment, and feed intake was recorded per cage every day. Average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F:G ratio) were calculated.

During the last four days of each phase, pigs were subjected to a four-day total faeces and urine collection period. The total amount of feed intake and excreta were recorded daily. During the four-day collection period, all faeces were collected, sealed in plastic bags, weighted daily, then one-tenth of the total of faeces was sampled, followed by addition of sulfuric acid (10% H_2SO_4) and two drops

Table 1 - Ingredient and chemical composition of experimental diets (as-fed basis)

Item	25-50 kg			50-75 kg			75-105 kg		
	HCP+ HNE[5]	LCP+ HNE[6]	LCP+ LNE[7]	HCP+ HNE[5]	LCP+ HNE[6]	LCP+ LNE[7]	HCP+ HNE[5]	LCP+ HNE[6]	LCP+ LNE[7]
Ingredient (g kg^{-1} dry matter)									
Corn	662.5	781.1	739.2	740.0	849.0	792.0	790.7	877.9	811.4
Soybean meal	293.9	174.1	149.7	226.5	116.2	97.4	183.4	68.7	48.0
Wheat bran			75.5			77.5		22.3	109.4
Soybean oil	18.7	9.7	0.0	10.0	1.8		4.0		
Limestone	6.5	6.0	7.7	5.8	5.3	7.0	5.5	5.5	7.3
Monocalcium phosphate	8.9	10.8	8.8	8.1	9.8	7.9	6.4	7.6	5.4
NaCl	3.0	3.0	3.0	3.0	3.0	3.0	4.0	4.0	4.0
L-Lys-HCl (78%)	1.3	4.9	5.2	1.9	5.1	5.3	1.6	4.8	5.0
DL-Met (99%)	1.0	1.9	1.9	0.7	1.6	1.6	0.4	1.3	1.3
Thr (98.5%)	0.5	1.9	2.1	0.3	1.7	1.8	0.3	1.7	1.8
Trp (98%)		0.5	0.5		0.5	0.5		0.5	0.5
Ile (99%)		0.9	1.1		0.9	1.1		0.9	1.1
Val (99%)		1.3	1.4		1.1	1.1		1.0	1.0
His (99%)		0.2	0.2		0.1	0.1		0.1	0.2
Vitamin premix[1]	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Mineral premix[2]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Choline chloride	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Nutrient									
Crude protein[3] (g kg^{-1} dry matter)	170.0	135.0	135.0	155.0	120.0	120.0	135.0	100.0	100.0
Crude protein[4] (g kg^{-1} dry matter)	170.4	137.8	135.1	154.3	122.0	121.6	136.2	103.4	102.0
Net energy (MJ kg^{-1})	10.36	10.36	9.86	10.36	10.36	10.01	10.36	10.36	10.01
SID Lys (g kg^{-1} dry matter)	9.8	9.8	9.8	8.5	8.5	8.5	7.3	7.3	7.3
SID (Met+Cys) (g kg^{-1} dry matter)	5.5	5.5	5.5	4.8	4.8	4.8	4.2	4.2	4.2
SID Thr (g kg^{-1} dry matter)	5.9	5.9	5.9	5.2	5.2	5.2	4.6	4.6	4.6
SID Trp (g kg^{-1} dry matter)	1.8	1.7	1.7	1.5	1.5	1.5	1.3	1.3	1.3
Calcium (g kg^{-1} dry matter)	6.6	6.6	6.6	5.9	5.9	5.9	5.2	5.2	5.2
Available phosphorous (g kg^{-1} dry matter)	3.4	3.6	3.4	3.1	3.4	3.1	2.7	2.9	2.5

