Journal of Animal Science, 2025, **103**, skaf349
https://doi.org/10.1093/jas/skaf349
Advance access publication 10 October 2025
Non Ruminant Nutrition

Feeding intact protein from soybean meal instead of corn and synthetic amino acids does not affect growth performance, carcass composition, blood cytokines, or mRNA abundance of intestinal amino acid transporters in growing pigs, but net energy tends to be greater in diets with soybean meal

Minoy Cristobal, Su A Lee, Carl M. Parsons, and Hans H. Stein[1]

Department of Animal Sciences, University of Illinois, Urbana, IL 61801

[1]Corresponding author: hstein@illinois.edu

Abstract

The objective was to test the hypothesis that reducing dietary crude protein in corn-soybean meal (SBM) diets will not increase dietary net energy (NE) and will not affect growth performance, carcass composition, nutrient deposition, intestinal morphology, blood cytokine concentrations, or the mRNA abundance of intestinal amino acid (AA) transporters. A corn-SBM-based diet was prepared, and three additional diets were formulated by reducing SBM inclusion, increasing corn, and adding three, four, or five synthetic AA (Lys, Met, Thr, Trp, and Val), resulting in diets with protein levels of 20.0, 16.4, 15.4, and 13.4%, respectively. All diets were formulated to meet requirements for standardized ileal digestible indispensable AA. A total of 176 pigs (initial weight: 32.2 ± 4.2 kg) were used. On day 1, 16 randomly chosen pigs were euthanized, and body nutrient composition was determined. The remaining 160 pigs were allotted to the four experimental diets with four pigs per pen and 10 replicate pens per diet. Diets were provided for 28 d. One pig per pen was slaughtered on day 29, and blood, carcass, and viscera were collected and analyzed for nitrogen, fat, and energy to calculate nutrient deposition. Samples of blood were also analyzed for total protein, albumin, plasma urea nitrogen, and cytokines. Samples of ileal mucosa, ileum and colon tissue, and ileum and colon digesta were collected, and tissue morphology, and mRNA abundance of AA transporters were determined. Results indicated that average daily gain, average daily feed intake, gain-to-feed ratio, carcass characteristics, and protein, lipid, and energy depositions were not affected by reducing dietary crude protein, but NE in diets tended to decrease (linear, $P = 0.051$). Blood urea nitrogen was reduced (linear, $P < 0.001$) as dietary protein levels were reduced, but blood total protein or albumin was not affected by dietary protein levels. Blood cytokines, jejunal and ileal morphology, ammonia in ileal and colon digesta, and mRNA abundance of AA transporters in ileal mucosa were also not affected by the treatments. Bacterial protein in colon digesta decreased (linear, $P = 0.030$) by reducing dietary protein levels. In conclusion, reducing dietary protein levels did not affect growth performance, carcass composition, nutrient deposition, intestinal morphology, blood cytokines, or mRNA abundance of AA transporters in growing pigs, but NE of diets tended to reduce as dietary protein levels were reduced.

Lay Summary

The hypothesis that reducing dietary crude protein in corn-soybean meal-based diets will not increase net energy (NE) in diets and will not affect growth performance, carcass composition, nutrient deposition, intestinal morphology, or immune status of pigs was tested. Four diets were formulated with decreasing soybean meal inclusion and increasing corn and synthetic amino acids (AA), resulting in protein levels of 20.0, 16.4, 15.4, and 13.4%, respectively. Pigs (initial weight: 32.2 ± 4.2 kg) were fed these diets for 28 days with samples collected to assess body composition, intestinal morphology, blood markers, and mRNA abundance of AA transporters. Results indicated that reducing dietary protein levels did not impact any of the analyzed pig characteristics, but NE tended to decrease as soybean meal in the diet was reduced. In conclusion, growth performance can be maintained on low-protein diets with synthetic AA supplementation, but reducing dietary protein levels may decrease NE values in diets.

Key words: amino acids, carcass composition, net energy, pigs, protein, soybean meal

Abbreviations: AA, amino acids; ADFI, average daily feed intake; ADG, average daily gain; cDNA, complementary deoxyribonucleic acid; EDTA, ethylenediaminetetraacetic acid; G:F, gain-to-feed ratio; IL, interleukin; ISG, initial slaughter group; NE, net energy; qRT-PCR, quantitative reverse-transcription polymerase chain reaction; RNA, ribonucleic acid; SBM, soybean meal; *SLC3A2*, solute carrier family 3 member 2; *SLC6A14*, solute carrier family 6 member 14; *SLC6A19*, solute carrier family 6 member 19; ; TNF-α, tumor necrosis factor alpha

Introduction

Diets for growing pigs based on soybean meal (SBM) and cereal grains typically meet amino acid (AA) requirements and maximize growth performance and protein synthesis. However, over the past few decades, the use of synthetic AA has increased, whereas the inclusion of SBM has been reduced, resulting in diets with lower protein levels (Pope et al., 2023). Historically, low-protein diets have been formulated using up to four

Received July 18, 2025. Accepted September 26, 2025

synthetic AA (i.e., Lys, Met, Thr, and Trp), but with advances in industrial AA production, more indispensable AA (i.e., Val, Ile, His, and Arg) are now commercially available in feed grade form and may be included in low-protein diets (Wang et al., 2018; Duarte et al., 2024).

Regardless of if AA are supplied by SBM or synthetic sources, it is assumed that protein and energy deposition occur at similar rates as long as AA requirements are met (Che et al., 2017; Wang et al., 2018). However, results of recent research indicate that daily nitrogen retention may decrease when pigs are fed diets with less SBM and more synthetic AA, compared with diets containing more SBM (Gloaguen et al., 2014; Zhao et al., 2019; Cristobal et al., 2025). This may indicate either an AA deficiency in diets with synthetic AA or that there are compounds in SBM, beyond AA, that are beneficial for pigs. In addition to AA, SBM provides other functional components, such as polyphenols, terpenoids, bioactive peptides, dietary fiber, oligosaccharides, and functional lipids, that may offer anti-inflammatory, antimicrobial, antioxidant, or immunomodulatory effects for pigs (Smith and Dilger, 2018; Boyd et al., 2023; Petry et al., 2024). Another possible explanation is that AA from intact proteins, such as SBM, are digested more slowly than synthetic AA, which improves synchronization of AA supply for protein synthesis. In contrast, free AA from synthetic sources may be absorbed rapidly, which may create temporary imbalances if not all indispensable AA needed for protein synthesis are available in the cells at the same time, thereby increasing their oxidation rather than incorporation into body protein (Trottier, 2006; Selle et al., 2020).

The shift from intact protein to more synthetic AA may result in a compensatory increase in AA transporters or changes in the intestinal morphology to facilitate the absorption of free AA (Morales et al., 2015). However, there is a lack of data for mRNA abundance of AA transporters or morphology in different sections of the small intestine of pigs fed diets based on either intact protein or synthetic AA (Morales et al., 2015; Wang et al., 2018).

Reducing crude protein in corn-SBM diets by decreasing SBM inclusion and increasing corn, while supplementing with synthetic AA, is believed to increase the net energy (NE) of the diets because corn contains more NE than SBM (NRC, 2012). However, results of recent experiments indicate that the NE in SBM may have been underestimated and is close to the NE in corn (Cemin et al., 2020; Boyd et al., 2023; Cristobal et al., 2024). Therefore, if the NE in SBM is close to that of corn, it is expected that dietary NE will not increase if more corn and less SBM is used in diets, but validation of this hypothesis is lacking.

Therefore, the objective of this experiment was to test the hypothesis that reducing crude protein by reducing the inclusion of SBM in corn-SBM-based diets will not increase dietary NE and will not affect growth performance, carcass composition, protein and energy deposition, intestinal morphology, blood cytokine concentrations, or the mRNA abundance of intestinal AA transporters.

Materials and Methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for the experiment before animal work was initiated. Pigs were the offspring of Line 800 boars and Camborough females (Pig Improvement Company, Hendersonville, TN, USA).

Dietary treatments

A control diet was formulated based on corn and SBM without synthetic AA. Three additional diets were formulated by reducing the inclusion rate of SBM and adding more corn and three synthetic AA (i.e., Lys, Met, and Thr), four synthetic AA (i.e., Lys, Met, Thr, and Trp), or five synthetic AA (i.e., Lys, Met, Thr, Trp, and Val) to the diet. Therefore, a total of four diets were used (Table 1). Concentrations of standardized ileal digestible indispensable AA and all other nutrients were at or above requirements for growing pigs in all diets (NRC, 2012), but the concentration of crude protein was reduced from 20.0% in the control diet to 16.4%, 15.4%, or 13.4% in diets containing three, four, or five synthetic AA.

