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Article

Dynamics Affect Nitrogen Retention, Ileal Amino Acid Digestibility, and Gene Expression Levels of Digestive Enzymes at Three Stages in Pigs Fed Two Levels of Low-Protein Diets

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Abstract: This study was conducted to determine the dynamic effects of dietary crude protein (CP) intake on nitrogen (N) balance, ileal amino acid digestibility, and gene expression levels of digestive enzymes at three stages in pigs. In Experiment 1, 18 growing pigs (average body weight (BW) = 9.5 kg) were randomly assigned to one of three treatments (n = 6/treatment group), including normal (20% CP), low (17% CP), and very low (14% CP) protein intake. In Experiment 2, 18 growing pigs (average BW = 30 kg) were allotted randomly to one of three treatments (n = 6/treatment group), including normal (18% CP), low (15% CP), and very low (12% CP) protein intake. In Experiment 3, 18 growing pigs (average BW = 45 kg) were assigned randomly to one of three treatments (n = 6/treatment group), including normal (16% CP), low (13% CP), and very low (10% CP) protein intake. Growing pigs fed the 14% CP and 17% CP diets had lower final BW ($P < 0.05$) and average daily gain (ADG) ($P < 0.05$) compared to pigs fed the 20% CP diet. Reducing the dietary CP level from 20 to 14% decreased urinary N excretion by 52.8% ($P < 0.001$) in Experiment 1. Reducing the dietary CP level from 18 to 12% decreased urinary N excretion by 55.3% ($P < 0.001$) and reduced fecal N excretion by 34% ($P < 0.05$) in Experiment 2. Reducing the dietary CP level from 16 to 10% decreased urinary N excretion by 56.4% ($P < 0.001$) and fecal N excretion by 47.1% ($P < 0.001$) in Experiment 3. Pigs fed the very low (14%, 12%, and 10% CP) diets showed higher digestibility for CP ($P < 0.05$), His ($P < 0.05$), Ile ($P < 0.05$), Phe ($P < 0.05$), Thr ($P < 0.05$), Trp ($P < 0.05$), Glu ($P < 0.05$), and Ser ($P < 0.05$) compared to pigs fed the normal (20%, 18%, and 16% CP) diets among the three experiments. Pigs fed the very low (14%, 12%, and 10% CP) diets showed higher mRNA levels for chymotrypsin C ($P < 0.01$ in Experiment 1 and 2; $P < 0.05$ in Experiment 3) compared to pigs fed the normal (20%, 18%, and 16% CP) diets among the three experiments. These results indicated that a reduction in dietary CP by 6% limited the growth performance of growing pigs, and a reduction of dietary CP by 3% supplemented with essential amino acids could reduce the excretion of N into the environment without affecting weight gain.

Keywords: amino acid; digestive enzyme; low protein diet; nitrogen balance; pigs

1. Introduction

Amino acid (AA) nutrition plays important roles in global animal production progress, such as facilitating increased growth and health status and stimulated immunity function[1]. Studies have shown that a diet containing low crude protein (CP) supplemented with AA may reduce diarrhea in weaning pigs, boost feed economy, repair digestive function (including gene expression alterations of digestive enzymes) by increasing the digestion and absorption of AA, and reduce nitrogen (N), which excretion contributes appreciably to environmental pollution[2-7]. However, there are discrepancies among these studies concerning the extent to which the CP level of diets from growing pigs to finishing pigs can be reduced. First, a CP reduction of 2- to 3% is can be made without affecting average daily gain (ADG) or feed efficiency when diets are supplemented with AA [8,9]. Second, some reports have shown that reductions exceeding 3% also have no effects on ADG, feed efficiency and mRNA levels of digestive enzymes (including trypsinogen, chymotrypsin B, and dipeptidase-II and III) [2,10,11], but that trend has not always been observed in other reports [12-14]. A possible explanation for these discrepancies is that in some studies, the standard diet may have exceeded the AA requirements for single- or multiple stages in pigs. However, in other studies, the standard diets may have only just met or been slightly below the AA requirements for single- or multiple stages in pigs. Therefore, there are limited reports related to the dynamics and continuous research for different stages of pigs.

To enhance the current understanding of the dynamics and continuous effects of reductions in CP, we devised an experiment to evaluate the dynamic effects on growth performance, N balance, ileal amino acid digestibility, and gene expression levels of digestive enzymes after feeding two levels of low CP diets in three stages of pigs.

2. Materials and Methods

2.1 Ethics statement

This study was conducted according to the guidelines of the Declaration of Institute, and all procedures involving animal subjects were approved by the animal welfare committee of the Institute of Subtropical Agriculture at the Chinese Academy of Sciences (Changsha, Hunan Province, China, No.: 20150607).

2.2 Experimental design and procedure

All of the cross-bred pigs [Yorkshire × (Duroc × Landrace)] (Hunan New Wellful Co., Ltd., Hunan Province, China) were assigned randomly to one of three diet treatments (6 pigs/group), including very low (Group A), low (Group B) and normal (Group C) dietary intake of CP. All of the pigs were housed individually in metabolism cages, and had free access to feedstuff and drinking water. The temperature in the metabolism room was maintained at approximately 24ºC and light was provided continuously. Titanium dioxide (TiQ2) (1 g/kg diet) was added to all experiment diets and served as an indigestion marker to calculate total tract N digestibility [15]. There were 3-separate experiments involving 18 growing pigs (Experiment 1), 18 growing pigs (Experiment 2), and 18 finishing pigs (Experiment 3). The experimental design and procedure are shown in Figure 1. All of the experiment diets were formulated according to the National Research Council (NRC, 2012) to meet the nutrient requirements for growing pigs and finishing pigs [16]. There was a 3-day acclimatization period prior the commencing of each experiment. The results of body weight (BW) and feed consumption, such as average daily gain (ADG), average daily feed intake (ADFI), and the ratio of feed and gain (F:G), were recorded at the beginning and end of the experiment period.

2.3 Experiment 1

A total of eighteen [Yorkshire × (Duroc × Landrace)] (Hunan New Wellful Co., Ltd., Hunan Province, China) cross-bred growing pigs with an average BW of 9.51 ± 0.75 kg were assigned randomly to one of three dietary CP levels: very low (14% CP, Group A), low (17% CP, Group B), and normal (20% CP, Group C) CP. The dietary treatments devised as Group A and B were supplemented with some AA that are not synthesized in a pig's body (L-lysine, L-methionine,

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L-threonine, and L-tryptophan) to meet the National Research Council (NRC, 2012) nutrient requirements for growing pigs[16]. The experiments lasted 45 days. The experimental diet ingredients and nutrient compositions in this experiment are shown in Table 1.