SID - standard ileal digestible; CP - crude protein; NE - net energy.
[1] Supplied the following per kg of diet: vitamin A, 5,512 IU; vitamin D3, 2,200 IU; vitamin E, 30 IU; vitamin K3, 2.2 mg; vitamin B12, 27.6 µg; riboflavin, 4 mg; pantothenic acid, 14 mg; niacin, 30 mg; choline chloride, 400 mg; folic acid, 0.7 mg; thiamin, 1.5 mg; pyridoxine, 3 mg; biotin, 44 µg.
[2] Supplied the following per kg of diet: 25-50 kg phase = Fe, 60 mg; Mn, 2 mg; Zn, 60 mg; Cu, 4 mg; I, 0.14 mg; Se, 0.2 mg; 50-75 kg phase = Fe, 50 mg; Mn, 2 mg; Zn, 50 mg; Cu, 3.5 mg; I, 0.14 mg ; Se, 0.15 mg; 50-75 kg phase = Fe, 40 mg; Mn, 2 mg; Zn, 40 mg; Cu, 3.5 mg; I, 0.14 mg; Se, 0.15 mg.
[3] Calculated values.
[4] Analysed values
[5] High protein + high NE diet = 17.0-15.5-13.5% CP and 10.36 MJ/kg NE diets for phases 25-50, 50-75, and 75-105 kg, respectively.
[6] Low protein + high NE diet = 13.5-12.0-10.0% CP and 10.36 MJ/kg NE diets for phases 25-50, 50-75, and 75-105 kg, respectively.
[7] Low protein + low NE diet = 13.5-12.0-10.0% CP and 9.86-10.01-10.01 MJ/kg NE for phases 25-50, 50-75, and 75-105 kg, respectively.

of toluene to the sample for each pig. On the 4th day of the collection period, a few fresh faeces were sampled into a cryopreservation tube and stored at –80 °C for further analysis. At the end of the collection period, faecal samples from each pig were pooled, and a 500-g subsample was taken, then dried in a forced-draft oven at 65 °C and subsequently grounded through a 0.45-mm screen. After drying and grinding, samples were kept at –20 °C for further analysis. Urine was collected daily at the same time as the faecal collection. At the end of the collection period, urine samples from each pig were pooled, and a 100-mL subsample was taken and stored at –20 °C for further analysis.

At the end of the trial, blood samples were collected via jugular vein puncture of each pig after 12 h of overnight fasting. Blood samples were centrifuged (3,000 × *g* for 15 min at 4 °C), and serum samples were stored at –20 °C for blood profile analysis.

For determination of nutrient digestibility and nitrogen balance, experimental diets and faecal and urine samples were analysed in triplicate for dry matter (DM; method 930.15), ash (method 942.05), ether extract (EE; method 920.39), and crude protein (CP; method 990.03) according to AOAC (2007).

Concentrations of serum urea nitrogen (SUN), total protein (TP), albumin (ALB) were analysed using assay kits according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, China). Ammonia nitrogen (NH_3-N) concentration in serum and faeces was determined spectrophotometrically according to Nessler reagent with yellow colouring and photometering at a wavelength of 420 nm.

The pH analysis of faeces was performed at room temperature (20 °C) with a glass electrode (model PHS-3C, INESA, Shanghai, China) directly submerged in the faecal diluent, which consisted of 1 g of faeces and 9 mL of ultrapure water.

The faecal *Escherichia coli*, *Lactobacillus spp*, and *Bifidobacterium spp* were determined by quantitative real-time polymerase chain reaction (PCR) as described by Diao et al. (2015). All primers and probes (Table 2) were referred by Chen et al. (2013), and commercially synthesised by Invitrogen Ltd (Shanghai, China). All measurements were determined in triplicate.

All data were compared among all three groups, being subjected to one-way ANOVA for a randomised complete block design using the SPSS statistical software package (SPSS 20.0). Statistical differences among treatments were separated by Duncan's multiple range tests. Probability values of $P \leq 0.05$ were considered significant, while values of $0.05 < P \leq 0.10$ were considered to constitute a tendency.