Animals, housing, feeding, and growth performance

A total of 176 growing pigs (average initial body weight: 32.2 ± 4.2 kg) were used in the experiment. Among these, 16 pigs (eight gilts and eight barrows) were randomly selected at the start of the experiment and designated as an initial slaughter group (ISG) to determine the initial body nutrient composition of pigs. The remaining 160 pigs were allotted to the four diets using a randomized complete block design with four pigs per pen (two gilts and two barrows) and 10 replicate pens per diet. Starting weight was used as the blocking factor. Pigs were housed in a mechanically ventilated grower unit, where pens had partly slatted concrete floors and were equipped with a feeder and a nipple drinker. Water and experimental diets were provided to pigs on an *ad libitum* basis for 28 d. Individual pig weights were recorded at the beginning of the experiment and at the conclusion of the experiment on day 29. Feed additions were recorded daily, and the weight of feed left in the feeder was recorded on day 29. Data were summarized to calculate final body weight, average daily feed intake (ADFI), average daily gain (ADG), and gain:feed ratio (G:F) within each pen and treatment group.

Sample collection and analysis

At the conclusion of the experiment, one gilt or barrow (average final body weight: 61.1 ± 8.4 kg) was randomly selected from each pen, for a total of 20 gilts and 20 barrows. In each pen, the selected pig was the one whose body weight was closest to the pen average. Two blood samples were collected from the jugular vein of each selected pig without prior fasting. One blood sample was collected in heparinized vacutainers, whereas the other sample was collected in vacutainers containing ethylenediaminetetraacetic acid (EDTA). After sample collection, the selected pigs were transported to the Meat Science Laboratory at the University of Illinois and slaughtered after an overnight fast. Pigs were euthanized via head to heart electric stunning followed by exsanguination. Blood was quantitatively collected from each pig. A sample of 50 mL of blood was collected for body compositional analysis.

Euthanized pigs were scalded, dehaired, and singed to remove all hair from the carcass; toenails, tail, and head were then removed. Weights of organs (i.e., heart, kidneys, liver, gall bladder, spleen, lungs, and gastrointestinal tract) were recorded. Within 15 min after euthanasia, the gastrointestinal tract was emptied and rinsed with water, and the empty weights of the stomach, small intestine, and large intestine were recorded. Before emptying the intestinal tract, digesta samples from the

Table 1. Ingredient composition of experimental diets and analyzed nutrient composition of ingredients and experimental diets formulated to different concentrations of soybean meal (SBM) and crude protein, as-fed basis[1]

Item	Ingredients		Diets			
	Corn	SBM[2]	20.0% crude protein	16.4% crude protein	15.4% crude protein	13.4% crude protein
Ingredient, %						
Ground corn	–	–	60.40	70.27	71.40	75.11
Soybean meal	–	–	34.50	24.00	22.80	18.75
Soybean oil	–	–	2.50	2.50	2.50	2.50
Dicalcium phosphate	–	–	0.90	1.10	1.10	1.15
Ground limestone	–	–	0.80	0.75	0.75	0.75
L-Lys-HCl	–	–	–	0.32	0.35	0.47
DL-Met	–	–	–	0.08	0.09	0.12
L-Thr	–	–	–	0.08	0.10	0.15
L-Trp	–	–	–	–	0.01	0.03
L-Val	–	–	–	–	–	0.07
Sodium chloride	–	–	0.40	0.40	0.40	0.40
Vitamin-mineral premix[3]	–	–	0.50	0.50	0.50	0.50
Analyzed nutrients						
Dry matter, %	86.95	89.80	88.49	89.73	88.16	88.55
Crude protein, %	7.80	45.18	20.02	16.36	15.38	13.40
Gross energy, kcal/kg	3,819	4,204	3,976	3,936	3,924	3,963
Acid-hydrolyzed ether extract, %	2.53	1.76	5.17	4.49	4.15	4.62
Starch, %	64.50	–	39.75	45.88	46.58	48.88
Total dietary fiber, %	15.10	23.10	17.70	18.20	18.50	17.00
Soluble dietary fiber, %	2.50	3.4	2.40	2.10	2.00	2.80
Insoluble dietary fiber, %	12.6	19.7	15.30	16.10	16.50	14.20
Ash, %	1.32	6.19	4.65	4.27	4.21	4.05
Indispensable amino acids, %						
Arg	0.35	3.31	1.25	0.94	0.98	0.93
His	0.23	1.23	0.50	0.40	0.41	0.39
Ile	0.27	2.05	0.85	0.69	0.69	0.65
Leu	0.91	3.48	1.58	1.35	1.39	1.32
Lys	0.24	2.84	1.07	1.09	1.08	1.11
Met	0.17	0.63	0.28	0.28	0.31	0.36
Phe	0.37	2.30	0.95	0.77	0.79	0.75
Thr	0.27	1.79	0.70	0.63	0.67	0.64
Trp	0.06	0.63	0.34	0.23	0.26	0.25
Val	0.36	2.14	0.92	0.75	0.75	0.80
Dispensable amino acids, %						
Ala	0.57	1.98	0.87	0.82	0.82	0.75
Asp	0.51	5.19	1.81	1.56	1.56	1.35
Cys	0.18	0.67	0.27	0.26	0.27	0.26
Glu	1.40	8.20	3.16	2.82	2.83	2.54
Gly	0.29	1.91	0.73	0.65	0.65	0.58
Pro	0.67	2.43	1.03	0.97	0.97	0.90
Ser	0.36	2.27	0.75	0.68	0.69	0.61
Tyr	0.25	1.53	0.58	0.52	0.52	0.47
Total amino acids, %	7.46	44.58	17.64	15.41	15.40	14.66

[1] Diets containing 20.0, 16.4, 15.4, or 13.4% crude protein were formulated to contain 3,401, 3,391, 3,389, and 3,382 kcal metabolizable energy per kg (as-fed basis), respectively, and the following quantities of standardized ileal digestible amino acids: Lys, 0.98, 0.98, 0.98, and 0.98%; Met, 0.28, 0.32, 0.32, and 0.33%; Thr, 0.65, 0.59, 0.59, and 0.59%; Trp, 0.23, 0.17, 0.17, and 0.17%, and Val, 0.82, 0.65, 0.64, and 0.64%.

[2] Sugar composition (%): glucose, 0.05; sucrose, 6.27; maltose, 0.13; fructose, 0.07; stachyose, 5.61; raffinose, 1.64. Trypsin inhibitor units per mg: 3.42.

[3] The vitamin–mineral premix provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,150 IU; vitamin D_3 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 B_{12}, 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.

small intestines and colon were collected for ammonia analysis. For each sample, 3 mL of digesta were collected in 25-mL tubes, and 3 mL of HCl were added as a preservative. Concentration of protein was also analyzed in colon digesta.

The carcass was divided down the midline from the groin to the chest cavity. One half of each carcass was used to determine hot carcass weight, dressing percentage, and chilled carcass weight. For body composition analysis, the body was partitioned into three components: carcass, blood, and viscera. The carcass consisted of skin, lean tissue, and fat tissue, without all bones. Blood included all blood collected during exsanguination. Viscera comprised the liver, heart, kidneys, lungs, spleen, and the emptied stomach, small intestine, and large intestine.

Jejunal and ileal tissue samples were collected (about 5 cm in length) approximately 200 cm distal to the pylorus and 80 cm caudal to the ileal-cecal junction, respectively. The jejunal and ileal tissue samples were opened longitudinally along the mesenteric attachment, rinsed with phosphate buffered saline, pinned serosa side down on a piece of cardboard (Nabuurs et al., 1993), and then fixed by immersion in 10% neutral buffered formalin. These tissue samples were delivered to the Veterinary Diagnostic Laboratory at the University of Illinois (Urbana, IL, USA) within 24 h post fixation to be sectioned and transferred to slides. After fixation, the intestinal tissues were embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin (Pluske et al., 1996). Villus height and crypt depth of the jejunum and ileum were measured from 10 straight and integrated villi and their associated crypts in each sample using Nanozoomer Digital Pathology View2 (Hammatsu, Bridgewater, NJ, USA) as described by Liu et al. (2018).

