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Non Ruminant Nutrition

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Effect of dietary crude protein level on growth performance, blood characteristics, and indicators of intestinal health in weanling pigs

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Abstract

An experiment was conducted to test the hypothesis that reducing crude protein (CP) in starter diets for pigs reduces post-weaning diarrhea and improves intestinal health. In total, 180 weanling pigs were allotted to 3 diets containing 22, 19, or 16% CP. Fecal scores were visually assessed every other day. Blood samples were collected from 1 pig per pen on days 1, 6, 13, 20, and 27, and 1 pig per pen was euthanized on day 12. Results indicated that reducing dietary CP reduced ($P < 0.01$) overall average daily gain, gain to feed ratio, final body weight, and fecal scores of pigs. Pigs fed the 16% CP diet had reduced ($P < 0.01$) serum albumin compared with pigs fed other diets. Blood urea nitrogen, haptoglobin, interleukin-1β, and interleukin-6 concentrations in serum were greatest ($P < 0.01$) on day 13, whereas tumor necrosis factor-α and interleukin-10 concentrations were greatest ($P < 0.01$) on day 6. Villus height in the jejunum increased ($P < 0.05$) and crypt depth in the ileum was reduced ($P < 0.01$) if the 19% CP diet was fed to pigs compared with the 22% CP diet. A reduction ($P < 0.05$) in mRNA abundance of interferon-γ, chemokine ligand 10, occludin, trefoil factor-2, trefoil factor-3, and mucin 2 was observed when pigs were fed diets with 16% CP. In conclusion, reducing CP in diets for weanling pigs reduces fecal score and expression of genes associated with inflammation.

Key words: blood characteristics, crude protein, fecal score, gene expression, growth performance, pigs

Introduction

Post-weaning diarrhea is one of the contributing causes to reduced growth performance and increased mortality in weanling pigs (Pluske et al., 1997; Rhouma et al., 2017). Diets with high concentration of crude protein (**CP**; i.e., 21% to 24%) may increase microbial colonization and fermentation due to the undigested protein that pass through the gastrointestinal tract (Diether and Willing, 2019). The undigested protein from high CP diets may subsequently increase intestinal permeability and favor proliferation of pathogenic bacteria resulting in increased incidence of diarrhea in weaned pigs (Moeser et al., 2017; Gao et al., 2019). High CP diets also have high buffering capacity, which may increase intestinal pH that favors an ideal environment for pathogen propagation (Partanen and Mroz, 1999). Therefore, reducing the concentration of CP in diets for nursery pigs is practiced to reduce post-weaning diarrhea and

Abbreviations

AA	amino acid
ADFI	average daily feed intake
ADG	average daily gain
BUN	blood urea nitrogen
CP	crude protein
G:F	gain to feed ratio
NH3	ammonia
VFA	volatile fatty acid

mortality (Stein and Kil, 2006). Opapeju et al. (2008) demonstrated that reduced levels of CP in the diet reduces the concentration of ammonia (NH_3) in the intestinal lumen, which was correlated with reduced fecal score.

The concern associated with the low CP approach is reduction in pig growth performance (Nyachoti et al., 2006; Wellock et al., 2006; 2008; Opapeju et al., 2008; Yue and Qiao, 2008; Lynegaard et al., 2021). By reducing dietary CP in the initial diets provided post-weaning, pig growth performance may be reduced, but it is possible that this reduction will be compensated by an improved intestinal health (Stein and Kil, 2006; Kil and Stein 2010). However, data demonstrating this are limited. Therefore, an experiment was conducted to test the hypothesis that reducing the concentration of dietary CP, either while meeting the amino acid (**AA**) requirement or not meeting AA requirement, reduces post-weaning diarrhea and improves intestinal health of weanling pigs.

Materials and Methods

The protocol for the experiment was approved by the Institutional Animal Care and Use Committee at the University of Illinois prior to initiation of the experiment. Pigs that were the offspring of Line 359 boars and Camborough females (Pig Improvement Company, Hendersonville, TN) were used.

Animals and treatments

In total, 180 weanling pigs (20 ± 2 d old; initial body weight: 5.53 ± 0.88 kg) were allotted to 1 of 3 dietary treatments. There were 5 pigs per pen (3 gilts and 2 castrates) and 12 replicate pens per treatment. The average barn temperature for the entire experimental period was 28.5 °C. The experiment was conducted for 28 d. A 2-phase feeding program was used with day 1 to day 7 as phase 1 and day 8 to day 28 as phase 2. Pigs were fed 1 of 3 experimental diets during phases 1 and 2; therefore, a total of 6 diets were formulated. The 3 dietary treatments were primarily consisted of corn, soybean meal, and fish meal (Table 1). Treatments were as follows: 1) high protein (22% CP) with adequate AA; 2) low protein (19% CP) with adequate AA; and 3) low protein (16% CP) with AA being provided below the requirement (Table 2). Via the use of crystalline AA, both the 22% and 19% CP diets provided AA to meet the requirement (NRC, 2012). However, not all AA requirements were met for the 16% CP diet. The concentration of standardized ileal digestible Phe, which was the first limiting AA in the phase 1 diet containing 16% CP, was 70% of the NRC (2012) requirement. The first limiting AA in the phase 2 diet was His, which was included at 80% of the NRC (2012) requirement. As a consequence, concentrations of all indispensable AA in the 16% CP Phase 1 and Phase 2 diets were provided to meet at least 70% and 80% of the requirement, respectively (NRC, 2012).

Table 1. Analyzed nutrient composition of corn, soybean meal, and fish meal, as-fed basis

Item	Corn	Soybean meal	Fish meal
Dry matter, %	85.89	87.54	91.39
Gross energy, kcal/kg	3,868	4,196	4,384
Crude protein, %	6.49	46.03	65.16
Acid-hydrolyzed ether extract, %	2.97	1.46	6.93
Ash, %	1.13	6.23	19.30
Indispensable amino acids, %			
Arg	0.33	3.37	3.57
His	0.20	1.22	1.34
Ile	0.25	2.26	2.60
Leu	0.75	3.59	4.28
Lys	0.25	2.95	4.32
Met	0.16	0.68	1.67
Phe	0.32	2.36	2.44
Thr	0.24	1.79	2.39
Trp	0.05	0.65	0.55
Val	0.33	2.30	2.98
Dispensable amino acids, %			
Ala	0.48	2.01	3.93
Asp	0.47	5.29	5.40
Cys	0.16	0.65	0.47
Glu	1.17	8.46	7.94
Gly	0.28	1.97	4.54
Pro	0.58	2.28	2.79
Ser	0.30	2.07	1.99
Tyr	0.20	1.72	1.88

Experimental procedures

Individual pig weights were recorded at the beginning of the experiment, and on days 7, 14, 21, and 28. Feed addition was recorded daily and the weight of feed left in the feeder was recorded on days 7, 14, 21, and 28. During the experiment, fecal scores were assessed visually every other day using a subjective score ranging from 1 to 5 according to the method of Espinosa et al. (2017): 1 = normal feces; 2 = moist feces; 3 = mild diarrhea; 4 = severe diarrhea; and 5 = watery diarrhea. At the conclusion of the experiment, data were summarized to calculate average daily feed intake (ADFI), average daily gain (ADG), and gain to feed ratio (G:F) within each pen and treatment group. Data were summarized for day 1 to day 7, day 8 to day 14, day 15 to day 21, day 22 to day 28, and for the entire experiment.