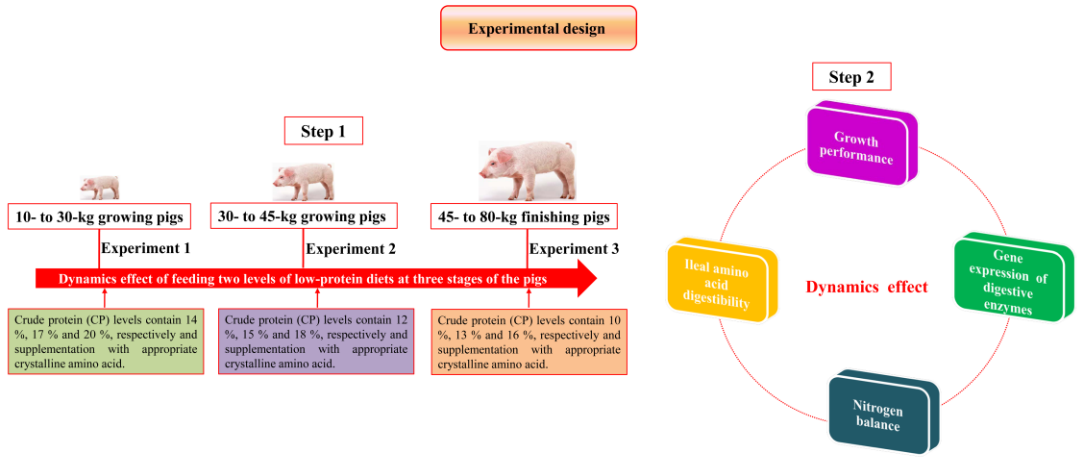

Figure 1 Experimental design. There were 3-separate experiments involving 18 [Yorkshire × (Duroc × Landrace)] growing pigs (Experiment 1), 18 [Yorkshire × (Duroc × Landrace)] growing pigs (Experiment 2), and 18 [Yorkshire × (Duroc × Landrace)] finishing pigs (Experiment 3). Experiment 1: Group A = 14% CP (very low CP level); Group B = 17% CP (low CP level); Group C = 20% CP (normal CP level). Groups A and B had diets supplemented with L-lysine, L-methionine, L-threonine, and L-tryptophan (see Table 1). Experiment 2: Group A = 12% CP (very low CP level); Group B = 15% CP (low CP level); Group C = 18% CP (normal CP level). Groups A and B had diets supplemented with L-lysine, L-methionine, L-threonine, and L-tryptophan (see Table 2). Experiment 3: Group A = 10% CP (very low CP level); Group B = 13% CP (low CP level); Group C = 16% CP (normal CP level). Groups A and B had diets supplemented with L-lysine, L-methionine, L-threonine, and L-tryptophan. CP = crude protein.

2.4 Experiment 2

A total of eighteen [Yorkshire × (Duroc × Landrace)] (Hunan New Wellful Co., Ltd., Hunan Province, China) cross-bred growing pigs with an average BW of 29.12 ± 2.30 kg were assigned randomly to one of three dietary CP levels: very low (12% CP, Group A), low (15% CP, Group B), and normal (18% CP, Group C) CP. The dietary treatments devised as Group A and B were supplemented with some AA that are not synthesized in a pig's (L-lysine, L-methionine, L-threonine, and L-tryptophan) to meet the National Research Council (NRC, 2012) nutrient requirements for growing pigs[16]. The experiments lasted 30 days. The experimental diets of ingredients and nutrient compositions in this experiment are shown in Table 2.

2.5 Experiment 3

A total of eighteen [Yorkshire × (Duroc × Landrace)] (Hunan New Wellful Co., Ltd., Hunan Province, China) cross-bred finishing pigs with an average BW of 45.00 ± 2.07 kg were assigned randomly to one of three dietary CP levels: very low (10% CP, group A), low (13% CP, group B), and normal (16% CP, group C) CP. The dietary treatments for Groups A and B were supplemented with some AA that are not synthesized in the pig body (L-lysine, L-methionine, L-threonine and L-tryptophan) to meet the National Research Council (NRC, 2012) nutrient requirements for finishing pigs[16]. The experiments lasted 50 days. The experimental diets of ingredients and nutrient compositions in this experiment are shown in Table 3.

2.6 Sample collection and preparation

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At the end of each experiment, pigs were anesthetized through an intravenous injection of 50 mg/kg sodium pentobarbital and then sacrificed. The collection period was divided into parts to conduct experiments on apparent digestibility and N balance (days 3-7), and all the samples of intestine (duodenum and jejunum) were removed, collected and immediately frozen in liquid nitrogen at -80oC for subsequent analysis of gene expression levels as previously described [2,17]. The digesta samples were collected from the ileum for analyzing the digestibility of energy (DE), dry matter (DM), CP, and AA as previously described [2].

Table 1. Feedstuff ingredients and nutrient composition of experimental diets for 10- to 30-kg growing pigs (%).

Feed ingredient	CP levels[a]		
	14% CP	17% CP	20% CP
Corn（43%CP）	71.80	66.50	63.70
Soybean meal	13.40	18.80	19.80
Whey powder	4.40	4.30	4.30
Fish meal（64%CP）	1.50	4.00	9.00
Soybean oil	4.10	2.60	0.80
Lysine hydrochloride	0.88	0.62	0.38
Hydroxy methionine	0.27	0.19	0.10
L-threonine	0.33	0.21	0.09
L-tryptophan	0.08	0.04	0.01
CaHPO3	1.15	0.74	0.00
Rock-powder	0.79	0.70	0.52
Salt	0.30	0.30	0.30
1 % Premix[b]	1.00	1.00	1.00
Total	100.00	100.00	100.00
Calculated and analyzed nutrient composition[c]			
DE (MJ/kg)	14.60	14.60	14.60
CP	14.14	17.32	20.27
Total Ca	0.70	0.71	0.69
Total P	0.53	0.55	0.57
Starch	45.16	41.95	40.22
NDF	8.40	8.66	8.54
ADF	3.05	3.30	3.29
Lys	1.26	1.25	1.26
Met + Cys	0.63	0.65	0.62
Thr	0.76	0.75	0.76
Trp	0.20	0.20	0.20
Arg	0.71	0.93	1.09
His	0.30	0.37	0.44
Ile	0.46	0.60	0.71
Leu	1.11	1.32	1.52
Phe	0.56	0.70	0.81
Val	0.54	0.64	0.72

[a]Diet treatment: Crude protein (CP) levels contain 14%, 17% and 20%, respectively and supplementation with appropriate crystalline AA. [b]Premix provided these amounts of vitamins and minerals per kilogram on an as-fed basis: 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, 12 mg; vitamin B_6, 6 mg; vitamin B_{12}, 0.05 mg; biotin, 0.2 mg; folic acid, 2 mg; niacin, 50 mg; D-calcium pantothenate, 25 mg; Fe,100 mg as ferrous sulfate; Cu, 150 mg as copper sulphate; Mn, 40 mg as manganese oxide; Zn, 100 mg as zinc oxide; I, 0.5 mg as potassium iodide; and Se, 0.3 mg as sodium selenite. The values are expressed as percentage (%), except for

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digestible energy (DE; MJ/kg), essential amino acid (EAA)/nonessential amino acid (NEAA). [c]The DE was calculated according to NRC (2012). [c]All other values represent analyzed values.