Ileal mucosa samples were also collected and mRNA abundance of AA transporters including solute carrier family 3 member 2 (*SLC3A2*; rBAT), solute carrier family 6 member 14 (*SLC6A14*; ATB$^{\circ,+}$), and solute carrier family 6 member 19 (*SLC6A19*; B°AT) were determined in these samples. Samples were washed with phosphate-buffered saline, scraped gently, snap-frozen in liquid nitrogen, and stored at −80°C until used for ribonucleic acid (RNA) extraction and quantitative reverse-transcription polymerase chain reaction (qRT-PCR) as described by Espinosa et al. (2021).

Weights of muscle and fat tissues and skin from the left side of the carcass were recorded the day after pigs were slaughtered. Because one side was used, the weights of muscle and fat tissues and skin were calculated by multiplying the recorded weights from the left half of the carcass by two, and the sum was assumed to represent the weight of the total carcass.

Samples of viscera, muscle, fat, skin, and blood from exsanguination were stored at −20°C until processing. Prior to lyophilization, samples were frozen at −80°C for 8 h and then lyophilized for 70 h. Lyophilized samples were ground using a swing-type grain mill (model: RRH-500, Zhejiang Winki Plastic Industry Co., Ltd, Zhejiang, China). Samples were analyzed for dry matter (method 930.15; AOAC Int, 2019) and for nitrogen using the combustion procedure (method 990.03; AOAC Int, 2019) on a LECO FP628 (LECO Corp., Saint Joseph, MI, USA). Crude protein was calculated as analyzed nitrogen × 6.25. Gross energy in these samples was analyzed using bomb calorimetry (Model 6400; Parr Instruments, Moline, IL, USA), and acid-hydrolyzed ether extract was analyzed by acid hydrolysis using 3 *N* HCl (Ankom HCl Hydrolysis System, Ankom Technology, Macedon, NY, USA) followed by fat extraction (Ankom XT-15 Extractor, Ankom Technology, Macedon, NY, USA).

The blood samples collected in the vacutainer with EDTA were centrifuged at 4,000×*g* for 13 min to recover the plasma, which was then stored at −20 °C until analyzed. Heparinized plasma samples were analyzed for plasma urea nitrogen, total protein, and albumin, whereas plasma samples that contained EDTA were analyzed for cytokines [e.g., interleukin (IL) 1β, IL-10, IL-4, and tumor necrosis factor alpha (TNF-α)]. The IL-1β and TNF-α were selected as representative pro-inflammatory cytokines, and IL-4 and IL-10 were selected as representative anti-inflammatory cytokines, because these four markers are among the most widely studied in swine and provide a general overview of the balance between pro- and anti-inflammatory immune responses in pigs (Limbach et al., 2021; Duarte et al., 2024).

Plasma urea nitrogen, total protein, and albumin were analyzed using a Beckman Coulter Clinical Chemistry AU analyzer (Beckman Coulter Inc., Brea, CA, USA). Cytokines in plasma samples were analyzed using a sandwich enzyme-linked immunosorbent assay kit according to manufacturer's instructions (R&D Systems Minneapolis, MN, USA; Invitrogen, MA, USA).

Ammonia in digesta samples from the small intestines and colon was analyzed by gas chromatography using a Hewlett-Packard 5890A Series II gas-liquid chromatograph (Agilent, Santa Clara, CA) and a glass column (180 cm by 64 mm i.d.). Digesta samples from the colon were also analyzed for microbial protein. Samples were fractionated using differential centrifugation (Metges et al., 1999) and centrifuged at 250 relative centrifugal force for 15 min at 4°C, which separated fractions that contained undigested feed particles in the precipitate and porcine cells in the supernatant (Miner-Williams et al., 2009). The supernatant was centrifuged at 14,500 relative centrifugal force for 30 min at 4°C, which resulted in a precipitate that contained microbial cells (Miner-Williams et al., 2009). This precipitate was then subjected to a lysis buffer, which contained 100 mM of tris(hydroxymethyl) aminomethane at pH 7.2, 0.5% sodium dodecyl sulfate, and 0.5% sodium deoxycholate. The protein concentration of the lysed microbial cells was analyzed using Pierce Bicinchoninic Acid Assay Kit (ThermoFisher Scientific, Waltham, MA).

The RNA was extracted from 30 ± 0.2 mg of frozen ileal mucosa using β-mercaptoethanol (Sigma-Aldrich, St Louis, MO, USA) according to the RNeasy Mini Kit (QIAGEN, Germantown, MD, USA) manufacturer's instructions and following the procedure described by Espinosa et al. (2021). Total RNA was quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The RNA quality was determined using a Fragment Analyzer Automated CE System (method DNF-471-33-SS Total RNA 15 nt; Advanced Analytical, Ankeny, IA, USA). The RNA samples with an RNA quality number greater than 7 were diluted to 100 ng/µL with DNase/RNase free water and used for complementary deoxyribonucleic acid (cDNA) synthesis. The cDNA was then diluted 1:4 with DNase/RNase-free water to conduct qRT-PCR analysis which was performed using 4 µL of diluted cDNA and 6 µL of a mixture including forward and reverse primers, SYBR Green master mix (Quanta Biosciences Inc., Gaithersburg, MD, USA), and DNase/RNase free water in a MicroAmpTM Optical 384-Well Reaction Plate (Applied Biosystems, Foster City, CA, USA). Two internal control genes, glyceraldehyde 3-phosphate dehydrogenase and beta-actin (Nygard et al., 2007), were used to normalize the mRNA abundance of target genes. Target genes (ie, *SLC3A2, SLC6A14*, and *SLC6A19*) were analyzed to determine if dietary crude protein influences regulation of AA absorption and transport in the small intestine.

Chemical analysis of diets and ingredients

All diet and ingredient samples were ground using a swing-type grain mill (model: RRH-500, Zhejiang Winki Plastic Industry Co., Ltd, Zhejiang, China) prior to chemical analyses. Diet and ingredient samples were analyzed for dry matter, nitrogen, acid-hydrolyzed ether extract, and gross energy as described for viscera, muscle, fat, skin, and blood. Crude protein in diet and ingredient samples was also calculated as analyzed nitrogen × 6.25. Diet and ingredient samples were also analyzed for ash (method 942.05; AOAC Int, 2019), and insoluble dietary fiber and soluble dietary fiber were analyzed on an Ankom Total Dietary Fiber Analyzer (Ankom Technology, Macedon, NY, USA) according to method 991.43 (AOAC Int, 2019). Total dietary fiber was calculated as the sum of insoluble and soluble dietary fiber. Amino acids in diet and ingredient samples were analyzed on a Hitachi Amino Acid analyzer (Model No. L8800; Hitachi High Technologies America, Inc., Pleasanton, CA, USA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard [method 982.30 E(a, b, and c); AOAC Int, 2019]. Total starch was analyzed in corn and in diet samples using the glucoamylase procedure (method 979.10; AOAC Int, 2019), whereas glucose, sucrose, maltose, fructose, stachyose, and raffinose were analyzed in SBM using high-performance liquid chromatography (method 977.2, AOAC Int, 2019). Soybean meal was also analyzed for trypsin inhibitors (method Ba 12-75; AOCS, 2006).

Calculations

The analyzed energy, protein, and lipids in blood, viscera, muscle, fat, and skin samples were used to estimate total energy, protein, and lipids of viscera and blood. Total energy, protein, and lipids in the carcass were calculated as the sum of these components in muscle, fat, and skin. The total amount of energy, protein, and lipids in each pig at the conclusion of the experiment was calculated from the sum of the energy, protein, and lipids in the blood, viscera, and carcass. All values were calculated on a dry matter basis. Retention of energy, protein, and lipids was calculated from the difference between the average quantity of energy, protein, and lipids in the 16 pigs from the ISG and the quantity of energy, protein, and fat in the treatment pigs at the end of the 28-d experimental period.

Net energy in protein and lipids was calculated by multiplying retained protein and lipids by 5.66 and 9.46 kcal/g, respectively (Ewan, 2001). Net energy for growth was calculated as the sum of NE from retained protein and lipids. Daily NE for maintenance was calculated by multiplying the mean metabolic body weight ($kg^{0.60}$) at the start and the conclusion of the experiment by 179 kcal (Noblet et al., 1994). Net energy per kg of diet was calculated by dividing the sum of NE for growth and NE for maintenance by daily feed intake.

To calculate energy efficiency, daily energy intake was calculated by multiplying ADFI of pigs by analyzed gross energy in diets. Energy efficiency for growth was then calculated by dividing retained energy in the total body by energy intake and multiplying by 100.

