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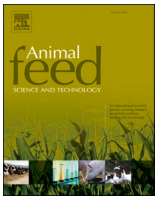

Spray-dried plasma titration effects on growth performance, intestinal morphology, and immune system indicators of weaned pigs housed in a sanitation-challenged environment

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ABSTRACT

An experiment was conducted to test the hypothesis that increasing inclusion of spray-dried plasma (SDP) in diets improves growth performance and intestinal morphology and reduces inflammation in newly weaned pigs. Four-hundred weaned pigs (body weight: 6.05 ± 0.80 kg) were allotted to a randomized complete block design with 5 diets and 2 blocks (16 pens/diet; 5 pigs/pen). Pigs were placed in pens that were not cleaned to create a sanitation challenge. Phase 1 diets containing 0, 20, 40, 60, or 80 g/kg SDP were formulated. One pig per pen was sacrificed on d 14 and samples of intestinal tissue and mucosa were collected. During phase 1, growth performance parameters and body weight of pigs on day 14 increased (linear, $P < 0.05$) with increasing dietary SDP. Villus width in the jejunum of pigs on day 14 tended to increase (linear, $P < 0.10$) with increasing inclusion of SDP, and villus height to crypt depth ratio tended to be the greatest (quadratic, $P < 0.10$) for pigs fed a diet with 80 g/kg SDP. The jejunal mucosa concentration of interleukin- (IL-) 2 tended to be least (quadratic, $P < 0.10$) at 80 g/kg inclusion of SDP and IL-8 tended to increase (linear, $P < 0.10$) as SDP inclusion increased in the diet. Secretory immunoglobulin A in the ileal mucosa was greatest at 20 g/kg inclusion of SDP to the diet and then decreased with increasing dietary SDP (quadratic, $P < 0.05$), and IL-10 tended to be least at 40 and 60 g/kg dietary SDP, but increased with 80 g/kg dietary SDP (quadratic, $P < 0.10$). Activated T cells and the ratio of activated to regulatory T cells tended to be greatest at 40 g/kg dietary SDP but then decreased as SDP increased in the diet (quadratic, $P < 0.10$), whereas systemic lymphocytes linearly decreased ($P < 0.05$) as SDP increased in the diet. The concentration of plasma urea N also linearly decreased ($P < 0.05$) as dietary SDP increased, indicating greater amino acid utilization with greater dietary SDP. In conclusion, the optimal inclusion of SDP in diets for weanling pigs was at least 80 g/kg as indicated by improvements in growth performance and utilization of amino acids, but data for intestinal morphology and mucosal and systemic inflammation did not result in a conclusive optimum concentration of dietary SDP.

Abbreviations: AA, amino acids; ADFI, average daily feed intake; ADG, average daily gain; BW, body weight; CD, cluster of differentiation; FoxP3, forkhead box protein 3; G:F, gain to feed ratio; IL-, interleukin-; IL-1Ra, interleukin-1 receptor antagonist; PUN, plasma urea nitrogen; SDP, spray dried plasma; SIgA, secretory immunoglobulin A.

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1. Introduction

The optimum concentration of spray-dried plasma (SDP) in phase 1 diets fed to weanling pigs is approximately 60 g/kg (van Dijk et al., 2001; Torrallardona, 2010). This inclusion level of SDP was determined by evaluating improvements in growth performance parameters of weaned pigs fed diets containing graded levels of SDP (van Dijk et al., 2001; Torrallardona, 2010). However, the mode of action of SDP is hypothesized to be related to its immunoglobulin concentration (Torrallardona, 2010). Immunoglobulins protect and maintain the function of the intestinal mucosa by identifying and neutralizing pathogens on the membrane (Torrallardona, 2010), thereby preventing para-cellular diffusion of pathogens, decreasing intestinal inflammation, and reducing villus atrophy in the small intestine post-weaning (Pierce et al., 2005; Pérez-Bosque et al., 2016b).

Because the antibody functions in preventing pathogen colonization on the mucosal membrane, SDP has a greater effect on improving intestinal barrier function when fed to pigs exposed to greater pathogen loads (Campbell et al., 2010). Feeding a diet with SDP increases anti-inflammatory cytokines and decreases pro-inflammatory cytokines in the mucosa (Pérez-Bosque et al., 2016b; Zhang et al., 2016), indicating that dietary SDP reduces activation of the immune system (Nofrarías et al., 2006). However, in most previous experiments, one level of SDP, usually around 60 g/kg, was used, and data for effects of graded levels of SDP in the diet on intestinal morphology and inflammation in weaned pigs are lacking. Therefore, this experiment was conducted to test the hypothesis that pigs exposed to greater pathogen loads have reduced prevalence of diarrhea, decreased concentrations of mucosal pro-inflammatory cytokines, and improved intestinal morphology and growth performance as inclusion rate of SDP in the diet increased.

2. Materials and methods

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

2.1. Diets, animals, and experimental design

Spray-dried plasma (Appetein B) was sourced from APC LLC (Ankeny, IA, USA), and the same batch of SDP was used in all diets containing SDP. Six diets were prepared (Tables 1, 2, and 3). A basal diet was formulated based on corn, soybean meal, and soy protein concentrate, and 4 additional diets were formulated by including 20, 40, 60, or 80 g/kg SDP to the basal diet at the expense of soy protein concentrate. A common phase 2 diet without SDP was also formulated. Vitamins and minerals were included in all diets to meet or exceed current nutritional requirement estimates of weaned pigs (NRC, 2012). Antimicrobials or pharmacological levels of Zn were not included in any of the diets.

Four-hundred pigs were weaned at 20 ± 2 days of age with an initial body weight (BW) of 6.05 ± 0.80 kg and randomly allotted to 1 of 5 dietary treatments in a randomized complete block design. Weaning group was used as the blocking factor, and there were 2 blocks of 40 pens with 5 pigs per pen. For each dietary treatment there were 4 pens of barrows and 4 pens of gilts in each block; therefore, there were a total of 16 replicate pens per dietary treatment. Pigs were placed into uncleaned pens using a sanitation challenge model and pens remained uncleaned throughout the experiment (Adewole et al., 2016). Pens had been occupied by a previous group of pigs

Table 1
Ingredient composition of experimental diets.

