Spray-dried porcine plasma enhances feed efficiency, intestinal integrity, and immune response of broilers challenged with necrotic enteritis

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ABSTRACT Re-emergence of enteric diseases in the postantibiotic era has imposed severe loss to the poultry industry leading to the urgent need for appropriate additives to maintain gut health. Recently, more attention has been paid to animal plasma due to its high concentrations of active components such as albumins and globulins. The objective of this study was to evaluate the effects of spray-dried porcine plasma (**SDP**) supplementation during the starter phase (d 0−10) on growth performance, intestine health, and immune response of broilers under necrotic enteritis (NE) challenge. A total of 720 day-old male broiler parental line chicks (Ross 308) were randomly assigned to a 2 (NE challenge: no, yes) × 2 (SDP: 0, 2%) factorial arrangement with 12 replications of 15 chicks each. To induce NE, birds were inoculated with live *Eimeria* vaccine on d 9 and *Clostridium perfringens* on d 14. The body weight of birds and feed consumption were measured per pen on d 8, 10, 24, and 29 to calculate performance parameters. On d 16, three birds per pen were sampled to analyse the intestinal lesion score, gut permeability, villi morphology, relative weight of organs, and immune response. Results showed that SDP improved ($P < 0.001$) FCR in the pre-challenge phase (d 0−8). The results indicated that supplementing SDP lowered ($P < 0.01$) FCR at the end of the experiment (d 29). Dietary SDP decreased ($P < 0.05$) the concentration of FITC-d in serum samples of challenged broilers, although it did not affect the intestinal morphology and lesion score. Birds fed with SDP had a higher ($P < 0.05$) relative weight of bursa (g/kg live body weight) compared to non-supplemented birds. Supplementing SDP reduced the concentration of interleukin-6 ($P < 0.05$) and α-1 acid glycoprotein ($P = 0.051$) in serum samples of broilers. In conclusion, supplementation of SDP in the starter phase enhanced feed efficiency and gut integrity in NE challenged broilers, possibly through manipulating the immune response, while further studies targeting intestinal microflora and key genes are required to explore the mode of action.

Key words: spray-dried porcine plasma (SDP), intestinal morphology, α-1 acid glycoprotein, immunoglobulin, broiler chicken

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INTRODUCTION

Since the intestine is the main site of nutrient absorption, any challenge disturbing the intestinal homeostasis can lead to enteric disease and consequently compromise nutrient absorption that negatively impacts the growth and health of broilers (Svihus, 2014). Among several enteric diseases in chickens, special attention has been paid to necrotic enteritis (**NE**) due to its huge economical cost of over $6 billion annually through decreased performance and increased mortality and management costs (Wade and Keyburn, 2015). It has been well-documented that the causative agent of NE is a gram-positive anaerobic bacterium, *Clostridium perfringens* producing toxin netB (Keyburn et al., 2008). In addition, dietary factors (e.g., fish meal) and pathogenic (e.g., *Eimeria spp.*) agents (Wu et al., 2014) can disrupt the intestinal integrity and predispose the gut environment to the proliferation of *C. perfringens* (>10^7 cfu). The higher number of *C. perfringens* results in a high number of necrotizing patches in the intestinal wall, consequently inducing NE in broilers (Van Immerseel et al., 2009; Timbermont et al., 2011). Adding antibiotics was the common remedy to control NE in broilers for the past decades, while emerging antibiotic-resistant bacteria (Aarestrup et al., 2008) has limited antibiotics use in poultry production in the outbreak of NE in recent

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years. To restrain the upsurge of NE, researchers have looked for various safe substitutions for antibiotic growth promoters, such as probiotics, prebiotics, acidifiers, and animal plasma.

Spray-dried plasma (**SDP**) is an ingredient obtained from healthy animal blood by separating plasma from whole blood via centrifugation (Blázquez et al., 2020). Then, clear plasma is spray-dried to; 1) preserve the biologically active components of plasma and 2) change the liquid plasma into fluffy yellowish powder (Blázquez et al., 2020). Spray-dried plasma contains active components including immunoglobulins, lipids, peptides, enzymes, amino acids, and other factors (Borg et al., 2002) that play important biological roles in various biochemical, immunological, and metabolic pathways (Pérez-Bosque et al., 2016). Results of previous studies on pigs showed that SDP improved the performance of piglets in the weaning period (van Dijk et al., 2001; Pierce et al., 2005) and under challenge conditions (Bosi et al., 2004). In addition, some researchers reported that SDP modulates the immune response in rats (Pérez-Bosque et al., 2004; Maijó et al., 2012) and pigs (Tran et al., 2014) under normal or challenging conditions. Campbell et al. (2004) demonstrated that dietary bovine SDP improved performance and lowered mortality in turkey poults under the *Pasteurella multocida* challenge. Beski et al. (2015) reported that dietary SDP increased villus height and crypt depth in the jejunum, improved the structure of the intestinal mucosa resulting in higher nutrient absorption and growth performance in broiler chickens. In the subsequent study, Beski et al. (2016) showed that *Salmonella sofia* challenged-broilers supplemented with SDP during the starter phase had better developed immune organs and healthier gut, resulting in better growth performance than non SDP-treated group. In a broiler trial, Campbell et al. (2006) found that SDP enhanced growth performance and attenuated the negative effect of naturally occurring NE in the broiler flock, while no experimental NE challenge trials have been performed to assess the effect of SDP on the disease so far. Therefore, the current study was conducted to evaluate the effects of SDP on growth performance, gut health, and immune response in NE challenged broilers. We hypothesized that SDP supplementation during the starter phase could alleviate the negative effects of NE in broilers by strengthening the immune system earlier, resulting in a healthy gut and, consequently higher growth performance.

MATERIALS AND METHODS

The University of New England's (**UNE**) Animal Ethics Committee reviewed and approved the experimental procedures of the current study (AEC19-093).

