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Research Article

Effects of the combination of spray dried plasma and reduced crude protein in diets on growth performance, diarrhea scores, gut morphology, and immune parameters of weanling pigs

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Abstract

The hypothesis that spray dried plasma (SDP) complements low crude protein (CP) phase 1 diets by improving growth performance, decreasing diarrhea, reducing immune system activation, and maintaining intestinal health of weanling pigs was tested. Four phase 1 diets were formulated (CP: 18.5% or 23.0%; SDP: 0% or 6%). One-hundred and sixty weaned pigs (5.89 ± 0.39 kg; 20 ± 2 days) were fed one of the four phase 1 diets (days 1–14) and a phase 2 diet (days 15–28). Results indicated that pigs fed the 18.5% CP diet instead of the 23.0% CP diet had reduced ($P < 0.05$) average daily gain (ADG) and gain to feed ratio (G:F), and pigs fed 6% SDP had greater ($P < 0.05$) ADG and G:F than pigs fed no SDP. Diarrhea scores were reduced ($P < 0.05$) for pigs fed the 18.5% CP diet. Villus height in the ileum and mucosa width in the colon increased if SDP was included in the 23.0% CP diet, but not in the 18.5% CP diet (interaction, $P < 0.05$). In conclusion, including 6% SDP in phase 1 diets resulted in improved ADG and G:F and reduced inflammatory responses.

Key words: cytokines, inflammation, low crude protein, spray dried plasma, weanling pigs

Introduction

During the initial 2 week post-weaning, when pigs are experiencing dietary, environmental, and social stressors, low feed intake and post-weaning diarrhea may result in decreased growth performance (van Dijk et al. 2001; Kil and Stein 2010; Campbell et al. 2013), but dietary approaches may be implemented to alleviate effects of weaning stress on pigs (van Dijk et al. 2001). Spray dried plasma (SDP) has greater palatability compared with dried milk and is a highly digestible animal protein with standardized ileal digestibility values that are greater than 96% for all indispensable amino acids (AA; Ermer et al. 1994; Almeida et al. 2013). Therefore, inclusion of up to 6% SDP in diets for newly weaned pigs may increase feed intake, which usually results in increased growth (van Dijk et al. 2001; Bailey et al. 2024, 2025). Inclusion of SDP in diets may ameliorate intestinal barrier dysfunction and mucosal inflammation by reducing ileal and colonic permeability and pro-inflammatory cytokine concentrations during the first week post-weaning (Peace et al. 2011), and as a result, overstimulation of the immune system may be prevented, and post-weaning diarrhea can be reduced.

Reducing crude protein (CP) in diets for weaned pigs is also a dietary approach to alleviate post-weaning diarrhea (Heo et al. 2008; Yue and Qiao 2008; Kil and Stein 2010; Bailey et al. 2024). By reducing CP in diets for newly weaned pigs, the concentration of undigested protein and AA entering the large intestine is reduced, which may reduce fermentation of CP by microorganisms and subsequently decrease diarrhea (Wang et al. 2018). A reduction in dietary CP may negatively affect growth performance (Hansen et al. 1993; Nyachoti et al. 2006; Limbach et al. 2021); however, there are no data for the combination of reduced dietary CP in phase 1 diets for pigs and addition of SDP. Therefore, the current experiment was conducted to test the hypothesis that SDP complements low CP concentrations in phase 1 diets by improving growth performance, reducing diarrhea, reducing stimulation of the immune system, and maintaining intestinal health of newly weaned pigs.

Materials and methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for this experiment (#19130). Pigs used in this experiment were the offspring of Line 359 boars and Camborough females (Pig Improvement Company, Hendersonville, TN, USA).

Diets, animals, and experimental design

Five diets were prepared (Tables 1, 2, and 3). Two phase 1 diets with normal concentrations of CP (i.e., approximately

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

Item, %	18.5% crude protein		23.0% crude protein		Phase 2
SDP, %	–	6.0	–	6.0	–
Spray dried plasma	–	6.00	–	6.00	–
Corn, ground	53.15	54.01	41.19	43.44	48.65
Soybean meal, 46% crude protein	12.70	14.20	25.00	25.00	25.00
Whey powder, dried	20.00	20.00	20.00	20.00	15.00
Soy protein concentrate	8.00	–	8.00	–	5.00
Soybean oil	3.10	3.10	3.10	3.10	3.50
Limestone, ground	0.91	1.17	0.95	1.20	0.99
Dicalcium phosphate	1.20	0.88	1.00	0.70	1.00
Sodium chloride	0.10	0.10	0.10	0.10	0.10
L-Lysine HCl	0.40	0.25	0.29	0.18	0.36
DL-Methionine	0.16	0.12	0.16	0.13	0.16
L-Threonine	0.11	0.02	0.06	–	0.09
L-Valine	0.02	–	–	–	–
Vitamin mineral premix*	0.15	0.15	0.15	0.15	0.15

*The vitamin–micromineral premix provided the following quantities of vitamins and microminerals per kg of complete diet: vitamin A as retinyl acetate, 11 136 mg; vitamin D_3 as cholecalciferol, 2208 mg; vitamin E as DL-alpha tocopheryl acetate, 66.0 mg; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20.0 mg as copper sulfate and copper chloride; Fe, 126.0 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

23.0% CP) and two phase 1 diets with reduced CP (i.e., approximately 18.5% CP) were formulated without or with 6% SDP (Appetein B) that was sourced from APC LLC., Ankeny, IA, USA. A common phase 2 diet without SDP was also prepared. Amino acids, vitamins, and minerals were included in all diets to meet or exceed current requirement estimates for nutrients and energy of weanling pigs (NRC 2012).

One-hundred and sixty pigs were weaned at 20 ± 2 days with an initial body weight (BW) of 5.89 ± 0.39 kg and randomly allotted to one of the four phase 1 diets in a randomized complete block design. There were two blocks of 16 pens for a total of 32 pens with five pigs per pen and eight split-sex pens per treatment (three barrows and two gilts or two barrows and three gilts in each pen). Pigs in the two blocks were weaned 2 weeks apart and the weaning group was the blocking factor. The four phase 1 diets were fed for 14 days after weaning, and pigs were allowed ad libitum access to feed and water throughout the experiment. After completing phase 1, all pigs were fed the common phase 2 diet for an additional 14 days.