Item, g/kg	Basal					Phase 2
Spray-dried plasma, g/kg:	—	20	40	60	80	—
Spray-dried plasma	—	20.0	40.0	60.0	80.0	—
Corn, ground	392.4	398.1	399.1	404.9	410.3	483.0
Soybean meal, 460 g/kg crude protein	250.0	250.0	250.0	250.0	250.0	250.0
Whey powder, dried	200.0	200.0	200.0	200.0	200.0	150.0
Soy protein concentrate	95.0	70.0	50.0	25.0	—	50.0
Soybean oil	31.4	31.4	31.4	31.4	31.4	35.0
Limestone, ground	9.5	10.0	11.0	11.7	12.6	9.9
Dicalcium phosphate	10.8	10.0	8.9	7.9	6.9	10.0
Sodium chloride	1.0	1.0	1.0	1.0	1.0	1.0
$_{L}$-Lys HCl, 78% lys	3.1	2.8	2.2	1.8	1.5	3.6
$_{DL}$-Met, 98% met	1.1	1.2	1.2	1.3	1.3	1.6
$_{L}$-Thr, 98% thr	0.7	0.5	0.2	—	—	0.9
Vitamin mineral premix[a]	5.0	5.0	5.0	5.0	5.0	5.0

[a] The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 10,622 mg; vitamin D_3 as cholecalciferol, 1660 mg; vitamin E as selenium yeast, 66.0 mg; vitamin K as menadione nicotinamide bisulfate, 1.40 mg; thiamin as thiamine mononitrate, 1.08 mg; riboflavin, 6.49 mg; pyridoxine as pyridoxine hydrochloride, 0.98 mg; vitamin B_{12}, 0.03 mg; $_{D}$-pantothenic acid as $_{D}$-calcium pantothenate, 23.2 mg; niacin, 43.4 mg; folic acid, 1.56 mg; biotin, 0.44 mg; Cu, 20.0 mg as copper chloride; Fe, 123.0 mg as iron sulfate; I, 1.24 mg as ethylenediamine dihydriodide; Mn, 59.4 mg as manganese hydroxychloride; Se, 0.27 mg as sodium selenite and selenium yeast; and Zn, 124.7 mg as zinc hydroxychloride.

for 7 weeks, but they had not been occupied for 7 days when pigs for the current experiment were placed. The 5 treatment diets were fed from day 1–14 for a total of 14 days (phase 1) and pigs were allowed ad libitum access to feed and water throughout the experiment. On day 14, a pig nearest to the average weight of the pen was euthanized, and all remaining pigs were fed the common phase 2 diet for an additional 14 days until day 28 post-weaning.

2.2. Sample collection

Diarrhea scores were assessed visually every other day for 28 days by 2 independent observers using a score from 1 to 5 according to the method of Espinosa et al. (2017): 1 = normal feces; 2 = moist feces; 3 = mild diarrhea; 4 = severe diarrhea; and 5 = watery diarrhea. Diarrhea frequency was obtained by totaling the number of pen days with diarrhea scores ≥ 3 divided by the total number of pen days multiplied by 100. Individual pig weights were recorded before weaning 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. If a pig was removed from a pen during the experiment, the feed left in the feeder was recorded and the weight of all remaining pigs in the pen was recorded for calculation of individual feed intake of the remaining pigs in the pen (Lindemann and Kim, 2007). Data for pig weights and feed allowance were summarized to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F) ratio for each pen and treatment group. Data were summarized for each phase and over the entire experiment.

The day before weaning, pigs were allotted to dietary treatments and 2 pigs per pen with a BW closest to the pen average were identified for blood collection. All blood samples were collected from the jugular vein using vacutainers. On the day before weaning, one blood sample from each of the identified pigs was collected, but on days 7 and 14 post-weaning, three blood samples from these pigs were collected. The first blood sample was collected in vacutainers with ethylenediaminetetraacetic acid and stored on ice

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

Item	Basal					Phase 2
Spray-dried plasma, g/kg:	—	20	40	60	80	—
Dry matter, g/kg	912.8	911.7	912.0	911.7	910.0	896.7
Crude protein, g/kg	240.8	234.5	236.7	239.8	232.0	196.9
Ash, g/kg	58.8	57.7	57.4	59.0	56.1	53.5
Acid hydrolyzed ether extract, g/kg	36.5	32.0	34.8	34.4	33.5	40.4
Gross energy, MJ/kg	17.2	17.2	17.3	17.3	17.4	17.0
Insoluble dietary fiber, g/kg	106.0	105.0	105.0	104.0	104.0	127.0
Soluble dietary fiber, g/kg	2.0	2.0	3.0	4.0	2.0	7.5
Total dietary fiber, g/kg	108.0	107.0	108.0	108.0	106.0	134.5
Starch, g/kg	279.3	283.1	284.4	309.8	283.1	336.5
Minerals, g/kg						
Ca	8.6	9.0	8.8	9.5	9.2	8.4
P	7.5	7.4	7.3	7.6	7.1	6.5
K	14.7	14.7	14.4	13.7	13.0	12.1
Mg	1.8	1.8	1.7	1.7	1.5	1.7
Na	2.3	2.5	3.5	3.8	4.4	1.6
Fe	0.2	0.2	0.2	0.2	0.2	0.2
Mn	0.1	0.1	0.1	0.1	0.1	0.1
Zn	0.2	0.2	0.2	0.1	0.1	0.2
Indispensable amino acids, g/kg						
Arg	15.2	14.0	14.2	14.3	12.9	12.3
His	6.1	5.9	6.0	6.2	5.8	5.2
Ile	11.7	10.8	11.0	10.6	9.8	9.3
Leu	20.3	19.6	20.4	20.8	20.1	17.0
Lys	19.3	16.0	16.0	18.3	16.1	14.6
Met	4.2	4.3	4.0	4.6	3.7	4.0
Phe	12.3	11.5	11.9	12.2	11.4	9.6
Thr	10.1	10.3	10.3	10.6	10.4	8.3
Trp	3.3	3.2	3.5	3.7	3.5	2.6
Val	12.3	12.0	12.6	12.9	12.4	10.0
Total	114.8	107.6	109.9	114.2	106.1	92.6
Dispensable amino acids, g/kg						
Ala	11.2	10.9	11.1	11.4	10.9	9.6
Asp	25.7	24.0	24.3	24.5	22.7	20.6
Cys	4.1	4.4	4.4	5.0	4.7	3.4
Glu	44.0	40.8	41.3	41.0	38.2	36.1
Gly	9.5	9.0	9.0	9.0	8.3	7.9
Pro	12.7	12.2	12.6	12.7	12.1	10.8
Ser	10.5	10.0	10.4	10.8	10.5	8.7
Tyr	8.8	8.3	8.5	9.1	8.7	7.0
Total	126.5	119.6	121.6	123.5	116.1	104.0
Total amino acids, g/kg	241.3	227.2	231.5	237.7	222.2	196.5

