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Early stage feeding of spray dried porcine plasma to improve health and performance of broilers in the presence and absence of challenge with necrotic enteritis

Milestone number 5

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Project Summary

Project Title	Early stage feeding of spray dried porcine plasma to improve health and performance of broilers in the presence and absence of challenge with necrotic enteritis
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Date	Start: 1 Dec 2019 End: 30 Aug 2020
Project Leader(s)	Robert A. Swick, Shubiao Wu, Nishchal Sharma, Ali Daneshmand
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Project Aim	Conduct a feeding study using the UNE necrotic enteritis model in broiler chickens to determine if starter feed supplemented with spray dried porcine plasma (SDPP) prevents necrotic enteritis (NE). *Eimeria* oocyst shedding, lesion scoring, fluorescein isothiocyanate dextran (FITC-d) passage across the gut and overall bird performance during the experiment were examined.
Background	Inclusion of SDPP in feed may represent an opportunity to control pathogens without the use of antibiotics. SDPP contains immunoglobulins and other components that limit pathogen growth but still allow immunity to develop. SDPP is not considered a rendered animal product and is not restricted for use in poultry feed in the European Union. SDPP is widely used in weanling piglet feed. The cost to use SDPP in broiler starter feed would be similar to using antibiotic growth promoters in starter, grower and finisher feeds.
Research Outcome	Early feeding of 2% SDPP in the starter period (first 10 days) improved feed conversion (feed:gain) by 4.5 points (1.078 to 1.033) from day 0 to day 8 and by 1.5 points (1.413 to 1.398) from day 0 to day 29. The statistical significance of improvement in FCR was lost on day 35 because of high variability during the last week. No SDPP by necrotic enteritis challenge (NE) interactions were detected for bird performance parameters at any time point. This indicated that early feeding of SDPP was effective in both unchallenged and NE challenged birds. An interaction was however detected for gut leakage indicating early feeding of SDPP to be effective in reducing passage of FITCd from gut to serum only in NE challenged birds. Economic analysis of SDPP inclusion indicated that the product would need to be priced between AUD 4 and 5 per kg to break even. Further work should examine shorter periods of inclusion perhaps at higher doses to reduce cost.
Impacts and Outcomes	This work indicates that early inclusion of SDPP enhances performance and improves gut health.
Publications	None as yet

Executive Summary

The early feeding of 2% spray dried porcine plasma protein (SDPP) in the starter feed from day 0 to day 10 improved feed conversion (feed:gain) by 4.5 points (1.078 to 1.033) from day 0 to day 8 ($P < 0.01$) and by 1.5 points (1.413 to 1.398) from day 0 to day 29 ($P < 0.01$). The FCR effect was not significant from day 0 to day 35 (1.510 to 1.500; $P = 0.186$) due to increased variability as a result of removal of birds from pens on day 29. No SDPP by NE challenge interactions were detected for bird performance (gain, FCR, feed intake or livability) indicating that SDPP was effective with or without NE challenge. An interaction however was detected for gut leakage as measured by serum FITC-d ($P < 0.009$). Birds fed diets with SDPP from 0 to 10 days had reduced the leakage of FITC-d dye from gut to blood serum on day 16 only with NE challenge. There was a strong tendency (main effect, $P = 0.051$) for early feeding of SDPP to decrease plasma alpha-1-acid glycoprotein on day 16. Early feeding of SDPP increased the relative weights of bursal tissue both on day 16 ($P < 0.01$) and on day 35 ($P < 0.05$). The NE challenge was effective in reducing performance throughout all the feeding periods, and shedding of *Eimeria* oocysts was evident on day 14 and again on day 16 in NE challenged birds. The NE challenge was effective in increasing lesion scores, gut leakage as indicated by increased FITC-d in serum, increasing relative bursa weight on day 16, and reducing relative breast yield on day 35 ($P < 0.05$). Challenge with NE decreased villus height, decreased villus surface area, increased crypt depth and decreased the VH:CD ratio on day 16 ($P < 0.001$). Challenge with NE increased serum IgA ($P < 0.001$) and alpha-1-glycoprotein ($P < 0.05$) when measured on day 16. Early feeding of SDPP decreased serum cytokine IL6 ($P < 0.05$) on day 16 indicating reduced inflammation. Economic analysis of SDPP inclusion indicated that the product would need to be priced between AUD 4 and 5 per kg to break even. Further work should examine shorter periods of inclusion perhaps at higher doses to reduce cost. Challenge with NE increased the cost to produce a kg of live weight by 7.4%.