Table 2 - Primers, probes, and annealed temperature for real time polymerase-chain reaction

Item	Nucleotide sequence (5'–3')	Annealing temperature (°C)	Product size (bp)
Lactobacillus			
Forward	GAGGCAGCAGTAGGGAATCTTC	55	126
Reverse	CAACAGTTACTCTGACACCCGTTCTTC		
Probe	AAGAAGGGTTTCGGCTCGTAAAACTCTGTT		
Bifidobacterium			
Forward	CGCGTCCGGTGTGAAAG	53	121
Reverse	CTTCCCGATATCTACACATTCCA		
Probe	ATTCCACCGTTACACCGGGAA		
Escherichia coli			
Forward	CATGCCGCGTGTATGAAGAA	53	96
Reverse	CGGGTAACGTCAATGAGCAAA		
Probe	AGGTATTAACTTTACTCCCTTCCTC		

Results

Within each phase and the overall period, no significant difference was observed for ADG, ADFI, and F:G among the three dietary treatments (P>0.05) (Table 3). During each phase, there were no significant differences in ATTD of DM, OM, EE, and CP among the three treatments (P>0.05) (Table 4).

According to the results of N balance (Table 5), during the 25-50 kg phase, both low protein + high NE and low protein + low NE diet groups had a lower (P<0.05) N intake, urine N excretion, and total N excretion than that of the high protein + high NE diet group. Furthermore, pigs fed low protein + low NE diet had a lower (P<0.05) faecal N excretion than pigs fed the high protein + high NE diet. However, no difference was found in N intake, N excretion, and N retention between low protein + high NE and low protein + low NE diet treatments. During the 50-75 and 75-105 kg phases, N intake, urine N excretion, and total N excretion followed a similar pattern to that of the 25-50 kg phase, but no obvious difference was detected in faecal N excretion among the three treatments. No significant difference was observed in N retention rate among the three treatments during the 25-50, 50-75, and 75-105 kg phases.

Blood profiles of finishing pigs at the end of the experiment including BUN, NH_3-N, TP, and ALB were determined (Table 6). The concentrations of BUN and NH_3-N were lower in pigs fed low protein + high NE and low protein + low NE diets than pigs fed high protein + high NE diet, and no significant difference was found between low protein + high NE and low protein + low NE diet groups. Serum TP and ALB contents were not affected by dietary treatments.

Table 3 - Effects of dietary treatments on growth performance of growing-finishing pigs

Item	HCP+HNE[1]	LCP+HNE[2]	LCP+LNE[3]	SEM	P-value
Phase I (25-50 kg)					
Initial weight (kg)	24.17	24.18	24.18	0.50	0.77
Final weight (kg)	52.42	53.08	51.83	0.67	0.77
ADFI (g)	1659.53	1713.36	1665.74	32.45	0.78
ADG (g)	807.14	825.95	790.24	10.00	0.36
F:G	2.06	2.07	2.11	0.03	0.84
Phase II (50-75 kg)					
Initial weight (kg)	52.42	53.08	51.83	0.67	0.77
Final weight (kg)	80.08	79.75	77.75	1.09	0.67
ADFI (g)	2666.69	2704.10	2642.64	47.06	0.88
ADG (g)	988.09	952.38	925.60	20.17	0.47
F:G	2.71	2.85	2.88	0.06	0.49
Phase III (75-105 kg)					
Initial weight (kg)	80.08	79.75	77.75	1.09	0.67
Final weight (kg)	105.75	105.08	104.48	1.42	0.94
ADFI (g)	3165.83	3289.67	3223.67	71.51	0.80
ADG (g)	950.62	938.27	990.13	30.43	0.79
F:G	3.44	3.52	3.25	0.11	0.63
Overall (25-105 kg)					
Initial weight (kg)	24.17	24.18	24.18	0.50	1.00
Final weight (kg)	105.75	105.08	104.48	1.42	0.94
ADFI (g)	2424.76	2494.48	2437.07	39.88	0.77
ADG (g)	906.48	898.98	892.32	13.80	0.93
F:G	2.69	2.78	2.73	0.04	0.83

SEM - standard error of the mean (n = 6, number of replicates); ADFI - average daily feed intake; ADG - average daily gain; F:G - feed to gain ratio; CP - crude protein; NE - net energy.
[1] High protein + high NE diet = 17.0-15.5-13.5% CP and 10.36 MJ/kg NE diets for phases I, II, and III, respectively.
[2] Low protein + high NE diet = 13.5-12.0-10.0% CP and 10.36 MJ/kg NE diets for phases I, II, and III, respectively.
[3] Low protein + low NE diet = 13.5-12.0-10.0% CP and 9.86-10.01-10.01 MJ/kg NE for phases I, II, and III, respectively.