Statistical analysis

Data were analyzed using the MIXED Procedure in SAS (version 9.4; SAS Inst. Inc., Cary, NC, USA). Homogeneity of the variances among treatments was confirmed using the UNIVARIATE procedure. The MIXED procedure was used to generate studentized residuals, and outliers were defined as means with residuals greater than 3 or less than −3. All outliers that were identified and removed included one pig fed the diet with 13.4% protein for the carcass composition analysis, one pig fed the diet with 20.0% protein for the IL-1β analysis, one pig fed the diet with 16.4% protein for the TNF-α analysis, one pig fed the diet with 15.4% for the ileal morphology analysis, and one pig fed the diet with 13.4% protein for the jejunal morphology analysis. In addition, four pigs fed each diet were identified as outliers for the mRNA abundance analysis. The pen was considered the experimental unit for growth performance, whereas the individual pig was considered the experimental unit for all other analyses. The statistical model included diet as fixed effect and replicate as random effects. Least square means were calculated, and orthogonal polynomial contrast coefficients were generated from the analyzed dietary crude protein using the Interactive Matrix Language procedure of SAS (version 9.4; SAS Inst. Inc., Cary, NC, USA). These coefficients were used to test linear and quadratic effects of reducing dietary crude protein. Statistical significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Results

Pigs remained healthy during the experiment and no mortality or feed refusals were observed. Final body weight of pigs was not affected by dietary treatment (Table 2). Average daily gain and ADFI of pigs were not affected by reducing SBM and increasing synthetic AA in diets, which resulted in no differences in G:F. Live weight, hot carcass weight, dressing percentage, viscera weights, and digesta-free body weight were also not different among treatments (Table 3). However, chilled carcass weight tended to decrease (quadratic, $P = 0.099$) as dietary protein levels were reduced.

Retention of nutrients and energy

Weights of carcass, viscera, and blood were not affected by dietary treatments (Table 4). Concentrations of protein and fat

Table 2. Growth performance of growing pigs fed experimental diets.[1]

Item	Dietary crude protein, %					*P*-value[2]	
	20.0	16.4	15.4	13.4	SEM	Linear	Quadratic
Initial body weight, kg	32.17	32.29	32.20	32.18	–	–	–
Final body weight, kg	61.31	60.64	60.62	61.70	2.20	0.908	0.378
ADG[3], kg/d	1.040	1.012	1.015	1.055	0.041	0.912	0.339
ADFI[3], kg/d	2.116	2.207	2.147	2.234	0.072	0.133	0.914
G:F[3]	0.493	0.460	0.473	0.474	0.014	0.296	0.275

[1] Least squares means represent 10 observations per dietary treatment.
[2] *P*-values for orthogonal polynomial contrast.
[3] ADG = average daily gain; ADFI = average daily feed intake; G:F = gain-to-feed ratio.

Table 3. Weights of carcass and viscera of growing pigs fed experimental diets.[1]

Item	Dietary crude protein, %					*P*-value[2]	
	20.0	16.4	15.4	13.4	SEM	Linear	Quadratic
Live weight, kg	58.85	59.47	58.47	59.80	1.96	0.542	0.658
Hot carcass weight[3], kg	42.50	43.11	43.66	42.34	1.60	0.896	0.305
Dressing percentage, %	72.10	72.42	73.32	72.02	0.63	0.721	0.172
Chilled carcass, kg	42.23	43.41	43.91	41.82	1.71	0.953	0.099
Full viscera[4], kg	8.28	8.47	8.29	8.51	0.25	0.514	0.920
Full viscera, % of live weight	14.14	14.28	14.22	13.97	0.35	0.790	0.548
Empty viscera, kg	7.09	6.99	7.00	6.97	0.20	0.562	0.881
Empty viscera, % of live weight	12.08	11.79	11.87	11.70	0.23	0.195	0.883
Digesta-free body weight[5], kg	52.32	53.27	53.61	51.54	1.98	0.825	0.171

[1] Least squares means represent 10 observations per each dietary treatment except that means for dressing percentage and empty viscera of diet containing 15.4% protein represent 9 observations and that means for dressing percentage and full viscera of diet containing 13.4% protein represent 9 observations.
[2] *P*-values for orthogonal polynomial contrast.
[3] Hot carcass weight does not include leaf fat.
[4] Full viscera include the combined weights of the liver, gall bladder, heart, kidneys, lungs, spleen, and the stomach, small intestine, and large intestine with their contents.
[5] Calculated as the sum of the weights of chilled carcass, empty viscera, and blood.

in carcass and viscera were also not affected by dietary treatments, but protein in blood increased and then decreased as dietary protein levels reduced (quadratic, $P < 0.034$). Concentration of energy in carcass, blood, and bone-free total body increased (quadratic, $P = 0.034$) or tended (quadratic, $P < 0.10$) to increase and then decreased as dietary protein levels were reduced. Retained protein, lipid, and energy were not affected by dietary treatment, but energy efficiency for growth tended to decrease (linear, $P = 0.83$; quadratic, $P = 0.087$) as dietary protein levels were reduced. Net energy in diets also tended to decrease (linear, $P = 0.051$) as dietary protein levels were reduced.

Blood characteristics, metabolites in digesta, and AA transporters

Plasma urea nitrogen was reduced (linear, $P < 0.001$) as dietary protein levels were reduced, but blood total protein was not affected by dietary treatment (Table 5). Albumin in blood tended to increase and then decrease (quadratic, $P = 0.072$) as dietary protein levels were reduced, but concentrations of cytokines were not affected by dietary protein levels. Ileal and jejunal morphologies were also not affected by dietary protein levels (Table 6). Ammonia concentrations in ileal digesta increased and then decreased (quadratic, $P = 0.043$) and ammonia in colon digesta tended to increase and then decrease (quadratic, $P = 0.074$) as dietary protein levels were reduced. Bacterial protein in colon digesta was reduced (linear, $P = 0.030$) as dietary protein levels were reduced, but mRNA abundance of related to AA transporters in the ileal mucosa was not affected by dietary protein levels (Table 7).

Discussion

The analyzed crude protein and AA in experimental diets were in close agreement with formulated values, which indicates that diets were mixed correctly. Likewise, analyzed concentration of nutrients in corn and SBM was in agreement with previous data (NRC, 2012).

It was expected that on average, all indispensable AA in diets, except for Met, would decrease by reducing the inclusion of SBM and increasing corn and synthetic AA in diets because of the increased digestibility of synthetic AA compared with AA in SBM, and this was also observed. However, the analyzed Met increased in the diets as SBM was reduced and synthetic AA were added, which is likely because synthetic DL-Met was added to diets to meet the requirements for both Met and Cys. The Lys to crude protein ratio in each diet increased as dietary crude protein was reduced, and the ratio ranged between 5.35 and 8.28% due to the gradual increase of synthetic Lys in diets.

Growth performance, carcass weights, and retained nutrients and energy

The lack of differences among treatments in final body weight, ADG, ADFI, and G:F is in agreement with results from previous experiments (Kerr et al., 1995; Le Bellego et al., 2001; Wang et al., 2018). The lack of differences in growth performance is likely a result of the fact that reduced protein diets supplemented with synthetic AA can provide enough AA to meet minimum requirements by pigs to maximize growth. However, reducing crude protein in corn-SBM diets by more than 4% may result in reduced growth performance due to a potential AA imbalance (Wang et al., 2018) or because something other than AA may become limiting to normal growth (Boyd et al., 2024). Feeding reduced protein diets may also result in increased fat deposition of pigs and reduced protein deposition (Ruusunen et al., 2007; Morazán et al., 2015; Ruiz-Ascacibar et al., 2017; Boyd et al., 2024).