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Introduction

Global poultry production is forecast to double by 2030 to satisfy the demand for human consumption of protein. There is current pressure to remove antibiotic growth promoters from broiler feed as such use has been linked to the occurrence of antibiotic resistant bacteria with ramifications for human health. The inclusion of plasma proteins in feed may be an opportunity to control pathogens without the use of antibiotics.

Plasma proteins contain immunoglobulins and other components that limit pathogen growth but still allow immunity to develop. Plasma is separated from blood collected in food grade pig and beef slaughter houses. It is then spray dried under controlled conditions to produce spray dried plasma protein. Spray dried porcine plasma (SDPP) is not considered a rendered animal product and is not restricted for use in poultry feed in the European Union where meat and bone meal is prohibited due to issues related to bovine spongiform encephalopathy due to contamination with prions. The SDPP has been used in weanling piglet feed for the past 25 years with high success. As reviewed by Perez-Bosque et al. (2016), SDPP has multiple modes of action on performance and immunity when fed to animals. Its effects on the immune system resulting in decreased inflammation have been regarded as the most relevant. Microorganisms, bacterial metabolites, toxins, and anti-nutritional factors present in the gut promote an immune response mediated by the gut-associated lymphoid tissue (GALT). This alters intestinal function increasing gut permeability and decreasing nutrient absorption. As GALT is part of the mucosa-associated-lymphoid tissue, its activation produces a systemic inflammatory state that is further exacerbated by infection with pathogens. These responses are mediated by circulating cytokines in the body. Feeding of SDPP increases the levels of anti-inflammatory cytokines such as IL-10, and reduces the key pro-inflammatory cytokines including TNF-α, IL-6 and IFN-γ (Campbell et al., 2019). These changes result in amelioration of the deleterious effects of immune activation at both local and systemic levels thereby reducing maintenance nutrient requirements. Immune activation requires nutrients to be diverted away from growth to support antibody production. When animals are fed SDPP, the duration of immune activation is lowered resulting in more nutrients being available to support growth. In 2008, the American Society of Animal Sciences identified SDPP as one of the top 10 most important discoveries in swine nutrition in the past 100 years. Reports from UNE (Beski et al., 2015) and elsewhere Campbell et al., 2006; Walters et al., 2018) have shown the effectiveness of SDPP in broilers.

The application of SDPP has not been tested during controlled necrotic enteritis (NE) infection and the objective of the research reported herein was to examine this.

Objectives

Conduct a feeding study to determine if early feeding of SDPP prevents NE using the UNE challenge model. Mode of action was examined by measuring feed intake, growth rate, FCR, gut morphology, relative carcass parts, relative lymphoid organ weight, *Eimeria* shedding, lesion scoring, FITCd absorption across the gut, and levels of serum interleukins and other marker proteins during the acute phase of the NE challenge.

Methodology

Birds

Ross 308 day-old cockerels (males from the male line obtained from the Aviagen breeder hatchery in Goulburn, NSW; n=816) were assigned to 4 treatments with 12 replications of 17 birds each (48 pens) at the Rob Cumming Poultry Innovation Centre at the Kirby Field Station.