Sample and data collection

Diarrhea scores were assessed visually every other day by two independent observers using a score from 1 to 5 (1 = normal feces; 2 = moist feces; 3 = mild diarrhea; 4 = severe diarrhea; and 5 = watery diarrhea). Individual pig weights were recorded at the beginning of the experiment and at the end of each phase. Daily feed allotments were recorded and feed left in the feeders was weighed at the end of each phase. Data collected for pig weights and feed allowance were summarized and used to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F) for each pen and treatment group. Data were summarized for each phase and over the entire experiment.

At the beginning of the experiment, the pig in each pen with the BW closest to the pen average was identified with four barrows and four gilts identified per treatment, and two blood samples were collected from the jugular vein of this pig on days 7, 14, and 28. One blood sample was collected in vacutainers with ethylenediaminetetraacetic acid (EDTA), and this sample was stored on ice immediately after collection and then delivered to the University of Illinois Veterinary Diagnostic Laboratory (Urbana, IL, USA) for white blood cell, neutrophil, and lymphocyte cell count analysis. The second blood sample was collected in heparinized vacutainers and centrifuged at 4000× *g* for 13 min to recover the plasma. Plasma samples were stored at −20 °C until analyzed at the University of Illinois Veterinary Diagnostic Laboratory for plasma urea nitrogen (PUN), albumin, and total protein using a Beckman Coulter Clinical Chemistry AU analyzer (Beckman Coulter, Inc., Brea, CA, USA). Globulin was calculated by subtracting albumin from total protein and the albumin:globulin ratio was also calculated. Heparinized plasma samples were also analyzed for immunoglobulin A (IgA) using Enzyme-Linked Immunosorbent Assay (ELISA) kits according to the recommendations of the manufacturer (Bethyl Laboratories, Inc., Montgomery, TX, USA), and for the following cytokines: interferon-gamma (IFN-γ), interleukin (IL)-1α, IL-1β, IL-1 receptor antagonist (IL-1Ra), IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, and tumor necrosis factor-α (TNF-α) using a MILLIPLEX MAP kit (MilliporeSigma, Burlington, MA, USA) in a MAGPIX instrument with ProcartaPlex- multiplex technology (R&D Systems, Inc., Minneapolis, MN, USA). Assay precision was acceptable with intra-assay coefficients of variation (CV) < 10% for all cytokines, except IL-1α, which had

Table 2. Analyzed nutrient composition of experimental diets (as-fed basis).

Item	18.5% crude protein		23.0% crude protein		Phase 2
SDP, %	–	6.0	–	6.0	–
Dry matter, %	87.52	87.59	88.20	87.96	87.30
Crude protein, %	18.96	18.91	23.17	22.93	21.17
Ash, %	5.36	5.36	5.82	5.75	5.32
Acid hydrolyzed ether extract, %	4.20	4.07	4.13	3.72	4.78
Gross energy, kcal/kg	3935	3969	3990	4031	3974
Starch, %	28.92	26.18	24.83	24.69	27.31
Minerals, %					
Ca	0.89	0.84	0.92	0.91	0.84
P	0.57	0.60	0.60	0.63	0.56
Na	0.22	0.36	0.25	0.39	0.17
Cl	0.40	0.50	0.40	0.40	0.30
K	1.10	0.97	1.47	1.25	1.16
Indispensable amino acids, %					
Arg	1.00	0.95	1.42	1.28	1.30
His	0.43	0.46	0.58	0.58	0.53
Ile	0.83	0.76	1.12	0.99	0.99
Leu	1.55	1.65	1.94	2.00	1.78
Lys	1.33	1.41	1.61	1.63	1.51
Met	0.39	0.24	0.42	0.48	0.40
Phe	0.80	0.84	1.09	1.06	1.00
Thr	0.80	0.88	0.98	1.04	0.91
Trp	0.14	0.19	0.27	0.29	0.23
Val	0.90	1.00	1.17	1.23	1.03
Total	8.17	8.38	10.60	10.58	9.68
Dispensable amino acids, %					
Ala	0.88	0.91	1.09	1.11	1.00
Asp	1.75	1.71	2.41	2.23	2.17
Cys	0.29	0.38	0.35	0.51	0.34
Glu	3.14	2.96	4.18	3.86	3.82
Gly	0.66	0.63	0.90	0.83	0.82
Pro	1.00	1.02	1.22	1.25	1.15
Ser	0.82	0.90	1.10	1.15	1.01
Tyr	0.54	0.62	0.75	0.78	0.69
Total	9.08	9.13	12.00	11.72	11.00
Total amino acids, %	17.25	17.51	22.60	22.30	20.68

an intra-assay CV < 15%. Inter-assay CVs were <20% for all cytokines.

On day 14, all pigs were weighed and the pig in each pen with the BW closest to the pen average was euthanized by captive bolt for tissue and mucosa collection with sex balanced among dietary treatments. If the pig with the BW closest to the pen average was the same pig being used for blood sampling, the pig with the next closest BW to the pen average was euthanized.

Intestinal morphology

Tissue samples of jejunum, ileum, and proximal colon were collected (about 5 cm in length). Jejunum and ileal tissue samples were collected approximately 150 cm distal to the pylorus and 80 cm caudal to the ileal–cecal junction, respectively. All intestinal 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 until analysis. After fixation, all tissue samples were delivered to Veterinary Diagnostic Pathology, LLC (Fort Valley, VA, USA), where they were embedded in paraffin, sectioned, and stained with hematoxylin and eosin before being transferred to slides. Slides were examined on a Meiji 5300 microscope using 40× magnification and photographed with a mounted 17-megapixel Canon Rebel 3 Ti camera, and for each slide, 10 intact villi and the associated crypts were measured using ImageJ software (National Institutes of Health, Bethesda, MD, USA), with the line tool calibrated from pixels to micrometers using an AmScope MR400 calibration slide. Villus height was measured from the villus tip to the base, and the crypt depth was measured

Table 3. Analyzed nutrient composition of ingredients (as-fed basis).