It has been speculated that there are fewer AA provided in excess of requirements in reduced protein diets, which require less energy for deamination of AA and excretion of nitrogen. Low-protein diets, therefore, may provide more NE, which can result in increased fat deposition (Smith et al., 1999). However, despite a reduction in dietary protein levels by more than six percentage units in this experiment, neither growth performance nor carcass weights were affected by treatment. It is, therefore, likely that the AA profile in the diets fed in the present experiment was closer to meeting the requirements of the pigs than in some previous experiments where reduced growth performance was observed in pigs fed low-protein diets (Ruusunen et al., 2007; Ruiz-Ascacibar et al., 2017). Another possibility is that the SBM was not reduced to the extent that it limited growth, as observed by Boyd et al. (2024). The current data are in agreement with data demonstrating that it is

Table 4. Analyzed composition of lyophilized carcass, retention of energy, protein, and lipids in pigs from the initial slaughter group (ISG) and pigs fed experimental diets, and net energy (as-fed basis) in experimental diets[1,2]

Item		Dietary crude protein, %					*P*-value[3]	
	ISG	20.0	16.4	15.4	13.4	SEM	Linear	Quadratic
Carcass[4]								
Weight, kg	8.82	21.60	21.91	20.99	20.33	1.14	0.163	0.261
Protein, g/kg	558	481	457	496	486	13	0.670	0.401
Lipids, g/kg	365	479	474	419	463	24	0.378	0.483
Energy, Mcal/kg	6.65	7.15	7.22	7.30	7.02	0.1	0.640	0.096
Viscera[5]								
Weight, kg	0.68	1.58	1.63	1.61	1.63	0.06	0.438	0.784
Protein, g/kg	696	626	632	598	609	19	0.357	0.866
Lipids, g/kg	194	237	245	244	250	13	0.482	0.976
Energy, Mcal/kg	5.52	5.60	5.76	5.72	5.70	0.08	0.346	0.311
Blood								
Weight, kg	0.25	0.53	0.50	0.46	0.49	0.03	0.120	0.544
Protein, g/kg	994	1,006	1,022	1,025	1,009	7	0.454	0.034
Lipids, g/kg	10	21	14	14	12	2	0.005	0.645
Energy, Mcal/kg	5.41	5.26	5.58	5.41	5.26	0.11	0.837	0.025
Total body[6]								
Weight, kg	9.74	23.71	24.04	23.07	22.45	1.19	0.167	0.270
Protein, g/kg	579	503	481	514	506	13	0.747	0.399
Lipids, g/kg	344	452	449	399	438	22	0.391	0.513
Energy, Mcal/kg	6.54	7.00	7.09	7.15	6.89	0.10	0.664	0.069
Retained protein[7], g/d	–	223	210	221	201	19	0.285	0.725
Retained lipid[7], g/d	–	265	266	234	236	30	0.213	0.751
Retained lipid: protein	–	1.06	1.09	0.94	1.01	0.07	0.491	0.976
Retained energy[7], Mcal/d	–	3.66	3.82	3.52	3.25	0.33	0.135	0.120
Energy intake[8], Mcal/d	–	8.41	8.68	8.42	8.85	0.29	0.184	0.609
Energy efficiency for growth[8], %	–	42.79	43.86	41.68	36.40	3.03	0.083	0.087
NE in diets								
NE from retained protein[9], kcal/d	–	1,263	1,187	1,250	1,138	107	0.285	0.725
NE from retained lipids[9], kcal/d	–	2,510	2,521	2,214	2,234	281	0.213	0.751
NE for growth[10], kcal/d	–	3,774	3,708	3,464	3,372	357	0.124	0.671
NE for maintenance[11], kcal/d	–	1,795	1,788	1,787	1,800	40	0.883	0.411
NE in diets[12], kcal/kg	–	2,605	2,488	2,436	2,305	134	0.051	0.667

[1] Least squares means represent 10 observations per dietary treatment.

[2] Concentrations of protein, lipid, and energy represent analyzed nitrogen × 6.25, acid-hydrolyzed ether extract, and gross energy in each body part, respectively.

[3] *P*-values for orthogonal polynomial contrast.

[4] Carcass includes skin, lean tissue, and fat tissue, excluding all bones.

[5] Viscera include the liver, heart, kidneys, lungs, spleen, and the empty stomach, small intestine, and large intestine.

[6] Total body includes bone-free carcass, viscera, and blood.

[7] Retained nutrients and energy in the body were calculated using the difference in body composition between ISG ($n = 16$) and body composition of pigs fed experimental diets for 28 d.

[8] Energy intake was calculated as multiplying average daily feed intake of pigs by analyzed gross energy in each respective diets; energy efficiency for growth was calculated dividing retained energy by the energy intake and multiplying it by 100.

[9] NE in protein and lipids was calculated by multiplying retained protein and lipids by 5.66 and 9.46 kcal/g, respectively (Ewan, 2001).

[10] NE for growth was calculated as the sum of NE from retained protein and lipids.

[11] Daily NE for maintenance was calculated by multiplying the mean metabolic body weight (kg$^{0.60}$) by 179 kcal (Noblet et al., 1994).

[12] NE in diets was calculated by dividing the sum of NE for growth and NE for maintenance by daily feed intake.

possible to feed reduced protein diets without negatively impacting growth performance or carcass characteristics (Kerr et al., 1995; Li et al., 2016; Suárez-Belloch et al., 2016).

One concern with formulating low-protein diets is that as diet SBM is reduced, the provision of dispensable AA and nitrogen to synthesize dispensable AA is also reduced and at some point, protein synthesis in pigs fed low-protein diets is reduced due to a lack of dispensable AA (Camire et al., 2023). However, total analyzed indispensable AA were between 46 and 49% of all AA in the four experimental diets, and the concentration of indispensable AA, therefore, stayed below 50% of all AA in all diets, which has been suggested to be necessary to maintain protein synthesis in pigs fed low-protein diets (Lenis et al., 1999). It is, therefore, unlikely that any of the diets were limited in dispensable AA and the observation that the concentration of carcass protein in pigs was not impacted by diet protein level supports this conclusion.

The values for hot carcass weight and digesta-free body weight in diets with 20.0% or 16.4% crude protein were in agreement with data from previous experiments in which corn-SBM diets with approximately 20% crude protein were fed to growing pigs (Kil et al., 2011; 2013; Stewart et al., 2013). The observed values for chilled carcass and the full viscera weight also agreed with previous data. However, empty viscera weight in kg or as percent of live weight was less in this experiment than previously reported (Stewart et al., 2013), which is

Table 5. Concentrations of plasma urea nitrogen, total protein, albumin, and cytokines in plasma of pigs fed experimental diets[1]

Item	Dietary crude protein, %					*P*-value[2]	
	20.0	16.4	15.4	13.4	SEM	Linear	Quadratic
Plasma urea nitrogen, mg/dL	16.10	10.30	8.10	5.00	0.93	<0.001	0.886
Total protein, g/dL	5.59	5.88	5.67	5.64	0.12	0.742	0.175
Albumin, g/dL	3.70	3.86	3.84	3.66	0.10	0.999	0.072
Cytokines[3], pg/mL							
IL-1β	33.22	13.87	18.75	20.77	7.70	0.197	0.211
IL-4	0.23	0.22	0.22	0.22	0.00	0.142	0.347
IL-10	0.09	0.09	0.09	0.09	0.00	0.996	0.236
TNF-α	118.14	92.87	105.26	96.58	11.20	0.182	0.539

[1] Least squares means represent 10 observations per each dietary treatment except that means for IL-1β of diet containing 20.0% protein represent 9 observations and that means for IL-1β and TNF-α of diet containing 16.4% protein represent 9 observations.
[2] *P*-values for orthogonal polynomial contrast.
[3] IL, interleukin; TNF, tumor necrosis factor alpha.

Table 6. Morphology of jejunal and ileal tissues, ammonia concentrations in ileal and colon digesta, and bacteria in colon digesta of pigs fed experimental diets[1]

Item	Dietary crude protein, %					*P*-value[2]	
	20.0	16.4	15.4	13.4	SEM	Linear	Quadratic
Jejunal morphology							
Villi height, µm	496.45	514.81	510.86	502.58	16.60	0.715	0.450
Crypt depth, µm	275.46	290.24	271.93	289.37	12.15	0.525	0.931
Villi height to crypt depth	1.83	1.79	1.90	1.75	0.07	0.631	0.426
Ileal morphology							
Villi height, µm	511.06	488.14	477.42	495.06	15.84	0.324	0.287
Crypt depth, µm	259.51	246.24	259.30	251.83	12.79	0.704	0.781
Villi height to crypt depth	2.02	2.02	1.87	1.97	0.08	0.401	0.673
Ammonia in ileum, mg/g	0.52	0.84	0.78	0.60	0.12	0.421	0.043
Ammonia in colon, mg/g	2.14	2.32	2.49	2.15	0.13	0.559	0.074
Bacteria protein in colon, µg/g	963.14	817.07	868.43	710.05	74.30	0.030	0.700

[1] Least squares means represent 10 observations per each dietary treatment except that means for ileal morphology of pigs fed diet containing 15.4% protein represent 9 observations and that means for jejunal morphology of pigs fed diet containing 13.4% protein represent 9 observations.
[2] *P*-values for orthogonal polynomial contrast.

