Effect of dietary protein content on ileal amino acid digestibility, growth performance, and formation of microbial metabolites in ileal and cecal digesta of early-weaned pigs

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J Anim Sci 2007.85:3303-3312.
doi: 10.2527/jas.2007-0105 originally published online Sep 4, 2007;

Effect of dietary protein content on ileal amino acid digestibility, growth performance, and formation of microbial metabolites in ileal and cecal digesta of early-weaned pigs[1,2]

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ABSTRACT: Diarrhea incidence in weaned pigs may be associated with the concentration of intestinal microbial metabolites (ammonia, amines, and VFA) that are influenced by dietary CP content. Three experiments were conducted to determine effects of a low-protein, AA-supplemented diet on ileal AA digestibility, growth performance, diarrhea incidence, and concentration of microbial metabolites in ileal and cecal digesta of pigs weaned at 14 d of age. In Exp. 1, 8 pigs fitted with a simple T-cannula at the distal ileum were assigned in a crossover design to 2 diets containing 24 or 20% CP using wheat, corn, full-fat soybeans, whey powder, fish meal, and blood plasma as the main ingredients. Supplemental AA were added to the diets to meet the AA standards according to the 1998 NRC recommendations. Chromic oxide was used as an indigestible marker. Diets were fed at 2.5 times the ME requirement for maintenance. The reduction of dietary CP decreased ($P < 0.05$) the apparent ileal digestibility of most AA, except Lys, Met, Thr, Val, and Pro. Dietary CP content did not affect the pH of ileal digesta or ileal concentrations of ammonia N, cadaverine, putrescine, or VFA. In Exp. 2, 8 pigs fitted with a simple T-cannula in the cecum were assigned to 2 diets, similar to Exp. 1. Dietary CP content did not affect the pH of cecal digesta. The reduction in CP content decreased ($P < 0.05$) cecal ammonia N, acetic acid, isobutyric acid, isovaleric acid, total VFA, and putrescine concentrations by 28 to 39%. In Exp. 3, 32 pigs were assigned to 2 diets, similar to Exp. 1, according to a randomized complete block design. Pigs had free access to feed and water. Dietary CP content did not affect growth performance or fecal consistency scores during the 3-wk study, and diarrhea was not observed. The results of these experiments indicate that lowering the dietary CP content combined with supplementation of AA markedly reduced the production of potentially harmful microbial metabolites in cecal digesta of early-weaned pigs without affecting growth performance.

Key words: amino acid, digestibility, early-weaned pig, growth, microbial metabolite, protein

J. Anim. Sci. 2007. 85:3303–3312
doi:10.2527/jas.2007-0105

INTRODUCTION

Early weaning of pigs is often associated with depressed growth and greater incidence of gut disorders such as diarrhea (Aherne et al., 1992) mainly due to an immature gastrointestinal tract. The content of dietary CP, individual AA, or both may affect the formation and concentrations of metabolites resulting from microbial fermentation (Hobbs et al., 1996). High dietary CP concentration, as is common in diets for early-weaned pigs, may increase microbial fermentation of undigested protein, and encourage proliferation of pathogenic bacteria in the gastrointestinal tract (Ball and Aherne, 1987). Bacterial fermentation of undigested protein produces VFA and potentially toxic substances such as ammonia and amines that can reduce growth (Gaskins, 2000). The incidence of diarrhea at weaning in pigs has been associated with increased production of amines (Porter and Kentworthy, 1969) and ammonia (Dong et al., 1996).

The use of antibiotic growth promoters has been effective in alleviating the incidence of diarrhea. Control of

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Received February 16, 2007.
Accepted August 28, 2007.

postweaning diarrhea may require using strategies other than dietary antibiotics (Wierup, 2000). Reducing the dietary CP concentrations and balancing with AA is hypothesized to reduce the formation of microbial metabolites in the gastrointestinal tract, and consequently reduce the incidence of diarrhea. However, performance of pigs should not be compromised as a result of feeding low-CP diets to enhance commercial adoption.

The objective of the present experiments was to determine the effects of reducing the dietary CP content from 24 to 20% while supplementing with limiting AA on apparent ileal digestibility (**AID**) of CP and AA, pH and concentrations of microbial metabolites in ileal and cecal digesta, growth performance, and incidence of diarrhea in early-weaned pigs. The approach was to feed diets based on wheat, corn, and full-fat soybeans that were formulated to differ by 4 percentage units in CP but with an equal standardized ileal digestible (**SID**) AA and NE content.

MATERIALS AND METHODS

The experimental proposals and procedures for the care and treatment of the pigs were approved by the Animal Care Committee of the University of Alberta, in accordance with the guidelines of the Canadian Council on Animal Care (1993).

Experimental Design and Diets

Three experiments were conducted at the Swine Research Technology Centre at the University of Alberta.