immediately after collection. Samples were analyzed for white blood cells, neutrophils, and lymphocytes at the University of Illinois Veterinary Diagnostic Laboratory (Urbana, IL, USA). Following this analysis, samples were centrifuged at room temperature at 4000 × g for 13 min to recover the plasma, which was stored at −20 °C until analysis for free amino acids (AA). The second blood sample was collected in heparinized vacutainers and centrifuged at room temperature at 4000 × g for 13 min to recover the plasma, which was stored at −20 °C until analysis for plasma urea nitrogen (PUN), albumin, and total plasma protein using a Beckman Coulter Clinical Chemistry AU analyzer (Beckman Coulter, Inc., Brea, CA, USA). Plasma globulin was calculated as the concentration of albumin subtracted from total protein, and the albumin:globulin ratio was calculated. The third blood sample was collected directly into sodium citrate mononuclear cell preparation vacutainers (BD Biosciences, San Jose, CA, USA) and centrifuged at 1650 relative centrifugal force for 20 min at 25 °C. The peripheral blood monocyte cells were re-suspended in the plasma layer by gentle inversion of the tube and samples were shipped overnight to Iowa State University (Ames, IA, USA) for staining for cluster of differentiation (CD) 4, forkhead box protein 3 (FoxP3), CD25, and CD8alpha and CD8beta. This enabled the identification of the following subsets in pigs: 1) Th naïve (CD4 +CD8alpha-); 2) Th effector or memory (CD4 +CD8alpha+ without or with FoxP3 +CD25 + to indicate current activation status); 3) Cytotoxic T cells (CD8beta+ without or with FoxP3 +CD25 + to indicate current activation status); 4) Regulatory T cells (CD4 +CD8alpha+, CD4 +CD8alpha-, and CD8beta+ with FoxP3 +CD25 +); and 5) Activated T cells (CD4 +CD8alpha+, CD4 +CD8alpha-, and CD8beta+ without FoxP3 +CD25 +). On the last day of phase 1, one of the pigs identified at the beginning of the experiment for blood sampling was euthanized via captive bolt stunning and samples for intestinal morphology assessment and secretory immunoglobulin A (sIgA) and cytokine analysis were collected.

2.3. Intestinal morphology

Samples of jejunum (about 5 cm in length) were collected approximately 150 cm from the pylorus of the pig that was sacrificed on day 14 post-weaning. 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 100 g/kg neutral buffered formalin. After fixation, jejunum samples were sent to Veterinary Diagnostic Pathology, LLC (Fort Valley, VA, USA) where they were sectioned (5 mm thick cross-sections) and embedded in paraffin for slide preparation. For each sample, 3–4 transverse sections were stained with hematoxylin and eosin for histological analysis. Slides were then scanned using a 2.0-HT NanoZoomer (Hammatsu, Bridgewater, NJ, USA), and for each slide, 10 intact villi and the associated crypts were measured using NDP.View2 (Hammatsu, Bridgewater, NJ, USA). Villus height was measured from the villus tip to the base, crypt depth was measured from the crypt-villus junction to the base of the crypt, and the villus height to crypt depth ratio was calculated. Villus width and lamina propria width were measured at the midpoint of the villus. Villus width was measured at the third top of the villus and at the level of the crypt-villus junction to calculate villus surface area. Inflammatory cell infiltration, edema, and misshaped tips of the villi were also

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

Item	Spray-dried plasma	Corn	Soybean meal	Whey powder	Soy protein concentrate
Dry matter, g/kg	905.9	871.1	879.7	898.6	921.5
Crude protein, g/kg	804.7	71.2	452.0	116.6	626.2
Ash, g/kg	70.8	9.3	63.0	72.0	64.0
Acid hydrolyzed ether extract, g/kg	1.2	28.8	19.0	1.1	5.3
Gross energy, MJ/kg	20.2	16.2	17.4	15.2	18.4
Starch, g/kg	N/A[a]	642.5	N/A	N/A	N/A
Indispensable amino acids, g/kg					
Arg	45.6	3.4	31.7	2.6	44.6
His	24.7	2.0	11.7	2.1	16.5
Ile	24.9	2.5	21.2	7.0	30.7
Leu	74.6	7.9	34.3	11.3	48.1
Lys	71.3	2.4	28.7	9.1	39.9
Met	9.0	1.7	5.8	1.8	8.4
Phe	41.9	3.3	22.6	3.6	32.2
Thr	51.4	2.4	17.5	7.1	23.8
Trp	15.8	0.6	6.6	2.2	8.9
Val	55.9	3.3	22.2	6.6	31.9
Total	415.1	29.5	202.3	53.4	285.0
Dispensable amino acids, g/kg					
Ala	38.7	5.0	19.4	5.4	26.9
Asp	79.6	4.7	50.5	11.6	69.9
Cys	27.8	1.7	6.7	2.9	9.4
Glu	111.1	12.2	83.4	19.4	114.9
Gly	27.7	2.9	19.0	2.4	26.1
Pro	40.5	5.7	21.5	6.2	31.1
Ser	47.1	3.1	21.1	4.6	26.8
Tyr	41.1	2.5	17.0	2.7	22.7
Total	413.6	37.8	238.6	55.2	327.8
Total amino acids, g/kg	828.7	67.3	440.9	108.6	612.8

[a] N/A = not analyzed.

determined on the transverse sections of tissue. A range of scores from 0 to 5 (0, normal; 1, minimal; 2, mild; 3, moderate; 4, marked; and 5, severe) was used to determine the severity of morphological damage.

2.4. Secretory immunoglobulin A and cytokine analysis

Samples of jejunum and ileum mucosa scrapings were collected approximately 150 cm from the pylorus and 80 cm from the ileal-cecal junction, respectively. 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) and the supernatant was collected and used for determination of sIgA using an Enzyme Linked Immuno Sorbent Assay 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: interleukin- (IL-) 1α, IL-1β, IL-1 receptor antagonist (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).