Sample collection

On day 1, a blood sample was collected from one pig in each pen. Within each treatment, the gilt with the body weight closest to the pen average was used in 6 of the pens, and the barrow with the body weight closest to the pen average was used in the other 6 pens. Blood was collected via vena puncture, and a blood sample was also collected on days 6, 13, 20, and 27 from the same pigs. Blood was collected in vacutainers without anticoagulant and serum was obtained by centrifuging blood samples at 4,000 × *g* at 4 °C for 13 min. Samples were stored at -20 °C until analyzed. Fecal samples from the pigs used for blood sampling were collected on days 13, 20, and 27, and these

Table 2. Composition of phase 1 and 2 experimental diets[1]

	Phase 1			Phase 2		
Item	22% CP	19% CP	16% CP[2]	22% CP	19% CP	16% CP[2]
Ingredient, %						
Ground corn	44.28	51.45	59.84	51.26	57.78	65.46
Soybean meal	21.00	13.00	5.50	28.50	21.50	14.00
Fish meal[3]	6.00	6.00	6.00	5.00	5.00	5.00
Blood plasma[3]	3.50	3.50	3.50	-	-	-
Dried whey[3]	20.00	20.00	20.00	10.00	10.00	10.00
Soybean oil	3.00	3.00	3.00	3.00	3.00	3.00
Limestone	1.05	1.12	1.20	1.15	1.20	1.28
L-Lys HCL, 78% Lys	0.32	0.57	0.24	0.29	0.51	0.41
DL-Met, 98% Met	0.12	0.19	0.04	0.09	0.15	0.07
L-Thr, 99% Thr	0.08	0.19	0.03	0.06	0.16	0.11
L-Trp, 99% Trp	-	0.04	-	-	0.02	0.02
L-Ile, 99 % Ile	-	0.08	-	-	-	-
L-Phe, 99% Phe	-	0.13	-	-	0.01	-
L-His, 99% His	-	0.08	-	-	0.02	-
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin–mineral premix[4]	0.15	0.15	0.15	0.15	0.15	0.15
Analyzed values						
Dry matter, %	88.82	88.28	88.20	87.92	87.88	87.86
Gross energy, kcal/kg	4,076	4,032	3,989	4,053	4,046	4,022
Crude protein, %	21.99	19.13	15.27	21.74	18.75	16.18
Acid-hydrolyzed ether extract, %	5.24	4.97	5.29	5.12	5.33	5.80
Ash, %	6.23	6.26	5.96	5.64	5.61	5.51
Indispensable amino acids, %						
Arg	1.26	0.94	0.83	1.34	1.07	0.91
His	0.54	0.51	0.39	0.53	0.46	0.39
Ile	0.96	0.82	0.67	0.94	0.79	0.70
Leu	1.81	1.53	1.38	1.72	1.49	1.34
Lys	1.61	1.53	1.17	1.48	1.42	1.20
Met	0.53	0.51	0.32	0.46	0.45	0.39
Phe	1.02	0.92	0.72	1.01	0.85	0.73
Thr	1.03	0.94	0.74	0.87	0.83	0.71
Trp	0.26	0.26	0.18	0.23	0.22	0.18
Val	1.11	1.01	0.81	1.01	0.94	0.79
Dispensable amino acids, %						
Ala	1.10	0.94	0.87	1.06	0.95	0.86
Asp	2.14	1.65	1.44	2.12	1.73	1.48
Cys	0.37	0.31	0.33	0.31	0.26	0.25
Glu	3.52	2.80	2.43	3.58	2.98	2.58
Gly	0.94	0.76	0.69	0.95	0.83	0.72
Pro	1.18	0.98	0.92	1.16	1.01	0.92
Ser	0.93	0.75	0.68	0.91	0.75	0.63
Tyr	0.72	0.59	0.53	0.71	0.61	0.52

[1]Phase 1 diets were formulated to contain 0.85% Ca, 0.40% standardized total tract digestible P, 0.56% Na, and 0.80% Cl. Phase 2 diets were formulated to contain 0.80% Ca, 0.37% standardized total tract digestible P, 0.37% Na, and 0.63% Cl.
[2]Phenylalanine was the first limiting AA in the Phase 1 diet with 16% CP at 70% of requirement. Histidine was the first limiting AA in the Phase 2 diet with 16% CP at 80% of requirement.
[3]Fish meal = Menhaden Select; Omega Protein, Houston, TX; Blood plasma = Appetein; APC Inc., Ankeny, IA; Dried whey = Land O'Lakes, Inc., Arden Hills, MN.
[4]Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,150 IU; vitamin D3 as cholecalciferol, 2,210 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 125 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

samples were analyzed for fecal NH_3 and volatile fatty acids (VFA). For VFA and NH_3 analysis, approximately 5 g of feces were collected. After collection, fecal samples were placed in 15 mL tubes and samples were stabilized in 2*N* HCl and stored at -20 °C until analyzed.

On day 12, the weight of all pigs was recorded, and 1 pig per pen (6 gilts and 6 barrows per treatment) was euthanized via captive bolt stunning. The pig to be euthanized was the pig of the specified sex that had a body weight closest to the pen average with the exception that the pig that had been used

for blood and fecal collections was not sacrificed. Samples from the cecum and colon were collected to determine concentrations of NH_3 and VFA. All cecal and colonic contents from each pig were collected and mixed, and approximately 5 g was subsampled and stored for analysis. The same sampling, stabilizing, and storage techniques were used for the contents of cecum and colon as for the fecal samples. The pH was measured twice *in-situ* by making a small incision for the pH electrode to penetrate in the stomach, duodenum, and ileum. The pH was measured immediately using a Benchtop pH meter (Orion Star A111, Fisher Scientific, Waltham, MA). Ileal mucosa samples that were collected 30 cm anterior to the ileocecal valve were scraped gently, snap frozen in liquid N, and stored at -80 °C until used for gene expression analysis. Jejunum and ileum samples between 2 and 3 cm long were collected approximately 2 m from the pylorus and 80 cm from the ileal–cecal junction, respectively. Samples were cut and pinned with the serosa side down on a piece of cardboard. Samples were then fixed in 10% neutral buffered formalin until processing for morphological evaluation and immunohistochemistry staining.

Chemical analyses

All ingredient and diet samples were ground through a 1-mm screen in a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ) prior to chemical analysis. Diets and ingredients were analyzed for dry matter (Method 930.15; AOAC Int., 2007) and ash (Method 942.05; AOAC Int., 2007), and gross energy was determined using bomb calorimetry (Model 6400; Parr Instruments, Moline, IL). Diets and ingredients were analyzed for acid-hydrolyzed ether extract by acid hydrolysis using 3*N* HCl (Ankom HCl Hydrolysis System, Ankom Technology, Macedon, NY) followed by fat extraction (Ankom XT-15 Extractor, Ankom Technology, Macedon, NY), and N was determined using the combustion procedure (Method 990.03; AOAC Int., 2007) on an FP628 N analyzer (Leco Corporation, St. Joseph, MI). Crude protein was calculated as N × 6.25. Amino acids were analyzed on a Hitachi amino acid analyzer (Model No. L8800; Hitachi High Technologies America, Inc., Pleasanton, CA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard [Method 982.30 E (a, b, c); AOAC Int., 2007].