Item	Spray dried plasma	Corn	Soybean meal	Whey powder	Soy protein concentrate
Dry matter, %	93.27	83.71	87.65	90.08	90.82
Crude protein, %	82.44	7.05	46.99	13.37	64.65
Ash, %	9.70	0.81	6.15	8.12	6.67
Acid hydrolyzed ether extract, %	0.18	2.97	1.43	0.51	0.96
Gross energy, kcal/kg	4931	3763	4176	3616	4394
Minerals, %					
Ca	0.13	0.01	0.28	0.63	0.44
P	1.46	0.24	0.52	0.68	0.70
Na	2.40	<0.02	<0.02	0.86	<0.02
Cl	0.70	<0.10	<0.10	1.50	<0.10
K	0.16	0.31	1.90	2.21	2.17
Indispensable amino acids, %					
Arg	4.63	0.32	3.34	0.30	4.55
His	2.46	0.20	1.21	0.24	1.65
Ile	2.48	0.27	2.27	0.81	3.14
Leu	7.53	0.81	3.56	1.31	4.97
Lys	7.27	0.24	2.98	1.14	4.01
Met	0.90	0.14	0.60	0.17	0.83
Phe	4.18	0.33	2.33	0.41	3.28
Thr	5.26	0.25	1.79	0.83	2.46
Trp	1.19	0.04	0.64	0.22	0.84
Val	5.71	0.35	2.35	0.77	3.23
Total	41.61	2.95	21.07	6.20	28.96
Dispensable amino acids, %					
Ala	3.92	0.50	2.00	0.64	2.76
Asp	7.90	0.47	5.18	1.31	7.05
Cys	2.58	0.17	0.65	0.30	0.87
Glu	11.37	1.25	8.63	2.20	11.76
Gly	2.80	0.28	1.95	0.25	2.65
Pro	4.18	0.55	2.29	0.72	3.15
Ser	5.31	0.31	2.21	0.57	3.07
Tyr	4.07	0.20	1.66	0.29	2.20
Total	42.13	3.73	24.57	6.28	33.51
Total amino acids, %	83.74	6.68	45.64	12.48	62.47

from the crypt–villus junction to the base of the crypt. Villus and lamina propria widths were also measured at the midpoint of the villus. Neutrophils were counted in five fields in a 10 × 10 grid with 250 mm per side at approximate equidistant points along the slide, using a Leica DM 3000 microscope at 400× magnification, and expressed as neutrophils/mm^2 for each pig (Li et al. 2016; Zhu et al. 2017).

Secretory immunoglobulin A and cytokine analysis

On day 14, samples of jejunum, ileum, and proximal colon mucosa were collected. Mucosa samples were washed with phosphate buffered saline, snap frozen in liquid N, and stored at −80 °C until analysis. Intestinal mucosa samples were homogenized in phosphate buffered saline containing protease inhibitors (SKU, P8340; Sigma–Aldrich, St. Louis, MO, USA). The supernatant was collected and used for determination of secretory IgA (sIgA) using an ELISA kit according to the manufacturer's recommended procedures (Bethyl Laboratories, Inc., Montgomery, TX, USA). Concentrations of sIgA were expressed on a per mg protein basis, and all values were normalized with total protein concentration in each sample quantified by a Pierce bicinchoninic acid Protein Assay Kit (Thermo Scientific, Woltham, MA, USA). The supernatant was also used to determine concentrations of the following cytokines: IL-1α, IL-1β, IL-1Ra, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, and IL-18 using a MILLIPLEX MAP kit (MilliporeSigma, Burlington, MA, USA) in a MAGPIX instrument with ProcartaPlex- multiplex technology (R&D Systems, Inc., Minneapolis, MN, USA).

Chemical analysis

All diet and ingredient samples were analyzed in duplicate for gross energy using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL, USA), and nitrogen was analyzed by combustion (method 990.03; Association of Official Analytical Chemists—AOAC Int. 2019) using a LECO

Table 4. Main effects of crude protein (CP) concentration and inclusion of spray dried plasma (SDP) in phase 1 diets on growth performance parameters of weaned pigs.*,†

Item	CP, %		SDP, %		Pooled	*P* value‡	
SDP, %	18.5	23.0	–	6.0	SEM	CP	SDP
Days 1 to 14							
Initial BW, kg	5.88	5.89	5.89	5.88	0.12	0.464	0.659
ADG, g	92[b]	118[a]	86[b]	123[a]	10.61	0.003	<0.001
ADFI, g	158[b]	180[a]	153[b]	185[a]	5.66	0.010	<0.001
G:F	0.56[b]	0.65[a]	0.55[b]	0.66[a]	0.08	0.039	0.009
Final BW, kg	7.19[b]	7.56[a]	7.12[b]	7.63[a]	0.12	0.003	<0.001
Days 15 to 28							
ADG, g	430	439	463[a]	406[b]	12.19	0.591	0.002
ADFI, g	548	575	574	549	16.65	0.160	0.176
G:F	0.78	0.76	0.80[a]	0.74[b]	0.03	0.311	<0.001
Final BW, kg	13.72[z]	14.25[y]	14.11	13.86	0.26	0.079	0.388
Days 1 to 28							
ADG, g	261[z]	278[y]	275	264	9.02	0.095	0.318
ADFI, g	353[z]	377[y]	364	367	10.91	0.071	0.792
G:F	0.73	0.73	0.75[a]	0.72[b]	0.04	0.934	0.031

[a,b]Means within a row lacking a common superscript letter differ ($P < 0.05$).
[y,z]Means within a row lacking a common superscript letter differ ($0.05 \leq P < 0.10$).
*Data are least square means of 16 observations for all treatments.
†ADFI, average daily feed intake; ADG, average daily gain; BW, body weight, G:F, gain to feed ratio.
‡There was no interaction between level of CP and SDP for any of the growth performance parameters; therefore, the interaction term was removed from the final model and only main effects are shown.