2.5. Chemical analysis

All diet and ingredient samples were analyzed in duplicate for concentrations of gross energy using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL, USA), and nitrogen was analyzed by combustion (method 990.03; AOAC Int, 2019) using a LECO FP628 analyzer (LECO Corp., Saint Joseph, MI, USA) with subsequent calculation of crude protein as nitrogen × 6.25. Diets and ingredients were also analyzed for dry matter by oven drying at 135° C for 2 h (method 930.15, AOAC Int, 2019), dry ash (method 942.05; AOAC Int, 2019), and 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). In addition, all diet samples were analyzed for insoluble- and soluble-dietary fiber (method 991.43; AOAC Int, 2019) using the Ankom Dietary Fiber Analyzer (Ankom Technology, Macedon, NY, USA), and total dietary fiber was calculated as the sum of insoluble- and soluble-dietary fiber. Minerals (i.e., Ca, P, K, Mg, Na, Fe, Mn, and Zn) were analyzed in diet samples using inductively coupled plasma-optical emission spectrometry (Avio 200, PerkinElmer, Waltham, MA, USA). Sample preparation included dry ashing at 600° C for 4 h (method 985.01 A, B and C; AOAC Int, 2019) and wet digestion with nitric acid (method 3050 B; U.S. Environmental Protection Agency, 2000). All diet and ingredient samples were also analyzed for AA on a Hitachi Amino Acid Analyzer, Model No. L8800 (Hitachi High Technologies America, Inc; Pleasanton, CA, USA) using ninhydrin for post column derivatization and norleucine as the internal standard [method 982.30 E (a, b, c); AOAC Int, 2019], and diets and corn were analyzed for total starch using the glucoamylase procedure (method 979.10; AOAC Int, 2019). Amino acids and starch were analyzed at the Agricultural Experiment Station Chemical Laboratories at the University of Missouri (Columbia, MO, USA), but all other analyses were conducted at the University of Illinois.

2.6. Statistical analysis

Normality of residuals was verified, and outliers were identified using the UNIVARIATE and BOXPLOT procedures, respectively (SAS Institute Inc, 2016). Outliers were removed if they were located outside of the lower and upper far fences, which are located at 3 times the interquartile range (Tukey, 1977). Data for growth performance, diarrhea scores, tissue morphology, and mucosa inflammation were analyzed by ANOVA using the PROC MIXED procedure of SAS in a randomized complete block design with wean group as the blocking factor and pen as the experimental unit. Contrast statements were used with coefficients for equally spaced treatments to determine linear and quadratic effects of inclusion of SDP in the diet on growth performance, diarrhea, tissue, mucosa, and blood data. The statistical model included the fixed effect of dietary treatment and the random effects of block and replicate within block. Data for tissue histological scores were analyzed using PROC GLIMMIX with poisson distribution. Data for the frequency of T regulatory cells were $\log_{10}$ transformed before statistical analysis to obtain a normal distribution and data were analyzed using PROC MIXED because blood samples for this analysis were not collected from pigs before weaning. For all other blood parameters, differences were calculated using baseline measurements collected from pigs before weaning, and the differences were analyzed as repeated measures using the PROC MIXED and REPEATED procedures in SAS. The model included diet, day, and the interaction between diet and day as the fixed effects, day as the time effect, block and replicate within block as the random effects, and pig as the subject. However, the interaction between diet and day was not significant and was, therefore, removed from the final model. Treatment means were calculated using the LSMEANS statement, and if significant, means were separated using the PDIFF option in the PROC MIXED procedure. The $\chi 2$ test was used to analyze frequency of diarrhea among treatments, but contrast statements were not included in this analysis. Statistical significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

3. Results

3.1. Growth performance and diarrhea scores

There was no difference among treatments for initial BW of pigs (Table 4), but at the end of phase 1 there was a linear increase

($P < 0.05$) in pig BW as the inclusion of SDP in the diet increased. There was also a linear increase ($P < 0.05$) in ADG, ADFI, and G:F of pigs in phase 1 as the inclusion of SDP in the diet increased. In phase 2, there was no difference among treatments for ADG, ADFI, G:F, or final BW. However, there was a trend for a linear increase ($P < 0.10$) in the overall ADG from d 1–28 of pigs as SDP in phase 1 diets increased, but no differences were observed for the overall ADFI and G:F of pigs. There was no effect of dietary treatment on diarrhea scores or the frequency of diarrhea of pigs in phase 1 or phase 2 (Table 5). The mortality rate was 2.25 %, but no differences among treatments were observed (data not shown). No medications were used on individual pigs, in diets, or in the water.

3.2. Tissue morphology

Villus width on day 14 tended to increase ($P < 0.10$) as the inclusion of SDP in the diet increased (Table 6), and villus height to crypt depth ratio tended to decrease (quadratic, $P < 0.10$) as SDP inclusion increased with the least value observed in pigs fed the diet with 60 g/kg SDP. Histological scores of the jejunum of pigs were not influenced by inclusion level of SDP in the diet.

3.3. Secretory immunoglobulin A and mucosal cytokines

The sIgA concentration in jejunum mucosa on day 14 was not influenced by dietary SDP (data not shown). The mucosal concentration of IL-2 in the jejunum tended to increase (quadratic, $P < 0.10$) as the level of SDP in the diet increased, with the greatest concentration observed in pigs fed diets with 40 or 60 g/kg SDP. Mucosal concentration of IL-8 in he jejunum also tended to increase (linear, $P < 0.10$) as dietary SDP increased, whereas the concentration of IL-12 in the jejunum tended to decrease (linear, $P < 0.10$) as dietary SDP increased.

In the ileum mucosa on day 14 post-weaning, there was a quadratic increase ($P < 0.05$) in sIgA, with the greatest concentration observed in pigs fed the diet with 20 g/kg SDP. A quadratic response was observed for IL-1β ($P < 0.05$) and IL-10 ($P < 0.10$) with concentrations in the mucosa being least in pigs fed the diet with 40 or 60 g/kg SDP. The concentration of IL-6 in ileal mucosa tended to increase (linear, $P < 0.10$) as the level of SDP in the diet increased.

3.4. Blood parameters

For T regulatory cells in the blood of pigs on d 7 post-weaning, the frequency of CD8beta+ T cells and CD4 +CD8alpha+ T cells tended to increase (quadratic, $P < 0.10$) for pigs with the greatest value observed at 20 g/kg SDP in the diet (data not shown). The frequency of CD4 +CD8alpha+ FoxP3negCD25 + cells also increased (quadratic, $P < 0.05$) as SDP was added to the diet with the greatest value observed at 40 g/kg SDP. Subsequently, the sum of activated T cells and the ratio of activated to regulatory T cells increased (quadratic, $P < 0.10$) as dietary SDP increased with the greatest value observed for pigs fed diets with 40 or 60 g/kg SDP. There were no differences among regulatory T cells in the blood collected from pigs on day 14 post-weaning (data not shown).

The change in white blood cells from weaning to days 7 and 14 was not influenced by inclusion of SDP in the diet (Table 7). The difference from weaning to days 7 and 14 in neutrophil concentration increased (linear, $P < 0.05$) as SDP inclusion in the diet increased, whereas a linear decrease was observed for the difference from weaning to days 7 and 14 for lymphocytes and PUN

Table 4
Growth performance of weaned pigs fed phase 1 diets with increasing inclusion of spray-dried plasma[1,2,3].