Table 7. Relative mRNA abundance of genes for amino acid transporters in the ileal mucosa of pigs fed experimental diets.[1,2]

Item[3]	Dietary crude protein, %					*P*-value[4]	
	20.0	16.4	15.4	13.4	SEM	Linear	Quadratic
SLC3A2	1.85	1.10	2.19	1.41	1.29	0.629	0.762
SLC6A14	2.02	2.11	1.26	1.30	1.46	0.269	0.759
SLC6A19	0.62	0.58	0.54	0.94	1.35	0.454	0.265

[1] Data are least squares means of 6 observations per treatment.
[2] Least squares means and SEM were log2-backtransformed after the statistical analysis.
[3] *SLC3A2*, solute carrier family 3 member 2; *SLC6A14*, solute carrier family 6 member 14; and *SLC6A19*, solute carrier family 6 member 19.
[4] *P*-values for orthogonal polynomial contrast.

likely because diets high in fiber were used by Stewart et al. (2013), and dietary fiber will increase intestinal mass (Henry, 1985; Pond et al., 1988).

The observed values for total protein, lipids, and energy in carcass, viscera, and blood were slightly greater than values reported with a 20% crude protein diet (Stewart et al., 2013). Retained protein, lipid, and energy were in agreement with previous data (Ruiz-Ascacibar et al., 2017). Compared with results of other experiments (Kil et al., 2011; 2013), retained protein and lipid were greater, but the lipid:protein ratio aligned closely. The lack of differences in retained protein and lipid among pigs fed experimental diets contrasts with results indicating a reduced protein retention and increased lipid retention in pigs fed low-protein diets compared with pigs fed diets with greater protein concentration (Ruiz-Ascacibar et al., 2017; Boyd et al., 2024). Results from the present experiment were also different from data indicating that energy retention is increased in pigs fed low-protein diets (Le Bellego et al., 2001; Noblet et al., 2001), but it is not clear if this is because of differences in diet composition or a difference in the genetic ability of pigs to retain energy.

The observed NE values in diets were on average greater than values determined in corn-SBM diets (Kil et al., 2011, 2013), but that is likely because more soybean oil was included in the diets fed in this experiment than in previous experiments. The observed NE in the current experiment was close to the determined NE in corn-SBM diets with a similar soybean oil inclusion (Stewart et al., 2013). It was expected that NE would increase as dietary crude protein decreased because the NE in SBM is believed to be less than in corn (Sauvant et al., 2004; NRC, 2012). Therefore, reducing SBM and increasing corn should have increased dietary NE. If reducing SBM increases dietary NE, then G:F should have been improved as well. However, the observation that no increase of NE in diets or improved G:F was observed as crude protein was reduced in diets indicates that NE in SBM is close to or greater than the NE of corn. In fact, the tendency for a reduction in NE as SBM in diets was reduced indicates that NE in SBM may be greater than in corn, which has also been reported in other experiments in which the energy value of SBM was estimated from growth performance of pigs (Cemin et al., 2020; Boyd et al., 2023; Ibagon et al., 2025). It is recognized that estimating the energy value of a feed ingredient or diet from growth performance does not take possible changes in body composition into account and such values should, therefore, not be called NE values (Zhang et al., 2020; Ibagon et al., 2025). To overcome the limitations of estimating diet energy values from growth performance of pigs, the comparative slaughter procedure was used in the present experiment. This procedure allows for taking differences in body composition into account and should, therefore, provide more reliable values than using growth performance data. However, the fact that results of the present experiment were in agreement with some recent values estimated from growth performance of pigs, indicates that NE of SBM indeed is greater than previously believed. Likewise, in recent experiments in which NE in SBM was determined using indirect calorimetry, the NE in SBM was between 87 and 100% of the NE in corn (Li et al., 2017; Cristobal et al., 2024) further indicating that current book values (Sauvant et al., 2004; NRC, 2012) may underestimate the NE in SBM. Results of this experiment, therefore, are in agreement with results of other recently conducted experiments.

Blood parameters, intestinal morphology, digesta analysis, and mRNA abundance of intestinal AA transporters

Observed values for plasma urea nitrogen were within the range of values determined in previous experiments (Kerr and Easter, 1995; Che et al., 2017), and the observed decrease in plasma urea nitrogen as crude protein in diets was reduced, is consistent with other experiments (Kerr and Easter, 1995; Che et al., 2017; Wang et al., 2018; Limbach et al., 2021). Plasma urea nitrogen is generated during AA metabolism and reflects the amount of excess nitrogen that pigs are consuming (Wang et al., 2018). In pigs fed high-protein diets, some AA are fed in excess of the requirement, and these AA are deaminated, producing ammonia that is then converted to urea prior to excretion, which increases plasma urea nitrogen. Pigs fed diets with reduced protein are expected to have less excess AA, and therefore, less deamination, less ammonia production, and less urea synthesis, which reduces plasma urea nitrogen. Thus, the observed reduction in plasma urea nitrogen in pigs fed diets with reduced crude protein was expected.

The observed values for total protein and albumin in blood agreed with values obtained by Che et al. (2017). The lack of differences in total protein and albumin in blood indicates that the provision of limiting indispensable AA was close to the requirements regardless of the diet being fed. Albumin synthesis relies on indispensable AA, and stable levels of albumin in blood indicate that sufficient AA were available for protein synthesis. Maintaining constant levels of total protein in blood, along with the observed reduction in blood urea nitrogen, also supports that diets supported efficient nitrogen utilization.

The observed lack of effects on pro-inflammatory (IL-1β and TNF-α) or anti-inflammatory (IL-10 and IL-4) cytokine levels indicates that reducing dietary crude protein does not confer immune advantages compared with high-protein diets in healthy non-disease-challenged pigs. This observation contrasts data indicating that low-protein diets reduced post-weaning diarrhea in newly weaned pigs, potentially due to improved intestinal health (Limbach et al., 2021). However, results from the present experiment indicate that reducing protein in diets may not necessarily modulate systemic immune responses as measured by cytokine levels in older pigs. The observed lack of treatment effects on villi height or crypt depth in ileal and jejunal morphology also supports this conclusion.

Reducing dietary crude protein intake decreases substrate availability for bacterial fermentation in the hindgut (colon), potentially lowering ammonia production, although low-protein diets do not always result in significant changes in intestinal ammonia levels (Tao et al., 2021). This may be due to compensatory mechanisms in nitrogen metabolism or variations in gut microbiota composition (Liu and Fan, 2022). Reducing dietary protein levels may result in a decrease in bacteria that thrive on undigested protein (Liu and Fan, 2022), which may reduce bacterial protein content in the colon, which was also observed in the present experiment. It is, however, acknowledged that the data presented for intestinal ammonia and bacterial protein are based on the concentration in intestinal contents. If dietary treatments affected total fecal mass, concentrations in intestinal contents would not necessarily represent daily synthesis.

It was hypothesized that a reduction in dietary protein levels may result in adaptive responses for AA transporters, which may increase the mRNA abundance of AA transporters to increase the uptake of more free AA (Morales et al., 2015; Li et al., 2024). The observed lack of effects of dietary treatments on SLC6A14 differs from results of a previous experiment indicating that the mRNA abundance of this gene was reduced in pigs fed diets containing 14% crude protein compared with pigs fed a 20% crude protein diet (Wang et al., 2017). However, the lack of differences observed for SLC3A2 does agree with the previous experiment (Wang et al., 2017). The overall lack of effects from dietary treatments on the mRNA abundance of AA transporters indicates that these specific transporters are not sensitive to dietary protein levels. This observation agrees with data demonstrating that the mRNA abundance of AA transporters is not impacted by diet crude protein level (Morales et al., 2015).

Conclusions

The hypothesis that reducing dietary protein levels by reducing dietary SBM and increasing corn and synthetic AA would not increase dietary NE was confirmed as results demonstrated that there was a tendency for a reduction in NE as crude protein was reduced. Likewise, the hypothesis that reducing diet crude protein would not impact growth performance or carcass composition of pigs was confirmed. Intestinal morphology, blood cytokine concentrations, and mRNA abundance of AA transporters were also not impacted by dietary treatments, which was also in agreement with the hypothesis. Overall, these results indicate no advantage on any of the measured responses of reducing diet SBM concentrations, but the observation that the NE in SBM is likely greater than current book values deserves further investigation.

Acknowledgments

Financial support for this research from The United Soybean Board (Project #2240-352-0514-M; Chesterfield, MO, USA) is greatly appreciated.

Author Contributions

Minoy Cristobal (Data curation, Investigation, Methodology, Writing—original draft), Su A Lee (Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing—review & editing), Carl M. Parsons (Conceptualization, Methodology, Project administration, Resources, Supervision, Writing—review & editing), and Hans H Stein (Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing—review & editing)

Conflict of interest statement. The authors have no conflicts of interest related to the conduct of the work reported in the present manuscript.