Exp. 1. Eight crossbred (Duroc × Large White) barrows with an average initial BW of 5.2 ± 0.51 kg were obtained immediately after weaning at 14 d of age. The pigs were provided creep feed while with the sow. To ease acclimation to the metabolism pens (height, 0.85 m; length, 0.70 m; and width, 0.70 m), pigs were kept in pairs for the first 3 d postweaning. Subsequently, pigs were housed individually in a room with the temperature controlled at 28 to 30°C for 8 d. On d 11 and 12 postweaning, the pigs (6.0 ± 0.62 kg of BW) were fitted with a simple T-cannula at the distal ileum, approximately 5 cm cranial to the ileo-cecal sphincter. A detailed description of cannula preparation and pre- and postoperative care was previously given by Li et al. (1993). Before surgery and during a 7-d recuperation period following surgery, the pigs were fed a 23% CP commercial starter diet. Each pen was equipped with plastic-coated, expanded metal flooring, an infrared heating lamp, and a single-space feeder. Water was freely available from a low-pressure drinking nipple. After the 7-d recuperation, the pigs were allotted on the basis of BW to the experimental diets according to a 2-period crossover design. Each experimental period consisted of 7 d. The pigs were weighed at the beginning and end of each period. The average BW of the pigs was 6.7 ± 0.72 and 8.2 ± 0.75 kg at the beginning of periods 1 and 2, respectively, and 9.8 ± 0.99 kg at the end of the experiment.

Two experimental diets, based on wheat, corn, and full-fat soybeans, were formulated to contain a high (24%) or low (20%) CP content (Table 1). The ingredients were ground through a 2-mm mesh sieve before mixing. The diets were formulated to contain 10.70 MJ/kg of NE and 1.30% SID Lys. Ingredients contributing to the AA content of the diet were analyzed for CP and AA before diet formulation. Diets were supplemented with Lys, Met, Thr, Ile, and Val (Degussa AG, Hanau-Wolfgang, Germany) to balance the ratio of SID of Met + Cys, Thr, and Trp relative to the SID of Lys, according to the ideal AA profile for 10- to 20-kg pigs (Rademacher et al., 1999). Soybean oil was included in the diets to increase the dietary NE content and to reduce the dustiness. Vitamins and minerals were supplied to meet or exceed the NRC (1998) standards for pigs ranging in BW from 5 to 10 kg. Chromic oxide was included in the diets at 0.3% as an indigestible marker. The diets were fed to the pigs as mash at a rate of 2.5 times the maintenance requirement for ME (i.e., 106 kcal/kg of $BW^{0.75}$; NRC, 1998) based on the BW of each pig that was determined at the beginning of each experimental period. Pigs were given 3 meals of equal amounts daily, at 0800, 1600, and 2400.

Ileal digesta were collected for two 12-h periods from 0800 to 2000 on d 6 and 7 of each experimental period, for a total of 24 h. Digesta were collected by fastening soft plastic tubes (diameter, 3 cm; length, 8 cm) to the barrel of the cannula. To minimize bacterial fermentation in digesta collected for nutrient digestibility analyses, 5 mL of 10% (vol/vol) formic acid was added to the plastic tube. For pH measurement and determination of VFA concentrations, digesta were collected without adding formic acid. Approximately 25% of total digesta collection was reserved for pH and VFA measurements; after 3 bags were collected with formic acid, 1 bag was collected without formic acid. The bags were removed when filled approximately half way with digesta, and immediately frozen at –20°C.

Exp. 2. Eight barrows of the same origin as in Exp. 1 were used, with an average initial BW of 4.9 ± 0.30 kg. With the exception of the cannulation procedure, Exp. 2 was carried out under the same conditions as those described for Exp. 1. These conditions included the experimental design, feed processing and diet formulation, feeding times and allowance, length of the experimental period, BW measurement, and digesta collection procedure. On d 6 and 7 postweaning, the pigs (average BW 5.29 ± 0.28 kg) were fitted with a simple T-cannula in the cecum (Sauer, 1976). The average BW of the pigs was 6.5 ± 0.36 and 7.7 ± 0.47 kg at the beginning of experimental periods 1 and 2, respectively, and 9.9 ± 0.59 kg at the end of Exp. 2. To obtain the same AA content as the diets used in Exp. 1, the supplementation of AA to the experimental diets was adjusted based on the analyzed AA contents in the main feed ingredients, which were from a different batch (Table 1).

Exp. 3. Thirty-two crossbred (Duroc × Large White/Landrace) mixed-sex pigs with an average initial BW of

Table 1. Ingredient composition and calculated energy and nutrient content of experimental diets in Exp. 1, 2 and 3 (as-fed basis)