Birds and Housing Management

A total of 720 day-old Ross 308 male parental line chicks were sourced from a commercial hatchery (Goulburn, NSW, Australia). On arrival, chicks were weighed and randomly allocated to four treatments with 12 replications of 15 chicks in each replicate. To avoid cross-contamination between challenged and unchallenged groups, two separate rooms with similar environmental conditions were designated for each group at Rob Cumming Poultry Innovation Centre (Kirby Research Station, UNE, Armidale, NSW, Australia). An automated lighting program and the temperature were set for both rooms based on the guidelines of Ross 308 (Aviagen, 2014). Each pen (87 cm × 118 cm) was considered an experimental unit and covered with wood shavings (5 cm). Birds had free access to feed in tube feeders and water supplied with nipple drinkers.

Experimental Design and Diets

Treatments were designed in a 2 × 2 factorial arrangement, and the factors were NE challenge (no, yes) and SDP (0, 2%). Three feeding phases were used: starter (d 0−10), grower (d 11−24), and finisher (d 25−35), and SDP was only supplemented during the starter phase. The birds were weighed on d 8 to evaluate the effects of SDP before the NE challenge and again on d 10, when the starter diet was changed to the grower diet. During the prechallenge period (i.e., 0−8 d), the experiment had only 2 treatments (0, 2% SDP) with 24 replications of 360 birds each. From d 9, the experimental design changed to a factorial arrangement of four treatments as described above.

The near-infrared reflectance spectroscopy (Evonik AminoProx, Frankfurt, Germany) was used to analyse the nutrient content of ingredients prior to diet formulation. Diets were based on wheat, soybean meal, and sorghum (Table 1), formulated to meet or exceed the minimum nutritional recommendations for Ross 308 broilers (Aviagen, 2014) and passed through a pellet press (Palmer Milling Engineers Pty. Ltd., Griffith, NSW, Australia) to provide pellet diets for birds.

NE Challenge

The dual challenge of *Eimeria* and *C. perfringens* previously established at UNE (Rodgers et al., 2015) was applied to induce NE in broilers. Briefly, challenged birds were inoculated with 1 mL live *Eimeria* vaccine (Eimeria Pty Ltd., Ringwood, VIC, Australia), including *E. acervulina* (n = 5,000), *E. maxima* (n = 2,500), and *E. brunetti* (n = 2,500) on d 9, and gavaged with 1 mL containing approximately 10^8 cfu/mL *C. perfringens* producing NetB toxin (EHE-NE18, CSIRO, Livestock Industries, Geelong, Australia) on d 14.

Performance Parameters

Average daily gain (**ADG**), average feed intake (**AFI**), and feed conversion ratio (**FCR**) of each pen were calculated based on the weight of birds and the remaining feed in each pen for the starter (d 0−10),

Table 1. Composition of experimental diets.

Ingredients (%)	Starter (d 0−10)		Grower (d 11−24)	Finisher (d 25−35)
	Control	SDPP[1]		
Wheat	39.85	41.36	35.22	40.19
Sorghum	20.00	20.00	30.00	30.00
Soybean meal (CP, 47.5%)	34.15	31.30	29.20	24.12
Canola oil	2.45	2.06	2.51	2.96
Limestone	1.31	1.38	1.21	1.13
Dicalcium phosphate	0.89	0.79	0.67	0.49
Salt	0.25	0.14	0.21	0.21
Sodium bicarbonate	0.11	0.10	0.10	0.10
Choline chloride	0.04	0.05	0.04	0.04
L-lysine HCl	0.23	0.17	0.22	0.20
D,L-methionine	0.36	0.32	0.31	0.28
L-threonine	0.15	0.10	0.12	0.09
Xylanase[2]	0.01	0.01	0.01	0.01
Phytase 10000 TPT[3]	0.01	0.01	0.01	0.01
Vitamin Premix[4]	0.09	0.09	0.08	0.08
Mineral premix[5]	0.11	0.11	0.10	0.10
SDPP	0.00	2.00	0.00	0.00
Calculated nutrients (%, otherwise as indicated)				
AMEn (kcal/kg)	3000	3000	3075	3150
Crude protein	23.2	23.3	21.38	19.44
Crude fat	4.47	4.08	4.65	5.13
Crude fiber	2.91	2.83	2.80	2.68
Digestible Lys	1.280	1.280	1.15	1.02
Digestible Met	0.648	0.608	0.59	0.53
Digestible Met + Cys	0.950	0.950	0.87	0.80
Digestible Thr	0.860	0.860	0.77	0.68
Calcium	0.96	0.96	0.86	0.78
Available phosphorus	0.48	0.48	0.43	0.39
Sodium	0.20	0.20	0.18	0.18
Potassium	1.01	0.96	0.92	0.84
Linoleic acid	1.56	1.45	1.63	1.74
Analysed nutritional content (%, otherwise as indicated)				
Dry Matter	87.18	87.49	87.69	86.70
GE, kcal/kg	3,979	3,994	4,004	3,972
Crude protein	24.30	25.25	20.35	18.95
Crude fiber	2.86	3.33	2.71	3.19

[1]Spray dried porcine plasma (SDPP) was supplemented in rate of 2% to the diet.
[2]Xylanase: Econase XT, 25, AB Vista, 16,000 BXU/kg of diet).
[3]Phytase: Quantum Blue, 5G, AB Vista, 500 FTU/kg of diet.
[4]Vitamin premix per kg diet: vitamin A, 12 MIU; vitamin D, 5 MIU; vitamin E, 75 mg; vitamin K, 3 mg; nicotinic acid, 55 mg; pantothenic acid, 13 mg; folic acid, 2 mg; riboflavin, 8 mg; cyanocobalamin, 0.016 mg; biotin, 0.25 mg; pyridoxine, 5 mg; thiamine, 3 mg; antioxidant, 50 mg.
[5]Trace mineral concentrate supplied per kilogram of diet: Cu (sulfate), 16 mg; Fe (sulfate), 40 mg; I (iodide), 1.25 mg; Se (selenate), 0.3 mg; Mn (sulfate and oxide), 120 mg; Zn (sulfate and oxide), 100 mg; cereal-based carrier, 128 mg; mineral oil, 3.75 mg.

grower (d 11−24), and finisher (d 25−29) phases. Feed conversion efficiency was adjusted based on mortality. It should be mentioned that the numbers of birds in several pens after the NE challenge were higher than AEC approved protocol, and thus, we had to remove some excessive birds from those pens to keep the approved stocking density. Therefore, the pen performance data was not used after d 29 due to the bias produced by removing birds.