Blood profile analyses

Serum samples were analyzed for blood urea nitrogen (BUN), total protein, and albumin using a Beckman Coulter Clinical Chemistry AU analyzer (Beckman Coulter, Inc., Brea, CA). Vitamins A and E in blood samples were also analyzed using a high-performance liquid chromatography unit coupled with fluorescence detection (Aebischer et al., 1999). Serum samples were analyzed for interleukin 1-beta (IL-1ß), interleukin 6 (IL-6), interleukin 10 (IL-10), and tumor necrosis factor-α (TNF-α) using individual enzyme-linked immunoabsorbent assay kits (R&D Systems, Inc., Minneapolis, MN). Serum samples were also analyzed for immunoglobulin G, peptide YY, and haptoglobin using enzyme-linked immunoabsorbent assay kits (Bethyl Laboratories Inc., Montgomery, TX; Phoenix Pharmaceuticals Inc., Burlingame, CA; GenWay Biotech Inc., San Diego, CA, respectively).

Intestinal morphology

Each jejunal and ileal sample was cut in 2- to 3-mm thick cross-sections and embedded in paraffin for slide preparation after fixation. From each sample, 3 to 4 transverse sections were selected and stained with hematoxylin and eosin. Slides were scanned using a 17-megapixel Canon Rebel camera mounted to a Meiji 5300 (Veterinary Diagnostic Pathology, LLC, Fort Valley, VA). Ten villi and the associated crypts were measured using the NIH Image J analysis software. Villus height was measured from the villus tip to the crypt mouth and the crypts were measured from the crypt mouth to the top of the crypt valley.

Volatile fatty acid and NH_3 analyses

Concentrations of VFA (i.e., acetate, propionate, butyrate, valerate, isovalerate, and isobutyrate) and NH_3 in samples from cecum, colon, and feces were analyzed according to the method of Erwin et al. (1961) using a Hewlett-Packard (Hewlett Packard, Avondale, PA) Model 5890A gas chromatograph equipped with a flame ionization detector on a column (1.8 m × 4 mm i.d.) packed with GP 10% SP-1200/1% H3P04 on 80/100 chromosorb W/AW (Chromosorb® W/AW-DMCS, Supelco, Bellefonte, PA). Nitrogen was the carrier gas used with a flow rate of 45 mL/min. The oven, injection port, and detector port temperatures were 125, 175, and 180 °C, respectively.

Gene expression in ileal mucosa

Total RNA was extracted from 40 ± 0.2 mg of frozen ileal mucosa using a Qiagen RNeasy Mini Kit (Qiagen Inc., Germantown, MD) as per manufacturer's instructions. A portion of the RNA was diluted to 100 ng/μL with DNase/RNase-free water for cDNA synthesis using 2 μL of diluted RNA from ileal mucosa. The cDNA was then diluted with DNase/RNase-free water prior to quantitative polymerase chain reaction analysis. Quantitative polymerase chain reaction was performed using 4 μL of diluted cDNA combined with 6 μL of a mixture composed of 5 μL of SYBR Green master mix (PerfeCTa SYBR Green FastMix, ROX; Quanta BioSciences, Beverly, MA), 0.4 μL each of 10 μM forward and reverse primers, and 0.2 μL DNase/RNase free water in a MicroAmp Optical 384-Well Reaction Plate (Applied Biosystems, Foster City, CA). Two internal control genes, glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*; Gonzalez et al., 2013) and hypoxanthine-guanine phosphoribosyl transferase (*HPRT*; Nygard et al., 2007) were used to normalize the abundance of tested genes (Table 3). The geometric mean of the internal control genes was determined for the target normalization and relative abundance of specified genes. Abundance of the following pro-inflammatory genes were measured: *TNF-α*, *IL-1β*, *IL-6*, interleukin-8 *(IL-8)*, interleukin-21 (*IL-21*), interferon-gamma (*IFN-γ*), chemokine ligand 2 (*CXCL2*), chemokine ligand 9 (*CXCL9*), and chemokine ligand 10 (*CXCL10*). The following anti-inflammatory genes were also measured: interleukin-4 (*IL-4*), *IL-10*, interleukin 11 (*IL-11*), interleukin 13 (*IL-13*), and transforming growth factor-β (*TGF-β*). Gut-protective protein target genes were also measured: occludin (*OCLN*), claudin-1 (*CLDN-1*), zonula occludens-1 (*ZO-1*), trefoil factor-1 (*TFF-1*), trefoil factor-2 (*TFF-2*), trefoil factor-3 (*TFF-3*), and mucin 2 (*MUC2*). Glucose transporter 2 (*GLUT2*), glucose transporter 5 (*GLUT5*), and solute carrier family 15 member 1 (*SLC15A1*) were quantified as well.

Statistical analysis

Data were analyzed using the PROC MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with pen as the experimental unit. Normality of residuals and assumptions of the model were tested using PROC GPLOT and influence options of SAS (SAS Inst. Inc.,

Table 3. Forward and reverse primer sequences used for quantitative reverse transcription-polymerase chain reaction