Item	Basal					Pooled SEM	*P*-value	
Spray-dried plasma, g/kg:	—	20	40	60	80		Linear	Quadratic
days 1–14								
Initial BW, kg	6.05	6.05	6.04	6.05	6.04	0.39	0.422	0.692
ADG, g	76	99	107	116	125	29.08	< 0.001	0.315
ADFI, g	152	179	180	178	188	24.78	0.004	0.219
G:F	0.47	0.54	0.57	0.64	0.65	0.09	< 0.001	0.376
Final BW, kg	7.06	7.44	7.54	7.67	7.79	0.80	< 0.001	0.192
days 15–28[4]								
ADG, g	520	526	519	519	508	24.10	0.485	0.637
ADFI, g	607	623	631	611	616	54.66	0.905	0.411
G:F	0.86	0.85	0.83	0.86	0.83	0.04	0.209	0.771
Final BW, kg	14.26	14.91	14.75	14.94	14.56	1.17	0.520	0.142
days 1–28								
ADG, g	296	313	318	317	317	26.95	0.097	0.210
ADFI, g	380	398	407	395	402	40.24	0.221	0.295
G:F	0.78	0.79	0.79	0.81	0.79	0.01	0.225	0.300

[1]Data are least square means of 15 or 16 observations per diet.
[2]ADFI = average daily feed intake; ADG = average daily gain; BW = body weight; G:F = gain-to-feed ratio.
[3]All pigs were fed phase 1 diets for 14 days post-weaning, and they were then fed the common phase 2 diet with no spray dried plasma from days 15–28 post-weaning.
[4]Growth performance parameters were based on 5 pigs per pen from days 1–14. One pig per pen was euthanized on day 14, and therefore, growth performance parameters from days 15–28 were based on 4 pigs per pen.

Table 5
Diarrhea scores and frequency of diarrhea for weaned pigs fed phase 1 diets with increasing inclusion of spray-dried plasma[a].

Item	Basal					Pooled SEM	*P*-value		
Spray-dried plasma, g/kg:	—	20	40	60	80		Diet	Linear	Quadratic
Diarrhea score[b]									
days 1–6	2.10	2.22	2.29	2.21	2.24	0.19	—	0.195	0.176
days 8–14	2.89	2.85	3.03	2.92	2.83	0.16	—	0.902	0.304
days 1–14	2.57	2.58	2.71	2.64	2.58	0.07	—	0.742	0.185
days 16–28	1.84	1.93	1.73	1.90	1.81	0.05	—	0.499	0.948
days 1–28	2.21	2.26	2.24	2.29	2.19	0.05	—	0.945	0.170
Frequency of diarrhea									
days 1–6									
Pen days[c]	48	48	48	48	48				
Frequency[d]	27.08	31.25	20.83	25.00	29.17	—	0.813	—	—
days 8–14									
Pen days	64	64	64	64	64				
Frequency	60.94	53.13	56.25	64.06	53.13	—	0.648	—	—
days 1–14									
Pen days	112	112	112	112	112				
Frequency	3.57	2.68	4.46	6.25	4.46	—	0.751	—	—
days 16–28									
Pen days	112	112	112	112	112				
Frequency	46.43	43.75	41.07	47.32	42.86	—	0.878	—	—
days 1–28									
Pen days	224	224	224	224	224				
Frequency	25.00	23.21	22.77	26.79	23.66	—	0.863	—	—

[a] Data are least square means of 15 or 16 observations per diet.
[b] Diarrhea scores were visually assessed every other day by 2 independent observers for 28 days. Diarrhea score: 1, normal feces; 2, moist feces; 3, mild diarrhea; 4, severe diarrhea; and 5, watery diarrhea.
[c] Pen days = number of pens × the number of days assessing diarrhea scores.
[d] Frequency = (number of pen days with diarrhea scores ≥ 3 / pen days) × 100.

Table 6
Morphology measurements and histology scores of the jejunum of pigs fed phase 1 diets with increasing inclusion of spray-dried plasma[a].

Item	Basal					Pooled SEM	*P*-value	
Spray-dried plasma, g/kg:	—	20	40	60	80		Linear	Quadratic
Villus height, μm	349	335	347	336	371	14.83	0.330	0.154
Villus width, μm	124	122	131	127	132	4.51	0.085	0.861
Crypt depth, μm	320	320	331	333	317	11.07	0.805	0.253
Villus height:crypt depth ratio	1.12	1.07	1.09	1.05	1.20	0.06	0.355	0.051
Lamina propria thickness, μm	81.4	80.8	87.5	84.0	86.5	5.47	0.167	0.720
Villus surface area, mm	138	129	144	136	154	8.81	0.143	0.346
Histological scores[b]								
Lymphoid infiltration	1.5	1.6	1.9	1.4	1.6	0.31	0.998	0.624
Neutrophilic infiltration	1.1	1.1	1.2	1.1	1.1	0.27	0.880	0.802
Edema	0.1	0.1	0.1	0.3	0.1	0.08	0.815	0.562
Misshapen tips	0.3	0.6	0.9	0.6	0.7	0.20	0.213	0.175

[a] Data are least square means of 14, 15, or 16 observations.
[b] A score of 0–5 was assigned: 0, normal; 1, minimal; 2, mild; 3, moderate; 4, marked; and 5, severe.

($P < 0.05$) and a tendency for a linear decrease in albumin ($P < 0.10$) was observed as the inclusion of dietary SDP increased. Concentrations of these blood parameters also changed over time with white blood cells, neutrophils, PUN, total protein, and albumin: globulin ratio being greater ($P < 0.05$) for pigs on day 14 than on day 7, whereas the concentration of lymphocytes and albumin were less ($P < 0.05$) for pigs on day 14 than on day 7.

Blood collected from pigs the day before weaning was used as a baseline measurement to calculate the change in plasma AA concentrations of pigs fed diets with increasing levels of SDP during phase 1 (Table 8). A quadratic increase ($P < 0.05$) was observed for differences from weaning to days 7 and 14 of Gly and Ser with the greatest concentrations in the plasma of pigs fed the diet with 40 g/kg SDP, and the change in concentration of Pro and total AA from weaning to days 7 and 14 tended to increase (quadratic, $P < 0.10$) as SDP was added to the diet. The change in concentration of Cys from weaning to days 7 and 14 increased (linear, $P < 0.05$) and the change in concentrations of Lys, Ala, and Tyr tended to increase (linear, $P < 0.10$) as dietary SDP increased, but the change in concentration of plasma Ile decreased ($P < 0.05$) as SDP was added to the diet. The concentration of most AA in plasma was greater ($P < 0.05$) on day 14 than on day 7, but the concentration of Met was less ($P < 0.05$) on day 14 than on day 7.