Sampling

Three birds from each pen were euthanized and the relative weights of the spleen, bursa of Fabricius, and liver on d 16, those of organs and carcass parts (breast and thigh-drumstick) on d 35 were measured. On d 16, sampled birds were electrically stunned, blood samples were collected via the jugular vein into Vacutainers (Beckton Dickinson, North Ryde, NSW, Australia). Serum was separated by centrifuging blood at 3,000 × g for 10 min, and immediately stored in a −20°C freezer for further analysis of gut permeability and immunological parameters (interleukin-6, IgA, IgM, IgG, α-1 acid glycoprotein, and ovotransferrin). The intestine was carefully removed and divided into duodenum, jejunum, and ileum to inspect NE lesions based on a scale of 0 to 6 (Keyburn et al., 2006). On d 16, approximately 5 cm jejunum tissue was sampled, flushed with phosphate buffer saline (**PBS**), and kept in 10% neutral buffered formalin (**NBF**) for villi morphology analysis.

Oocyst Enumeration

The oocyst shedding was quantified following the methods previously described by Kumar et al. (2022) and expressed as oocysts per gram (**OPG**) of excreta samples. Briefly, fresh excreta samples were collected from all pens on 5 and 7 d postinfection. Collected samples were stored at 4°C for *Eimeria* oocyst counts. One hundred milligrams of excreta samples were diluted with

900 μL saturated salt solution, vortexed to thoroughly mix and left for 2 h in the fridge to float oocysts and to settle sample debris. Then, 600 μL saturated salt solution was added to the Whitlock chamber (Whitlock universal slides, JA Whitlock & Co., NSW 2122, Australia) and 150 μL of diluted samples were pipetted and added to the Whitlock chamber. *Eimeria* oocysts were counted using a 40 × objective lens (Nikon Eclipse Ci-l, Tokyo, Japan) and the counts were multiplied by 100 as the dilution factor.

Gut Permeability

Fluorescein isothiocyanate dextran (FITC-d, Sigma-Aldrich, Stockholm, Sweden) was used to determine the effects of SDP on gut permeability following the procedure previously described by Barekatain et al. (2019). Three birds per pen were selected on d 16 and gavaged with FITC-d. Blood samples were collected 2 h postinoculation. Then, serum samples were separated from blood and diluted (1:1 v/v) with PBS to determine the fluorescence concentration at an excitation wavelength of 485 nm using a microplate reader (Molecular Devices, San Jose, CA). The concentration of FITC-d (μg/mL) in serum samples was calculated based on a standard curve obtained with standard FITC-d concentrations following the procedures previously described by Prado-Rebolledo et al. (2017).

Jejunal Morphology

The jejunal samples kept in 10% NBF were processed, fixed in paraffin, sectioned with a microtome at 5 μm thickness, placed on the slide, and stained using standard hematoxylin-eosin dyes (Golder et al., 2011). Villus height (**VH**), villus width (**VW**), and crypt depth (**CD**) of 20 well-oriented villi per slide were measured under the microscope with a 10 × objective lens. The microscope (Nikon Eclipse Ci-l, Tokyo, Japan) was equipped with a camera taking slide photos and transferring them into a computer operated by the software NIS-Elements Documentation (Nikon, Tokyo, Japan) for measurements. The results of VH, VW, and CD were used to calculate the VH:CD ratio and villus surface area (**VSA**) using the formula introduced by Mayhew (1984).

Immunological Parameters

The collected blood samples were centrifuged at 3,000 × g for 10 min to obtain serum samples and then kept at −20°C for further analysis. Serum samples were used to analyse the concentration of IgA (ab157691, Abcam, Cambridge, MA), IgM (ab157692, Abcam, Abcam, Cambridge, MA), IgG (KT-619, Kamiya Biomedical Company, Seattle, WA), α-1 acid glycoprotein (ab157690, Abcam, Cambridge, MA), ovotransferrin (ab157694, Abcam, Cambridge, MA), and interleukin-6 (IL-6; ELG-IL6-1, Raybiotech, Peachtree Corners, GA) with the related commercial assay kits assisted with an ELISA reader (Epoch Microplate Spectrophotometer, BioTek Instruments, Winooski, VT) according to the manufacturers' instructions.

Statistical Analysis

Normally distributed data during 0 to 8 d were analyzed using a Student's t-test, and those involved challenges were analyzed in a completely randomized design using a 2 × 2 factorial arrangement using JMP 14.0 (SAS Institute). Kruskal−Wallis nonparametric test was used to analyse the lesion score data. Mean differences were compared using Tukey's and Student's t-test, and $P < 0.05$ was considered to be statistically significant.