Table 4 - Effects of dietary treatments on apparent total tract digestibility of nutrients of growing-finishing pigs (%)

Item	HCP+HNE[1]	LCP+HNE[2]	LCP+LNE[3]	SEM	P-value
Phase I (25-50 kg)					
Dry matter	87.00	87.38	87.15	0.39	0.93
Organic matter	89.30	89.60	89.38	0.33	0.93
Crude protein	84.33	84.71	84.55	0.55	0.96
Ether extract	71.19	74.49	75.26	1.07	0.34
Phase II (50-75 kg)					
Dry matter	86.24	86.66	86.43	0.31	0.88
Organic matter	88.91	89.14	88.88	0.25	0.91
Crude protein	84.63	82.30	84.66	0.63	0.31
Ether extract	60.95	58.38	57.40	1.03	0.37
Phase III (75-105 kg)					
Dry matter	87.72	87.22	86.85	0.33	0.82
Organic matter	90.17	90.34	89.87	0.23	0.72
Crude protein	83.99	84.01	84.69	0.50	0.83
Ether extract	57.73	53.46	54.44	0.93	0.12

SEM - standard error of the mean (n = 6, number of replicates); CP - crude protein; NE - net energy.
[1] High protein + high NE diet = 17.0-15.5-13.5% CP and 10.36 MJ/kg NE diets for phases I, II, and III, respectively.
[2] Low protein + high NE diet = 13.5-12.0-10.0% CP and 10.36 MJ/kg NE diets for phases I, II, and III, respectively.
[3] Low protein + low NE diet = 13.5-12.0-10.0% CP and 9.86-10.01-10.01 MJ/kg NE for phases I, II, and III, respectively.

Table 5 - Effects of dietary treatments on N retention and excretion of growing-finishing pigs

Item	HCP+HNE[1]	LCP+HNE[2]	LCP+LNE[3]	SEM	P-value
Phase I (25-50 kg)					
N intake (g/day)	61.20a	51.35b	47.42b	1.76	<0.01
Fecal N excreted (g/day)	10.41a	8.88ab	8.24b	0.39	0.05
Urine N excreted (g/day)	10.17a	5.55b	5.40b	0.90	0.04
Total N excretion (g/day)	20.58a	14.42b	13.64b	1.18	0.02
Retained N (g/day)	40.62a	36.93ab	33.77b	1.11	0.03
N retention rate (%)	66.71	72.02	71.00	1.42	0.28
Phase II (50-75 kg)					
N intake (g/day)	75.46a	61.96b	58.97b	2.29	<0.01
Fecal N excreted (g/day)	11.11	9.84	9.41	0.44	0.28
Urine N excreted (g/day)	14.43a	10.79b	10.46b	0.76	0.04
Total N excretion (g/day)	25.54a	20.63b	19.86b	1.00	0.03
Retained N (g/day)	49.92a	41.33b	39.11b	1.89	0.03
N retention rate (%)	65.92	66.33	66.25	1.19	0.98
Phase III (75-105 kg)					
N intake (g/day)	71.45a	59.48b	59.16b	2.42	0.05
Fecal N excreted (g/day)	12.00	9.71	9.94	0.53	0.15
Urine N excreted (g/day)	15.19a	11.54b	11.41b	0.73	0.04
Total N excretion (g/day)	27.19a	21.26b	21.35b	1.11	0.03
Retained N (g/day)	44.26	38.23	37.81	2.16	0.42
N retention rate (%)	60.81	64.00	63.92	1.69	0.71

SEM - standard error of the mean (n = 6, number of replicates); CP - crude protein; NE - net energy.
[1] High protein + high NE diet = 17.0-15.5-13.5% CP and 10.36 MJ/kg NE diets for phases I, II, and III, respectively.
[2] Low protein + high NE diet = 13.5-12.0-10.0% CP and 10.36 MJ/kg NE diets for phases I, II, and III, respectively.
[3] Low protein + low NE diet = 13.5-12.0-10.0% CP and 9.86-10.01-10.01 MJ/kg NE for phases I, II, and III, respectively.
a-b - Values with different letters in the same row are different (P<0.05).