Table 2. Feedstuff ingredients and nutrient composition in experimental diets for 30- to 45-kg growing pigs (%).

Feed ingredient	CP levels[a]		
	12%CP	15%CP	18%CP
Corn(43%CP)	77.60	67.50	58.60
Soybean meal	10.00	19.50	29.00
Wheat bran	5.06	6.94	7.80
Soybean oil	3.00	2.38	1.55
Lysine hydrochloride	0.74	0.46	0.18
Hydroxy methionine	0.17	0.09	0.00
L-threonine	0.26	0.14	0.01
L-tryptophan	0.07	0.02	0.00
CaHPO3	0.90	0.78	0.69
Rock-powder	0.90	0.89	0.87
Salt	0.30	0.30	0.30
1 % Premix[b]	1.00	1.00	1.00
Total	100.00	100.00	100.00
Calculated and analyzed nutrient composition [c]			
DE (MJ/kg)	14.20	14.20	14.20
CP	12.35	15.16	18.27
Total Ca	0.61	0.63	0.60
Total P	0.45	0.48	0.51
Starch	49.87	44.16	38.96
NDF	10.09	11.09	11.87
ADF	3.53	4.14	4.68
Lys	0.94	0.97	0.97
Met + Cys	0.55	0.56	0.57
Thr	0.60	0.61	0.61
Trp	0.17	0.17	0.17
Arg	0.57	0.82	1.08
His	0.25	0.33	0.41
Ile	0.35	0.49	0.64
Leu	0.94	1.14	1.35
Phe	0.46	0.62	0.77
Val	0.44	0.56	0.66

[a]Diet treatment: Crude protein (CP) levels contain 12 %, 15 % and 18 %, respectively and supplementation with appropriate crystalline AA. [b]Premix provided these amounts of vitamins and minerals per kilogram on an as-fed basis: 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, 12 mg; vitamin B_6, 6 mg; vitamin B_{12}, 0.05 mg; biotin, 0.2 mg; folic acid, 2 mg; niacin, 50 mg; D-calcium pantothenate, 25 mg; Fe,100 mg as ferrous sulfate; Cu, 150 mg as copper sulphate; Mn, 40 mg as manganese oxide; Zn, 100 mg as zinc oxide; I, 0.5 mg as potassium iodide; and Se, 0.3 mg as sodium selenite. The values are expressed as percentage (%), except for digestible energy (DE; MJ/kg), essential amino acid (EAA)/nonessential amino acid (NEAA). [c]The DE was calculated according to NRC (2012). [c]All other values represent analyzed values.

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Table 3. Feedstuff ingredients and nutrient composition in experimental diets for 45- to 80-kg finishing pigs (%).

Feed ingredient	CP levels[a]		
	10%CP	13%CP	16%CP
Corn（43%CP）	87.40	78.36	67.00
Soybean meal	5.50	15.00	23.76
Wheat bran	2.00	3.00	6.00
Soybean oil	1.71	0.90	0.88
Lysine hydrochloride	0.55	0.27	0.01
Hydroxy methionine	0.09	0.00	0.00
L- threonine	0.19	0.06	0.00
L-tryptophan	0.06	0.01	0.00
CaHPO3	0.65	0.55	0.50
Rock-powder	0.55	0.55	0.55
Salt	0.30	0.30	0.30
1 % Premix[b]	1.00	1.00	1.00
Total	100.00	100.00	100.00
Calculated and analyzed nutrient composition[c]			
DE (MJ/kg)	14.20	14.20	14.20
CP	10.26	13.17	16.30
Total Ca	0.51	0.50	0.52
Total P	0.38	0.40	0.45
Starch	55.22	49.97	43.71
NDF	9.37	10.18	11.33
ADF	3.14	3.69	4.34
Lys	0.73	0.72	0.72
Met + Cys	0.43	0.42	0.50
Thr	0.49	0.50	0.56
Trp	0.13	0.13	0.17
Arg	0.44	0.70	0.94
His	0.22	0.31	0.39
Ile	0.30	0.45	0.60
Leu	0.91	1.13	1.32
Phe	0.41	0.57	0.71
Val	0.36	0.50	0.61

[a]Diet treatment: Crude protein (CP) levels contain 10%, 13% and 16%, respectively and supplementation with appropriate crystalline AA. [b]Premix provided these amounts of vitamins and minerals per kilogram on an as-fed basis: 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, 12 mg; vitamin B_6, 6 mg; vitamin B_{12}, 0.05 mg; biotin, 0.2 mg; folic acid, 2 mg; niacin, 50 mg; D-calcium pantothenate, 25 mg; Fe,100 mg as ferrous sulfate; Cu, 150 mg as copper sulphate; Mn, 40 mg as manganese oxide; Zn, 100 mg as zinc oxide; I, 0.5 mg as potassium iodide; and Se, 0.3 mg as sodium selenite. The values are expressed as percentage (%), except for digestible energy (DE; MJ/kg), essential amino acid (EAA)/nonessential amino acid (NEAA). [c]The DE was calculated according to NRC (2012). [c]All other values represent analyzed values.

2.7 N balance

The N balance experiment was maintained for seven days. During the 3-day collection period, pigs were placed in metabolism cages, and all of their fresh feces and urine were collected as previously described [18]. Fresh urine was collected in a plastic cask containing 30 mL of 6 mol/L HCl, and a 50-mL aliquot of fresh urine was stored daily at 4oC until the end of the experiment and was subsequently frozen at 20oC for further analyses. TiQ2 (1 g/kg diet) was used as an inert marker to identify feces from the initial diet during the collection period as previously described [15]. At the end of the experiment, all of the fresh feces were desiccated in a 55oC air-forced drying oven. The feces were sub-sampled and ground through a 1-mm screen, then weighed and stored in plastic bags. All of the feces and urine samples were analyzed for N as previously described [15]. The apparent N balance and other N balance items were calculated as the difference between consumption and excretion.

Table 4. Primers used for real-time PCR analysis.