RESULTS

Performance

Results showed that supplementing broilers with SDP decreased ($P < 0.001$) FCR but did not affect ($P > 0.05$) ADG and ADFI at the first 8 d of age before the onset of NE challenge (Table 2). When considering the main effects of the whole starter phase (first 8 d without challenge plus 2 d following the *Eimeria* inoculation), the challenge group showed higher ($P < 0.001$) weight gain and consequently led to lower ($P < 0.001$) FCR compared to unchallenged birds (Table 3). Interestingly, supplementing SDP decreased ($P < 0.001$) FCR without any negative effects on performance parameters compared to non-supplemented birds. During the grower phase (d 11−24) coinciding with NE induction, the challenge decreased ($P < 0.001$) feed intake and weight gain and increased ($P < 0.001$) FCR, while SDP did not affect ($P > 0.05$) performance parameters. During the finisher phase (d 25−29), NE challenge reduced ($P < 0.001$) feed intake and weight gain and increased ($P < 0.001$) FCR, while SDP fed birds showed higher ($P < 0.05$) weight gain and lower ($P < 0.05$) FCR compared to untreated birds as main effects. Overall performance results (d 0 −29) showed that SDP fed chickens had lower ($P < 0.01$) FCR compared to non-supplemented birds, while NE challenged birds showed lower ($P < 0.001$) feed intake and weight gain and higher ($P < 0.001$) FCR compared to unchallenged birds. No interaction between

Table 2. Effects of porcine plasma supplementation on growth performance of broilers at first 8 d before necrotic enteritis challenge.

Treatments	Feed intake (g)	Weight gain (g)	FCR (g/g)
Control	210	195	1.078[a]
Porcine plasma (2%)	207	199	1.033[b]
SEM[1]	2.28	1.97	0.008
P-value	0.287	0.218	<0.001

[a-b]values within a column with different letters differ significantly ($P < 0.05$).

[1]SEM: standard error of means (the values are means of 24 replicates/treatment).

Table 3. Effects of porcine plasma supplementation on growth performance of broilers challenged with necrotic enteritis.

Interaction effect		D 0–10			D 11–24			D 25–29			D 0–29		
Porcine plasma (%)	Challenge	ADFI[1] (g)	ADG (g)	FCR (g/g)	ADFI (g)	ADG (g)	FCR (g/g)	ADFI (g)	ADG (g)	FCR (g/g)	ADFI (g)	ADG (g)	FCR (g/g)
0	No	330	307	1.077	1409	1015	1.391	713	451	1.581	2447	1779	1.376
	Yes	330	352	0.937	1121	700	1.587	678	409	1.659	2129	1461	1.451
2	No	316	303	1.042	1405	1021	1.376	718	466	1.543	2441	1791	1.362
	Yes	331	358	0.908	1136	725	1.566	690	421	1.641	2157	1504	1.434
SEM		3.70	3.71	0.006	14.7	11.7	0.013	8.19	6.08	0.012	22.4	15.0	0.005
Main effect													
Porcine plasma (%)													
0		330	329	1.007[a]	1265	857	1.489	695	430[b]	1.620[a]	2288	1620	1.413[a]
2		324	331	0.975[b]	1270	873	1.471	704	443[a]	1.592[b]	2299	1648	1.398[b]
SEM		2.65	2.65	0.004	10.7	8.3	0.009	5.79	4.30	0.008	15.8	10.6	0.004
Challenge													
No		323	305[b]	1.059[a]	1407[a]	1018[a]	1.383[b]	715[a]	458[a]	1.562[b]	2444[a]	1785[a]	1.369[b]
Yes		331	355[a]	0.922[b]	1128[b]	713[b]	1.576[a]	684[b]	415[b]	1.650[a]	2143[b]	1483[b]	1.442[a]
SEM		2.65	2.65	0.004	10.7	8.3	0.009	5.79	4.30	0.008	15.8	10.6	0.004
P-value													
Porcine plasma		0.082	0.727	< 0.001	0.727	0.183	0.186	0.303	0.038	0.026	0.648	0.078	< 0.01
Challenge		0.053	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Porcine plasma × Challenge		0.055	0.215	0.636	0.546	0.432	0.829	0.673	0.812	0.403	0.461	0.326	0.703

[a-b]values within a column with different letters differ significantly ($P < 0.05$).
[1]ADFI: average daily feed intake, ADG: average daily gain, FCR: feed conversion ratio.

SDP and NE treatments was observed for the performance data over the entire study.

Lesion Score and Oocyst Shedding

The induction of NE challenge in broilers produced mild lesions in the duodenum of challenged birds; however, the difference between challenge and unchallenged birds was not significant in this segment (Figure 1). Necrotic enteritis challenge as the main effect induced more lesions ($P < 0.05$) in the jejunum and ileum compared to the unchallenged control group.

Table 4 shows the effects of experimental treatments on excreta OPG count on d 14 and 16. No interaction between SDP and NE was observed. Necrotic enteritis challenged birds had a higher ($P < 0.001$) OPG count compared to the unchallenged birds, while SDP supplementation had no significant effects ($P > 0.05$) on OPG counts on both d 14 and 16.

Gut Permeability

Results showed that there was an SDP × NE challenge interaction on the serum FITC-d concentration (Table 5). Spray-dried porcine plasma supplementation reduced ($P < 0.01$) serum FITC-d concentration only in challenged birds suggesting the improved gut integrity by SDP when birds were challenged with NE, but not ($P > 0.05$) when birds were not challenged owing to already healthy intestine.

Organ and Carcass Weight

The effects of treatments on the relative weight of organs on d 16 and relative organ and carcass weights on d 35 are shown in Table 6. In regard to the main effects, SDP supplementation increased ($P < 0.05$) the relative weight of the bursa of Fabricius at 16 and 35 d of age. Challenged birds had higher ($P < 0.05$) bursa weight on d 16, and higher ($P < 0.01$) thigh-drumstick weight and lower ($P < 0.001$) breast weight on d 35 compared to the unchallenged birds. Adding SDP to the diet of NE challenged birds decreased ($P < 0.05$) the relative weight of fat pad compared to non-supplemented challenged birds. No differences were observed for other organs on both days and no other interaction between SDP and NE challenge was observed.