Faecal NH_3-N concentration and pH value were also determined (Table 7). During each phase, pigs fed the low protein + high NE and low protein + low NE diets had a lower (P<0.05) faecal NH_3-N concentration than those fed the high protein + high NE diet. At the same time, the faecal pH value of pigs fed low protein + high NE diet was lower (P<0.05) than that pigs fed high protein + high NE diet during the 25-50 and 75-100 kg phases. Within each phase, no difference was detected in faecal pH value between low protein + high NE and low protein + low NE groups.

From the results of the faecal microbiota of finishing pigs at the end of the experiment (Table 8), it was found that based on the same dietary NE density, reduced CP level had no significant impact on faecal *Lactobacillus* counts. However, the population of faecal *Bifidobacterium* of pigs fed the low protein + high NE diet tended to increase (P = 0.07) when compared with pigs fed the high protein +

Table 6 - Effects of dietary treatments on blood profiles of finishing pigs

Item	HCP+HNE[1]	LCP+HNE[2]	LCP+LNE[3]	SEM	P-value
BUN (mmol/L)	4.92a	3.44b	3.22b	0.22	<0.01
TP (g/L)	58.13	60.55	58.10	1.07	0.59
ALB (g/L)	41.32	44.89	41.80	0.90	0.23
NH_3-N (mg/L)	53.34a	47.36b	48.33b	1.02	0.02

BUN - serum urea nitrogen; TP - total protein; ALB - albumin; SEM - standard error of the mean (n = 6, number of replicates); CP - crude protein; NE - net energy.
[1] High protein + high NE diet = 17.0-15.5-13.5% CP and 10.36 MJ/kg NE diets for phases I, II, and III, respectively.
[2] Low protein + high NE diet = 13.5-12.0-10.0% CP and 10.36 MJ/kg NE diets for phases I, II, and III, respectively.
[3] Low protein + low NE diet = 13.5-12.0-10.0% CP and 9.86-10.01-10.01 MJ/kg NE for phases I, II, and III, respectively.
a-b - Values with different letters in the same row are different (P<0.05).

Table 7 - Effects of dietary treatments on faecal pH value and NH_3-N concentration of pigs

Item	HCP+HNE[1]	LCP+HNE[2]	LCP+LNE[3]	SEM	P-value
Phase I (25-50 kg)					
NH_3-N (mg/g)	1339.42a	941.34b	911.76b	72.59	0.02
pH	6.73a	6.27b	6.49ab	0.07	0.01
Phase II (50-75 kg)					
NH_3-N (mg/g)	1197.66a	823.57b	731.88b	77.06	0.02
pH	6.91a	6.59ab	6.42b	0.08	0.01
Phase III (75-105 kg)					
NH_3-N (mg/g)	1163.32a	665.68b	672.32b	3.03	<0.01
pH	6.91a	6.70b	6.55b	0.05	<0.01

SEM - standard error of the mean (n = 6, number of replicates); CP - crude protein; NE - net energy.
[1] High protein + high NE diet = 17.0-15.5-13.5% CP and 10.36 MJ/kg NE diets for phases I, II, and III, respectively.
[2] Low protein + high NE diet = 13.5-12.0-10.0% CP and 10.36 MJ/kg NE diets for phases I, II, and III, respectively.
[3] Low protein + low NE diet = 13.5-12.0-10.0% CP and 9.86-10.01-10.01 MJ/kg NE for phases I, II, and III, respectively.
a-b - Values with different letters in the same row are different (P<0.05).