	Exp. 1		Exp. 2		Exp. 3	
	CP content, %		CP content, %		CP content, %	
Item	24	20	24	20	24	20
Ingredient, %						
Wheat	30.00	30.96	30.00	30.96	30.00	30.00
Corn	19.04	36.89	19.04	36.89	17.77	32.48
Full-fat soybean	23.94	3.54	23.94	3.54	25.72	9.69
Whey powder	10.00	10.00	10.00	10.00	10.00	10.00
Fish meal, 64% CP	6.00	6.00	6.00	6.00	6.00	6.00
Blood plasma	5.00	5.00	5.00	5.00	5.00	5.00
Skim milk powder	2.50	2.50	2.50	2.50	2.50	2.50
Soybean oil	1.84	2.09	1.84	2.09	1.83	2.03
Limestone	0.77	0.65	0.77	0.65	0.78	0.69
Dicalcium phosphate	0.08	0.43	0.08	0.43	0.05	0.33
Premix[1]	0.25	0.25	0.25	0.25	0.25	0.25
Biolys[2]	0.18	0.76	0.18	0.76	0.02	0.56
DL-Met	0.08	0.16	0.08	0.16	0.08	0.16
L-Thr	—	0.15	—	0.13	—	0.10
L-Ile	0.02	0.20	0.02	0.20	—	0.16
L-Trp	—	—	—	0.04	—	0.02
L-Val	—	0.12	—	0.10	—	0.03
Chromic oxide	0.30	0.30	0.30	0.30	—	—
Calculated nutrient and energy contents[3]						
ME, MJ/kg	14.87	14.63	14.87	14.63	14.89	14.71
NE, MJ/kg	10.70	10.70	10.70	10.70	10.70	10.70
CP, %	24.00	20.00	24.00	20.00	24.00	20.00
Total AA, %						
Lys	1.48	1.43	1.56	1.46	1.50	1.44
Met	0.47	0.49	0.48	0.49	0.47	0.49
Thr	0.97	0.92	1.03	0.93	1.02	0.93
Trp	0.37	0.29	0.31	0.27	0.31	0.26
Ile	0.91	0.86	0.97	0.88	0.93	0.87
Val	1.13	1.02	1.19	1.03	1.21	1.04
Standardized ileal digestible AA, %						
Lys	1.30	1.30	1.36	1.33	1.30	1.30
Met + Cys	0.79	0.78	0.82	0.80	0.79	0.78
Thr	0.83	0.82	0.87	0.83	0.86	0.82
Trp	0.32	0.25	0.27	0.24	0.27	0.23
Ile	0.78	0.78	0.83	0.80	0.79	0.78
Val	0.97	0.91	1.02	0.92	1.03	0.91
ADF, %	4.08	2.81	4.08	2.81	4.19	3.18
NDF, %	8.82	7.90	8.82	7.90	8.92	8.16
Total NSP	11.36	9.60	11.36	9.60	11.56	10.15
Fermentable NSP	8.09	5.92	8.09	5.92	8.31	6.59
Ash, %	4.88	4.34	4.88	4.34	4.92	4.50
Total P, %	0.65	0.65	0.65	0.65	0.65	0.65
Ca, %	0.80	0.80	0.80	0.80	0.80	0.80

[1]Provided per kilogram of diet: vitamin A, 10,000 IU; vitamin D_3, 1,000 IU; vitamin E, 80 IU; vitamin K_3, 2.0 mg; vitamin B_{12}, 0.03 mg; riboflavin, 12 mg; niacin, 40 mg; D-pantothenic acid, 25 mg; biotin, 0.25 mg; folic acid, 1.6 mg; thiamine, 3.0 mg; pyridoxine, 2.25 mg; choline chloride, 300 mg; Fe (ferrous sulfate), 150 mg; Zn (zinc carbonate), 150 mg; Mn (manganese sulfate), 40 mg; Cu (copper sulfate), 25 mg; I (potassium iodate), 0.21 mg; Co (cobalt sulfate), 0.5 mg; Se (sodium selenite), 0.3 mg; and ethoxyquin, 5.0 mg.

[2]Contained 50.7% L-Lys (Degussa AG, Hanau-Wolfgang, Germany).

[3]Based on analyzed AA content in protein sources and standardized ileal digestibility coefficients (Rademacher et al., 1999), and feed composition data for NE, ADF, NDF, ash, total P, and Ca (Degussa AG, Hanau-Wolfgang, Germany), and for total and fermentable nonstarch polysaccharides (NSP; CVB, 2004).

7.5 ± 0.78 kg were obtained immediately after weaning at 19 d of age. The pigs were provided creep feed while with the sow. Pigs were housed in floor pens (length = 1.50 m; width = 1.10 m) in a room with the temperature controlled at 28 to 30°C. During a 6-d adaptation period, the pigs were fed a 23% CP commercial starter diet. At d 25 of age, the pigs (average BW of 8.2 ± 0.77 kg) were blocked by BW, litter, and sex, and allotted to 2 experimental diets according to a randomized complete block design. There were 4 pen replicates per treatment and 4 pigs per pen. Each pen was equipped with plastic-coated expanded metal flooring, a 4-hole self-feeder, and

a single automatic drinking nipple. The experimental diets were formulated to contain similar nutrient and energy contents as the diets used in Exp. 1, but the main feed ingredients were obtained from a different batch and were analyzed for AA before diet formulation. Pigs had free access to feed and water; diets were fed as mash for 21 d. Individual BW and feed disappearance were recorded weekly to calculate ADG, ADFI, and G:F. The average BW of the pigs was 8.2 ± 0.77 and 17.0 ± 2.13 kg at the beginning and end of Exp. 3, respectively. The incidence and severity of diarrhea were monitored and assessed based on a daily visual score (Ball and Aherne, 1987).

Chemical Analyses

Digesta were thawed at room temperature (21°C), pooled within each pig and period, and mixed before chemical analysis. A portion of mixed digesta was freeze-dried and ground through a 0.5-mm screen for CP and AA analysis. Diets and ingredients were ground similarly. In diets and digesta, DM and ash were determined according to AOAC (1990), and OM was calculated as DM minus ash. Chromic oxide was determined by spectrophotometry (Fenton and Fenton, 1979). The N content was determined with a Leco FP-428 nitrogen determinator (Leco Corporation, St. Joseph, MI). The pH was measured directly from homogenized digesta by using a pH meter with a glass electrode (Accumet Basic AB15; Fisher Scientific, Fair Lawn, NJ).

The AA contents in the diets and ileal digesta were determined by ion-exchange chromatography with post-column derivatization with ninhydrin. Amino acids were oxidized with performic acid, which was neutralized with sodium metabisulfite (Llames and Fontaine, 1994; Commission Directive, 1998). Amino acids were liberated from the protein by hydrolysis with 6 *N* HCl for 24 h at 110°C. Amino acids were quantified with the internal standard method by measuring the absorption of reaction products with ninhydrin at 570 nm. Tryptophan was determined by HPLC with fluorescence detection (extinction 280 nm, emission 356 nm), after alkaline hydrolysis with barium hydroxide octahydrate for 20 h at 110°C (Commission Directive, 2000). Tyrosine was not determined. Supplemented AA were determined by extraction with 0.1 *N* HCl (Commission Directive, 1998).