Item[1]	Primer sequences (5′→3′)		Reference
	Forward	Reverse	
Internal control genes			
GAPDH	ATCCTGGGCTACACTGAGGAC	AAGTGGTCGTTGAGGGCAATG	Gonzalez et al., 2013
HPRT	GGACTTGAATCATGTTTGTG	CAGATGTTTCCAAACTCAAC	Nygard et al., 2007
Pro-inflammatory target genes			
TNF-α	AGCACTGAGAGCATGATCCG	GACATTGGCTACAACGTGGG	Ferrandis Vila et al., 2018
IL-1β	CCAATTCAGGGACCCTACC	CATGGCTGCTTCAGAAACCT	Lapthorne et al., 2015
IL-6	TGAACTCCCTCTCCACAAGC	GGCAGTAGCCATCACCAGA	Lapthorne et al., 2015
IL-8	AAGCTTGTCAATGGAAAAGAG	TGATTCTCATCAAGCAGGTCTCC	Petrov et al., 2014
IL-21	GGCACAGTGGCCCATAAATC	GCAGCAATTCAGGGTCCAAG	Kiros et al., 2011
IFN-γ	GCTTTTCAGCTTTGCGTGACT	TCACTCTCCTCTTTCCAATTCTTC	Ferrandis Vila et al., 2018
CXCL2	CCGAAGCTTGAATCCTCATC	TAGCAGCAGGTGACTGGAGA	Ondrackova et al., 2013
CXCL9	AGCAGTGTTGCCTTGCTTTTGGGTATCATC	GCTGGTGTTGATGCAGGAACAACGTCC	Ondrackova et al., 2013
CXCL10	CCCACATGTTGAGATCATTGC	CATCCTTATCAGTAGTGCCG	Ondrackova et al., 2013
Anti-inflammatory target genes			
IL-4	CCAACCCTGGTCTGCTTACTG	TTGTAAGGTGATGTCGCACTTGT	Sweeney et al., 2012
IL-10	CACTGCTCTATTGCCTGATCTTCC	AAACTCTTCACTGGGCCGAAG	Xun et al., 2015
IL-11	CAAATTCCCAGCTGACGGAGA	GTAGGAAAACAGGTCTGCTCG	Ferrandis Vila et al., 2018
IL-13	CTGACCACCAGCATGCAGTACT	GCTGCAGTCGGAGATGTTGA	Royaee et al., 2004
TGF-β	CACCCCAGATCCTCCTACCT	GTCAGCACTAGCAGCCACAG	Chen et al., 2018b
Gut-protective protein target genes			
OCLN	TCCTGGGTGTGATGGTGTTC	CGTAGAGTCCAGTCACCGCA	Hu et al., 2013
CLDN-1	AGAAGATGCGGATGGCTGTC	CCCAGAAGGCAGAGAGAAGC	Hu et al., 2013
ZO-1	AAGCCCTAAGTTCAATCACAATCT	ATCAAACTCAGGAGGCGGC	Hu et al., 2013
TFF-1	CCATGGAGCACAAGGTGA	AGGGTGGAAGCACCACGGGA	Scholven et al., 2009
TFF-2	CAAGAGTCTGAGGAGTGCGTCA	GACATGGGGAAGAAGCACC	Scholven et al., 2009
TFF-3	GGGAGTATGTGGGCCTGTC	AGGTGCATTCTGTTTCCTGC	Scholven et al., 2009
MUC2	GGCTGCTCATTGAGAGGAGT	ATGTTCCCGAACTCCAAGG	Ferrandis Vila et al., 2018
Miscellaneous target genes			
GLUT2	TTTTGGGTGTTCCGCTGGAT	GAGGCTAGCAGATGCCGTAG	Saqui-Salces et al., 2017
GLUT5	TGTGTGGCTCCTGGTAACAC	TCGGCCATGTTCGATTCCTT	Saqui-Salces et al., 2017
SLC15A1	CAGACTTCGACCACAACGGA	TTATCCCGCCAGTACCCAGA	Fiesel et al., 2014

[1]*GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; *HPRT*, hypoxanthine-guanine phosphoribosyl transferase; *TNF-α*, tumor necrosis factor-alpha; *IL-1β*, interleukin-1beta; *IL-6*, interleukin-6; *IL-8*, interleukin-8; *IL-21*, interleukin-21; *IFN-γ*, interferon- gamma; *CXCL2*, chemokine ligand 2; *CXCL9*, chemokine ligand 9; *CXCL10*, chemokine ligand 10; *IL-4*, interleukin-4; *IL-10*, interleukin-10; *IL-11*, interleukin-11; *IL-13*, interleukin-13; *TGF-β*, transforming growth factor-beta; *OCLN*, occludin; *CLDN-1*, claudin-1; *ZO-1*, zonula occludens-1; *TFF-1*, trefoil factor-1; *TFF-2*, trefoil factor-2; *TFF-3*, trefoil factor-3; *MUC2*, mucin 2; *GLUT2*, glucose transporter 2; *GLUT5*, glucose transporter 5; *SLC15A1*, solute carrier family 15 member 1.

Cary, NC). For growth performance, fecal scores, NH_3 and VFA production, and gene abundance analyses, the statistical model included diet as fixed effect, and replicate was considered a random effect. Treatment means were calculated and separated using the LSMEANS statement and the PDIFF option of PROC MIXED, respectively. For the blood profile analyses, results for all treatment groups were analyzed as repeated measures using the PROC MIXED of SAS (Littell et al., 1998). Fixed effects included day and diet, and the interaction between day and diet. Statistical significance and tendencies were considered at $P < 0.05$ and $0.05 < P < 0.10$, respectively.

Results

Growth performance and fecal score

No differences among treatments were observed for ADG, ADFI, G:F, and final body weight of pigs from day 1 to day 7 (Table 4). From day 8 to day 14, pigs fed the 16% CP diet tended ($P < 0.10$) to have reduced ADG and reduced ($P < 0.05$) G:F compared with the other treatments, which resulted in a tendency for a reduction ($P < 0.10$) in final body weight of pigs on day 14. Reducing dietary CP from 22% to 16% reduced ($P < 0.01$) the ADG, G:F, and final body weight of pigs from day 15 to day 21 and from day 22 to day 28. During the overall experimental period, pigs fed the 22% CP diet had greater ($P < 0.01$) ADG compared with pigs fed diets containing 19% or 16% CP. A reduction ($P < 0.01$) in overall G:F was also observed if pigs were fed the diet containing 16% CP.

A tendency for a reduction in fecal scores was observed if pigs were fed diets containing 16% CP from day 1 to day 7 (Table 5). Reducing the level of dietary CP from 22% to 16% CP also reduced ($P < 0.05$) the fecal scores in pigs from day 8 to day 14, but no differences in fecal scores were observed during the following 2 wk. Overall, fecal scores of pigs were reduced ($P < 0.01$) by reducing the level of dietary CP from 22 to 16%.

Blood characteristics

Reduction in CP had a greater effect on reducing BUN on day 20 and day 27 than on day 13 (dietary CP × day interaction, $P < 0.05$; Table 6). A tendency for an interaction ($P < 0.10$) between dietary CP and day was observed for the concentration of albumin where greater reduction in albumin concentration

Table 5. Fecal scores of pigs fed diets containing high, medium, and low levels of crude protein[1]

Item	Dietary crude protein level, %			SEM	P-value
	22	19	16		
Fecal score[2]					
Days 1 to 7	1.67[x]	1.50[xy]	1.25[y]	0.13	0.053
Days 8 to 14	1.67[a]	1.42[ab]	1.21[b]	0.12	0.037
Days 15 to 21	1.64	1.44	1.31	0.16	0.333
Days 22 to 28	1.17	1.15	1.04	0.06	0.283
Days 1 to 28	1.52[a]	1.36[ab]	1.19[b]	0.08	0.020

[1]Data are means of 10 to 12 observations per measurement.
[2]Fecal score = 1, normal feces; 2, moist feces; 3, mild diarrhea; 4, severe diarrhea; 5, watery diarrhea.
[a,b]Means within a row lacking a common letter are different ($P \leq 0.05$).
[x,y]Means within a row lacking a common letter tend to be different ($P \leq 0.10$).

was observed with reducing dietary CP on day 27 compared with day 6, day 13, and day 20. The concentration of total protein was less ($P < 0.01$) on day 13 compared with the total protein concentrations analyzed on day 1, day 6, day 20, and day 27.