Table 7
Differences from weaning to day 7 and 14 for blood parameters of weaned pigs fed phase 1 diets with increasing inclusion of spray-dried plasma[1,2].

Item	Basal					Pooled SEM	*P*-value[4]				Pooled SEM	*P*-value[5]
Spray-dried plasma, g/kg:	—	20	40	60	80		Linear	Quadratic	day 7	day 14		
White blood cells, $\times 10^3/\mu l$												
Weaning	10.07	11.00	10.73	9.71	9.74							
Difference	7.63	6.73	7.14	8.86	8.70	4.01	0.134	0.420	0.17	15.46	3.96	< 0.001
Neutrophils, % of WBC[3]												
Weaning	34.96	38.79	34.31	30.97	33.44							
Difference	4.82	3.95	7.58	13.98	10.22	5.39	0.023	0.780	−0.79	17.01	4.81	< 0.001
Lymphocytes, % of WBC												
Weaning	58.31	53.86	60.06	62.10	59.03							
Difference	−5.52	−3.94	−11.14	−14.84	−10.72	5.67	0.009	0.372	0.20	−18.66	5.25	< 0.001
Plasma urea N, mg/dL												
Baseline	5.44	6.08	5.33	5.53	6.33							
Difference	9.00	9.97	6.00	7.69	5.19	1.82	0.004	0.747	6.49	8.65	1.60	0.004
Albumin, g/dL												
Weaning	2.77	2.69	2.90	2.78	2.88							
Difference	0.06	0.04	−0.09	0.01	−0.13	0.15	0.093	0.994	0.11	−0.16	0.13	< 0.001
Total protein, g/dL												
Weaning	4.49	4.24	4.58	4.48	4.47							
Difference	0.14	0.28	−0.01	0.11	0.03	0.19	0.227	0.946	0.23	−0.01	0.17	< 0.001
Albumin:Globulin												
Weaning	1.64	1.84	1.76	1.68	1.89							
Difference	−0.06	−0.26	−0.15	−0.10	−0.29	0.08	0.125	0.850	−0.08	−0.27	0.06	< 0.001

[1]Blood samples that were collected the day before pigs were weaned were used to calculate the difference between day 7 and weaning and between day 14 and weaning. The day 7 and day 14 differences were then analyzed using repeated measures of SAS.
[2]Data are least square means of 15 or 16 observations.
[3]WBC = white blood cells.
[4]The P-value refers to the effect of adding increasing levels of spray-dried plasma to the diets. Values represent the average of day 7 and day 14 measurements.
[5]The P-value represents the difference between day 7 and day 14 measurements.

Table 8
Differences from weaning of plasma amino acid concentrations (μM/mL) of pigs fed phase 1 diets with increasing inclusion of spray-dried plasma[1,2].

Item	Basal					Pooled SEM	*P*-value[3]				Pooled SEM	*P*-value[4]
Spray-dried plasma, g/kg:	—	20	40	60	80		Linear	Quadratic	d 7	d 14		
Indispensable amino acids												
Arg	−24.5	−12.7	−13.7	−13.7	−24.2	8.40	0.985	0.151	−38.8	3.3	3.87	< 0.001
His	8.7	12.0	9.7	4.7	6.2	2.64	0.106	0.478	4.8	11.7	1.38	< 0.001
Ile	176.2	182.5	144.6	71.7	34.2	42.08	< 0.001	0.134	117.5	126.1	30.94	0.477
Leu	95.5	122.5	114.2	73.3	86.8	36.39	0.324	0.456	75.7	121.3	26.06	0.002
Lys	−40.0	19.2	−17.1	34.1	4.8	19.64	0.087	0.215	−11.0	11.4	8.81	0.019
Met	−34.7	−30.6	−20.8	−27.5	−24.2	36.83	0.450	0.628	−14.1	−41.0	29.25	< 0.001
Phe	33.2	45.7	50.4	55.1	49.3	10.10	0.139	0.272	38.8	54.7	5.56	0.002
Thr	−2.7	0.0	37.5	43.3	42.5	92.64	0.338	0.814	−47.8	96.0	69.12	< 0.001
Trp	−25.9	−18.8	−13.6	−16.3	−15.8	5.02	0.146	0.247	−21.0	−15.1	2.26	0.011
Val	180.7	207.9	292.4	206.5	259.0	114.93	0.329	0.523	165.6	293.0	87.29	< 0.001
Total	367.4	518.2	601.9	431.2	418.6	251.46	0.973	0.282	273.2	661.8	179.57	< 0.001
Dispensable amino acids												
Ala	−292.6	−183.4	−145.2	−60.8	−101.7	128.47	0.098	0.477	−135.3	−178.2	81.84	0.423
Asn	−25.4	−22.8	−23.5	−21.9	−26.1	3.53	0.951	0.347	−34.6	−13.3	1.81	< 0.001
Asp	−2.5	5.9	−1.4	−12.8	−13.4	12.28	0.233	0.583	−6.6	−3.1	6.41	0.177
Cys	−1.0	−0.8	−0.4	4.3	2.3	1.65	0.004	0.956	−0.8	2.5	1.04	< 0.001
Gln	−17.7	20.7	−2.0	18.9	1.5	14.72	0.383	0.172	−9.8	18.3	7.66	< 0.001
Glu	52.0	54.8	69.5	60.8	56.9	14.77	0.705	0.458	26.6	91.0	8.45	< 0.001
Gly	25.5	172.7	268.1	210.3	79.8	93.63	0.585	0.029	187.5	115.1	52.51	0.273
Pro	−642.5	−580.8	−537.7	−522.9	−635.8	140.90	0.684	0.071	−636.5	−531.3	107.99	< 0.001
Ser	−70.1	−46.8	−34.6	−51.8	−70.8	21.70	0.845	0.006	−62.6	−47.0	16.05	< 0.001
Tyr	−175.0	−145.9	−140.5	−133.2	−120.1	21.24	0.072	0.708	−174.9	−111.0	8.44	< 0.001
Total	−1158.5	−732.2	−601.8	−502.0	−827.4	211.41	0.182	0.054	−870.0	−658.8	98.46	0.123
Total amino acids	−782.5	−214.0	−18.3	−70.8	−408.8	377.93	0.361	0.077	−604.1	6.4	223.40	< 0.001
Ornithine	−29.1	−26.8	−28.5	−25.5	−30.7	3.90	0.868	0.449	−39.2	−17.0	1.89	< 0.001
Citrulline	−35.1	−38.7	−39.2	−44.3	−32.2	6.04	0.989	0.142	−42.6	−33.2	3.54	< 0.001

[1]Blood samples that were collected the day before pigs were weaned were used to calculate the difference between day 7 and weaning and between day 14 and weaning. The day 7 and day 14 differences were then analyzed using repeated measures of SAS.
[2]Data are least square means of 14–16 observations per treatment.
[3]The P-value refers to the effect of adding increasing levels of spray-dried plasma to the diets. Values represent the average of day 7 and day 14 measurements.
[4]The P-value represents the difference between day 7 and day 14 measurements.