Table 8 - Effects of dietary treatments on faecal *Lactobacillus*, *Bifidobacterium*, and *Escherichia coli* counts of finishing pigs

Item	HCP+HNE[1]	LCP+HNE[2]	LCP+LNE[3]	SEM	P-value
Lactobacillus (log[copies/g])	7.54	7.88	7.50	0.14	0.12
Bifidobacterium (log[copies/g])	8.68	9.48	9.58	0.18	0.07
Escherichia coli (log[copies/g])	9.11a	8.38b	8.35b	0.14	0.03

SEM - standard error of the mean (n = 6, number of replicates); CP - crude protein; NE - net energy.
[1] High protein + high NE diet = 17.0-15.5-13.5% CP and 10.36 MJ/kg NE diets for phases I, II, and III, respectively.
[2] Low protein + high NE diet = 13.5-12.0-10.0% CP and 10.36 MJ/kg NE diets for phases I, II, and III, respectively.
[3] Low protein + low NE diet = 13.5-12.0-10.0% CP and 9.86-10.01-10.01 MJ/kg NE for phases I, II, and III, respectively.
a-b - Values with different letters in the same row are different (P<0.05).

high NE diet. Decrease of dietary CP level led to a significant decrease in faecal *Escherichia coli* counts of pigs (P<0.05). There was no difference in populations of faecal *Lactobacillus, Bifidobacterium*, and *Escherichia coli* between low protein + high NE and low protein + low NE groups.

Discussion

There is growing environmental awareness on animal production due to the increasingly negative impact of animal production on the environment (Portejoie et al., 2002). Traditionally, over-supplementation of diets with nutrients to ensure the maximisation of pig performance can result in an excessive amount of nutrients being excreted in the faeces and urine. Nitrogen is one of the primary sources of environmental pollution caused by livestock manure. Level of dietary CP may affect water intake and subsequent excretion and manure output. Furthermore, most of the N loss is related to the low efficiency of digestion and absorption of N source (Otto et al., 2003). Pigs fed low CP diets supplemented with crystalline AA have been shown to achieve the same performance as those fed normal CP level diets (Kerr et al., 2003; Shriver et al., 2003; Deng et al., 2007), while the amount of N excretion was reduced dramatically. Growth response of pigs fed reduced CP diets varies among the results of published studies (Figueroa et al., 2003; Shrive et al., 2003; Knowles et al., 1998); usually, a reduction of four percentage points or less of CP supplement with AA did not hamper growth performance (Figueroa et al., 2002; Kerr et al., 2003). In the current study, reduced CP level by 3.5 percentage points had no difference in growth performance, which was consistent with previous studies (Figueroa et al., 2002; Kerr et al., 2003). For growing-finishing pigs, growth performance and lean tissue deposition will not be influenced if crystalline AA is provided to balance any dietary AA limitation, because the essence of protein nutrition is to meet the needs of amino acids of growing-finishing pigs.

Reducing dietary CP level will result in a decrease in protein and AA deamination, urea excretion, and heat production of pigs (Noblet et al., 2001). Furthermore, the weight of viscera associated with protein metabolism is reduced for pigs that received low CP diets (Kerr et al., 2003; De La Llata et al., 2007). Size of viscera and energy intake were positively correlated; viscera accounted for only 10% of body weight, but the total calories consumed was more than 50% (Gómez et al., 2002). Protein has a higher heat increment than that of carbohydrate and fat (Noblet et al., 1994). Consequently, low CP diets will save more dietary energy, which could increase fat deposition, but lead to a negative effect on carcass quality. Previous research has shown that formulation of low-protein diets using NE system and appropriately decreasing dietary NE density could regulate pig carcass fat deposition (Kerr et al., 2003; De La Llata et al., 2007). However, protein degradation and synthesis are processes that consume energy. Thus, different dietary energy levels would affect nitrogen deposition and emission. However, in the present study, we found that, based on the same low dietary CP level, decreasing dietary NE density by 0.5, 0.35, and 0.35 MJ/kg for the 25-50, 50-75, and 75-105 kg phases, respectively, had no effect on growth performance, which was consistent with previous studies (Knowles et al., 1998; Kerr et al., 2003).