Serum immunoglobulin G concentrations analyzed on day 1 were greater ($P < 0.01$) compared with the concentrations on day 6, day 13, day 20, or day 27; however, no influence of dietary CP was observed for serum immunoglobulin G concentration. Greater ($P < 0.01$) serum peptide YY concentrations were observed on day 20 compared with peptide YY concentrations analyzed on day 13 or day 27. An interaction ($P < 0.01$) between dietary CP and day was observed for serum haptoglobin with concentrations being reduced with reduced dietary CP on day 6, day 13, and day 20, but not on day 27. Serum TNF-α and IL-10 concentrations were greatest ($P < 0.01$) on day 6, whereas serum IL-1β and IL-6 were greatest ($P < 0.01$) on day 13. Vitamins A and E concentrations were reduced ($P < 0.01$) on day 13 regardless of dietary treatment, but then increased ($P < 0.01$) on day 20 and day 27. Reducing dietary CP also tended to reduce ($P < 0.10$) serum vitamin E concentration of pigs.

Table 4. Growth performance of pigs fed diets containing high, medium, and low levels of crude protein[1]

Item	Dietary crude protein level, %			SEM	P-value
	22	19	16		
Days 1 to 7					
Initial body weight, kg	5.54	5.52	5.54	0.23	0.353
ADG[2], kg	0.08	0.07	0.08	0.01	0.729
ADFI[2], kg	0.13	0.13	0.14	0.01	0.621
G:F[2]	0.63	0.60	0.57	0.03	0.190
Final body weight, kg	6.10	6.06	6.06	0.24	0.833
Days 8 to 14					
ADG, kg	0.11[x]	0.11[x]	0.09[y]	0.01	0.084
ADFI, kg	0.21	0.20	0.20	0.01	0.440
G:F	0.55[a]	0.55[a]	0.44[b]	0.03	0.010
Final body weight, kg	6.93[x]	6.82[xy]	6.66[y]	0.27	0.064
Days 15 to 21					
ADG, kg	0.29[a]	0.22[b]	0.19[c]	0.01	<0.001
ADFI, kg	0.44[a]	0.39[b]	0.38[b]	0.02	0.019
G:F	0.65[a]	0.58[b]	0.50[c]	0.02	<0.001
Final body weight, kg	8.93[a]	8.45[b]	7.99[c]	0.36	<0.001
Days 22 to 28					
ADG, kg	0.44[a]	0.43[a]	0.34[b]	0.02	<0.001
ADFI, kg	0.67	0.65	0.62	0.03	0.437
G:F	0.64[a]	0.67[a]	0.53[b]	0.02	<0.001
Final body weight, kg	12.05[a]	11.46[a]	10.35[b]	0.46	<0.001
Days 1 to 14					
ADG, kg	0.10	0.09	0.09	0.00	0.197
ADFI, kg	0.16	0.16	0.17	0.01	0.640
G:F	0.59[a]	0.57[a]	0.50[b]	0.02	0.021
Days 15 to 28					
ADG, kg	0.36[a]	0.33[a]	0.26[b]	0.02	<0.001
ADFI, kg	0.56	0.52	0.51	0.02	0.364
G:F	0.65[a]	0.63[a]	0.51[b]	0.01	<0.001
Days 1 to 28					
ADG, kg	0.24[a]	0.21[b]	0.17[c]	0.01	<0.001
ADFI, kg	0.37	0.34	0.34	0.01	0.147
G:F	0.64[a]	0.62[a]	0.51[b]	0.01	<0.001

[1]Data are means of 10 to 12 observations per measurement.
[2]ADG, average daily gain; ADFI, average daily feed intake; G:F, gain to feed ratio.
[a-c]Means within a row lacking a common letter are different ($P \leq 0.05$).
[x,y]Means within a row lacking a common letter tend to be different ($P \leq 0.10$).

Table 6. Blood characteristics of pigs fed diets containing high, medium, and low levels of crude protein (CP)[1]

		Experimental day						P-value		
Item	% CP	1	6	13	20	27	SEM	CP	Day	CP × Day
Blood urea nitrogen (mg/dL)	22	5.83^{fg}	9.33^{bc}	11.50^{ab}	8.33^{cde}	9.33^{c}	0.80	0.008	<0.001	0.011
	19	5.42^{fg}	8.50^{cd}	12.28^{a}	7.25^{cdef}	5.25^{fg}				
	16	5.81^{fg}	6.67^{defg}	9.42^{bc}	5.25^{fg}	4.83^{g}				
Total protein (g/dL)	22	4.36	4.14	3.84	4.28	4.46	0.10	0.601	<0.001	0.870
	19	4.37	4.24	3.89	4.34	4.41				
	16	4.31	4.25	3.76	4.19	4.32				
Albumin (g/dL)	22	2.55^{rst}	2.48^{rstu}	2.19^{xyz}	2.21^{wxyz}	2.56^{rst}	0.08	<0.001	<0.001	0.080
	19	2.64^{r}	2.55^{rst}	2.25^{vwx}	2.25^{vwx}	2.35^{tuvwx}				
	16	2.45^{rstuv}	2.41^{stuvw}	2.00^{z}	2.03^{yz}	2.21^{x}				
Peptide YY (ng/mL)	22	5.28	4.95	4.01	5.81	4.47	0.53	0.392	<0.001	0.148
	19	5.44	4.84	3.34	5.07	4.70				
	16	5.03	6.52	3.58	6.58	5.38				
Immunoglobulin G (mg/mL)	22	13.34	6.58	5.99	4.84	4.41	0.91	0.984	<0.001	0.597
	19	12.18	7.61	5.73	5.84	4.65				
	16	11.92	7.77	5.80	5.66	4.70				
Haptoglobin[2] (mg/mL)	22	--	0.54^{cde}	1.42^{a}	0.71^{bc}	0.70^{bc}	0.11	0.426	<0.001	0.008
	19	--	0.57^{cde}	1.22^{a}	0.65^{bcd}	0.37^{de}				
	16	--	0.30^{e}	1.19^{a}	0.55^{cde}	0.88^{b}				
Tumor necrosis factor-α (pg/mL)	22	77.70	147.15	114.79	122.06	121.19	8.49	0.700	<0.001	0.276
	19	85.47	152.35	117.33	129.03	131.97				
	16	86.51	140.92	121.70	141.43	107.60				
Interleukin 1-beta (pg/mL)	22	13.42	13.25	28.79	6.70	8.44	1.65	0.649	<0.001	0.960
	19	13.26	12.16	27.58	5.75	6.88				
	16	13.98	11.78	26.68	5.29	6.52				
Interleukin-6 (pg/mL)	22	7.06	4.22	23.43	3.48	2.63	1.08	0.872	<0.001	0.656
	19	6.94	4.23	23.51	4.83	2.93				
	16	6.81	5.26	24.39	3.79	3.47				
Interleukin-10 (pg/mL)	22	9.74	13.68	12.75	10.69	7.06	0.99	0.108	<0.001	0.338
	19	10.03	14.33	12.04	8.42	5.94				
	16	8.85	11.18	9.64	7.75	5.99				
Vitamin A[3] (ng/mL)	22	-	186.07	133.48	286.90	324.82	15.32	0.222	<0.001	0.851
	19	-	167.53	124.26	250.96	303.08				
	16	-	172.33	108.24	249.50	288.06				
Vitamin E[3] (ng/mL)	22	-	1,415.55	797.05	1,340.52	1,163.88	79.43	0.068	<0.001	0.439
	19	-	1,396.78	730.64	1,037.64	1,010.28				
	16	-	1,183.94	717.83	1,145.56	1,042.69				

[1]Data are means of 9 to 12 observations per treatment.
[2]Day 1 data were detected at low levels.
[3]Day 1 data were analyzed as a covariate due to imbalance of values across treatment. Original values (ng/mL) for vitamin A were: 22% CP = 148.29; 19% CP = 137.16; 16% CP = 175.46; Original values (ng/mL) for vitamin E were: 22% CP = 4,497.50; 19% CP = 3,503.73; 16% CP = 3,477.75.
[a-g]Means within a column lacking a common letter are different ($P \leq 0.05$).
[r-z]Means within a column lacking a common tend to differ ($P \leq 0.10$).