The ammonia N concentration in digesta was determined using spectrophotometry with a method adapted from Novozamsky et al. (1974). Briefly, 2 mL of phenate solution and 3 mL of 0.01% sodium nitroprusside were added to 40 μL of digesta in a 16- × 100-mm glass tube and vortexed. A 3% sodium hypochlorite solution (3 mL) was then added, covered with parafilm, mixed well and placed in complete darkness for 1 h, followed by reading of absorbance at 600 nm using a microplate spectrophotometer (Spectra Max 190, Molecular Devices Corporation, Sunnyvale, CA). An ammonium sulfate (A4418, Sigma-Aldrich, St. Louis, MO) standard solution (100 μL of ammonia N/mL) was used in preparing a standard curve. Ammonia N concentrations were determined by calculating the concentrations from a regression equation of the standard curve.

The concentrations of 2 amines, cadaverine and putrescine, in digesta were determined by HPLC with a method adapted from Sedgwick et al. (1991). Briefly, 2 g of digesta was centrifuged at 2,500 × *g* for 10 min at 4°C. The supernatant of the sample (100 μL) was diluted with distilled water (400 μL), and 150 μL of internal standard [32 mg of 1,8 diaminooctane (D22401, Sigma-Aldrich) in 50 mL of distilled water] was added. After mixing, 50 μL of the solution was transferred into a vial and analyzed using a Varian 5000 HPLC system with a reverse-phase column and a Varian Fluorichrom detector (Varian Canada Inc., Mississauga, Ontario, Canada). Cadaverine and putrescine were derivatized with an *o*-phthaldialdehyde reagent solution. Peaks were recorded and integrated using the Ezchrom Chromatography Data System (version 4.2, Shimadzu Scientific Instruments Inc., Columbia, MD). Cadaverine and putrescine concentrations were identified and quantitated from standard curves constructed from a standard solution [50 mg of cadaverine dihydrochloride (C8561, Sigma, St. Louis, MO) and 40 mg of putrescine dihydrochloride (P5780, Sigma) in 50 mL of distilled water].

The concentrations of VFA in digesta were determined using gas chromatography with a method adapted from Erwin et al. (1961). Briefly, 0.75 mL of 25% phosphoric acid and 3 mL of digesta fluid were added to 0.6 mL of internal standard solution (150 mg of 4-methyl-valeric acid, S381810, Sigma-Aldrich) in 50 mL of distilled water in a 16- × 100-mm glass tube, mixed well, and kept at room temperature for 30 min. A portion (1.2 mL) of the mixture was transferred into a centrifuge tube and centrifuged at 2,500 × *g* for 10 min at 4°C. The supernatant was analyzed for VFA (i.e., acetic, propionic, butyric, isobutyric, valeric, isovaleric, and caproic acids) using a Varian model 3400 Gas Chromatograph (Varian, Walnut Creek, CA) with a Stabilwax-DA column (30-m × 0.25-mm i.d.; Restek, Bellefonte, PA). A flame-ionization detector was used with an injector temperature of 170°C and a detector temperature of 190°C.

Calculation and Statistical Analyses

In Exp. 1, the AID of DM, OM, CP, and AA in the experimental diets were determined using chromic oxide as indigestible marker. The AID coefficients were calculated according to the following equation: $AID = \{100 - [(A_F \times I_D) / (A_D \times I_F)]\} \times 100\%$, where A_F is the concentration of a component in ileal digesta (%), I_D is the chromic oxide concentration in the diet (%), A_D is the concentration of a component in the diet (%), and I_F is the chromic oxide concentration in ileal digesta (%). The SID of CP and indispensable AA in the experimental diets were calculated by correcting AID values for basal endogenous AA losses (Rademacher et al., 1999). The daily flow of OM, CP, Arg, Glu, and Lys in ileal digesta was calculated using the fed amount of chromic oxide and concentra-

tions of chromic oxide and the specific nutrient in the digesta.

In Exp. 1 and 2, the data were analyzed statistically for a crossover design using the MIXED procedure (SAS Inst. Inc., Cary, NC). The following linear equation was applied:

$$Y_{ijk} = \mu + T_i + S_j + P_k + A_l + \varepsilon_{ijk},$$

where Y_{ijk} is the dependent variable; μ is the overall mean effect; T_i is the fixed effect of treatments and i = 1, 2; S_j is the random effect of sequences and j = 1, 2; P_k is the random effect of experimental periods and k = 1, 2; Al is the random effect of animals and l = 1, 2, 3, 4, 5, 6, 7, or 8; and ε_{ijk} is the residual experimental error, with $N(0, \sigma^2)$. The pig was used as the experimental unit.

In Exp. 3, the data were subjected to statistical analysis for a randomized complete block design using the MIXED procedure of SAS. Pen was used as the experimental unit for determining the treatment effects.

In all experiments, differences between treatments were determined using the LSMEANS statement. Differences were considered significant if $P < 0.05$ and were described as tendencies if $0.05 < P < 0.10$.