4. Discussion

Weaning exposes pigs to many stressors, such as environmental, social, and dietary changes, that may result in anorexia or infections that negatively affect the health and growth performance (Pluske et al., 1997; Lallès et al., 2004; Kil and Stein, 2010). Including SDP in diets fed to pigs immediately after weaning stimulates feed intake, and therefore, improves growth performance (Torrallardona, 2010; Bailey et al., 2024). Improvements in growth performance have also been reported in dogs (Quigley et al., 2004), calves (Henrichs et al., 2021), broiler chickens (Campbell et al., 2019), and mice (Thomson et al., 1995) fed a diet with SDP compared with animals fed a diet without SDP. A review of experiments with pigs where SDP was included in a dose-dependent manner concluded that 60 g/kg SDP inclusion in diets for pigs was most consistent in improving G:F and increased ADG and ADFI (van Dijk et al., 2001). However, growth performance continues to increase as SDP inclusion increases in the diet (Torrallardona, 2010), which was also observed in the current experiment. Diets have been supplemented with up to 250 g/kg SDP (Torrallardona, 2010) but including more than 100 g/kg SDP in a diet may result in an imbalance of AA, because SDP is low in Met and Ile, or has a high salt content that could affect electrolyte balance (Kats et al., 1994; Torrallardona, 2010). Therefore, inclusion of 20–80 g/kg SDP in the diet has been recommended (Torrallardona, 2010).

Spray-dried plasma is often included in diets fed to pigs during the initial 2 weeks after weaning, but after 2 weeks, improvements in growth performance are usually not observed for pigs fed a diet with SDP (Torrallardona, 2010). Therefore, in the current experiment, diets with SDP were fed to pigs for 2 weeks after weaning and then a common diet without SDP was provided, and results of the experiment agree with van Dijk et al. (2001) who concluded that growth performance of pigs was not further improved in weeks 3, 4, or 5 after weaning. The observation that there was no effect of phase 1 dietary treatments on performance in phase 2 is in agreement with data demonstrating no carry-over effect of SDP from phase 1 to phase 2 (van Dijk et al., 2001; Bailey et al., 2023a). However, the tendency for an improved ADG that was observed for the overall post-weaning period may be a result of the sanitation challenge environment the pigs were housed in. Pigs experiencing greater antigen exposure have greater improvements in feed intake, weight gain, and feed efficiency if fed a diet with SDP than pigs not exposed to antigens (Coffey and Cromwell, 1995; Torrallardona, 2010). The sanitation challenge model that was used resulted in an ADG for pigs fed the control diet during the initial two weeks post-weaning that was 30–50 g less than the ADG obtained recently for pigs placed in cleaned pens in the same facility and using diets without SDP fed to the same genotype of pigs (Mallea et al., 2023; Bailey et al., 2024; Acosta et al., 2025). It therefore appears that the challenge model worked and that this sanitation challenge reduced ADG of pigs.

Post-weaning diarrhea in pigs may be a result of the underdeveloped intestinal tract producing insufficient amounts of digestive enzymes, and therefore, digestion and absorption are incomplete in the small intestine resulting in greater undigested material in the large intestine that is fermented by microorganisms causing diarrhea (Kil and Stein, 2010; Zhang et al., 2015; Balan et al., 2021). However, including SDP in the phase 1 diet may increase nutrient digestibility (Bailey et al., 2023b), and as a result, feeding pigs a diet with SDP decreases the incidence of post-weaning diarrhea (Gatnau and Zimmerman, 1991; Kil and Stein, 2010), but no differences among treatments were observed in frequency of diarrhea in the current experiment. Spray-dried plasma may not be as effective in reducing *E. coli* in the small intestine compared with the antibiotic colistin (Pérez-Bosque et al., 2016b), but when fed in combination with antimicrobials, sometimes an interaction between SDP and antimicrobial inclusion has been observed (Torrallardona et al., 2003; Bikker et al., 2004). In contrast, SDP included in a diet fed to pigs challenged with rotavirus reduced the incidence of diarrhea and enhanced mucosal immunity (Campbell et al., 2010; Yan et al., 2024), indicating that SDP may be more effective in protecting pigs against viral pathogens than bacterial pathogens, but because we did not include a viral challenge in this experiment, we cannot confirm this hypothesis.

In some countries in the world, it is common practice to control post-weaning diarrhea by inclusion of antibiotic growth promoters or pharmacological levels of Zn in diets, whereas this practice is prohibited in other countries. In the present work, no antibiotic growth promoters were used, and Zn was provided only in quantities that met the requirement (NRC, 2012). As a consequence, possible interactions between the use of SDP and antibiotic growth promoters or elevated inclusion of Zn were not investigated in this work and it is not known if the same results would have been obtained if antibiotics or high levels of Zn had been included in the diets.

The increased villus width as SDP inclusion increased may be a result of the increased ADFI that was observed, because post-weaning anorexia is a primary factor in the alteration of tissue morphology often observed after weaning (Lallès et al., 2004). Feeding a diet with 50 g/kg SDP to weaned pigs increased villus height and villus height to crypt depth ratio in the duodenum and increased the ratio of villus height to crypt depth in the jejunum, but decreased crypt depth compared with pigs fed a diet without SDP (Tran et al., 2014; Zhang et al., 2015). However, villus height and crypt depth in the jejunum, ileum, and colon were not influenced by 60 g/kg SDP in the diet (Nofrarías et al., 2007). The tendency for an increase in the villus height to crypt depth ratio that was observed in this experiment at the greatest inclusion of SDP is, therefore, in agreement with previous data.