Literature Cited

AOAC Int. 2019. Official methods of analysis of AOAC Int. 21st ed. Rockville, MD, USA: AOAC Int.

AOCS. 2006. Official methods and recommended practices. 5th ed. Urbana, IL: AOCS. 21st century.

Boyd, R. D., M.E. Johnston, J. Usry, P. Yeske, and A. Gaines. 2023. Soybean meal mitigates respiratory disease-impaired growth in pigs. Feedstuffs, October. [Accessed February 24, 2025]. https://informamarkets.turtl.co/story/feedstuffs-october-2023/page/2

Boyd R. D., M. E. Johnston, J. Usry, and R.E. Austic. 2024. Foundation study for low protein diet research: SBM depletion impaired growth in pigs despite restoration of all essential amino acids and non-essential amino acid nitrogen. Feedstuffs, April. [Accessed April 24, 2025]. https://www.soymeal.org/soy-meal-articles/sbm-depletion-impaired-growth-in-pigs-despite-restoration-of-all-essential-amino-acids-and-non-essential-amino-acid-nitrogen/

Camire, C. M., M. O. Wellington, J. C. Panisson, L. A. Rodrigues, A. K. Shoveller, and D. A. Columbus. 2023. Effect of the essential amino acid-nitrogen to total nitrogen ratio on lysine requirement for nitrogen retention in growing pigs. J. Anim. Sci. 101: skad298. https://doi.org/doi.org/10.1093/jas/skad298

Cemin, H. S., H. E. Williams, M. D. Tokach, S. S. Dritz, J. C. Woodworth, J. M. DeRouchey, R. D. Goodband, K. F. Coble, B. A. Carrender, and M. J. Gerhart. 2020. Estimate of the energy value of soybean meal relative to corn based on growth performance of nursery pigs. J. Anim. Sci. Biotechnol. 11:70. https://doi.org/10.1186/s40104-020-00474-x

Che, L. Q., X. Peng, L. Hu, C. Wu, Q. Xu, Z. F. Fang, Y. Lin, S. Y. Xu, J. Li, B. Feng, *et al.* 2017. The addition of protein-bound amino acids in low-protein diets improves the metabolic and immunological characteristics in fifteen- to thirty-five-kg pigs. J. Anim. Sci. 95(3):1277–1287. https://doi.org/10.2527/jas.2016.0990

Cristobal, M., S. A. Lee, A. P. Mallea, L. J. Torres-Mendoza, C. M. Parsons, and H. H. Stein. 2024. 380 Diet protein concentration does not influence dietary net energy by group-housed growing pigs offered ad libitum access to feed. J. Anim. Sci. 102(Supplement_3):250–251. (Abstr.) https://doi.org/10.1093/jas/skae234.288

Cristobal M., S. A. Lee, C. M. Parsons, and H. H. Stein. 2025. Replacement of soybean meal in diets for growing pigs with corn and synthetic amino acids results in reduced energy and nitrogen retention and reduced daily nitrogen retention, but metabolizable energy is not changed. J. Anim. Sci. 103: skaf197. https://doi.org/10.1093/jas/skaf197

Duarte, M. E., W. Parnsen, S. Zhang, M. L. T. Abreu, and S. W. Kim. 2024. Low crude protein formulation with supplemental amino acids for its impacts on intestinal health and growth performance of growing-finishing pigs. J. Anim. Sci. Biotechnol. 15(1):55. https://doi.org/10.1186/s40104-024-01015-6

Espinosa, C. D., J. K. Mathai, L. Blavi, Y. Liu, J. K. Htoo, J. C. Gonzalez-Vega, and H. H. Stein 2021. Effects of supplemental d-methionine in comparison to l-methionine on nitrogen retention, gut morphology, antioxidant status, and mRNA abundance of amino acid transporters in weanling pigs. J. Anim. Sci. 99(9):1–10. https://doi.org/10.1093/jas/skab248

Ewan, R.C. 2001. Energy utilization in swine nutrition. In: Lewis, A. J. and L. L. Southern, editors, Swine nutrition. 2nd ed. Washington, DC: CRC Press; p. 85–94.

Gloaguen, M., N. Le Floc'h, E. Corrent, Y. Primot, and J. van Milgen. 2014. The use of free amino acids allows formulating very low crude protein diets for piglets. J. Anim. Sci. 92(2):637–644. https://doi.org/10.2527/jas.2013-6514

Henry, Y. 1985. Dietary factors involved in feed intake regulation in growing pigs: a review. Livest. Prod. Sci. 12(4):339–354. https://doi.org/10.1016/0301-6226(85)90133-2

Ibagon, J. A., H. H. Stein, and S. A. Lee. 2025. The soybean oil equivalency of soybean meal indicates a high energy value of soybean meal when fed to growing pigs. Can. J. Anim. Sci. 105:1–7. https://doi.org/10.1139/cjas-2025-0002

Kerr, B. J., F. K. McKeith, and R. A. Easter. 1995. Effect on performance and carcass characteristics of nursery to finisher pigs fed reduced crude protein, amino acid-supplemented diets. J. Anim. Sci. 73(2):433–440. https://doi.org/10.2527/1995.732433x

Kerr, B. J., and R. A. Easter. 1995. Effect of feeding reduced protein, amino acid-supplemented diets on nitrogen and energy balance in grower pigs. J. Anim. Sci. 73(10):3000–3008. https://doi.org/10.2527/1995.73103000x

Kil, D. Y., F. Ji, L. L. Stewart, R. B. Hinson, A. D. Beaulieu, G. L. Allee, J. F. Patience, J. E. Pettigrew, and H. H. Stein. 2011. Net energy of soybean oil and choice white grease in diets fed to growing and finishing pigs. J. Anim. Sci. 89(2):448–459. https://doi.org/10.2527/jas.2010-3233

Kil, D. Y., F. Ji, L. L. Stewart, R. D. Hinson, A. D. Beaulieu, G. L. Allee, J. F. Patience, J. E. Pettigrew, and H. H. Stein. 2013. Effects of dietary soybean oil on pig growth performance, retention of protein, lipids, and energy, and the net energy of corn in diets fed to growing or finishing pigs. J. Anim. Sci. 91(7):3283–3290. https://doi.org/10.2527/jas.2012-5124

Le Bellego, L., J. van Milgen, S. Dubois, and J. Noblet. 2001. Energy utilization of low-protein diets in growing pigs. J. Anim. Sci. 79(5):1259–1271. https://doi.org/10.2527/2001.7951259x

Lenis, N. P., H. T. van Diepen, P. Bikker, A. W. Jongbloed, and J. van der Meulen. 1999. Effect of the ratio between essential and nonessential amino acids in the diet on utilization of nitrogen and amino acids by growing pigs. J. Anim. Sci. 77(7):1777–1787. https://doi.org/10.2527/1999.7771777x

Li, Y., F. Li, L. Wu, H. Wei, Y. Liu, T. Li, B. Tan, X. Kong, K. Yao, S. Chen, *et al.* 2016. Effects of dietary protein restriction on muscle fiber characteristics and mTORC1 pathway in the skeletal muscle of growing-finishing pigs. J. Anim. Sci. Biotechnol. 7(1):47. https://doi.org/10.1186/s40104-016-0106-8

Li, Z., Y. Li, Z. Lv, H. Liu, J. Zhao, J. Noblet, F. Wang, C. Lai, and D. Li. 2017. Net energy of corn, soybean meal and rapeseed meal in growing pigs. J. Anim. Sci. Biotechnol. 8:44. https://doi.org/10.1186/s40104-017-0169-1

Li, J., Y. Chen, Y. Yang, Y. Yang, and Z. Wu. 2024. High-level L-Gln compromises intestinal amino acid utilization efficiency and inhibits protein synthesis by GCN2/eIF2α/ATF4 signaling pathway in piglets fed low-crude protein diets. Anim. Nutr. 19:480–487. https://doi.org/10.1016/j.aninu.2024.06.008

Limbach, J. R., C. D. Espinosa, E. Perez-Calvo, and H. H. Stein. 2021. Effect of dietary crude protein level on growth performance, blood characteristics, and indicators of intestinal health in weanling pigs. J. Anim. Sci. 99(6):1–14. https://doi.org/10.1093/jas/skab166

Liu, Y., J. Choe, S. Kim, B. Kim, J. M. Campbell, J. Polo, J. D. Crenshaw, J. E. Pettigrew, and M. Song. 2018. Dietary spray-dried plasma improves intestinal morphology of mated female mice under stress condition. J. Anim. Sci. Technol. 60:10. https://doi.org/10.1186/s40781-018-0169-5