Intestinal morphology, pH, VFA, and NH_3 concentrations

In the jejunum, villus height and villus height:crypt depth ratio increased ($P < 0.05$) as the concentration of dietary CP decreased from 22% to 19% in the diet (Table 7). Reducing dietary CP from 22% to 19% or 16% also resulted in a reduction ($P < 0.05$) in crypt depth in the ileum.

A reduction ($P < 0.05$) in ileal pH was observed as CP in the diet was reduced from 22% to 16% (Table 8). Pigs fed diets containing 19% CP tended to have reduced ($P < 0.05$) pH in the colon compared with pigs fed the other diets. In the cecum and in the colon, there was no effect of dietary CP level on NH_3 and VFA concentrations.

On day 13, no effect of dietary CP on NH_3 or total VFA in feces was observed (Table 9). On day 20, there was a reduction ($P < 0.01$) in butyrate concentration as CP decreased from 22% to 16% in the diet, but concentrations of other VFA were not affected by dietary CP level. On day 27, there was a tendency for an increased ($P < 0.05$) concentration of NH_3 when pigs consumed the 19% CP diet, but dietary CP level had no effect on the concentration of individual VFA or on total VFA concentration.

Gene expression in ileal mucosa

A reduction ($P < 0.05$) in the mRNA abundance of *IL-8*, *IFN-γ*, and *CXCL10* was observed in pigs fed the 16% CP diet compared with pigs fed the 22% CP diet (Table 10). Pigs fed the 16% CP diet also tended to have reduced ($P < 0.10$) mRNA abundance of *CXCL9* compared with pigs fed the diet containing 19% CP. There was a tendency for an increased ($P < 0.10$) *IL-10* and *TGF-β* abundance

Table 7. Intestinal morphology in the duodenum and jejunum of pigs fed diets containing high, medium, and low levels of crude protein[1]

	Dietary crude protein level, %				
Item	22	19	16	SEM	P-value
Jejunum					
Villus height, µm	278.33[b]	327.96[a]	300.82[ab]	11.82	0.023
Crypt depth, µm	212.67	192.30	209.81	13.10	0.504
Villus height:crypt depth ratio	1.44[b]	1.85[a]	1.54[ab]	0.11	0.040
Ileum					
Villus height, µm	242.14	207.39	248.14	20.52	0.310
Crypt depth, µm	200.03[a]	161.00[b]	171.71[b]	7.89	0.002
Villus height:crypt depth ratio	1.22	1.30	1.37	0.12	0.684

[1]Data are means of 11 to 12 observations per treatment.
[a,b]Means within a row lacking a common letter are different ($P \leq 0.05$).

Table 8. pH and concentration of volatile fatty acids (VFA) in cecal and colonic contents of pigs fed diets containing high, medium, and low levels of crude protein[1]

	Dietary crude protein level, %				
Item	22	19	16	SEM	P-value
pH					
Stomach	2.90	2.97	3.43	0.22	0.196
Ileum	7.07[a]	6.99[ab]	6.68[b]	0.11	0.046
Colon	6.70[x]	6.52[y]	6.69[x]	0.07	0.071
Cecum					
Ammonia, mg/g	0.20	0.17	0.19	0.02	0.615
Volatile fatty acids					
Acetate	74.76	67.12	77.67	6.28	0.448
Propionate	35.25	30.97	39.39	2.93	0.146
Butyrate	16.39	15.91	14.88	1.85	0.845
Isobutyrate	0.27	0.29	0.41	0.07	0.496
Isovalerate	0.32	0.34	0.46	0.07	0.171
Valerate	2.92	3.11	3.50	0.51	0.726
Total [VFA], µmol/g	130.64	118.06	137.17	10.51	0.424
Colon					
Ammonia, mg/g	0.34	0.31	0.31	0.04	0.827
Volatile fatty acids					
Acetate	78.48	73.22	73.11	5.71	0.682
Propionate	26.67	27.35	28.70	2.18	0.744
Butyrate	10.97	14.05	11.12	1.58	0.205
Isobutyrate	0.84	0.83	0.86	0.14	0.956
Isovalerate	1.05	1.13	1.14	0.21	0.951
Valerate	2.45	3.04	2.77	0.39	0.529
Total [VFA], µmol/g	120.42	119.61	117.69	9.27	0.969

[1]Data are means of 9 to 12 observations per measurement.
[a,b]Means within a row lacking a common letter are different ($P \leq 0.05$).
[x,y]Means within a row lacking a common letter tend to be different ($P \leq 0.10$).

in pigs fed the 16% CP diet compared with pigs fed the 22% CP diet.

Abundance of *OCLN* decreased ($P < 0.05$), and a tendency for a reduced ($P < 0.10$) *ZO-1* abundance was observed with the reduction of dietary CP from 22% to 16%. Abundance of *TFF-2*, *TFF-3*, and *MUC-2* were ($P < 0.05$) reduced in pigs fed the 16% CP diet compared with pigs fed the 22% or the 19% CP diet. The abundance of *GLUT2* tended to decrease ($P < 0.05$) and *GLUT5* abundance was reduced ($P < 0.05$) in pigs fed the 16% CP diet compared with pigs fed the 22% CP diet.

Discussion

The weaning process is a critical period for pigs, and a decrease in growth performance and an increase in mortality during this period is often observed (Campbell et al., 2013). Therefore, strategies to reduce diarrhea occurrence and post-weaning mortality have to be identified, and feeding low CP diets is one such strategy (Wang et al., 2018). The lack of differences in growth performance among diets during the initial week of the experiment is in agreement with published data (Heo et al., 2008),

Table 9. Concentrations of ammonia and volatile fatty acids (VFA) in feces of nursery pigs fed diets containing high, medium, and low levels of crude protein[1]