FP628 analyzer (LECO Corp., Saint Joseph, MI, USA) with the subsequent calculation of CP as nitrogen × 6.25. Dry matter was analyzed in all diet and ingredient samples by oven-drying at 135 °C for 2 h (method 930.15; AOAC Int. 2019), and dry ash (method 942.05; AOAC Int. 2019) was analyzed as well. All diet and ingredient samples were analyzed for acid hydrolyzed ether extract using the acid hydrolysis filter bag technique (Ankom^{HCl} Hydrolysis System; Ankom Technology, Macedon, NY, USA) followed by crude fat extraction using petroleum ether (Ankom^{XT15} Extractor; Ankom Technology, Macedon, NY, USA). At the Agricultural Experiment Station Chemical Laboratories at the University of Missouri, Columbia, MO, USA, minerals (i.e., Ca, P, Na, Cl, and K) were analyzed using inductively coupled plasma optical emissions spectrometry (method 985.01 A, B, and C; AOAC Int. 2019). Diets and ingredients were also analyzed for AA (method 982.30 E (a, b, c); AOAC Int. 2019) and all diets were analyzed for total starch using the glucoamylase procedure (method 979.10; AOAC Int. 2019).

Statistical analysis

Normality of residuals was verified and outliers were identified using the UNIVARIATE and BOXPLOT procedures, respectively (SAS Inst. Inc., Cary, NC, USA). Outliers were removed if they were located outside the lower and upper far fences, which are located at 3× the interquartile range (Tukey 1977). Data for growth performance, diarrhea scores, tissue morphology, and mucosa were analyzed by ANOVA using the PROC MIXED procedure of SAS (SAS Inst. Inc. Cary, NC, USA) in a randomized complete block design with weaning group as the blocking factor and pen as the experimental unit. The model was a 2 × 2 factorial and included the fixed effects of CP, SDP, and the interaction between CP and SDP, and the random effects of block and replicate within block. Blood samples were collected from the same pig each collection day; therefore, data were analyzed as repeated measures with unstructured variance based on the likelihood ratio test using PROC MIXED and REPEATED procedures of SAS. The model included the fixed effects of CP, SDP, and the interaction between CP and SDP, day as the time effect, the random effects of block and replicate within block, and pig was the subject. Contrast statements were used with coefficients for unequally spaced treatments being generated using the PROC IML statement in SAS to determine linear and quadratic effects of day on blood cell counts and plasma AA. Treatment means were calculated using the LSMEANS statement, and if an interaction was significant, means were separated using the PDIFF option in the PROC MIXED procedure. Statistical significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Results

Growth performance

There were no interactions between dietary CP and inclusion of SDP in the phase 1 diet for growth performance (Table 4). At the end of phase 1, greater ($P < 0.05$) BW was observed for pigs fed the 23.0% CP diet compared with pigs fed the 18.5% CP diet, and greater ($P < 0.05$) BW was observed for pigs fed a diet containing 6% SDP compared with pigs fed a diet without SDP. During phase 1, pigs fed the diet with 18.5% CP had reduced ($P < 0.05$) ADG, ADFI, and G:F

Table 5. Main effects of crude protein (CP) concentration and inclusion of spray dried plasma (SDP) in phase 1 diets on diarrhea scores of weaned pigs.*

Item	CP, %		SDP, %			*P* value†	
SDP, %	18.5	23.0	–	6.0	Pooled SEM	CP	SDP
Diarrhea score[3]							
Days 1 to 7	1.89[z]	2.11[y]	2.05	1.94	0.23	0.092	0.398
Days 8 to 14	2.34[z]	2.60[y]	2.33[z]	2.60[y]	0.64	0.086	0.071
Days 1 to 14	2.14[b]	2.39[a]	2.21	2.33	0.46	0.025	0.260

[a,b]Means within a row lacking a common superscript letter differ ($P < 0.05$).
[y,z]Means within a row lacking a common superscript letter differ ($0.05 \leq P < 0.10$).
*Data are least square means of 16 observations for all treatments.
†There was no interaction between level of CP and SDP for any of the growth performance parameters; therefore, the interaction term was removed from the final model and only main effects are shown.

compared with pigs fed the diet with 23.0% CP. A greater ($P < 0.01$) ADG, ADFI, and G:F was also observed for pigs fed the diet containing 6% SDP compared with pigs fed a diet without SDP.

During phase 2, when pigs were fed the common diet without SDP, no differences were observed for ADG, ADFI, and G:F between pigs that had been fed the phase 1 diet with 18.5% and 23.0% CP, but pigs that had been fed the phase 1 diets without SDP had greater ($P < 0.01$) ADG and G:F in phase 2 than pigs that had been fed the phase 1 diets with 6% SDP. At the end of phase 2, there was a tendency for greater ($P < 0.10$) BW of pigs fed the phase 1 diets with 23.0% CP compared with pigs fed the phase 1 diets with 18.5% CP. During the overall experiment, there was a tendency for reduced ($P < 0.10$) ADG and ADFI for pigs that had been fed the phase 1 diets with 18.5% CP compared with pigs that had been fed the phase 1 diets with 23.0% CP. Pigs fed phase 1 diets without SDP had greater ($P < 0.05$) overall G:F compared with pigs fed the phase 1 diets with 6% SDP, regardless of CP concentration.

Diarrhea scores

There was no interaction between dietary CP and inclusion of SDP in the phase 1 diet for diarrhea scores, and only main effects are, therefore, presented (Table 5). During the initial 7 days post-weaning, there was a tendency ($P < 0.10$) for a reduction in diarrhea scores for pigs fed diets with 18.5% CP compared with pigs fed diets with 23.0% CP, but no differences were observed in diarrhea scores for pigs fed diets without or with 6% SDP. From days 8–14, a tendency for a reduction ($P < 0.10$) in diarrhea scores was observed for pigs fed the diets with 18.5% CP compared with the 23.0% CP diets, and there was also a tendency for a reduction ($P < 0.10$) in diarrhea scores for pigs fed the diets without SDP compared with pigs fed the diets with 6% SDP. Overall, in phase 1, there was a reduction ($P < 0.05$) in diarrhea scores for pigs fed the diets with 18.5% CP compared with diets with 23.0% CP.