An increase in crypt hyperplasia post-weaning results in a greater number of immature cells, and therefore, pathogens can more easily migrate through the intestinal barrier (Pluske et al., 1997; Lallès et al., 2004; Zhang et al., 2016). Maintenance of the intestinal barrier is important for nutrient absorption and prevention of para-cellular diffusion of toxins and microorganisms to reduce over-stimulation of the immune system (Pérez-Bosque et al., 2016b). The immune system is also activated by weaning (Nofrarías et al., 2006), which may result in greater allocation of nutrients to support the immune response than to stimulate growth of pigs (Goodband et al., 2014; Campbell et al., 2019). However, the continued improvement in growth performance of pigs observed as SDP was included in the diet indicates that SDP may have prevented over-stimulation of the immune system, and one of the hypothesized modes of action for SDP is the modulation of intestinal immune response (Campbell et al., 2019). In contrast to results of the current experiment, mice fed a diet with 60 g/kg SDP or rats fed a diet with 80 g/kg SDP had increased synthesis of anti-inflammatory cytokine IL-10 in the intestinal mucosa (Nofrarías et al., 2006; Pérez-Bosque et al., 2010). Expression of the pro-inflammatory cytokine IL-8 in the jejunal

mucosa of rats or pigs fed 60 g/kg dietary SDP and expression of IL-6 in the jejunal mucosa of rats fed 80 g/kg dietary SDP were reduced compared with animals fed a diet without SDP (Bosi et al., 2004; Pérez-Bosque et al., 2010, 2016b). However, Peace et al. (2011) reported that IL-10 and IL-6 in the ileal and colonic mucosa of pigs were not influenced by 25 or 50 g/kg dietary SDP, although growth performance of pigs fed 25 or 50 g/kg dietary SDP was greater than of control pigs. Therefore, even though the present data indicate an increase in a few pro-inflammatory cytokines and a decrease in anti-inflammatory cytokines in the jejunal and ileal mucosa of pigs fed increasing dietary SDP, the immune cell infiltration in the villi of the jejunum were minimal to mild in severity, indicating that the local inflammatory response may not have been over-stimulated, which is in agreement with Bosi et al. (2004). The pro-inflammatory cytokine IL-1β functions in the development and prolongation of intestinal inflammation (Al-Sadi et al., 2012), and an increase in IL-1β in the mucosa may result in an increase in intestinal tight junction permeability (Rawat et al., 2020). Tight junction proteins were increased in the jejunum, ileum, and colon of pigs fed 50 g/kg dietary SDP compared with pigs fed a diet without SDP (Peace et al., 2011; Zhang et al., 2016). Therefore, reduction in IL-1β as dietary SDP increased in the current experiment may indicate reduced para-cellular permeability of pathogens, which agrees with previous data (Moretó and Pérez-Bosque, 2009).

Secretory IgA is a component of the innate immune system with decreased concentrations in the mucosa correlated with decreased activation of the innate immune response (Mantis et al., 2011). Secretory IgA is secreted by cells in the lamina propria and can prevent pathogen attachment to epithelial cells protecting intestinal barrier function (Ushida et al., 2008; Mantis et al., 2011). In contrast with data from the current experiment, a reduction in lamina propria cell density and reduced sIgA concentration in the intestinal mucosa of pigs fed a diet with 50 g/kg SDP have been reported (Peace et al., 2011; Zhang et al., 2016). Neutrophils are also a component of the innate immune system and are among the initial cells recruited to sites of inflammation to function in phagocytosis and inflammation (Jones et al., 2016). Increased neutrophils in the blood of pigs fed increased dietary SDP agree with data from Nile tilapia fed graded levels of SDP (Araújo et al., 2017). The increase in neutrophils may be a result of the increase in IL-8, which can stimulate neutrophils to migrate to the sites of inflammation (Bosi et al., 2004). However, the increased sIgA and neutrophil concentrations that were observed as dietary SDP increased indicate activation of the innate immune system, which can result in subsequent activation of the adaptive immune system consisting of T lymphocytes (Moretó and Pérez-Bosque, 2009).

Regulatory T cells are responsible for maintaining immune homeostasis and suppressing intestinal inflammation (Pérez-Bosque et al., 2016a). However, IL-2 can activate regulatory T cells in pigs (Käser et al., 2011), and once activated, T cells can stimulate the release of pro-inflammatory cytokines (Pérez-Bosque et al., 2016a). Therefore, the changes in IL-2 activated T cells and pro-inflammatory cytokines that were observed are consistent with previous data (Käser et al., 2011; Pérez-Bosque et al., 2016a). In contrast with data from the current experiment, supplementation of dietary SDP has also been observed to reduce the percentage of activated T lymphocytes and to reduce release of IL-2 (Pérez-Bosque and Moretó, 2015). However, dietary SDP may also reduce systemic lymphocyte concentration (Pérez-Bosque and Moretó, 2015), which was observed in the current experiment.

Increased activation of the immune response, innate or adaptive, results in increased AA requirements of the animal to support the synthesis of immune cells (Goodband et al., 2014). The PUN is positively correlated with urinary nitrogen excretion and, therefore, PUN is an indicator of AA utilization efficiency (Kohn et al., 2005). Decreased PUN observed with increasing dietary SDP in the current experiment indicates that protein utilization of the pig became more efficient, contributing to the better performance of pigs fed dietary SDP, which agrees with published data (Jiang et al., 2000; Hernández et al., 2010; Weaver et al., 2014). It is possible that this was related to fewer antinutritional factors being supplied by SDP than by the soy protein concentrate that was replaced, but because we did not measure antinutritional factors in the diets, we cannot confirm this speculation.

5. Conclusions

Increasing inclusion of dietary spray-dried plasma is positively correlated with improvements in growth performance of weanling pigs, indicating that inclusion of at least 80 g/kg spray-dried plasma in diets for weanling pigs from d 1–14 post-weaning, linearly increases average daily feed intake, average daily gain, gain to feed ratio and final body weight. However, because pigs were kept in a sanitation challenge model, average daily gain was less than in pigs kept under less challenging conditions. Increasing levels of dietary spray-dried plasma may stimulate the innate immune system through increased pro-inflammatory cytokine synthesis and increased sIgA concentration in the intestinal mucosa, as well as increased circulating neutrophils. In contrast, the adaptive immune response was not stimulated when 80 g/kg spray-dried plasma was included in the diet as observed by reduced circulating lymphocytes and no change in the frequency of regulatory T cells.

CRediT authorship contribution statement

Joy M. Campbell: Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Stein Hans H:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization. **Hannah M. Bailey:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Natalia S. Fanelli:** Writing – review & editing, Resources.

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Declaration of Competing Interest

Joy M. Campbell is an employee of APC, which is a supplier of spray dried plasma protein. Hannah M. Bailey, Natalia S. Fanelli, and Hans H. Stein have no conflicts of interest.

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