RESULTS

All pigs remained healthy and consumed their feed allowances throughout the experiments. Postmortem examinations conducted at the end of Exp. 1 and 2 did not reveal intestinal adhesions. The analyzed dietary CP and AA contents were similar to the calculated contents (Tables 1 and 2). In Exp. 2, the analyzed CP content in the high-CP diet was 1.5 percentage units greater and the analyzed Thr content in the low CP diet was 0.13 percentage units lower than calculated contents. Diets with a reduced CP content had a lower content of ADF, NDF, and total and fermentable fiber (Table 1).

Exp. 1. The AID of DM and OM were not affected by reducing the dietary CP content (Table 3). Reducing the dietary CP content tended to decrease the AID of CP by 4.7 percentage units ($P = 0.060$). Reducing the dietary CP content decreased ($P < 0.05$) the AID of Arg, Asp, Glu, Gly, His, Leu, Phe, and Ser and tended to decrease ($P < 0.10$) the AID of Ala and Ile. There were no changes in the AID of Lys, Met, Thr, Val, and Pro. Reducing the dietary CP content decreased ($P < 0.05$) the SID of Arg and Phe, tended to decrease ($P < 0.10$) the SID of His and Leu, and did not change the SID of the other AA.

The pH in ileal digesta did not differ between the high- and low-CP diets (Table 4). Reducing the dietary CP content did not affect the concentrations of ammonia N, cadaverine, and putrescine in ileal digesta. Reducing the dietary CP content tended to decrease ($P < 0.10$) the concentration of butyric acid in ileal digesta but did not affect the concentrations of the other VFA. Reducing the dietary CP content did not alter the daily flow of OM, CP, Arg, and Lys in ileal digesta, and tended to increase ($P < 0.10$) daily flow of Glu.

Exp. 2. The pH in cecal digesta did not differ between the high- and low-CP diets (Table 5). Reducing the dietary CP content decreased ($P < 0.001$) the ammonia N concentration in cecal digesta by 32%. The concentration of putrescine, one of the major amines in cecal digesta, was decreased ($P < 0.01$) by 39% by lowering the dietary CP content. The concentration of cadaverine in cecal digesta was not affected by the dietary CP content. Reducing the dietary CP content decreased ($P < 0.05$) the concentrations of acetic, isobutyric, and isovaleric acids in cecal digesta, and tended to decrease ($P < 0.10$) the concentrations of butyric and propionic acids. The concentration of total VFA in cecal digesta was decreased ($P < 0.05$) by 28% by reducing the dietary CP content.

Exp. 3. During wk 1 and 2 and during the entire period (d 0 to 21), the ADG, ADFI, and G:F were not affected by reducing the dietary CP content (Table 6). During wk 3, the ADG of pigs fed the low-CP diet tended to increase ($P = 0.058$), but there were no differences in ADFI, G:F, and final BW. Diarrhea was not observed throughout the experiment. The fecal consistency scores were close to normal and were not affected by the dietary CP content.

DISCUSSION

The present experiments were conducted primarily to determine the effect of a reduction in the dietary CP content on the pH, concentrations of microbial metabolites (i.e., ammonia, putrescine, cadavarine, and VFA) in ileal and cecal digesta, and incidence of diarrhea in early-weaned pigs. In addition, the effect of dietary CP content on the growth performance and the AID of CP and AA in the experimental diets were determined.

Digestibility of CP and AA

Reducing the dietary CP content and supplementing with indispensable AA tended to decrease the AID of CP. The AID of the AA supplemented to both diets to contain similar SID supplies of AA (Lys, Met, Thr, Val, and Ile) did not differ between both diets. However, the AID of AA that were not supplemented to the low CP diet (i.e., Arg, His, Leu, and Phe) were decreased by lowering the dietary CP content. Similarly, the AID of CP in weanling pigs was decreased by reducing the dietary CP content from 21.7 to 15.3% by replacing SBM in a high-CP diet with wheat in the low-CP diet (Bikker et al., 2006). The AID of CP and AA will increase curvilinearly with increasing dietary AA content (Fan et al., 1994). The lower AID of CP and AA in the current study were mainly because of changes in the ratio of dietary ingredients and the contribution of endogenous CP and AA in ileal digesta. The content of full-fat soybeans was lower and the content of corn was greater in the low-CP diest compared with the high-CP diet, which thereby reduced the content of fiber in the low-CP diet. The AID of CP and most AA in full-fat soybeans is greater than in corn (Rademacher et al., 1999), providing an explana-

Table 2. Analyzed CP and AA composition of experimental diets in Exp. 1, 2, and 3 (on an as-fed basis)[1]

	Exp. 1		Exp. 2		Exp. 3	
	CP content, %		CP content, %		CP content, %	
Item, %	24	20	24	20	24	20
CP	24.11	19.76	25.48	19.87	23.93	20.01
Indispensable AA						
Arg	1.42	1.01	1.44	1.00	1.43	1.10
His	0.63	0.49	0.58	0.44	0.60	0.50
Ile	0.96	0.89	0.99	0.89	0.96	0.91
Leu	1.92	1.59	1.96	1.61	1.87	1.63
Lys	1.53	1.42	1.57	1.48	1.46	1.49
Met	0.51	0.51	0.48	0.48	0.45	0.48
Met + Cys	0.97	0.89	0.93	0.85	0.88	0.87
Phe	1.14	0.87	1.24	0.94	1.09	0.90
Thr	1.06	0.97	0.99	0.80	1.00	0.95
Val	1.20	1.09	1.21	0.96	1.18	1.03
Dispensable AA						
Ala	1.17	0.98	1.14	0.96	1.14	1.02
Asp	2.21	1.54	2.22	1.51	2.20	1.71
Cys	0.46	0.38	0.45	0.37	0.43	0.38
Glu	4.46	3.58	4.78	3.81	4.33	3.60
Gly	1.08	0.84	1.07	0.83	1.07	0.90
Pro	1.53	1.33	ND[2]	ND	1.51	1.32
Ser	1.24	0.93	1.16	0.88	1.16	0.98