	Dietary crude protein level, %				
Item	22	19	16	SEM	P-value
Feces					
Day 13					
Ammonia, mg/g	0.62	0.58	0.60	0.05	0.854
Volatile fatty acids					
Acetate	73.53	70.39	70.75	6.05	0.923
Propionate	22.31	19.68	19.51	2.37	0.645
Butyrate	8.89	8.89	6.94	1.33	0.487
Isobutyrate	1.79	1.34	1.45	0.16	0.126
Isovalerate	2.42	1.91	2.11	0.23	0.262
Valerate	2.47	2.22	2.28	0.29	0.813
Total [VFA], µmol/g	111.41	105.95	103.41	9.86	0.843
Day 20					
Ammonia, mg/g	0.62	0.78	0.59	0.08	0.200
Volatile fatty acids					
Acetate	108.93	112.12	104.50	4.47	0.355
Propionate	37.97	39.51	34.02	2.39	0.212
Butyrate	17.08[a]	15.66[ab]	12.80[b]	1.08	0.032
Isobutyrate	2.38	3.00	2.13	0.34	0.201
Isovalerate	3.31	4.47	3.04	0.51	0.136
Valerate	4.29	4.84	4.53	0.48	0.721
Total [VFA], µmol/g	174.70	179.17	162.15	8.29	0.252
Day 27					
Ammonia, mg/g	0.66[y]	0.85[x]	0.67[y]	0.07	0.093
Volatile fatty acids					
Acetate	88.44	87.52	89.69	3.31	0.824
Propionate	28.24	28.98	31.61	2.29	0.458
Butyrate	12.69	12.46	12.25	0.68	0.862
Isobutyrate	1.84	2.28	2.02	0.23	0.322
Isovalerate	2.36	3.25	2.84	0.34	0.159
Valerate	3.87	4.10	4.22	0.36	0.706
Total [VFA], µmol/g	138.95	137.62	142.63	6.74	0.782

[1]Data are means of 9 to 12 observations per measurement.
[a,b]Means within a row lacking a common letter are different ($P \leq 0.05$).

and this indicates that low CP diets can be fed during the immediate post-weaning period without reducing growth performance. The small reduction in growth performance observed during the second week post-weaning may allow for compensatory gain later in the nursery period if pigs are provided access to diets containing adequate concentrations of AA after the period with restricted intake of AA (Menegat et al., 2020). The observation that growth performance of pigs was reduced when diets containing 16% CP were fed for more than 2 wk post-weaning is in agreement with previous data (Opapeju et al., 2008; Yue and Qiao, 2008; Wellock et al., 2008; Lynegaard et al., 2021). Diets containing 16% CP in phases 1 and 2 were limiting in most indispensable AA; therefore, protein synthesis was likely not maximized in pigs fed this diet, which subsequently resulted in reduced overall ADG and final pig body weight. However, the observation that reducing dietary CP resulted in a reduced fecal score is in agreement with reported data (Yue and Qiao, 2008; Lynegaard et al., 2021). It was not the objective of this experiment to determine if the pigs on the 16% CP diet were able to catch up to pigs on the other diets after the nursery period, but results of other experiments have indicated that pigs that were deprived of protein will have compensatory gain later (Menegat et al., 2020; Hou et al., 2021).

Blood urea nitrogen is an indicator of AA utilization efficiency (Coma et al., 1995), and the observed reduction in BUN as dietary CP was reduced is in agreement with previous data (Cho et al., 2008; Yue and Qiao, 2008). This indicates that diets containing 19 or 16% CP provided a balanced supply of SID AA close to or below the requirement, and consequently, pigs fed these diets were more efficient in utilizing AA. However, the observed reduction in albumin on day 27 in pigs fed the 16% CP diet may be due to reduced absorption of nutrients. Albumin binds and transports fatty acids and amino acids in the blood (Quinlan et al., 2005); therefore, the reduced ADFI in pigs fed the 16% CP diet may have reduced the need for albumin to transport nutrients. The observed increase in BUN on day 13 compared with day 6 is likely a result of the reduced feed intake of pigs in this period (Moehn et al., 2004). Peptide YY plays a critical role in the regulation of food intake and energy homeostasis (Ueno et al., 2008), and the observed increase in peptide YY on day 20 and day 27 may have contributed to the observed increase in ADFI of pigs.

Analyzed values for serum immunoglobulin G concentration were in agreement with published data (Gomez et al., 1998; Yin et al., 2008). The decrease in serum immunoglobulin G concentration that was observed after weaning indicates that

Table 10. Least squares means (log_2-backtransformed) for expression of genes in the ileal mucosa of pigs fed diets containing high, medium, and low levels of crude protein[1]

	Dietary crude protein level, %				
Item[2]	22	19	16	SEM	P-value
Pro-inflammatory					
TNF-α	1.04	1.15	1.19	0.07	0.292
IL-1β	0.71	0.73	0.71	0.11	0.990
IL-6	1.01	0.81	0.98	0.17	0.580
IL-8	0.87^{ab}	1.07^{a}	0.69^{b}	0.12	0.046
IL-21	1.07	1.04	1.05	0.14	0.985
IFN-γ	1.46^{a}	1.04^{ab}	0.69^{b}	0.19	0.030
CXCL2	0.85	0.99	0.75	0.11	0.235
CXCL9	1.20^{xy}	1.44^{x}	0.90^{y}	0.19	0.077
CXCL10	1.41^{a}	1.07^{a}	0.67^{b}	0.16	0.001
Anti-inflammatory					
IL-4	0.87	1.11	1.48	0.28	0.317
IL-10	0.84^{y}	0.94^{x}	0.98^{x}	0.04	0.063
IL-11	0.58	0.81	0.96	0.17	0.246
IL-13	0.92	1.34	1.19	0.27	0.508
TGF-β	0.86^{y}	0.92^{xy}	1.09^{x}	0.08	0.096
Gut-protective proteins					
OCLN	1.08^{a}	1.09^{a}	0.63^{b}	0.14	0.020
CLDN-1	1.04	1.23	1.09	0.51	0.952
ZO-1	0.96^{xy}	1.01^{x}	0.88^{y}	0.04	0.057
TFF-1	0.33	0.58	0.39	0.24	0.772
TFF-2	1.47^{a}	1.11^{a}	0.55^{b}	0.17	0.001
TFF-3	1.22^{a}	1.03^{a}	0.59^{b}	0.12	<0.001
MUC2	1.23^{a}	1.27^{a}	0.73^{b}	0.15	0.012
Miscellaneous					
GLUT2	1.25^{x}	1.06^{xy}	0.60^{y}	0.21	0.055
GLUT5	1.31^{a}	1.10^{ab}	0.77^{b}	0.15	0.042
SLC15A1	0.85	0.71	0.55	0.18	0.482

[1]Data are means of 10 to 12 observations per measurement.
[2]*TNF-α*, tumor necrosis factor-alpha; *IL-1β*, interleukin-1beta; *IL-6*, interleukin-6; *IL-8*, interleukin-8; *IL-21*, interleukin-21; *IFN-γ*, interferon-gamma; *CXCL2*, chemokine ligand 2; *CXCL9*, chemokine ligand 9; *CXCL10*, chemokine ligand 10; *IL-4*, interleukin-4; *IL-10*, interleukin-10; *IL-11*, interleukin-11; *IL-13*, interleukin-13; *TGF-β*, transforming growth factor-beta; *OCLN*, occludin; *CLDN-1*, claudin-1; *ZO-1*, zonula occludens-1; *TFF-1*, trefoil factor-1; *TFF-2*, trefoil factor-2; *TFF-3*, trefoil factor-3; *MUC2*, mucin 2; *GLUT2*, glucose transporter 2; *GLUT5*, glucose transporter 5; *SLC15A1*, solute carrier family 15 member 1.
[a,b]Means within a row lacking a common letter are different ($P \leq 0.05$).
[x,y]Means within a row lacking a common letter tend to be different ($P \leq 0.10$).

pigs likely had reduced immune ability, or have not restored their immune status to pre-weaning levels (Cho et al., 2006). The observation that serum haptoglobin was reduced with reducing concentration of dietary CP during the initial 3 wk post-weaning indicates that pigs fed the 16% CP diet had greater immune response than pigs fed diets containing 22% or 19% CP. Haptoglobin is an acute phase protein that increases during an inflammatory response (Dritz et al., 1995; Eckersall et al., 1996). Therefore, it appears that although pigs fed the 16% CP diet had reduced ADG, these pigs had less inflammation caused by dietary CP, which is in agreement with the observed reduction in fecal score of pigs.