Target gene	Primer sequence	Accession NO.
Trypsinogen	Sense 5'-AGCAATTCATCAATGCCGCC-3'	NM_001162891.1
	Antisense 5'- CAGGAGCGAAGGGTAGCTG-3'	
Pancreatic α-Amylase 2A	Sense 5'-TAAGCACATGTGGCCTGGAG-3'	XM_001929136.5
	Antisense 5′-AAGGGCTCTATCAGAGGGCA-3'	
Pancreatic carboxypeptidase B1	Sense 5'-AGGTGAGAAGGTGTTCCGTG-3′	NM_214169.1
	Antisense 5'-TGCGAGAGATGAGGTCTGGA-3'	
Duodenal enterokinase	Sense 5'-TCTCCATACGGAGGAAGCCA-3'	XM_005668172.1
	Antisense 5'-TGGGCCAGTCATCCCATTTC-3'	
Chymotrypsin B	Sense 5'-AACAGGCTTCCACTACTGCG-3'	XM_003472038.1
	Antisense 5'-TGGTCAGTAGCAAAGGGCAG-3'	
Chymotrypsin C	Sense 5'-GCGGCACCTTAATCACCTCT-3'	NM_001244379.1
	Antisense 5'-GGCAGGCATAACACCTGGAT-3'	
Pancreatic α-amylase 2B	Sense 5'-TGCTCTTGAATGTGAGCGGT-3'	NM_214195.1
	Antisense 5'-TACGGACGCCAACGTTGTTA-3'	
Pancrelipase	Sense 5'-AAGGTGGAGAGCGTGAACTG-3'	NM_001177912.2
	Antisense 5'-TCCAGCCCTGTGATTCGTTC-3'	
Jejunal maltase	Sense 5'-GCACAGATCAGCCGATGAGA-3'	XM_005657730.1
	Antisense 5'-CAAATGACCGTCCAGCTCCT-3'	
Jejunal sucrase	Sense 5'-TGGTGGCACTGTTATCCGAC-3'	XM_005657098.1
	Antisense 5'-GAGCAGGCTCTTGACATGGT-3'	
Jejunal dipeptidase-II (DPP-II)	Sense 5'-TGTGGCAGATCACTTCGACC-3'	XM_003355779.3
	Antisense 5'-CTCTTCCTCACTCCAGCCAC-3'	

2.8 Analysis of conventional items

All samples of ideal digesta were pooled and homogenized in a blender, sub-sampled, and freeze-dried, then finely ground in grinder, and thoroughly mixed for analysis as previously described [2]. All samples and nine diets were analyzed for conventional items, such as DM, DE, N and concentrations of AA. DM was analyzed using an AOAC protocol (1990; method 925.09)[19], and gross energy (GE) was analyzed using an oxygen bomb calorimeter as previously described [2,20]. The N was analyzed using a previously described method [2]. Samples of AA determination were prepared with acid hydrolysis using a previously described protocol [2]. The tryptophan was not analyzed because it was destroyed during the preparation with acid hydrolysis[21].

2.9 Relative Quantification PCR analysis of gene expression levels for digestive enzymes

The software program (Primer 5.0; Primer-E Ltd., Plymouth, UK) was used to design primers, which are listed in Table 4. The β-actin gene was used as an internal control to normalize expression of target gene transcript levels. Total intestine tissue RNA was isolated using TRIzol regent (Invitrogen, Carlsbad, CA, USA) as previously described [22,23]. The cDNA was reverse transcribed and amplified by quantitative real-time PCR using the ABI 7900 PCR system (ABI Biotechnology, Eldersburg, MD, USA) as previously described [22,23]. Each sample had a total volume of 10 μL, including 1 μL of 4 times diluted cDNA, 5 μL SYBR Green mix, 0.2 μL ROX Reference Dye (50 times), and 0.2 μL each of forward and reverse primers. After predenaturation (10s at 95oC), 40 cycles of amplification were performed, and each cycle consisted of 95oC for 5s, and 60oC for 20s, followed by a melting curve program (60°C to 99°C with a heating rate of 0.1°C/s and fluorescence measurement). The relative levels of genes were expressed as a ratio of mRNA as R=2−(ΔΔCt). The efficiency of real-time PCR was determined by amplification of a dilution series of cDNA according to equation 10(−1/slope), and the results for target mRNA were consistent with the targets for β-actin. Negative controls were created by replacing cDNA with water.

2.10 Statistical analysis

All results are expressed as the mean ± standard error of the mean (SEM). Statistical analyses were subjected to one-way analysis of variance in the SAS 8.2 software program (Version 8.2; SAS Inst. Inc., Cary, NC). The differences among group means were compared using the Duncan multiple comparison test. Probability values < 0.05 were used to indicate statistical significance.

3. Results

3.1 Dynamics affect growth performance

As shown in Table 5, growing pigs fed the 14% CP and 17% CP diets had a lower final BW (P < 0.05) and ADG (P < 0.05) compared to pigs fed a 20% CP diet in Experiment 1. Growing pigs fed the control diet (20% CP) had no difference for F: G compared to 14% CP and 17% CP diets (P > 0.05). However, the ADFI of growing pigs was not affected (P > 0.05) after feeding 14% CP, 17% CP, and 20% CP diets. In Experiment 2, growing pigs fed the control diet (18% CP) had no differences in final BW (P > 0.05), ADG (P > 0.05), ADFI (P > 0.05), and F: G (P > 0.05) among animals that were fed 12% CP, 15% CP, and 18% CP diets. In Experiment 3, the finishing pigs fed the control diet (16% CP) had no differences in final BW (P > 0.05), ADG (P > 0.05), ADFI (P > 0.05), and F: G (P > 0.05) among animals fed the 10% CP, 13% CP, and 16% CP diets. Significantly different effects are clearly shown in Experiment 1, and there was a slightly higher value compared to the control (Group C) for ADG, ADFI, and F: G in Experiment 3.

Table 5. Dynamics alterations of low-protein diets affect growth performance for growing and finishing pigs.

Items	Group A	Group B	Group C	P value	SEM ±
Growing pigs (Experiment 1[5])					
Initial BW[1], kg	9.51	9.51	9.52	0.00	1.00
Final BW, kg	27.71[b]	29.29[ab]	30.36[a]	0.03	0.77
ADG[2], g/d	404.45[b]	439.55[ab]	463.1[a]	0.02	17.04
ADFI[3], g/d	690.55[b]	736.4[ab]	775.8a	0.06	24.63
F:G[4]	1.71	1.68	1.68	0.94	0.01
Growing pigs (Experiment 2[6])					
Initial BW, kg	27.71[b]	29.29[ab]	30.36[a]	0.03	0.77
Final BW, kg	43.07	45.37	45.77	0.22	0.84
ADG, g/d	448.3	478.5	483.8	0.23	11.06
ADFI, g/d	1291	1359	1379	0.26	26.63
F:G	2.88	2.84	2.85	0.92	0.01
Finishing pigs (Experiment 3[7])					
Initial BW, kg	43.07	45.37	45.77	0.22	0.84
Final BW, kg	84.22	82.78	82.38	0.40	0.56
ADG, g/d	598.3	586.5	583.3	0.90	4.56
ADFI, g/d	2196	2170	2164	0.96	9.82
F:G	3.67	3.7	3.71	0.93	0.01

Data are means with the pooled means ± standard error of the mean (SEM), n = 6/treatment group. Within a row, values with different superscript letters differ (P < 0.05). [1]BW, body weight; [2]ADG, average daily gain; [3]ADFI, average daily feed intake; [4]F: G, the ratio of feed and gain. CP = crude protein. [5] Experiment 1: Group A = 14 % CP (very low CP level); Group B = 17 % CP (low CP level); Group C = 20% CP (normal CP level). Groups A and B were supplemented with L-lysine, L-methionine, L-threonine, and L-tryptophan (see Table 1). [6] Experiment 2: Group A = 12% CP (very low CP level); Group B = 15% CP (low CP level); Group C = 18% CP (normal CP level). Groups A and B were supplemented with L-lysine, L-methionine, L-threonine, and L-tryptophan (see Table 2). [7] Experiment 3: Group A = 10% CP (very low CP level); Group B = 13% CP (low CP level); Group C = 16% CP (normal CP level). Groups A and B were supplemented with L-lysine, L-methionine, L-threonine, and L-tryptophan (see Table 3).