Tissue morphology

Pigs fed diets with 6% SDP had greater ($P < 0.05$) villus height and crypt depth in the jejunum on day 14 compared with pigs fed diets without SDP, and pigs fed diets with 18.5% CP tended to have reduced ($P < 0.10$) villus height and crypt depth in the jejunum compared with pigs fed diets with 23.0% CP (Table 6). Villus height in the ileum increased if SDP was included in the diet with 23.0% CP, but that was not the case if SDP was included in the diet with 18.5% CP (interaction, $P < 0.05$). The mucosa width in the colon was less for pigs fed the 23.0% CP diet without 6% SDP compared with pigs fed the 23% CP diet with SDP, but no effect of SDP was observed when SDP was included in the 18.5% CP diet (interaction, $P < 0.05$). However, pigs fed the diets with 23.0% CP had greater ($P < 0.05$) mucosa width in the colon than pigs fed the diets with 18.5% CP.

Secretory immunoglobulin A and mucosal cytokines

In the jejunal mucosa on day 14, sIgA concentration was not influenced by SDP inclusion in phase 1 (Table 7), but sIgA concentration decreased ($P < 0.05$) in the jejunum of pigs fed the diets with 18.5% CP compared with pigs fed the diets with 23.0% CP. The mucosal concentration of IL-12 tended to increase if 6% SDP was included in the diet with 23.0% CP, but inclusion of SDP in the 18.5% CP diet did not affect IL-12 (interaction, $P < 0.10$). Pigs fed the diets with 18.5% CP had decreased ($P < 0.05$) mucosal concentration of IL-1β and tended to have decreased ($P < 0.10$) IL-6 compared with pigs fed the diets with 23.0% CP. In addition, the IL-6 concentration tended to decrease ($P < 0.10$) if 6% SDP was included in the diet compared with the diet without SDP, regardless of CP concentration. In the ileum mucosa on day 14, IL-12 concentration was reduced in pigs fed the 23.0% CP diet with 6% SDP compared with pigs fed the same diet without SDP, but no effect of SDP was observed in pigs fed the 18.5% CP diets (interaction, $P < 0.05$). The concentration of IL-1β had a tendency to decrease in pigs fed the 23.0% CP diet if SDP was included in the diet compared with pigs fed the 18.5% CP diet, but if SDP was not included, no difference between the two protein levels was observed (interaction, $P < 0.10$). In contrast, IL-4 concentration increased in pigs fed the 23.0% CP diet with SDP compared with pigs fed the 18.5% diet with SDP, but that was not the case if no SDP was included in the diet (interaction, $P < 0.05$). In the colon, the mucosal concentration of IL-2 and IL-6 tended to decrease ($P < 0.10$) if 6% SDP was included in the diet, regardless of CP concentration.

Blood parameters

Systemic IgA on days 7 and 14 was not influenced by dietary CP or SDP (Table 8). Whereas systemic concentrations

Table 6. Morphology of the jejunum, ileum, and colon of pigs fed low crude protein (CP) or normal CP phase 1 diets without or with spray dried plasma (SDP).*

Item	18.5% CP		23.0% CP			*P* values		
SDP, %	–	6.0	–	6.0	Pooled SEM	CP	SDP	CP × SDP
Jejunum								
Villus height, μm	255	306	304	332	21.82	0.053	0.047	0.543
Villus width, μm	135	131	129	134	4.95	0.803	0.907	0.372
Crypt depth, μm	323	339	331	401	22.88	0.061	0.027	0.148
Villus height:crypt depth ratio	0.83	0.92	0.97	0.88	0.07	0.410	0.974	0.190
Lamina propia thickness, μm	80.9	74.0	74.9	79.7	4.34	0.976	0.786	0.147
Neutrophils, cells/mm^2	13.1	13.3	12.0	16.3	2.34	0.647	0.304	0.350
Ileum								
Villus height, μm	297^{ab}	254^{b}	268^{b}	337^{a}	27.69	0.247	0.564	0.019
Villus width, μm	137	137	137	141	6.34	0.728	0.743	0.759
Crypt depth, μm	278	281	284	323	16.13	0.107	0.151	0.207
Villus height:crypt depth ratio	1.10	0.93	0.94	1.08	0.11	0.952	0.865	0.118
Lamina propia thickness, μm	77.0	79.5	78.8	85.0	4.81	0.442	0.356	0.691
Neutrophils, cells/mm^2	28.8	26.1	29.6	33.8	3.86	0.279	0.854	0.385
Colon								
Mucosa width, μm	410^{b}	408^{b}	423^{b}	501^{a}	25.02	0.004	0.029	0.021
Lamina propia thickness, μm	62.9	53.5	61.1	58.7	3.75	0.647	0.132	0.356
Neutrophils, cells/mm^2	3.6	1.8	1.9	2.9	0.94	0.703	0.655	0.131

^{a,b}Means within a row lacking a common superscript letter differ ($P < 0.05$).
*Data are least square means of eight observations for all treatments, with the exception that the 18.5% CP diet with 6.0% SDP was least square means of seven observations.

decreased (interaction, $P < 0.05$) for IL-2 and tended to decrease (interaction, $P < 0.10$) for IFN-γ, IL-6, IL-10, and IL-18 if 6% SDP was included in the diet with 18.5% CP, the concentration of these cytokines was not affected by dietary SDP if pigs were fed the 23.0% CP diet. In contrast, the concentration of TNF-α on days 7 and 14 tended to increase if 6% SDP was included in the diet with 23.0% CP, but this was not the case if SDP was included in the diet with 18.5% CP (interaction, $P < 0.10$). If 6% SDP was included in the diet, regardless of dietary CP, concentrations of IL-1α, IL-1β, and IL-4 decreased ($P < 0.05$) compared with the diets without SDP. Concentrations of systemic cytokines also changed over time with most cytokines decreasing ($P < 0.05$) from days 7–14, but IL-12 and IgA increased ($P < 0.05$) from days 7–14.

White blood cells, and the neutrophils and lymphocytes that make up white blood cells, were not influenced by dietary CP or SDP on days 7, 14, or 28 post-weaning (Table 9). Plasma urea nitrogen was reduced in the 18.5% CP diet without SDP compared with the 23.0% CP diet without SDP, but if SDP was included in these diets, PUN did not differ (interaction, $P < 0.05$). In contrast, total protein in plasma was not influenced by dietary CP, but total protein was reduced ($P < 0.05$) if 6% SDP was included in the diet compared with the diet without SDP. These blood cell counts also changed from days 7–28 post-weaning, with white blood cells and neutrophils having the greatest concentration on day 14 (quadratic, $P < 0.05$), but lymphocytes, albumin, and the ratio between albumin and globulins had the least concentration on day 14 (quadratic, $P < 0.05$), and PUN linearly decreased ($P < 0.05$) from days 7 to 28.