It is well known that energy density is the first determinant of ADFI in most instances (Henry, 1985). However, most studies have assumed that, over a wide range of dietary energy density, pigs will adjust feed intake to maintain a constant or nearly constant daily energy intake (Ellis and Augspurger, 2001). It is noteworthy that the result of Yi et al. (2010) showed that there was a significant increase first and then a decrease in ADG as the NE level of the low-protein diet decreased. On the other hand, Quiniou et al. (1995) reported a significant increase in ADG as the NE level of the low-protein diet increased.

The inconsistent results on ADFI and ADG may be associated with different levels of NE in various studies. Pigs must be supplied with adequate levels of energy, which should be in an optimum ratio to dietary protein content, to maximise growth performance and lean deposition. The relationship between energy intake and tissue growth is that lean tissue and growth rate respond linearly to energy intake up to a point where the protein deposition rate is at a maximum. Any additional energy supplied beyond this point will result in a huge increase in lipid deposition with a modest rise in lean tissue (Lunen et al., 2001). In the research of Yi et al. (2010), the appropriate NE level was within the dietary

NE levels set by the test; however, Quiniou et al. (1995) could not find the appropriate NE level within the NE levels set by their trial.

In the current study, the low dietary NE level was based on the adequate NE level of Yi et al. (2010). The range from low to a high level was narrow (0.35-0.5 MJ/kg); thus, there was no noticeable difference in ADG between the low protein + high NE and low protein + low NE diets.

It was reported that nutrient digestibility of pigs fed reduced CP diets was varied depending on the extent of CP reduction, the number of indispensable AA supplemented, and the energy level of the diets. In the current study, reduced CP level had no significant effect on the ATTD of DM, OM, CP, and EE. Similar results were also observed by Kerr et al. (2003), who reported that there was no difference in N digestibility and crude fat digestibility between pigs fed a 16% CP and 12% CP diet. However, the results of Noblet et al. (1987) showed an increase in N digestibility as dietary CP levels increased. In contrast, Jin et al. (1998) reported that feeding a low CP diet with supplemental crystalline AA could improve CP and DM digestibility compared with feeding a high CP diet. There is no simple linear relationship between nutrient digestibility and protein content in the feed. De Silva and Perera (1984) pointed out that the apparent digestibility of protein increased first and then declined in the continued increase of feed protein content. At the same time, in the present study, dietary NE density also did not influence the ATTD of DM, OM, CP, and EE.

Formulation of reduced CP diets and supplementation with synthetic AA may mitigate the environmental impact of swine production by reducing N excretion (Dourmad et al., 1993; Kerr, 2003). In the current study, we found that within each growth period, decreased dietary CP level dramatically reduced N intake, urine N excretion, and total N (faecal + urine) excretion. As a result, it can be calculated that the total N excretion of low protein + high NE and low protein + low NE groups decreased by 23.17 and 25.13% in the whole experimental period, respectively, when compared with that of the high protein + high NE group. This was in good agreement with previous reports (Dourmad et al., 1993; Kerr, 2003), which indicated that for each percentage point reduction in dietary CP combined with AA supplementation, the total N excretion (urinary N + faecal N) was reduced by approximately 8%. Decreasing dietary NE density by 0.5, 0.35, and 0.35 MJ/kg for the 25-50, 50-75, and 75-105 kg phases, respectively, did not affect N excretion and N retention in low-protein diet. Nitrogen retention or N balance reflects the utilisation of proteins and balance between the body protein synthesis and degradation. The balance between dietary protein and energy affects the deposition and utilisation of protein (Campbell and Taverner, 1988). Only with the sufficient dietary energy, reducing the protein level could decrease N emissions, as protein metabolism is quite active and consume much energy; if the energy intake is lower than the requirement of maximum N deposition, N excretion in the urine will increase, while the biological value of N will decrease (Close, 1996). The result of the current study indicated that decreasing dietary NE density by 0.35-0.5 MJ/kg during the whole experiment could meet the energy requirement of the protein metabolism and utilisation when pigs fed a low-protein diet.