3.2 Dynamics affect N balance

As shown in Table 6, a reduction in dietary CP levels from 20% to 14% in growing pigs resulted in a significant decrease in N intake ($P < 0.01$), urinary N excretion ($P < 0.01$), total N excretion ($P < 0.01$) and relatively reduced N excretion ($P < 0.01$). There was also significantly increased N net utilization ($P < 0.01$) and apparent biological value ($P < 0.05$). There was no difference in fecal N excretion ($P > 0.05$), apparent N digestibility ($P > 0.05$) and N retained ($P > 0.05$) in Experiment 1.

Reduction in dietary CP levels from 18% to 12% in growing pigs resulted in a significant decrease in N intake ($P < 0.01$), urinary N excretion ($P < 0.01$), fecal N excretion ($P < 0.05$), total N excretion ($P < 0.01$), relatively reduced N excretion ($P < 0.01$), apparent N net utilization ($P < 0.01$) and N retention ($P < 0.05$). There was also a significantly increased apparent biological value ($P < 0.05$) in Experiment 2 (Table 6). There was no difference in apparent N digestibility ($P > 0.05$) in Experiment 2.

Reduction in dietary CP levels from 16% to 10% in finishing pigs resulted in significantly decreased N intake ($P < 0.01$), urinary N excretion ($P < 0.01$), fecal N excretion ($P < 0.05$), total N excretion ($P < 0.01$), relatively reduced N excretion ($P < 0.01$), apparent N net utilization ($P < 0.01$) and N retained ($P < 0.01$). There was also significantly increased apparent biological value ($P < 0.05$) in Experiment 3 (Table 6). There was no difference in apparent N digestibility ($P > 0.05$) in Experiment 3.

3.3 Dynamics affect ileal digestibility

As shown in Table 7, the low CP diet had no effect ($P > 0.05$) on its DE and DM from Experiment 1 to Experiment 3. In Experiment 1, growing pigs fed the 14% CP diet showed higher digestibility of CP ($P < 0.05$), Arg ($P <0.001$), His ($P < 0.05$), Ile ($P < 0.05$), Lys ($P <0.001$), Met ($P < 0.05$), Phe ($P < 0.05$), Thr ($P < 0.05$), Trp ($P < 0.05$), Val ($P < 0.05$), Asp ($P < 0.05$), Glu ($P < 0.05$), Pro ($P < 0.05$), and Ser ($P < 0.05$) compared to pigs fed the 20% CP diet (Table 7). Lys digestibility increased ($P <0.001$) from 77.45% in growing pigs fed the 20% CP diets to 87.7% in growing pigs fed the 14% CP diet. Similar results were obtained for Arg. There were no differences ($P > 0.05$) in the digestibility of other AA among the three groups of finishing pigs (Table 7).

In Experiment 2, growing pigs fed the 12% CP diet showed higher digestibility of CP ($P < 0.05$), His ($P < 0.05$), Ile ($P < 0.05$), Lys ($P <0.05$), Met ($P < 0.001$), Cys ($P < 0.001$), Phe ($P < 0.05$), Thr ($P < 0.05$), Trp ($P < 0.05$), Gly ($P < 0.05$), and Ser ($P < 0.05$) compared to pigs fed the 18% CP diet (Table 7). Met digestibility increased ($P <0.001$) from 70.48% in growing pigs fed the 18% CP diet to 80.42% in growing pigs fed the 12% CP diet. Similar results were obtained for Cys. There were no differences ($P > 0.05$) in the digestibility of other AA among the three groups of finishing pigs (Table 7).

Results for ileal AA digitibility in finishing pigs (Experiment 3) had a similar tendency in Table 7. Finishing pigs fed the 10% CP diet showed higher digestibility of CP ($P < 0.05$), Arg ($P < 0.05$), His ($P < 0.05$), Ile ($P < 0.05$), Lys ($P <0.001$), Met ($P < 0.05$), Phe ($P < 0.05$), Thr ($P < 0.05$), Trp ($P < 0.05$), Gly ($P < 0.05$), and Ser ($P < 0.05$) compared to pigs fed the 16% CP diet (Table 7). Lys digestibility increased ($P <0.001$) from 77.54% in finishing pigs fed the 16% CP diets to 87.91% in finishing pigs fed the 10% CP diet. There were no differences ($P > 0.05$) in the digestibility of other AA among the three groups of finishing pigs (Table 7).

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Table 6. Dynamics alterations of low-protein diets affect nitrogen balance for growing and finishing pigs.

Items	Group A	Group B	Group C	P value	SEM ±
Growing pigs (Experiment 1[6])					
N intake (g/d)	23.59[b]	26.76[ab]	29.47[a]	0.01	1.70
Urinary N excretion, (g/d)	4.85[b]	6.78[b]	10.27[a]	< 0.00	1.59
Fecal N excretion, (g/d)	2.61	3.72	4.06	0.11	0.44
Total N excretion[1], (g/d)	7.46[c]	10.5[b]	14.33[a]	< 0.00	1.99
Relative reduced of N excretion[2], (%)	47.94[a]	26.73[b]	100[c]	< 0.0001	6.14
N Retained[3], (g/d)	16.13	16.26	15.14	0.43	0.35
N net utilization[9], (%)	68.38[a]	60.76[a]	51.37[b]	0.00	4.92
Apparent N digestibility[4], (%)	88.94	86.1	86.22	0.67	0.93
Apparent biological value[5], (%)	76.88[a]	70.57[ab]	59.58[b]	0.01	5.05
Growing pigs (Experiment 2[7])					
N intake, (g/d)	27.51	34.03	39.95	< 0.00	3.59
Urinary N excretion, (g/d)	5.03	7.62	11.25	< 0.00	1.8
Fecal N excretion, (g/d)	4.09[b]	5.19[ab]	6.18[a]	0.03	0.6
Total N excretion, (g/d)	9.12[c]	12.81[b]	17.43[a]	< 0.00	2.4
Relative reduced of N excretion, (%)	47.68[a]	26.51[b]	100[c]	< 0.00	4.37
N Retained, (g/d)	18.39[b]	21.22[a]	22.52[a]	0.01	1.22
N net utilization, (%)	66.85[a]	62.36[ab]	56.37[b]	0.05	3.04
Apparent N digestibility, (%)	85.13	84.75	84.53	0.98	0.18
Apparent biological value, (%)	78.52[a]	73.58[ab]	66.69[b]	0.01	3.43
Finishing pigs (Experiment 3[8])					
N intake, (g/d)	47.95[c]	60.82[b]	74.42[a]	< 0.00	7.64
Urinary N excretion, (g/d)	9.15[c]	15.42[b]	20.97[a]	< 0.00	3.41
Fecal N excretion, (g/d)	5.94[b]	9.04[a]	11.22[a]	0.00	1.53
Total N excretion, (g/d)	15.09[c]	24.46[b]	32.19[a]	< 0.00	4.94
Relative reduced of N excretion, (%)	53.12	24.01	100	< 0.00	8.01
N Retained, (g/d)	32.86[b]	36.36[b]	42.23[a]	0.00	2.73
N net utilization, (%)	68.53[a]	59.78[b]	56.74[b]	0.00	3.53
Apparent N digestibility, (%)	87.61	85.14	84.92	0.48	0.86