Villus Morphology

No SDP × NE challenge interaction was detected for villus morphology on d 16 (Table 7). Necrotic enteritis challenge as the main effect decreased ($P < 0.001$) jejunal VH, VH/CD, and VSA and increased ($P < 0.001$) CD on d 16. Spray-dried porcine plasma supplementation as the main effect did not affect ($P > 0.05$) jejunal villi morphology on d 16.

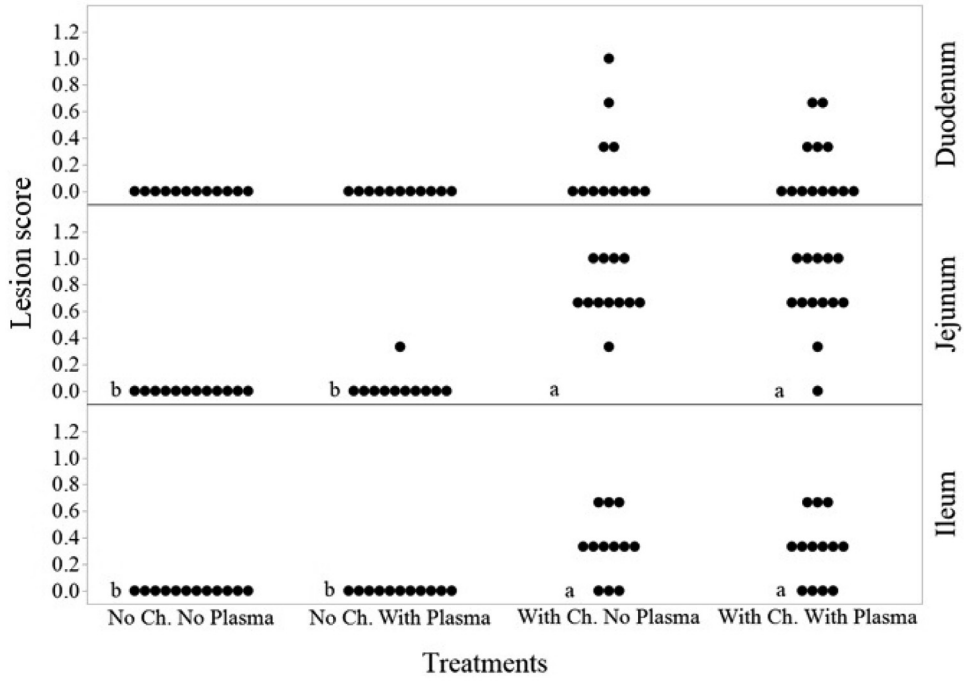

Figure 1. Effects of porcine plasma supplementation on lesion scores (d 16) of different segments of the intestine of broilers challenged with necrotic enteritis (No Ch.: No challenge, With Ch.: with NE challenge). The letters next to means show significant difference ($P < 0.05$).

Serum Immunological Parameters

There were no SDP × NE challenge interactions on the serum concentration of IL-6, IgA, IgM, IgG, α-1 acid glycoprotein, and ovotransferrin on d 16, as shown in Table 8. Necrotic enteritis challenge as the main effect increased the serum concentration of IgA ($P < 0.001$) and α-1 acid glycoprotein ($P < 0.05$) on d 16. Birds receiving SDP had a lower ($P < 0.05$) IL-6 and tended ($P = 0.051$) to have a lower α-1 acid glycoprotein concentration in the serum compared to the non-supplemented birds. No differences were observed for other serum immunological variables.

Table 4. Effects of porcine plasma supplementation on oocysts counts (oocysts per gram of excreta) of broilers challenged with necrotic enteritis on d 14 and 16.

Interaction effects		Days of age	
Porcine plasma (%)	Challenge	**14**	**16**
0	No	0	0
	Yes	5.57	4.73
2	No	0	0
	Yes	5.67	4.87
SEM[1]		0.086	0.075
Main effect			
Porcine plasma (%)			
0		2.78	2.36
2		2.84	2.43
SEM		0.061	0.053
Challenge			
No		0[b]	0[b]
Yes		5.62[a]	4.80[a]
SEM		0.061	0.053
***P*-value**			
Porcine plasma		0.533	0.350
Challenge		<0.0001	<0.0001
Porcine plasma × Challenge		0.533	0.350

[a-b]Values within a column with different letters differ significantly ($P < 0.05$).

[1]SEM: standard error of means (the values are means of 12 replicates/treatment).

DISCUSSION

The current study investigated the effects of porcine SDP on performance, immunity, and intestinal health in broilers, particularly under NE challenge conditions. Birds challenged with NE showed lesions in their intestine, impaired villi growth, compromised gut integrity, and altered immune response, indicating successful NE challenge. The present study demonstrated that porcine SDP improved the feed efficiency of broilers in both NE challenged and unchallenged birds. Furthermore, SDP supplementation decreased the serum IL-6 and α-1 acid glycoprotein concentrations, increased the relative bursa weight, and increased gut integrity in NE-challenged birds. Therefore, the results of this study accept the hypothesis that SDP supplementation in the diet improves the growth performance of broilers by modulating the immune response and increasing gut integrity.

Supplementing SDP to the broilers diet improved growth performance in broilers, as shown by lowered FCR at different feeding phases in the current study. Previous studies showed the beneficial effects of SDP on performance parameters in broiler chickens under normal and challenging conditions. For example, Beski et al. (2015) and Walters et al. (2019) supplemented broilers with different levels of SDP for the first 10 d of age and reported that increasing dietary SDP level increased body weight during the first 10 d and market weight at different ages (d 35 and d 42, respectively). The authors concluded that adding SDP to the starter diets would benefit the long-term growth of

Table 5. Effects of porcine plasma supplementation on concentration of FITC-d in serum samples of broilers challenged with necrotic enteritis on d 16.