The contents of serum TP and ALB reflect the level of protein in the diet and the degree of digestion, absorption, and utilisation of protein by the animal. The concentration of BUN was affected by the quantity and quality of dietary protein and the dietary amino acid balance condition. There was a significant negative correlation between the content of BUN and the utilization rate of protein or amino acid (Coma et al., 1995). Ammonia nitrogen is the product of intestinal microbiota-fermented protein and amino acid that also can reflect the utilisation of protein and AA. In the present study, there was no difference in serum TP and ALB concentration among the three treatments. However, pigs fed the low protein + high NE diet had a significantly lower serum BUN concentration and serum NH_3-N concentration than pigs fed the high protein + high NE diet. This result is consistent with previous research (Figueroa et al., 2003; Kerr et al., 2003). It indicated that degradation of protein and amino acids were reduced when pigs fed low CP diet. However, decreased dietary NE density in low CP diet did not affect serum BUN and NH_3-N concentrations.

Concentration of NH_3-N in the excrement reflects the presence of N in the form of free ammonia (NH_3) and ammonium (NH_4^+). During the discharge and storage process, 50 to 75% of the N in the excrement would decompose to NH_3-N by urease (Aarnink and Verstegen, 2007). Urease is a cytoplasmic enzyme primarily presented in faeces and feed, whose activity is affected by optimum pH value ranging from 6 to 9. When pH<7, NH_3-N is mainly in the form of NH_4^+; when pH>7, NH_3 begins to be produced; when pH≥9.25, the vast majority of NH_3-N separate into NH_3. It is essential to maintain NH_3-N in the NH_4^+ state, because NH_3 would be difficult to control once released into the air. Emission of NH_3 was reduced by approximately 45% for one-point reduction in slurry pH (Canh et al., 1998). In the current study, pigs fed low CP diet had a lower faecal NH_3-N concentration and pH value, which was consistent with the studies of Portejoie et al. (2004). The result was caused by less protein fermented in the hind gut and lower acid-binding capacity of low protein ingredients. However, decreasing the dietary NE density in low CP diet had no significant impact on faecal NH_3-N concentration and pH value. It is suggested that decreasing dietary NE density by 0.35-0.5 MJ/kg did not affect the digestion of protein in the foregut.

In the current study, we found that pigs fed the low-protein diet had a smaller population of faecal *Escherichia coli*, which corroborates Opapeju et al. (2009). *Escherichia coli* has a strong ability to hydrolyse and ferment proteins, which has a competitive advantage over other bacteria in amino acid-rich environments and can use AA as an energy source (Wellock et al., 2006; Rist et al., 2014). Meanwhile, low protein diet has a lower acid-binding capacity; the lower pH environments inhibited the proliferation of *Escherichia coli*. However, the faecal *Bifidobacterium* counts of pigs tended to increase by decreased dietary CP content. *Bifidobacterium* has the ability to degrade polysaccharides; the amount of *Bifidobacterium* in feaces depends on the content of carbohydrates in the diet (Mikkelsen and Jensen, 2004), and the low pH environment is beneficial to the proliferation of *Bifidobacterium*. In the present study, to reduce the dietary protein levels, the proportion of soybean meal in the formulation was reduced and the ratio of corn was increased. At the same time, wheat bran was used to replace partial corn to reduce dietary NE density. Therefore, the proportion of corn or wheat bran in the low-protein diet was higher than that of the high-protein diet. Pigs fed the low-protein diets would have more resistant starch or non-starch polysaccharide entered into the hindgut and utilised by microbiota. Therefore, more carbohydrates in the low-protein diets promoted the increase of the faecal *Bifidobacterium* count.

Conclusions

Reducing dietary crude protein level by 3.5 percentage points does not affect the growth performance of growing-finishing pigs, but drastically reduces N excretion and faecal *Escherichia coli* count. Additionally, decreasing net energy density of low protein diets by 0.50, 0.35, and 0.35 MJ/kg for the 25-50, 50-75, and 75-105 kg phases, respectively, has no significant effect on growth performance, N balance, blood profiles, and faecal bacteria population. Therefore, from the perspectives of animal production and environmental protection, it is feasible to formulate a low-protein diet with appropriately decreased dietary net energy level for growing-finishing pigs.

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