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Apparent biological value, (%)	78.22^{a}	70.22^{ab}	66.82^{b}	0.02	3.38

Data are means with the pooled means ± standard error of the mean (SEM), n = 6/treatment group. Within a row, values with different superscript letters differ (P < 0.05). The average initial weight for the four periods was showed in Table 4. Each period was 7 d (28 d total). 1Total N excretion = urinary N excretion + fecal N excretion; 2Relative reduced of N excretion = N excretion of Group A (or Group B) / N excretion of Group C × 100, the N excretion value of Group C (normal crude protein (CP) level) was set to 100%; 3N Retained = N intake - N output; 4Apparent N digestibility = (N absorbed (N absorbed = N intake - fecal N)×100)/ N intake; 5 Apparent biological value = (N intake- total N excretion)/(N intake- fecal N excretion); 6 Experiment 1: Group A = 14% CP (very low CP level); Group B = 17% CP (low CP level); Group C = 20% CP (normal CP level). Groups A and B were supplemented with L-lysine, L-methionine, L-threonine, and L-tryptophan (see Table 1). 7 Experiment 2: Group A = 12% CP (very low CP level); Group B = 15% CP (low CP level); Group C = 18% CP (normal CP level). Groups A and B were supplemented with L-lysine, L-methionine, L-threonine, and L-tryptophan (see Table 2). 8 Experiment 3: Group A = 10% CP (very low CP level); Group B = 13% CP (low CP level); Group C = 16% CP (normal CP level). Groups A and B were supplemented with L-lysine, L-methionine, L-threonine, and L-tryptophan (see Table 3). [9] N net utilization = N Retained/N intake × 100.

3.4 Dynamic changes affect gene expression levels of digestive enzymes

In Experiment 1, pigs fed the 14% CP diet showed higher mRNA levels for pancreatic carboxypeptidase B1 (P < 0.01), chymotrypsin C (P < 0.01), and jejunal dipeptidase-II (P < 0.05) compared to pigs fed the 20% CP diet (Table 8). There were no differences in the expression levels of pancreatic carboxypeptidase B1, chymotrypsin C, or jejunal dipeptidase-II between the 14 and 17% CP diet groups. The mRNA expression levels of trypsinogen, pancreatic α-amylase 2A, duodenal enterokinase, chymotrypsin B, pancreatic α-amylase 2B, jejunal maltase, and jejunal sucrose did not differ among the three groups of growing pigs (P > 0.05) (Table 8).

In Experiment 2, pigs fed the 12% CP diet showed higher mRNA expression levels of duodenal enterokinase (P < 0.05), chymotrypsin B (P < 0.01), and chymotrypsin C (P < 0.01) compared to pigs fed the 18% CP diet. The lowest mRNA expression levels of trypsinogen (P < 0.05) and jejunal dipeptidase-II (P < 0.05) occurred in pigs fed the 12% CP diet (Table 8). The mRNA expression levels of pancreatic α-amylase 2B, pancrelipase, jejunal maltase, and jejunal sucrose did not differ among three groups of growing pigs (P > 0.05).

In Experiment 3, pigs fed the 10% CP diet showed lower mRNA expression levels of trypsinogen (P < 0.05), chymotrypsin C (P < 0.05), and jejunal dipeptidase-II (P < 0.05) compared to pigs fed the 16% CP diet. The lowest mRNA expression levels of trypsinogen (P < 0.05) occurred in pigs fed the 10% CP diet. However, the highest value for chymotrypsin B (P < 0.05) occurred in pigs fed the 10% CP diet (Table 8). The mRNA expression levels of pancreatic α-amylase 2A, pancreatic carboxypeptidase B1, duodenal enterokinase, pancreatic α-amylase 2B, pancrelipase, jejunal maltase, and jejunal sucrose did not differ among the three groups of finishing pigs (P > 0.05).

Table 7. Dynamics alterations of low-protein diets affect ileal digestibilities of DE, DM, and IDAA of growing and finishing pigs.

Items	Group A	Group B	Group C	P value	SEM ±
<u>Growing pigs (Experiment 1[1])</u>					
Energy	86.08	84.02	83.24	0.21	0.93
DM[2]	80.4	82.4	81.1	0.20	0.89
Protein	92.51[a]	88.86[ab]	85.03[b]	0.037	2.11
Arg	88.02[a]	86.63[a]	80.34[b]	<0.001	2.34
His	83.28[a]	78.11[b]	78.22[b]	0.02	1.68
Ile	84.56[a]	82.17[ab]	77.18[b]	0.01	1.88
Leu	83.78	80.26	80.73	0.30	2.41
Lys	87.70[a]	82.23[a]	77.45[b]	<0.001	3.54
Met	86.89[a]	82.79[ab]	80.25[b]	0.03	1.53
Cys	84.03	83.68	80.22	0.14	1.47
Phe	83.87[a]	81.56[ab]	77.78[b]	0.04	2.79
Tyr	87.28	85.75	84.79	0.51	2.19
Thr	80.53[a]	77.15[ab]	74.58[b]	0.04	3.08
Trp	80.28[a]	77.28[ab]	73.57[b]	0.02	1.86
Val	79.19[a]	72.36[b]	72.54[b]	0.02	2.46
Ala	82.76	80.08	80.19	0.31	0.84
Asp	80.63[a]	79.67[ab]	74.27[b]	0.02	2.77
Glu	87.59[a]	86.71[a]	80.64[b]	0.02	1.97
Gly	77.61	76.48	74.40	0.54	1.06
Pro	76.42[a]	72.38[ab]	70.89[b]	0.04	1.73
Ser	82.21[a]	79.77[ab]	75.52[b]	0.01	3.73
<u>Growing pigs (Experiment 2[3])</u>					