[1]Values are adjusted to a dietary DM content of 88%.
[2]Not determined.

tion for the greater AID of AA in the high-protein diet. In pigs fed a diet with a lower dietary CP content, endogenous CP and AA form a relatively greater contribution of ileal digesta (Fan and Sauer, 1997) relative to undigested CP and AA of dietary origin, and thereby influence the AID of CP and AA. Therefore, the AID coefficients are an

Table 3. Apparent ileal digestibility (AID) of DM and OM, and the apparent and standardized ileal digestibility of CP and AA in the experimental diets (Exp. 1)

	Apparent ileal digestibility				Standardized ileal digestibility[1]			
	CP content, %				CP content, %			
Item, %	24	20	SEM[2]	*P*-value	24	20	SEM[2]	*P*-value
DM	79.8	80.7	2.07	0.541				
OM	81.0	82.3	1.91	0.473				
CP	82.9	78.2	1.61	0.060	87.3	83.6	1.61	0.128
Indispensable AA								
Arg	90.0	82.8	1.50	0.003	92.5	86.3	1.50	0.007
His	87.8	83.1	1.76	0.034	90.6	86.6	1.76	0.063
Ile	87.2	83.5	1.55	0.095	90.8	87.4	1.55	0.118
Leu	88.0	83.8	1.40	0.044	90.4	86.6	1.40	0.065
Lys	88.2	85.2	1.28	0.112	90.5	87.7	1.28	0.133
Met	89.8	88.7	1.01	0.484	91.7	90.7	1.01	0.483
Met + Cys	87.1	83.9	1.30	0.101	90.1	87.2	1.30	0.127
Phe	85.7	79.5	1.82	0.015	88.4	83.0	1.82	0.030
Thr	81.9	79.1	1.48	0.207	87.1	84.8	1.48	0.292
Val	86.0	82.6	1.66	0.112	90.1	87.1	1.66	0.152
Dispensable AA								
Ala	83.8	79.7	1.72	0.099				
Asp	84.8	74.0	2.18	0.001				
Glu	90.7	85.6	1.13	0.009				
Gly	81.6	74.2	2.73	0.009				
Pro	86.3	83.2	1.31	0.125				
Ser	85.2	77.7	1.90	0.004				

[1]Calculated by correcting the AID values for minimum endogenous AA losses (Rademacher et al., 1999).
[2]SE of least squares means (n = 8).

Table 4. Effect of dietary CP content on pH, concentrations of ammonia N, amines, and VFA in ileal digesta, and daily flow of nutrients in ileal digesta of early-weaned pigs (Exp. 1)

	CP content, %			
Item	24	20	SEM[1]	*P*-value
pH	7.15	7.36	0.34	0.663
Ammonia N, mg/L	51.89	48.51	12.24	0.650
Amines, mmol/L				
Putrescine	0.14	0.10	0.02	0.288
Cadavarine	0.06	0.06	0.02	0.953
VFA, mmol/L				
Acetic	14.12	12.92	2.75	0.595
Butyric	1.34	0.95	0.39	0.076
Caproic	0.02	0.02	0.01	0.530
Isobutyric	0.22	0.26	0.04	0.454
Isovaleric	0.06	0.04	0.03	0.447
Propionic	1.98	2.42	0.34	0.293
Valeric	0.08	0.07	0.03	0.701
Total	17.80	16.67	3.04	0.686
Digesta flow, g/d				
OM	54.07	51.64	2.57	0.508
CP	14.09	14.73	1.08	0.684
Arg	0.48	0.60	0.05	0.124
Glu	1.41	1.76	0.14	0.060
Lys	0.62	0.72	0.06	0.235

[1]SE of least squares means (n = 8).

inaccurate assessment of AA digestibility values because they are dependent on the dietary AA content (Rademacher et al., 1999). The dietary CP content did not affect the measured SID of CP and AA when balanced for a similar SID supply. The SID coefficients, obtained by correcting AID coefficients for basal endogenous AA losses, are a more accurate reflection of AA supply to the pig and thus provide a better prediction of AA digestibility values of feedstuffs (Rademacher et al., 1999; Mosenthin et al., 2000).

Table 5. Effect of dietary CP content on pH, concentrations of ammonia N, amines and VFA in cecal digesta of early-weaned pigs (Exp. 2)

	CP content, %			
Item	24	20	SEM[1]	*P*-value
pH	6.33	6.07	0.29	0.473
Ammonia N, mg/L	149.76	101.42	11.19	<0.001
Amines, mmol/L				
Putrescine	0.88	0.54	0.07	0.002
Cadavarine	0.58	0.58	0.08	0.969
VFA, mmol/L				
Acetic	51.94	36.52	3.54	0.016
Butyric	8.28	5.63	1.11	0.099
Caproic	0.09	0.06	0.02	0.286
Isobutyric	0.20	0.11	0.03	0.021
Isovaleric	0.30	0.14	0.04	0.005
Propionic	23.13	17.94	2.37	0.095
Valeric	1.32	1.37	0.29	0.890
Total	85.27	61.78	7.12	0.036

[1]SE of least squares means (n = 8).