Fat-soluble vitamins A and E are coenzymes for nutrient metabolism (NRC, 2012). These vitamins are involved in the antioxidant system, which can be overwhelmed by free radical production linked to immune activation (Buchet et al., 2017). The observation that serum vitamin E concentration is reduced during the post-weaning period compared with the pre-weaning period is in agreement with previous data (Sivertsen et al., 2007; Lauridsen et al., 2011; Kim et al., 2016), and may indicate that humoral and cell-mediated immune responses are reduced during this period (Bonnette et al., 1990). The current data indicate that serum concentrations of vitamin E are reduced after weaning, which supports the hypothesis that pigs are utilizing vitamin E to support their active immune system. This also agrees with the results obtained for immunoglobulin G, indicating that pigs had reduced immune response after weaning.

Soy products contain isoflavones that have anti-inflammatory and antioxidative properties (Smith et al., 2020); however, weanling pigs cannot ferment the oligosaccharides in soybean meal efficiently (Rojas and Stein, 2013). Therefore, the reduced concentration of soybean meal, and, therefore, reduced concentration of non-digestible oligosaccharides in the diet, is likely the reason for the observed increase in villus height of pigs fed the 19% CP diet compared with the 22% CP diet (Li et al., 1990, 1991). The observed increase in villus height of pigs fed the 19% CP diet may also be due to reduced proliferation of pathogenic bacteria in the intestine caused by undigested protein from the high CP diet. The observed reduction in villus height from pigs fed the 16% CP diet is in contrast with data reported by Larsen et al. (2021) indicating that reducing dietary CP did not influence intestinal morphology of weaned pigs. However, the decrease in villus height of pigs fed the 16% CP diet may be associated with

a reduction in protein synthesis in these pigs due to limited AA supply that may be needed for cell proliferation (Núñez et al., 1996; Gu and Li, 2004).

The observed increase in ileal pH of pigs fed the 22% CP diet is in agreement with the observed increase in fecal score. Diets with high concentration of CP have high buffering capacity, which results in increased intestinal pH and a more favorable environment for proliferation of bacteria (Partanen and Mroz, 1999; Htoo et al., 2007). In the present experiment, diets were formulated to contain reduced levels of CP to address the possibility that reduced dietary CP reduces NH_3 and VFA synthesis, but results did not indicate that reducing dietary CP influenced total VFA synthesis. However, pigs fed the high CP diet may excrete more products of proteolytic fermentation than pigs fed low CP diets (Heo et al., 2008; Rist et al., 2013), but because a quantitative collection of feces was not possible in this experiment, this hypothesis cannot be confirmed.

Pro-inflammatory cytokines are produced by macrophages, lymphocyte cell lines, and monocytes in response to diseases and infections (Pauli, 1995). Therefore, the observation that the mRNA abundance of *IL-8*, *IFN-γ*, *CXCL9*, and *CXCL10* was reduced by decreasing the amount of CP in the diet may indicate less inflammation in the intestinal tract of pigs. Transforming growth factor-β is an anti-inflammatory cytokine involved in the suppression of infiltrating cells (Chen et al., 2018a) and containment of inflammation (Al-Sadi et al., 2009). The increase in the mRNA abundance of *IL-10* and *TGF-β* observed in the mucosa of pigs fed low CP diets indicates a reduction in inflammation. However, upregulation of anti-inflammatory genes was not consistently observed in this experiment. It is possible that feeding reduced levels of CP in the diet enhances the immune system, and the immune system had no need to turn on transcription of other anti-inflammatory genes (Hu et al., 2013). This may also explain the observed reduction in *MUC2* abundance in the ileal mucosa of pigs fed the 16% CP diet. Mucins are important for the local defense against pathogens and enteric bacteria (Betscher et al., 2010); therefore, less mucin secretion is needed if pigs fed lower CP diets had reduced inflammation. The observed reduction in *MUC2* abundance in pigs fed the 16% CP diet may also be due to restriction in protein synthesis for cell turnover because the diet was limiting in AA.

Tight junctions are multi-protein complexes that are needed to maintain epithelial permeability (Nusrat et al., 2000). The TFF peptides (i.e., TFF-1, TFF-2, and TFF-3) are released by mucus-secreting cells and have a protective effect on epithelial cells in the gastrointestinal tract (Hoffmann et al., 2001; Kim et al., 2009). The observed reduction in the mRNA abundance of gut-protective protein appears to be in contrast with the observed reduction in the mRNA abundance of pro-inflammatory cytokines. Increased pro-inflammatory cytokines causes disruption of the intestinal barrier, resulting in increased permeation of antigens in the intestinal lumen (Bruewer et al., 2006; Shen and Turner, 2006; Al-Sadi et al., 2009; Moeser et al., 2017). However, it is possible that pigs fed the 22% CP diet had increased inflammation, which subsequently resulted in upregulation of gut-protective proteins as a control mechanism to alleviate potential infection.

Glucose and galactose may be transported into the enterocytes via facilitated diffusion using GLUT2, whereas fructose and xylose are transported via facilitated diffusion using GLUT5 (Helliwell et al., 2000). The observed linear reduction in *GLUT2* and *GLUT5* abundance in the ileal mucosa of pigs fed reducing levels of dietary CP may indicate a reduced glucose and/or fructose availability for pigs (Vigors et al., 2014). The hypothesized reduction in glucose availability for pigs fed the 16% CP diet due to reduced *GLUT2* and *GLUT5* abundance may be explained by the observed reduction in feed intake of the pigs. However, further research is needed to confirm this.

Conclusion

The results of this experiment indicate that feeding diets with reduced CP to pigs reduced fecal scores during the initial 2 wk post-weaning. However, feeding reduced CP diets for more than 2 wk post-weaning reduced pig growth performance. Blood urea nitrogen and albumin were reduced, and villus height increased in the jejunum of pigs fed reduced CP diets. The decrease in dietary CP decreased pH in the ileum. Reducing dietary CP also reduced the abundance of pro-inflammatory genes and increased the abundance of the anti-inflammatory gene *TGF-β* and *IL-10*. Therefore, feeding low CP diets to pigs for the initial 2 wk post-weaning may be an alternative feeding strategy to reduce post-weaning diarrhea and improve intestinal health of weanling pigs.

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Conflict of interest statement

The authors declare no real or perceived conflicts of interest.

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