Liu, S., and Z. Fan. 2022. Effects of dietary protein restriction on colonic microbiota of finishing pigs. Animals. 13(1):9. https://doi.org/10.3390/ani13010009

Metges, C. C., K. J. Petzke, A. E. El-Khoury, L. Henneman, I. Grant, S. Bedri, M. M. Regan, M. F. Fuller, and V. R. Young. 1999. Incorporation of urea and ammonia nitrogen into ileal and fecal microbial proteins and plasma free amino acids in normal men and ileostomates. Am. J. Clin. Nutr. 70(6):1046–1058. https://doi.org/10.1093/ajcn/70.6.1046

Morales, A., L. Buenabad, G. Castillo, N. Arce, B. A. Araiza, J. K. Htoo, and M. Cervantes. 2015. Low-protein amino acid–supplemented diets for growing pigs: Effect on expression of amino acid transporters, serum concentration, performance, and carcass composition. J. Anim. Sci. 93(5):2154–2164. https://doi.org/10.2527/jas.2014-8834

Morazán, H., J. Alvarez-Rodriguez, A. R. Seradj, J. Balcells, and D. Babot. 2015. Trade-offs among growth performance, nutrient digestion and carcass traits when feeding low protein and/or high neutral-detergent fiber diets to growing-finishing pigs. Anim. Feed Sci. Technol. 207:168–180. https://doi.org/10.1016/j.anifeedsci.2015.06.003

Miner-Williams, W., P. J. Moughan, and M. F. Fuller. 2009. Endogenous components of digesta protein from the terminal ileum of pigs fed a casein-based diet. J. Agric. Food Chem. 57(5):2072–2078. https://doi.org/10.1021/jf8023886

Nabuurs, M. J., A. Hoogendoorn, E. J. van der Molen, and A. L. van Osta. 1993. Villus height and crypt depth in weaned and unweaned pigs, reared under various circumstances in The Netherlands. Res. Vet. Sci. 55(1):78–84. https://doi.org/10.1016/0034-5288(93)90038-H

Noblet, J., H. Fortune, X. S. Shi, and S. Dubois. 1994. Prediction of net energy value of feeds for growing pigs. J. Anim. Sci. 72(2):344–354. https://doi.org/10.2527/1994.722344x

Noblet, J., L. Le Bellego, J. van Milgen, and S. Dubois. 2001. Effect of reduced dietary protein level and fat addition on heat production and nitrogen and energy balance in growing pigs. Anim. Res. 50(3):227–238. https://doi.org/doi.org/10.1051/animres : 2001129

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Washington, DC: Natl. Acad. Press.

Nygard, A. B., C. B. Jørgensen, S. Cirera, and M. Fredholm. 2007. Selection of reference genes for gene expression studies in pig tissues using SYBR green qPCR. BMC Mol. Biol. 8:67. https://doi.org/10.1186/1471-2199-8-67

Pond, W. G., H. G. Jung, and V. H. Varel. 1988. Effect of dietary fiber on young adult genetically lean, obese, and contemporary pigs: body weight, carcass measurements, organ weights, and digesta content. J. Anim. Sci. 66(3):699–706. https://doi.org/10.2527/jas1988.663699x

Petry AL, L. Weaver, and R. D. Boyd. 2024. Functional compounds in soybean meal: Implications for pig health and physiology. *Feedstuffs*, February. [Accessed February 24, 2025]. https://informamarkets.turtl.co/story/feedstuffs-february-2024/page/1

Pluske, J. R., I. H. Williams, and F. X. Aherne. 1996. Maintenance of villous height and crypt depth in piglets by providing continuous nutrition after weaning. Anim. Sci. 62(1):131–144. https://doi.org/10.1017/S1357729800014417

Pope, M., B. Borg, R. D. Boyd, D. Holzgraefe, C. Rush, and M. Sifri. 2023. Quantifying the value of soybean meal in poultry and swine diets. J. Appl. Poult. Res. 32(2):100337. https://doi.org/10.1016/j.japr.2023.100337

Ruiz-Ascacibar, I., P. Stoll, M. Kreuzer, V. Boillat, P. Spring, and G. Bee. 2017. Impact of amino acid and CP restriction from 20 to 140 kg BW on performance and dynamics in empty body protein and lipid deposition of entire male, castrated and female pigs. Animal. 11(3):394–404. https://doi.org/10.1017/s1751731116001634

Ruusunen, M., K. Partanen, R. Pösö, and E. Puolanne. 2007. The effect of dietary protein supply on carcass composition, size of organs, muscle properties and meat quality of pigs. Livest. Sci. 107(2–3):170–181. https://doi.org/10.1016/j.livsci.2006.09.021

Sauvant, D., J. M. Perez, and G. Tran. 2004.Tables of composition and nutritional value of feed ingredients. INRA Editions, Versailles, France: Wageningen Academic Publishers; p. 25–35.

Selle, P. H., J. C. de Paula Dorigam, A. Lemme, P. V. Chrystal, and S. Y. Liu. 2020. Synthetic and crystalline amino acids: Alternatives to soybean meal in chicken-meat production. Animals. 10(4):729. https://doi.org/10.3390/ani10040729

Smith, J. W., II., P. R. O'Quinn, R. D. Goodband, M. D. Tokach, and J. L. Nelssen. 1999. Effects of low-protein, amino acid-fortified diets formulated on a net energy basis on growth performance and carcass characteristics of finishing pigs. J. Appl. Anim. Res. 15(1):1–16. https://doi.org/10.1080/09712119.1999.9706225

Smith, B. N., and R. N. Dilger. 2018. Immunomodulatory potential of dietary soybean-derived isoflavones and saponins in pigs. J. Anim. Sci. 96(4):1288–1304. https://doi.org/10.1093/jas/sky036

Stewart, L. L., D. Y. Kil, F. Ji, R. B. Hinson, A. D. Beaulieu, G. L. Allee, J. F. Patience, J. E. Pettigrew, and H. H. Stein. 2013. Effects of dietary soybean hulls and wheat middlings on body composition, nutrient and energy retention, and the net energy of diets and ingredients fed to growing and finishing pigs. J. Anim. Sci. 91(6):2756–2765. https://doi.org/10.2527/jas.2012-5147

Suárez-Belloch, J., M. A. Latorre, and J. A. Guada. 2016. The effect of protein restriction during the growing period on carcass, meat and fat quality of heavy barrows and gilts. Meat Sci. 112:16–23. https://doi.org/10.1016/j.meatsci.2015.10.006

Tao, X., B. Deng, Q. Yuan, X. Men, J. Wu, and Z. Xu. 2021. Low crude protein diet affects the intestinal microbiome and metabolome differently in barrows and gilts. Front. Microbiol. 12:717727. https://doi.org/10.3389/fmicb.2021.717727

Trottier, N. L. 2006. Utilization of crystalline amino acids by the gut in growing pigs. National Pork Board. [Accessed: September 25, 2025]. https://live.porkcheckoff.org/research/utilization-of-crystalline-amino-acids-by-the-gut-in-growing/

Wang, D., X. Wan, J. Peng, Q. Xiong, H. Niu, H. Li, J. Chai, and S. Jiang. 2017. The effects of reduced dietary protein level on amino acid transporters and mTOR signaling pathway in pigs. Biochem. Biophys. Res. Commun. 485(2):319–327. https://doi.org/10.1016/j.bbrc.2017.02.084

Wang, Y., J. Zhou, G. Wang, S. Cai, X. Zeng, and S. Qiao. 2018. Advances in low-protein diets for swine. J. Anim. Sci. Biotechnol. 9:60. https://doi.org/10.1186/s40104-018-0276-7

Zhao, Y., G. Tian, D. Chen, P. Zheng, J. Yu, J. He, X. Mao, Z. Huang, Y. Luo, J. Luo, and B. Yu. 2019. Effect of different dietary protein levels and amino acids supplementation patterns on growth performance, carcass characteristics, and nitrogen excretion in growing-finishing pigs. J. Anim. Sci. Biotechnol. 10:75. https://doi.org/10.1186/s40104-019-0381-2

Zhang, S., Z. Li, and L. Liu. 2020. Concerns about the misleading conclusions regarding estimation of the net energy value of soybean meal relative to corn using the caloric efficiency approach in pigs. J. Anim. Sci. Biotechnol. 11(1):113. https://doi.org/10.1186/s40104-020-00519-1