Energy	85.62	86.82	85.23	0.69	0.48
DM	84.43	83.78	82.15	0.31	0.68
Protein	80.15[a]	78.41[ab]	76.53[b]	0.05	1.05
Arg	85.36	85.65	84.27	0.78	0.42
His	75.68[a]	73.73[ab]	70.24[b]	0.02	1.59
Ile	73.72a	70.37b	69.66b	0.01	1.25
Leu	73.38	72.25	71.34	0.58	0.59
Lys	79.47[a]	75.26[ab]	72.32[b]	0.01	2.07
Met	80.42[a]	77.13[a]	70.48[b]	<0.001	2.92
Cys	81.63[a]	77.39[ab]	72.36[b]	<0.001	2.68
Phe	79.38[a]	78.74[ab]	73.79[b]	0.03	1.77
Tyr	78.71	78.24	76.97	0.72	0.52
Thr	79.35[a]	75.2[ab]	73.98[b]	0.02	1.63
Trp	78.85[a]	75.27[ab]	73.24[b]	0.01	1.64
Val	74.83	73.68	72.94	0.66	0.55
Ala	72.15	71.73	70.52	0.74	0.49
Asp	84.97	85.14	82.76	0.53	0.77
Glu	87.24	86.73	86.15	0.88	0.31
Gly	78.36[a]	75.21[ab]	73.19[b]	0.02	1.50
Pro	75.24	75.15	74.84	0.97	0.12
Ser	77.69[a]	75.23[ab]	73.97[b]	0.03	1.09
Finishing pigs (Experiment 3[4])					
Energy	86.58	84.12	83.64	0.20	0.91
DM	80.26	82.17	81.25	0.46	1.16
Protein	81.74[a]	80.36[a]	76.95[b]	0.005	1.42
Arg	88.52[a]	86.83[a]	80.64[b]	0.01	2.40
His	83.39[a]	78.23[b]	78.32[b]	0.02	1.71
Ile	84.78[a]	82.26[ab]	78.21[b]	0.01	1.91
Leu	83.89	80.37	80.89	0.29	2.09
Lys	87.91[a]	83.46[a]	77.54[b]	<0.001	3.60
Met	87.73[a]	83.66[ab]	80.1[b]	0.04	1.64
Cys	84.43	83.98	80.26	0.14	1.32
Phe	83.91[a]	81.97[ab]	77.95[b]	0.03	2.89
Tyr	87.54	85.85	84.86	0.40	2.24
Thr	80.57[a]	77.27[ab]	74.64[b]	0.03	3.62
Trp	80.46[a]	77.12[ab]	73.63[b]	0.01	1.97
Val	79.36[a]	72.58[b]	72.28[b]	0.01	2.31
Ala	82.87	80.14	80.23	0.26	0.90
Asp	80.96[a]	78.52[ab]	74.22[b]	0.02	2.82
Glu	87.67[a]	86.68[a]	80.88[b]	0.01	2.12
Gly	77.52	76.54	74.43	0.52	0.91
Pro	76.3[a]	72.47[ab]	70.93[b]	0.04	1.60
Ser	82.1[a]	79.89[ab]	75.72[b]	0.01	3.64

Data are means with the pooled means ± standard error of the mean (SEM), n = 6/treatment group. Within a row, values with different superscript letters differ ($P < 0.05$). The average initial weight for the four periods was showed in Table 4. [2] DM = dry matter; CP= crude protein; IDAA = ileal digesta of amino acids; [1] Experiment 1: Group A = 14% CP (very low CP level); Group B = 17% CP (low CP level); Group C = 20% CP (normal CP level). Groups A and B were supplemented with L-lysine, L-methionine, L-threonine, and L-tryptophan (see Table 1). [3] Experiment 2: Group A = 12% CP (very low CP level); Group B = 15% CP (low CP level); Group C = 18% CP (normal CP level). Groups A and B were supplemented with L-lysine, L-methionine, L-threonine, and L-tryptophan (see Table 2). [4] Experiment 3: Group A = 10% CP (very low CP level); Group B = 13% CP (low CP level); Group C = 16%

CP (normal CP level). Groups A and B were supplemented with L-lysine, L-methionine, L-threonine, and L-tryptophan (see Table 3).

Table 8. Dynamics alterations of low-protein diets affect gene expression levels of digestive enzymes from growing and finishing pigs.

Items	Group A	Group B	Group C	*P* value	SEM ±
Growing pigs (Experiment 1[1])					
Trypsinogen	1.18	1.31	1.00	0.35	0.09
PA2A[2]	1.29	1.35	1.00	0.07	0.11
PCPB15	1.36[a]	1.31[a]	1.00[b]	0.005	0.11
Duodenal enterokinase	0.91	0.94	1.00	0.64	0.03
Chymotrypsin B	0.78	0.74	1.00	0.12	0.08
Chymotrypsin C	0.79[b]	0.85[ab]	1.00[a]	0.04	0.06
Pancreatic α-amylase 2B	1.15	1.12	1.00	0.34	0.05
Pancrelipase	0.81	0.77	1.00	0.06	0.07
Jejunal Maltase	1.01	1.00	1.00	0.99	0.00
Jejunal Sucrase	1.13	1.05	1.00	0.58	0.04
Jejunal Dipeptidase-II	1.23[a]	1.19[ab]	1.00[b]	0.04	0.07
Growing pigs (Experiment 2[3])					
Trypsinogen	0.72[a]	1.06[b]	1.00[b]	<0.001	0.23
PA2A2	0.89[a]	1.12[ab]	1.00[b]	0.06	0.12
PCPB15	0.90	1.11	1.00[b]	0.10	0.11
Duodenal enterokinase	1.65[a]	1.12[b]	1.00[b]	0.01	0.12
Chymotrypsin B	1.75[a]	1.25[b]	1.00[b]	0.005	0.11
Chymotrypsin C	1.78[a]	1.24[b]	1.00[b]	<0.001	0.13
Pancreatic α-amylase 2B	1.15	1.33	1.00	0.38	0.06
Pancrelipase	1.14	1.02	1.00	0.44	0.05
Jejunal Maltase	1.05	0.88	1.00	0.23	0.11
Jejunal Sucrase	1.14	0.91	1.00	0.14	0.08
Jejunal Dipeptidase-II	0.88[a]	1.08[ab]	1.00[b]	0.02	0.13
Finishing pigs (Experiment 3[4])					
Trypsinogen	0.76[a]	0.95[b]	1.00[b]	0.01	0.08
PA2A2	0.78	1.04	1.00	0.11	0.10
PCPB15	0.77	1.03	1.00[b]	0.13	0.11
Duodenal enterokinase	1.12	0.87	1.00	0.09	0.07
Chymotrypsin B	1.29[a]	1.01[ab]	1.00[b]	0.04	0.07
Chymotrypsin C	0.79[b]	0.85[ab]	1.00[a]	0.04	0.06
Pancreatic α-amylase 2B	0.82	1.00	1.00	0.24	0.07
Pancrelipase	1.02	0.86	1.00	0.34	0.14
Jejunal Maltase	0.89	0.94	1.00	0.77	0.29
Jejunal Sucrase	0.99	0.92	1.00	0.81	0.54
Jejunal Dipeptidase-II	0.83[b]	1.00[a]	1.00[a]	0.05	0.07