Interaction effects		FITC-d
Porcine plasma (%)	Challenge	(μg/mL serum)
		0.037[c]
0	No	0.105[a]
	Yes	0.040[c]
2	No	0.088[b]
	Yes	0.037[c]
SEM[1]		0.004
Main effects		
Porcine plasma (%)		
0		0.071
2		0.064
SEM		0.003
Challenge		
No		0.038
Yes		0.097
SEM		0.003
***P*-value**		
Porcine plasma		0.073
Challenge		<0.001
Porcine plasma × Challenge		0.009

[a-b]Values within a column with different letters differ significantly ($P < 0.05$).
[1]SEM: standard error of means (the values are means of 12 replicates/treatment).

broilers. Beski et al. (2016) evaluated the effect of SDP on performance and some physiological responses during *Salmonella sofia* challenge in broilers. They demonstrated that supplementing *Salmonella*-challenged broilers with SDP increased body weight compared to the control group in the starter and grower phases, and the body weight of birds fed 2% SDP was similar to the unchallenged control. Cherian et al. (2019) evaluated the effects of SDP in commercial farms with a history of hepatitis and reported that the inclusion of 2% SDP for the first 10 d decreased mortality, increased weight gain, and improved FCR compared to the control group. In the current study, symptoms of subclinical NE including mild lesions in the jejunum and ileum, reduction of feed intake and depression of weight gain were observed in challenged broilers, as shown in the other studies (Gharib-Naseri et al., 2021; Keerqin et al., 2021; Kumar et al., 2021). The negative effects of NE challenge have been attributed to the destructive role of *Eimeria* and *C. perfringens* on epithelial cells that may consequently reduce luminal nutrient absorption, decrease feed consumption, and compromise FCR. In the current study, SDP supplementation significantly improved FCR during the starter, finisher phases, and overall period regardless of the NE challenge. The suggested mode of action of supplementing SDP on growth performance has been attributed to the modulation of the immune system as the rate of immune activation can determine the availability of energy for growth, transform the structural integrity of the intestinal barrier, and limit productive performance (Campbell et al., 2008; Balan et al., 2019). For example, it was reported that acute-phase protein production, secretion, and circulation are costly for the host cells because the production of these proteins diverts nutrient flow from growth performance to the expensive immune response leading to the growth retardation in broilers (Johnson, 1997; Peebles et al., 2014). Therefore, if an additive restores the immune system to its homeostasis, it may divert the energy for growth performance. Campbell et al. (2019) concluded that supplementing SDP in the starter phase improves overall broiler performance, and the response of birds to feeding SDP may depend on the severity of various diseases and stress challenges. In the current study, supplementation of SDP improved FCR at the first 8 d of age (before challenge) and overall period (d 0 −29), suggesting that the role of SDP may be related to the maintenance of immune homeostasis in broilers.

Supplementing broiler chickens with SDP during d 0 to 8 improved immunity balances, as shown by the decreased cytokines and immune-related proteins. It has

Table 6. Effects of porcine plasma supplementation on carcass and organ weights (g/kg live bodyweight) of broilers challenged with necrotic enteritis on d 16 and 35.

Interaction effects		D 16			D 35					
Porcine plasma (%)	Challenge	Liver	Bursa	Spleen	Liver	Bursa	Spleen	Breast	Thigh and drumstick	Fat pad
0	No	29.41	2.076	0.683	22.11	1.30	0.806	191.69	200.42	9.58[a,b]
	Yes	28.67	2.320	0.699	23.24	1.41	0.782	176.36	205.97	10.51[a]
2	No	28.60	2.345	0.651	21.27	1.67	0.785	194.23	197.65	10.15[a,b]
	Yes	28.41	2.416	0.714	22.49	1.56	0.762	181.07	204.71	9.34[b]
SEM[1]		0.4828	0.0715	0.0342	0.867	0.120	0.0294	2.507	2.005	0.417
Main effects										
Porcine plasma (%)										
0		29.04	2.198[b]	0.691	22.68	1.35[b]	0.794	184.02	203.19	10.05
2		28.50	2.381[a]	0.683	21.88	1.61[a]	0.773	187.65	201.18	9.75
SEM		0.3414	0.0505	0.0242	0.613	0.085	0.0208	1.773	1.418	0.295
Challenge										
No		29.00	2.211[b]	0.667	21.69	1.48	0.796	192.96[a]	199.03[b]	9.87
Yes		28.54	2.368[a]	0.707	22.86	1.48	0.772	178.71[b]	205.34[a]	9.93
SEM		0.3414	0.0505	0.0242	0.613	0.085	0.0208	1.773	1.418	0.295
P-value										
Porcine plasma		0.272	0.014	0.809	0.361	0.035	0.487	0.155	0.321	0.480
Challenge		0.346	0.033	0.266	0.184	0.990	0.430	<0.0001	0.003	0.880
Porcine plasma × Challenge		0.572	0.232	0.511	0.961	0.366	0.967	0.668	0.707	0.027

[a-b]Values within a column with different letters differ significantly ($P < 0.05$).
[1]SEM: standard error of means.

Table 7. Effects of porcine plasma supplementation on jejunal villus morphology of broilers challenged with necrotic enteritis on d 16.