Table 6. Effect of dietary CP content on ADG, ADFI, and G:F of early-weaned pigs (Exp. 3)

	CP content, %			
Item	24	20	SEM[1]	*P*-value
Initial BW, kg	8.2	8.1	0.26	0.194
Final BW, kg	17.3	16.7	0.67	0.403
ADG, g/d				
d 0 to 7	272	243	23.7	0.332
d 7 to 14	481	422	40.1	0.374
d 14 to 21	531	565	14.1	0.058
d 0 to 21	429	410	23.0	0.529
ADFI, g/d				
d 0 to 7	379	373	20.8	0.794
d 7 to 14	612	555	37.8	0.342
d 14 to 21	789	788	40.4	0.979
d 0 to 21	593	572	29.7	0.491
G:F, g/g				
d 0 to 7	0.72	0.65	0.04	0.231
d 7 to 14	0.79	0.75	0.03	0.455
d 14 to 21	0.68	0.72	0.02	0.161
d 0 to 21	0.72	0.72	0.01	0.783
Fecal score[2]				
d 0 to 21	0.17	0.18	0.04	0.690

[1]SE of least squares means (n = 4).
[2]Fecal score: 0 = normal, 1 = slight diarrhea, 2 = moderate diarrhea, and 3 = severe diarrhea.

pH and Concentrations of Ammonia, VFA, and Amines

The pH and concentrations of microbial metabolites (ammonia, cadaverine, putrescine, and VFA) in digesta were used as indicators of intestinal health and microbial activity (Nyachoti et al., 2006). Diets with a high CP content have a high buffering capacity (Partanen and Mroz, 1999) and will increase gastric pH, thereby favoring the proliferation of bacteria. To compensate, more HCl has to be produced in the stomach to lower the gastric pH for efficient protein digestion (Schutte, 2000). One of the causes of postweaning gut disorders (e.g., diarrhea) is the inability of early-weaned pigs to produce and secrete sufficient HCl in the stomach (Cranwell, 1995). The pH of ileal digesta decreased quadratically in pigs fed diets in which the dietary CP content was reduced from 23 to 17% (Nyachoti et al., 2006). In contrast, in the current study, the pH of ileal and cecal digesta were not affected when the pigs were fed the high- or low-CP diet (Table 4). The pH of digesta was within the normal range of values for 4- to 5-wk-old weanling pigs (Franklin et al., 2002).

In pigs, fermentation occurs in the caudal ileum and large intestine (Decuypere and Van der Heyde, 1972). Lowering the dietary CP content was expected to reduce fermentation and formation of microbial metabolites in the ileum and cecum, but also reduced dietary fiber content in the current study. A lower dietary CP content will reduce the N and fermentable energy supply to the intestinal microbes. Lowering the dietary CP content reduced the concentrations of ammonia (Bikker et al., 2006; Nyachoti et al., 2006) and individual VFA (Nya-

choti et al., 2006) in ileal digesta of pigs weaned at 18 d of age. In the current study, the dietary CP content did not affect the concentrations of ammonia, cadaverine, putrescine, and VFA in ileal digesta, and the concentrations of these metabolites were markedly lower in the ileum than in the cecum. The reduced dietary CP content in the low-CP diet coincided with reduced CP and AA digestibility compared with the high-CP diet, resulting in a similar daily flow of nutrients in the ileum for the 2 diets, thereby explaining the lack of a difference in microbial metabolites in the ileum. The effect of dietary CP content on ileal VFA concentrations is not consistent among studies. Reducing the dietary CP content from 21.7 to 15.3% did not affect the concentrations of individual and total VFA in ileal digesta of pigs weaned at 26 d of age (Bikker et al., 2006). The values for the concentrations of VFA were within the range of values observed by Bach Knudsen et al. (1991) who reported that the total VFA concentrations in ileal digesta of 42-d-old pigs fed high- or low-fiber diets based on wheat flour and casein were approximately 15 and 17 mmol/L, respectively. Possible explanations for the absence of an effect of dietary CP content on the concentrations of microbial metabolites in the ileum might be the rapid passage rate (2 to 6 h) of digesta through the small intestine (Low and Zebrowska, 1989) limiting bacterial fermentation in the ileum, or the role of ingredient selection and nutrient digestibility, thereby determining the flow of nutrients in digesta serving as a substrate for bacterial fermentation. The implications of a persistent low concentration of microbial metabolites in the ileum on small intestine health are not clear for pigs with free access to feed.

Information on the production of microbial metabolites in the cecum of the pig is scarce. The reduction of dietary CP content reduced the ammonia concentration in cecal digesta (Table 5). A reduction in ammonia concentration has a beneficial effect on the health of the gastrointestinal tract because ammonia can negatively affect growth and differentiation of intestinal epithelial cells (Gaskins, 2000) and increase the incidence of diarrhea (Dong et al., 1996). The reduction in ammonia production is a primary mechanism for the growth response induced by antibiotics (Visek, 1978).