Discussion

The post-weaning period is often characterized by a high occurrence of intestinal disturbances with increased diarrhea incidence and depressed growth performance of pigs (Heo et al. 2013). Diarrhea caused by bacterial or viral pathogens is a vital problem post-weaning leading to economic losses due to pig mortality, morbidity, and decreased efficiency (Wang et al. 2011). Reducing CP in diets fed to weanling pigs is a strategy used to decrease post-weaning diarrhea by reducing the amount of nitrogen from undigested protein and AA reaching the hindgut for fermentation (Stein and Kil 2006; Wang et al. 2018). Inclusion of SDP in diets for weanling pigs increases post-weaning performance and improves intestinal function of pigs (van Dijk et al. 2001; Zhang et al. 2016). Dietary SDP may also reduce inflammation throughout the intestine (Peace et al. 2011), which may result in reduced post-weaning diarrhea. However, research on the complementary effects of low dietary CP and inclusion of SDP in diets is limited.

The observed decrease in diarrhea incidence for pigs fed the low CP diets is in agreement with previous data indicating that reducing dietary CP to around 17% decreased post-weaning diarrhea and improved fecal consistency (Lordelo et al. 2008; Yue and Qiao 2008; Bhandari et al. 2010; Limbach et al. 2021). Addition of 5% SDP to a diet reduces diarrhea of pigs challenged with *Escherichia coli* (Zhang et al. 2015), and the influence of dietary SDP on intestinal health is greater when pigs are exposed to increased pathogen loads (Campbell et al. 2010). Therefore, the observation that diarrhea incidence during the initial 2 week post-weaning generally was low and

Table 7. Influence of crude protein (CP) concentration and inclusion of spray dried plasma (SDP) in phase 1 diets fed to weaned pigs on intestinal mucosa concentrations of secretory immunoglobulin A (μg/mg of protein) and cytokines (ng/mL).[*,†]

Item	18.5% CP		23.0% CP		Pooled SEM	*P* value		
SDP, %	–	6.0	–	6.0		CP	SDP	CP × SDP
Jejunum								
sIgA	2.06	2.42	2.90	3.82	0.68	0.034	0.211	0.576
IL-1α	0.09	0.11	0.10	0.11	0.02	0.616	0.110	0.832
IL-1β	1.99	1.97	2.99	3.34	0.41	0.001	0.610	0.578
IL-1Ra	0.55	0.56	0.60	0.66	0.09	0.178	0.552	0.579
IL-2	0.04	0.04	0.06	0.04	0.01	0.608	0.241	0.127
IL-4	0.03	0.03	0.03	0.03	0.01	0.789	0.222	0.823
IL-6	0.03	0.01	0.06	0.03	0.01	0.052	0.057	0.410
IL-8	32.40	19.54	19.21	19.87	6.55	0.125	0.144	0.108
IL-10	0.04	0.04	0.05	0.05	0.01	0.215	0.885	0.722
IL-12	0.16^{yz}	0.16^{yz}	0.13^{z}	0.20^{y}	0.03	0.849	0.094	0.098
IL-18	15.71	15.75	16.69	14.32	0.95	0.818	0.228	0.214
Ileum								
sIgA	2.58	1.70	2.33	2.26	0.64	0.807	0.445	0.518
IL-1α	0.11	0.14	0.12	0.13	0.02	0.953	0.153	0.552
IL-1β	3.39^{yz}	4.32^{y}	3.55^{yz}	2.85^{z}	0.40	0.112	0.774	0.051
IL-1Ra	0.62	0.69	0.61	0.60	0.05	0.326	0.477	0.382
IL-2	0.05	0.05	0.05	0.05	0.01	0.780	0.301	0.608
IL-4	0.05^{ab}	0.04^{b}	0.04^{ab}	0.06^{a}	0.01	0.656	0.996	0.033
IL-6	0.04^{z}	0.07^{yz}	0.07^{y}	0.06^{yz}	0.01	0.245	0.609	0.061
IL-8	22.44	19.80	22.14	20.14	2.22	0.992	0.304	0.886
IL-10	0.05	0.06	0.07	0.07	0.01	0.101	0.828	0.651
IL-12	0.24^{ab}	0.29^{a}	0.28^{ab}	0.18^{b}	0.03	0.290	0.449	0.045
IL-18	12.75	12.51	11.69	11.74	0.83	0.107	0.859	0.797
Colon								
IL-1α	0.34	0.31	0.42	0.31	0.24	0.722	0.536	0.745
IL-1β	3.61	2.18	3.76	2.77	2.46	0.726	0.252	0.830
IL-1Ra	0.61	0.55	0.66	0.60	0.16	0.616	0.583	0.986
IL-2	0.04	0.03	0.04	0.03	0.01	0.278	0.060	0.522
IL-4	0.04	0.04	0.04	0.04	0.01	0.444	0.779	0.779
IL-6	0.03	0.02	0.03	0.02	0.01	0.669	0.095	0.650
IL-8	2.56	2.73	2.46	2.36	0.54	0.656	0.947	0.800
IL-10	0.02	0.02	0.04	0.02	0.01	0.353	0.224	0.288
IL-12	0.05	0.03	0.05	0.04	0.03	0.906	0.337	0.648
IL-18	4.73	5.03	4.66	5.89	2.43	0.735	0.516	0.690

[a,b]Means within a row lacking a common superscript letter differ ($P < 0.05$).
[y,z]Means within a row lacking a common superscript letter differ ($0.05 \leq P < 0.10$).
[*]Data are least square means of 6–8 observations per treatment.
[†]IL-, interleukin-; IL-1Ra, interleukin-1 receptor antagonist; sIgA, secretory immunoglobulin A.

not influenced by dietary SDP indicates that pigs used in this experiment were of high health status.