Interaction effects		Measurement				
Porcine plasma (%)	Challenge	Villus height (μm)	Crypt depth (μm)	Villus width (μm)	Villus height/ crypt depth	Villus surface area (μm^2)
0	No	117.7	13.7	20.8	8.8	8392.0
	Yes	80.3	25.0	19.9	3.2	5661.7
2	No	122.1	13.2	20.8	9.5	8698.4
	Yes	87.3	26.4	19.9	3.3	6096.4
SEM		3.76	0.97	0.82	0.29	401.20
Main effects						
Porcine plasma (%)						
0		98.9	19.4	20.4	6.0	7026.8
2		104.7	19.8	20.3	6.4	7397.4
SEM		2.66	0.69	0.58	0.20	283.71
Challenge						
No		119.9[a]	13.5[b]	20.8	9.1[a]	8545.2[a]
Yes		83.8[b]	25.7[a]	19.9	3.3[b]	5879.0[b]
SEM		2.66	0.69	0.58	0.20	283.71
P-values						
Porcine plasma		0.134	0.665	0.959	0.177	0.361
Challenge		<0.001	<0.001	0.307	<0.001	<0.001
Porcine plasma × Challenge		0.726	0.332	0.954	0.312	0.874

[a-b]Values within a column with different letters differ significantly ($P < 0.05$).

been demonstrated that the oral administration of SDP and other plasma proteins can improve immune response in animals through different mechanisms such as increasing immunoglobulins (Igs, Balan et al., 2010), increasing the systemic immune response and modulating immunocompetent cell function (Sugisawa et al., 2001; Ulfman et al., 2018), augmenting proliferation of lymphocytes, enhancing phagocytic activity and improving the concentration of antibodies in the gut (Cummings et al., 2004). In addition, other investigators reported the beneficial effects of oral administration of SDP and other plasma proteins on immune response in animals under different pathogenic challenges. For example, Pérez-Bosque et al. (2004, 2008) examined the effects of SDP on the immune response of rats challenged with *Staphylococcus aureus* toxin (SAT). The authors demonstrated that SDP could modulate the immune system by preventing the SAT-induced activation of T-helper cells (i.e., CD4 cells), inhibiting the effects of SAT on the release of pro-inflammatory cytokines, and increasing the production of anti-inflammatory cytokines (i.e., IL-10) in the mucosal layer of the intestine (Pérez-Bosque et al., 2004, 2008). Bosi et al. (2004) reported that SDP decreased salivary IgA secretion and pro-inflammatory cytokine expression in the gut of early-weaned piglets challenged with *E. coli* K88. Similarly, Maijó et al. (2012) indicated that oral administration of Igs reduced *E. coli* activation of T-helper lymphocytes (Th1 and Th2) and cytokines (IFN-γ, IL-5, IL-12, IL-13, and IL-17) in blood and lungs and

Table 8. Effects of porcine plasma supplementation on serum immunological parameters of broilers challenged with necrotic enteritis on d 16.

Interaction effects							
Porcine plasma (%)	Challenge	Interleukin-6 (ng/mL)	IgA (μg/mL)	IgM (μg/mL)	IgG (μg/mL)	α-1 acid glycoprotein (μg/mL)	Ovotransferrin (μg/mL)
0	No	0.244	142.2	159.5	1223.1	190.5	1345.8
	Yes	0.231	214.3	171.9	1167.3	227.8	1362.7
2	No	0.143	144.0	142.2	1112.5	171.4	1312.9
	Yes	0.194	204.3	194.8	1111.9	197.8	1479.9
SEM		0.026	18.62	19.07	117.2	12.25	88.61
Main effects							
Porcine plasma (%)							
0		0.237[a]	178.3	165.7	1195	209.1[a]	1354.2
2		0.169[b]	174.2	168.5	1112	184.6[b]	1396.4
SEM		0.019	13.17	13.48	82.89	8.66	61.80
Challenge							
No		0.189	143.1[b]	150.8	1168	180.9[b]	1329.4
Yes		0.212	209.3[a]	183.4	1140	212.8[a]	1421.3
SEM		0.020	13.17	13.48	82.89	8.66	61.80
P-values							
Porcine plasma		0.015	0.826	0.886	0.483	0.051	0.633
Challenge		0.239	<0.001	0.095	0.811	0.013	0.301
Porcine plasma × Challenge		0.494	0.753	0.297	0.815	0.661	0.397

[a-b]Values within a column with different letters differ significantly ($P < 0.05$).

increased gene expression and concentration of the anti-inflammatory cytokine such as IL-10 in a mouse model. Previous research showed that SDP supplementation decreased IL-6 level in rats and pigs compared to challenged groups (Touchette et al., 2002; Pérez-Bosque et al., 2007). In broilers, inconsistent findings have been reported about the effects of NE on interleukin production. For example, Lee et al. (2018) observed that NE increased the expression of IL-6 in the intestine of broilers, while others showed that NE had no significant effect on IL-6 (Park et al., 2008; Johnson et al., 2020). In addition, Beski et al. (2016) demonstrated that SDP increased the relative weight of bursa, but not other organs under challenging conditions. Similarly, Campbell et al. (2006) reported that broilers fed SDP had a heavier bursa, which is in line with the current results. The mucosal immune system stretches throughout the intestinal lumen. It is mainly composed of gut-associated lymphoid tissue (**GALT**), exhibiting crucial roles in protecting the epithelial cells from various challenges like pathogens, antinutritional factors, and multiple stressors (Kiyono and Fukuyama, 2004; Granger et al., 2006). Generally, the pathogen's invasion activates the mucosal immune response by initiating the correlated inflammatory process resulting in the secretion of pro-inflammatory cytokines (Petersen et al., 2004). Various mechanisms of action have been suggested for the immunomodulatory effects of SDP and other plasma proteins. The most likely mechanism has been attributed to Igs structural segments of F_{ab} and F_c (Balan et al., 2019). It has been hypothesized that the F_{ab} regions distinguish antigenic targets and facilitate antibody adherence, while the F_c region interacts with F_c gamma receptors on monocytes, macrophages, and neutrophils to stimulate phagocytic activity (Han et al., 2009; Balan et al., 2019). Another hypothetical mode of action of SDP on the immune system is the passive pathway of supporting the growth of lactic acid bacteria, which play crucial roles as immuno-modulatory and -stimulatory agents in the host gut (Jain et al., 2008; Balan et al., 2019). In the current study, the NE challenge did not affect the concentration of IL-6, while dietary SDP significantly decreased the concentration of IL-6 in the serum. Pro-inflammatory cytokines, specifically IL-6, were reported to stimulate hepatocytes to produce and secrete acute phase proteins (APPs), fulfilling several essential functions under challenging conditions (Petersen et al., 2004). Acute phase proteins such as ovotransferrin and α-1 acid glycoprotein are a group of proteins serving as nonspecific effectors of the innate immune system to maintain gut homeostasis and restore the intestinal environment after pathogenic invasion through various metabolic and immunological mechanisms, including opsonization, neutralizing enzymes, scavenging free radicals, and playing antibacterial and antioxidant roles (Murata et al., 2004; O'Reilly and Eckersall, 2014). In the current study, NE challenge increased the α-1 acid glycoprotein concentration, and SDP supplementation decreased ovotransferrin and α-1 acid glycoprotein concentration in the serum samples. Although the mechanism under the modulating effects of SDP on APPs is not well-documented, it could be postulated that the Igs content of SDP can bind to bacterial cell wall constituents and eliminate them from the gut resulting in the modification of immune response and subsequent decrease in the production of IL-6 and APPs. Campbell et al. (2008) concluded that SDP reduces attachment and replication of the pathogens (antigen-antibody interactions) resulting in the reduction of overall inflammatory response locally and systemically. Collectively, it is suggested that SDP modulates the immune response by reducing the numbers of pathogens in the intestine of challenged birds resulting in the reduction of IL-6 and α-1 acid glycoprotein in the serum samples. Furthermore, it could also be speculated that SDP improved performance in NE challenged broilers by reducing costs of the immune response through decreasing IL-6 production, lowering α-1 acid glycoprotein secretion, and shifting nutrient flow into the growing process, as observed in lower FCR.