Lowering the dietary CP content with supplementation of AA decreased the concentration of one of the major amines (putrescine) in the cecum (Table 5). Similarly, reducing the dietary CP content from 24 to 18% reduced the concentrations of amines (mainly cadaverine) in the large intestine of pigs by 49% (Schneider et al., 1989). Amines are formed via decarboxylation of the corresponding AA precursors that is preceded by the hydrolysis of protein into AA (Urlings et al., 1992). An increased production of amines, in particular cadaverine and putrescine, may result from an increase in the concentrations of precursor AA. Ornithine, Glu, and Arg are precursors for putrescine and Lys is the precursor for cadaverine (Urlings et al., 1993). However, data from the current study indicate that daily flow of CP and the precursors Arg, Glu, and Lys from the ileum into the cecum might not be the sole explanation for reduced amine and ammonia concentration. This flow was similar between diets differing by 4 percentage units in CP in Exp. 1 due to an offset between changes in content and digestibility of CP and AA, but the concentration of amines and ammonia was markedly reduced in digesta from pigs fed the low-CP diet in Exp. 2. Possible explanations for the observed reduction in microbial metabolites in Exp. 2 might be a carryover effect from reduced dietary fiber profiles in the low CP diet, thereby reducing bacterial fermentation in the cecum, the role of measurements of microbial metabolites in wet digesta vs. nutrient concentrations in dried digesta, or less of a difference in CP and AA digestibility between pigs fed the high- and low-CP diets for Exp. 2. An increase in the production of amines was associated with an increase in the incidence of diarrhea at 4 to 7 d after weaning in 3-wk-old pigs (Porter and Kentworthy, 1969). Ewtushick et al. (2000) also reported that dietary supplementation of polyamines (0.08% putrescine, 0.13% spermidine, 0.18% spermine) to a typical early-weaning diet was detrimental to intestinal growth in early-weaned pigs. Therefore, the reduction in the concentration of putrescine in cecal digesta of pigs fed the low-CP diet can be considered beneficial for maintaining gut health.

Lowering the dietary CP content decreased the concentrations of acetic, isobutyric, and isovaleric acids, and total VFA in cecal digesta up to 28% (Table 5). The values for the cecal VFA concentrations were lower than in a previous study by Canibe et al. (2001) who reported a total VFA concentration of 108 mmol/kg in the cecum of 35-d-old pigs that were fed a starter diet containing 20% CP. The VFA are products of bacterial fermentation of carbohydrates and protein residues in the large intestine (Le et al., 2005). In the cecum where the pH is neutral, deamination is the major pathway for metabolism of AA (Mackie et al., 1998). The quantity of VFA produced depends on the amount and composition of the substrate and on the type of microbes present in the cecum (van Beers-Schreurs et al., 1998). Lower VFA concentrations indicate that the amount of substrate fermented and the microbial activity were also lower in the cecum of pigs fed the low CP diet. The reduction in dietary CP content affects both N and energy metabolism of the cecal microbes, but the reduced VFA concentration in pigs fed the low CP diet was also most likely caused by the reduced fermentable fiber content of the diet.

The reduction in dietary CP content, while balancing with AA, will reduce N excretion (Zervas and Zijlstra, 2002; Shriver et al., 2003; Htoo et al., 2007) and ammonia emission (Canh et al., 1998) from swine manure. Our results show that the reduction in dietary CP content also reduces the concentrations of microbial metabolites in the cecum. Furthermore, the markedly greater concentrations of microbial metabolites in cecal than in ileal digesta also show that the cecum is the major site of microbial fermentation of undigested protein in pigs,

which is mainly due to a much longer retention time of digesta (20 to 38 h; Low and Zebrowska, 1989).

Growth Performance and Diarrhea Incidence

It is crucial that growth performance not be impaired when the dietary CP content is reduced. The ADG, ADFI, and G:F were not affected by reducing the dietary CP content (Table 6), which is in agreement with studies by Hansen et al. (1993) who reported that growth performance of 5- to 20-kg pigs fed either a 21 or 17% CP diet balanced for AA were similar. In contrast, Nyachoti et al. (2006) reported a lower ADG and ADFI of pigs fed a 17 or 19% CP diet compared with a 23% CP diet, which may have been due to a deficiency of Ile and Val. Previous studies (Kerr et al., 1995; Le Bellego and Noblet, 2002; Kerr et al., 2003; Shriver et al., 2003) with growing-finishing pigs also showed that the reduction in dietary CP content while balancing with limiting AA results in similar performance to those fed a high-CP diet. Correct balancing for limiting AA and NE (Le Bellego et al., 2001) will play a key role in maintaining growth performance.

The impact of dietary CP content on diarrhea is less clear. Both the severity and incidence of diarrhea may be greater in pigs fed diets high in CP (Ball and Aherne, 1987). Diarrhea was not observed in the current study and fecal consistency scores were not affected by dietary CP content (Table 6), similar to results in previous studies (Le Bellego and Noblet, 2002; Nyachoti et al., 2006). In this study, the pigs were housed in a clean environment, which may have contributed to an overall high intestinal health status and minimized diarrhea. The current study was thus not sensitive to test if dietary CP affects incidence of diarrhea, and this hypothesis should be tested again using an experimental design that includes pigs with diarrhea. However, the decrease in dietary CP content clearly reduced the concentrations of microbial metabolites. A decrease in dietary CP content could reduce diarrhea in commercial production systems with diarrhea problems (Nyachoti et al., 2006).

In conclusion, the dietary protein content in pig starter diets can be reduced safely from 24 to 20%, while balancing with limiting AA according to ideal protein ratios, without reducing performance. A planned reduction in dietary protein content combined with a concomitant reduction in fermentable fiber will reduce the production of potentially harmful microbial metabolites in the cecum. Therefore, a reduction in protein content can be used as a nutritional strategy to optimize intestinal health of early-weaned pigs. Finally, although the AID of some AA decreased with a reduction in the dietary protein content, the SID of AA was not affected, providing further evidence that feed formulation based on SID of AA will ensure equal growth performance and equal supporting dietary digestible AA content.

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