A reduction in dietary CP can result in dispensable AA becoming limiting in the diet, which negatively affects growth performance of pigs (Hansen et al. 1993; Nyachoti et al. 2006; Wang et al. 2018). Despite addition of some indispensable AA, if CP was reduced to 17.2%, ADG and G:F of weanling pigs decreased (Yue and Qiao 2008). Therefore, the observed reduction in growth performance of pigs fed the low CP diet the initial 2 week post-weaning is in agreement with previous data. In contrast, SDP included in diets from 2% to 8% results in a linear increase in ADG, ADFI, and G:F of pigs during the initial 2 week post-weaning (Torrallardona 2010; Bailey et al. 2025). The observed improvement in growth performance of pigs fed 6% dietary SDP is, therefore, in agreement with previous data. The greater phase 2 efficiency of pigs that were fed the diet without SDP in phase 1 may indicate compensatory weight gain. Pigs adapt to reduced feed intake immediately after weaning, and once they have adapted, protein turnover increases leading to compensatory growth (Remus et al. 2013). Nevertheless, feeding SDP to pigs immediately after weaning (phase 1) supports intestinal health, reduces

Table 8. Influence of crude protein (CP) concentration and inclusion of spray dried plasma (SDP) to phase 1 diets fed to weaned pigs on plasma concentrations of immunoglobulin A (g/mL) and cytokines (ng/mL).*,†

Item	18.5% CP		23.0% CP		Pooled	*P* value‡			Day		Pooled	
SDP, %	–	6.0	–	6.0	SEM	C	S	C × S	7	14	SEM	*P* value
IgA	145.8	112.1	148.0	158.8	19.8	0.175	0.517	0.215	124.9	157.5	14.57	0.028
Cytokines												
IFN-γ	39.65^{y}	8.08^{z}	14.35^{yz}	12.01^{yz}	8.29	0.209	0.051	0.090	28.27	8.77	4.42	<0.001
IL-1α	0.22	0.10	0.17	0.11	0.03	0.396	<0.001	0.136	0.23	0.07	0.03	<0.001
IL-1β	0.72	0.15	0.45	0.15	0.10	0.169	<0.001	0.161	0.45	0.29	0.06	0.008
IL-1Ra	1.33	1.49	1.25	0.94	0.16	0.056	0.648	0.141	1.42	1.09	0.11	0.044
IL-2	1.19^{a}	0.15^{b}	0.64^{b}	0.16^{b}	0.13	0.044	<0.001	0.040	0.62	0.45	0.10	0.215
IL-4	4.07	0.62	2.45	0.88	0.63	0.281	<0.001	0.140	3.01	1.00	0.42	<0.001
IL-6	0.50^{y}	0.10^{z}	0.31^{yz}	0.13^{z}	0.07	0.230	<0.001	0.084	0.37	0.15	0.05	<0.001
IL-8	0.25	0.19	0.17	0.24	0.04	0.687	0.967	0.153	0.33	0.10	0.02	<0.001
IL-10	1.89^{y}	0.40^{z}	1.19^{yz}	0.42^{z}	0.22	0.121	<0.001	0.090	1.24	0.71	0.16	0.008
IL-12	0.87	0.92	0.91	0.72	0.09	0.290	0.301	0.102	0.80	0.91	0.07	0.035
IL-18	2.89^{y}	0.77^{z}	1.87^{yz}	0.85^{z}	0.30	0.134	<0.001	0.079	2.06	1.13	0.21	0.001
TNF-α	0.85^{y}	0.63^{yz}	0.49^{z}	0.83^{y}	0.18	0.630	0.737	0.089	1.23	0.17	0.12	<0.001

[a,b]Means within a row lacking a common superscript letter differ ($P < 0.05$).
[y,z]Means within a row lacking a common superscript letter differ ($0.05 \leq P < 0.10$).
*Data are least square means of 6–8 observations per treatment.
†IFN-γ, interferon-gamma; IgA, immunoglobulin A; IL-, interleukin-; IL-1Ra, interleukin-1 receptor antagonist; TNF-α, tumor necrosis factor-α.
‡*P* values were calculated to test the main effect of crude protein concentration (C) and inclusion of spray dried plasma (S) and the interaction between crude protein and spray dried plasma (C × S).

Table 9. Influence of crude protein (CP) concentration and inclusion of spray dried plasma (SDP) in phase 1 diets fed to weaned pigs on blood cell counts.*,†

Item	18.5% CP		23.0% CP		Pooled	*P* value‡			Day			Pooled	*P* value§	
SDP, %	–	6.0	–	6.0	SEM	C	S	C × S	7	14	28	SEM	L	Q
White blood cells	16.21	15.94	15.92	16.17	0.83	0.966	0.990	0.739	10.54	19.81	17.82	0.76	<0.001	<0.001
Neutrophils	35.21	35.29	30.37	35.85	4.06	0.432	0.310	0.322	28.14	47.80	26.60	3.66	0.028	<0.001
Lymphocytes	57.03	57.36	61.14	56.43	4.06	0.579	0.447	0.382	63.69	44.74	65.54	3.62	0.037	<0.001
Plasma urea N	8.25^{b}	10.77^{ab}	11.58^{a}	9.45^{ab}	0.71	0.167	0.788	0.003	11.53	10.88	7.63	0.57	<0.001	0.337
Albumin	2.80	2.80	2.92	2.78	0.06	0.377	0.252	0.284	2.99	2.58	2.90	0.05	0.842	<0.001
Total protein	4.97	4.77	5.08	4.79	0.11	0.556	0.029	0.693	4.86	4.81	5.03	0.08	0.050	0.212
AGR‖	1.33	1.61	1.38	1.44	0.13	0.627	0.205	0.391	1.63	1.19	1.51	0.10	0.821	<0.001

[a,b]Means within a row lacking a common superscript letter differ ($P < 0.05$).
[y,z]Means within a row lacking a common superscript letter differ ($0.05 \leq P < 0.10$).
*Data are least square means of 6–8 observations per treatment.
†Units for the blood cell counts: white blood cells, $\times 10^3$ per μL; neutrophils, % of white blood cells; lymphocytes, % of white blood cells; plasma urea N, mg per dL; albumin, g per dL; total protein, g per dL.
‡*P* values were calculated to test the main effect of crude protein concentration (C) and inclusion of spray dried plasma (S) and the interaction between crude protein and spray dried plasma (C × S).
§*P* values were calculated to test the linear (L) and quadratic (Q) effects of day.
‖AGR, albumin to globulin ratio.

immune activation, and improves feed intake, thereby facilitating adaptation to the stress that characterizes the first 2 week following weaning (Campbell et al. 2010; Peace et al. 2011).