Supplementing NE challenged broilers with SDP during the first 8 d of age improved gut integrity. The intestinal epithelium consists of various interlocking proteins, called tight junction proteins including claudins. Claudins link the scaffolding proteins like Zonula occludens-1 (ZO-1) to the cytoskeleton and laterally seal epithelial cells against different destructing stimuli like pathogens and toxins (Ulluwishewa et al., 2011; Saitoh et al., 2015). Any factors damaging these proteins can increase intestinal permeability and ease pathogens' penetration to deeper layers of the intestine (Ulluwishewa et al., 2011; Saitoh et al., 2015). SDP may play a role in alleviating such permeability. It has been shown that SDP could alleviate the negative effects of *Crytosporidial enteritis* (Hunt et al., 2002) and coronavirus (Arthington et al., 2002) in calves. Pérez-Bosque et al. (2006) challenged rats with SAT and found that the bacterial toxin increased the concentration of FITC-d in the Ussing chamber model; however, SDP supplementation mitigated the negative impact of the toxin, decreased the concentration of FITC-d and increased gut integrity in SAT challenged rats. The passive antibacterial effects of SDP may explain its benefit in increasing gut integrity. Bacterial toxins can directly attack junctional proteins (Chen et al., 2002) and decrease the number of strands in the tight junction (Sonoda et al., 1999) or indirectly excite lymphocytes to produce inflammatory cytokines like IFN-γ and TNF-α, detaching tight junction proteins such as ZO-1 and ß-catenin (Nusrat et al., 2000) and down-regulating their expression (Bruewer et al., 2003; Pérez-Bosque et al., 2006) at the epithelial level. Disseminating and down-regulating junctional proteins might cause a decrease in the tightness of the epithelial junction complex, increase paracellular permeability of microvascular endothelial cells (Nusrat et al., 2000), and result in an increased concentration of transmural flux of permeability markers like FITC-d (Moretó and Pérez-Bosque, 2009). On the other hand, it was reported that

dietary SDP reduced bacteria by a mechanism called immune exclusion, as above mentioned and described elsewhere (Balan et al., 2019). The detachment of bacteria subsequently decreased the expression of mucosal inflammatory cytokines (Moretó et al., 2008) and the T-helper subsets in the lamina propria and epithelium (Pérez-Bosque et al., 2008) resulting in the restoration of junctional protein expression (Pérez-Bosque et al., 2006) and consequently lowering the concentration of FITC-d in the serum samples. It was reported that *C. perfringens* adheres to the epithelial layer and disseminates junctional proteins to disintegrate the intestinal barrier (Saitoh et al., 2015; Hashimoto et al., 2017) as observed with high FITC-d concentration in NE challenged birds in the current study. In agreement with the previous findings, the present study showed that dietary SDP decreased the concentration of inflammatory cytokine (i.e., IL-6) and reduced the serum FITC-d level of NE challenged birds, possibly by increasing the expression of tight junction proteins in the epithelial level. It was concluded that SDP supplementation might change the mucosal balance of various cytokines and reduce mucosal inflammation (Moretó et al., 2008) resulting in the alteration of tight junction protein expression and gut permeability (Pérez-Bosque et al., 2006). Overall, the present study demonstrated that dietary SDP improved the gut integrity of NE challenged birds, as shown by the lower serum FITC-d concentration compared to the non-supplemented birds.

The present study indicated that SDP modulates the immune response by reducing the concentration of inflammatory cytokines and acute-phase proteins in NE challenged birds resulting in less energy cost for immunity and more energy for growth as shown by the lower FCR at the early age (before challenge) and overall experimental period (d 0−29). In addition, SDP modification of the immune system improves the gut integrity of NE challenged birds, as shown by the lower serum FITC-d concentration. However, further research is required to investigate the effects of SDP on gut microflora and the expression of genes related to intestinal tight junction that may provide more evidence for the mode of action of SDP in NE challenged broilers.

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DISCLOSURES

The authors declared that there are no conflicts of interest.

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