Data are means with the pooled means ± standard error of the mean (SEM), n = 6/treatment group. Within a row, values with different superscript letters differ ($P < 0.05$). The average initial weight for the four periods was showed in Table 4. [2]PA2A = pancreatic α-amylase 2A; 5PCPB1 = pancreatic carboxypeptidase B1; CP= crude protein; IDAA = ileal digesta of amino acids; [1] Experiment 1: Group A = 14% CP (very low CP level); Group B = 17% CP (low CP level); Group C = 20% CP (normal CP level). Groups A and B were supplemented with L-lysine, L-methionine, L-threonine, and L-tryptophan (see Table 1). [3] Experiment 2: Group A = 12% CP (very low CP level); Group B = 15% CP (low CP level); Group C = 18% CP (normal CP level). Groups A and B were supplemented with L-lysine, L-methionine, L-threonine, and L-tryptophan (see Table 2). [4] Experiment 3: Group A = 10% CP (very low CP level); Group B = 13% CP (low CP level); Group C = 16% CP (normal CP level). Groups

A and B were supplemented with L-lysine, L-methionine, L-threonine, and L-tryptophan (see Table 3).

4. Discussion

The results of this study support the concept that a substantial reduction of three percentage units of dietary CP, while maintaining adequate supplies of essential AA allowed for a significant decrease in total N excretion without negative effect on ADG, ADFI or F: G. The supplementary essential AA were L-lysine, L-methionine, L-threonine and L-tryptophan. However, maximal growth performance and ADFI in different stages of pigs depended on adequate provision of AA that can be synthesized by pigs [24,25]. In the present study, we sought to evaluate the dynamic effects of a 3- or 6-percentage unit reduction in dietary protein, while supplementing the diets with essential AA on weight gain and digestibility in pigs. The results indicated that a reduction in dietary CP by 3-percentage units with a concomitant addition of essential AA would support similar growth performance and feed efficiency from Experiment 1 to Experiment 3. However, a substantial reduction on in dietary CP by 6-percentage units had a negative impact on growth performance in Experiment 1 and Experiment 2, but there was a slight enhancement in growth performance in Experiment 3. Similar results were obtained by some other researchers [9,11,26], and the results were not consist with consistent with previous results that showed finishing pigs fed the 16% CP diet had higher ($P < 0.01$) final BW compared to pigs those fed the 10% CP diet [2]. In addition to this result, the reduction in total N excretion was mainly caused by a reduction in urinary excretion. In our experiment, reducing the dietary CP level from 20 to 14% decreased urinary N excretion by 52.8% ($P < 0.001$) in Experiment 1. Reducing the dietary CP level from 18 to 12% decreased urinary N excretion by 55.3% ($P < 0.001$) and fecal N excretion by 34% ($P < 0.05$) in Experiment 2. Reducing the dietary CP level from 10 to 16% decreased urinary N excretion by 56.4% ($P < 0.001$) and fecal N excretion by 47.1% ($P < 0.001$) in Experiment 3 (Table 6). This finding was not consistent with previous results showing there was a decrease in urinary N but not in fecal N excretion when lowering dietary CP from 16.9-15.6 to 14.6-13.5% for growing and finishing pigs [27]. The outcomes were consistent with previous results showing that a decrease in urinary N by 65% and fecal N excretion by 31% occurred when dietary CP was lowered from 20 to 12% for fattening pigs [28]. A possible explanation for discrepancies between this study and our present study could be that the effects of growth performance and N balance depend on the pig species, different diet formulations and different stages.

The results for the ileal digestibility of CP and certain AA in diets used for the 3 stages in the present study differed significantly. A reduction of 6-percentage units of dietary CP produced the highest ileal digestibility for CP and AA (Table 7). This finding is consistent with previous results [2,29]. The CP and AA ileal digestibility mainly depend on relative increases in the rates of protein digestion and absorption of the resulting products by the small intestine. Several previous studies also support the concept that synthetic crystalline AA (including essential AA) are fully available to the small intestine, whereas not all AA in dietary proteins are released by digestive proteases in the gut lumen [2,30-32]. In the present study, a reduction of 3-percentage units and supplementation with adequate amounts of AA just met the nutritional requirements for pigs. However, when dietary CP is reduced by 6-percentage units and supplemented with adequate amounts of AA, growth performance and feed efficiency are affected in growing pigs and finishing pigs, even though the AID of AA improved.

Some studies on pigs have shown that the activity of the digestive enzymes and their response to different dietary formulations evaluate how effectively a given diet can promote animal growth [33,34]. The digestion of dietary CP and carbohydrates in pigs depends on different kinds of digestive enzymes in the gastrointestinal tract, such as trypsinogen, pancreatic α-amylase 2A, pancreatic carboxypeptidase B1, duodenal enterokinase, chymotrypsin B, chymotrypsin C, pancreatic α-amylase 2B, pancrelipase, jejunal maltase, jejunal sucrose, and jejunal dipeptidase-II. In the present study, pigs fed the very low (14%, 12%, and 10% CP) diets showed higher mRNA levels for chymotrypsin C ($P < 0.01$ in Experiment 1 and 2; $P < 0.05$ in Experiment 3) compared to pigs fed the normal (20%, 18%, and 16% CP) diets among the three experiments. Thus, our results indicate that dynamic intestinal expression of the genes for protein digestion is reduced in response to two levels of low-protein diets. We also observed an increase in intestinal mRNA levels for chymotrypsin C in Experiment 1 and Experiment 3 after animals were fed low-protein diets. However, this value decreased in Experiment 2. This finding is not consistent with previous results showing that an increase in intestinal mRNA levels for chymotrypsins B and C as well as duodenal enterokinase in growing and finishing pigs that were fed low-protein diets [2]. A possible explanation for the discrepancies between the previous study and our present study could be that the mRNA levels for digestive enzymes depend on the pig species and that different stages are subjected to complex regulation at both transcriptional and translational levels by a series of factors, including the content and balance of dietary AA. Future studies are needed to determine the protein abundance of digestive enzymes in pigs fed low-protein and adequate CP diets.

5. Conclusions

In conclusion, two levels of low-protein diets supplemented with essential AA can dynamically affect N balance, digestive enzymes and ileal AA digestibility from growing pigs to finishing pigs, and there were gains in weight and feed efficiency in 30-45 kg (Experiment 2) and 45-80 kg (Experiment 3) pigs when dietary CP was reduced by 3-percentage units. Low-protein diets supplemented with nutritionally essential AA alone cannot maintain weight gains or feed efficiency in growing pigs alone (Experiment 1). These novel findings have potential implications for the development of methods to ameliorate dietary protein shortage and N-triggered environmental pollution from swine production.

Supplementary Materials: The following are available online at www.mdpi.com/link, Figure S1: title, Table S1: title, Video S1: title.

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