Villus atrophy and local inflammation are characteristic of weaning (Lallès et al. 2004). A decrease in villus height indicates less surface area for nutrient absorption (Yin et al. 2020), and inflammation of the intestine may result in increased intestinal permeability (Peace et al. 2011). Dietary SDP can reduce villus atrophy and mucosal cell production of pro-inflammatory cytokines by inhibiting pathogen colonization on the mucosal membrane (Peace et al. 2011; Tran et al. 2014). The observed increase in villus height in the jejunum of pigs fed SDP is in agreement with previous data (Torrallardona 2010; Zhang et al. 2015). Reducing dietary CP to 19% increased the villus height to crypt depth ratio (Opapeju et al. 2008), whereas a decrease in the villus height to crypt depth ratio were reported for pigs fed diets where CP was reduced from 18% to 15% (Chen et al. 2018). Previous data are inconclusive on the effect of reducing dietary CP on intestinal morphology of pigs, but reductions of dietary CP by more than 3% compared with NRC (2012) can have detrimental effects on intestinal morphology (Yu et al. 2019). The observed reduction in villus height in the jejunum of pigs fed diets with reduced CP is in agreement with Yue and Qiao (2008) and may be associated with reduced protein synthesis due to an insuf-

ficient supply of AA required to maintain the structure of the intestinal epithelium (Gu and Li 2004; Wang et al. 2018). The tendency for an increased crypt depth in pigs fed the 23.0% CP diet compared with pigs fed the 18.5% CP diet may be a response to increased intestinal inflammation, which may have been the reason pigs fed the 23.0% CP diet had more diarrhea. However, the increased crypt depth in the jejunum of pigs fed a diet containing SDP compared with pigs fed no SDP may be the reason for the increased villus height that were observed in the pigs fed the diets with SDP.

The increase in mucosa width of the colon for pigs fed 6% SDP may indicate increased inflammation in the intestine (Fernandes et al. 2014). However, SDP may reduce intestinal wall thickness (Jang et al. 2016), and with a tendency for a reduction in IL-2 and IL-6 in the colon of pigs fed 6% SDP, the increase in colonic mucosa width that was observed is not indicative of increased inflammation.

The mucosa membrane lining the intestine is important for protecting the host against pathogens (Xun et al. 2018). Additionally, sIgA, secreted by plasma cells in the lamina propria and transported across the intestinal epithelium, functions to protect the membrane by preventing adhesion and colonization of pathogens (Hansen et al. 2019), and synthesis of pro-inflammatory cytokines is regulated by sIgA (Hansen et al. 2019). Pigs fed a diet with 5% SDP had decreased sIgA in the ileum compared with pigs fed a diet without SDP (Zhang et al. 2016), but sIgA in the jejunum and ileum mucosa was not influenced by dietary SDP in the current experiment. However, decreased sIgA in the jejunum of pigs fed reduced dietary CP may indicate that local inflammation was reduced, which is in agreement with Limbach et al. (2021). Decreased inflammation in the jejunum of pigs fed a diet with reduced CP is also indicated by the decrease in pro-inflammatory cytokines IL-1β and IL-6. Decreased expression of pro-inflammatory cytokines in the intestine of pigs fed low CP diets has also been observed in the past (Limbach et al. 2021; Wang et al. 2021). SDP improves intestinal barrier function of pigs (Peace et al. 2011) and maintains local immune homeostasis by suppressing synthesis of pro-inflammatory cytokines IL-6, IFN-γ, and TNF-α, or the anti-inflammatory cytokines IL-4 and IL-10 that regulate pro-inflammatory cytokine production (Pérez-Bosque et al. 2010; Zhang et al. 2016). Inconsistencies in the response of cytokine synthesis to dietary SDP have also been reported (Peace et al. 2011), which was observed in the current experiment as well. However, inclusion of 6% SDP in diets generally reduced mucosal and systemic concentrations of pro-inflammatory cytokines, although some cytokines responded differently depending on dietary CP concentration, indicating that the immunomodulatory effects of SDP may vary according to protein level. SDP is hypothesized to mainly elicit its effects in the intestinal tract of pigs (Campbell et al. 2019), and therefore, cytokine synthesis by intestinal mucosa has been more widely researched compared with systemic cytokines for pigs fed SDP. However, cytokines may act in the brain and cause reduced feed intake (Johnson 1997), and therefore, the increased feed intake observed with inclusion of dietary SDP may be a result of decreased systemic cytokines. Additionally, the observation that plasma anti- and pro-inflammatory cytokines decreased for pigs fed the combination of reduced CP and inclusion of SDP indicates that stimulation of the systemic immune response was reduced in pigs fed this diet. It is possible that this is a consequence of pigs fed the 18.5% diet having greater or tending to have greater plasma concentrations of some cytokines compared with pigs fed the 23.0% diet indicating increased systemic immune activation and possibly inflammation. However, the observation that addition of SDP to the diets reduced the concentration of the majority of plasma cytokines indicates that SDP reduced systemic inflammation and activation of the immune system.

Conclusions

Inclusion of SDP in diets for newly weaned pigs improved growth performance parameters, whereas reduced dietary CP decreased efficiency of pigs during the initial 2 week post-weaning. Reducing dietary CP from 23.0% to 18.5% decreased diarrhea incidence of weanling pigs but also decreased villus height in the jejunum unless pigs were fed a combination of 18.5% CP and 6% SDP, in which case, villus height in the ileum was maintained. Reduced dietary CP decreased synthesis of pro-inflammatory cytokines and sIgA in the jejunum, whereas inclusion of SDP did not influence intestinal cytokine production. However, inclusion of SDP in the diet resulted in decreased systemic pro-inflammatory cytokines.

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Data availability

All data from this research are included in the manuscript.

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Competing interests

JMC is an employee of APC LLC. (Ankeny, IA, USA), which is a supplier of spray dried plasma protein to the global feed industry. HMB, NSF, and HHS have no conflicts of interest.

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