Treatments

The treatments were assigned as a 2 × 2 factorial arrangement with *NE* challenge (no, yes) and SDPP (0 or 2% from day 0 to day 10) as factors:

1. Unchallenged with 0/0/0 % SDPP in starter/grower/finisher
2. Unchallenged with 2/0/0 % SDPP in starter/grower/finisher
3. Challenged with 0/0/0 % SDPP in starter/grower/finisher
4. Challenged with 2/0/0 % SDPP in starter/grower/finisher

Diet formulation

Birds were fed respective diets ad libitum. Feed was provided as crumbled pellets in the starter phase and 3 mm pellets thereafter. Starter feed was fed to 10 days, grower from 10 to 24 days and finisher from 24 to 35 days. Table 1 shows the nutrient composition of SDPP. Table 2 shows the prices of ingredients used and their percent inclusion and final content of nutrients in each diet. The SDPP was formulated into the starter diet using its recommended nutrient composition obtained from FeedWorks Pty Ltd. Because of the high protein content of SDPP and its effect nutrient density, less SBM and canola oil were needed in the starter diet containing SDPP. Diets were formulated to the minimum standard ileal digestible amino acid (SIDAA) specifications for Ross 308 broilers (Aviagen, 2019). Xylanase was used without

matrix values but with +100 kcal/kg uplifts in AMEn of wheat and phytase was used with the recommended matrix values of Dupont.

Necrotic enteritis challenge

The NE challenge was performed based on previous reports with modifications (Wu et al., 2014; Rodgers et al., 2015). The *Eimeria acervulina, E. brunetti* ("Roybru") and *E. maxima* were vaccine strains obtained from Eimeria Pty. Ltd. (Ringwood, VIC, Australia). *Clostridium perfringens* type G strain EHE-NE18 obtained from CSIRO Livestock Industries (Geelong, Australia) was incubated overnight at 39˚C in 100 mL of sterile thioglycollate broth (USP alternative; Oxoid) followed by subsequent overnight incubations of 1 mL of the previous culture in 100 mL of cooked meat medium (Oxoid), and then in 700 mL of thioglycollate broth (USP alternative; Oxoid) containing starch (10 g/L) and pancreatic digest of casein (5 g/L) to obtain the challenge inoculum. On day 9, challenged birds were inoculated with 5,000 sporulated oocysts each of *E. maxima* and *E. acervulina* and 2,500 sporulated oocysts of *E. brunetti* in 1 mL of 1% (w/v) sterile saline. Non-challenged birds received 1 mL of 1% (w/v) thioglycollate broth. On day 14, birds were inoculated twice at an interval of 5 h with 2 mL of *C. perfringens* (EHE-NE18, CSIRO) suspension (3.8×10^8 CFU/mL).

Table 1. Nutrient composition of SDPP, 92% DM basis (percent unless otherwise noted)

Nutrient	Percent
AMEn kcal/kg	3830
Crude protein	78
Crude fat	0.3
Crude fibre	0.5
Ash	10
d Arg	4.7
d Lys	6.5
d Met	0.7
d Cys	2.8
d Thr	4.8
d Val	5.2
d Ile	2.9
d Leu	7.8

Growing conditions and measurements

Birds were grown under simulated commercial conditions with wood shavings as bedding material, tube feeders and nipple waterers. Birds and feed intake were weighed on days 8, 10, 16, 24, 29 and 35. The measurements undertaken included:

1. Gain, feed intake, FCR, livability
2. Carcass yield (breast, thigh, drumstick), abdominal fat, liver weight, intestine weight, bursa

3. Villi height, crypt depth
4. NE lesion scoring on day 16.
5. Oocyst shedding from collected faeces on days 14 and 16
6. Gut permeability on day 16 using FITC dextran.
7. Inflammatory cytokines and marker proteins in serum.
8. Cost-benefit analysis based on cost per kg live birds (excluding sampled birds) upon termination of the experiment.

Table 2. Ingredient and nutrient composition of diets (percent unless otherwise noted)

Ingredients	Ing price AUD/mt	Starter Control	Starter SDPP	Grower common	Finisher common
Wheat 10.5	300	39.85	41.36	35.22	40.19
Sorghum 11.2	300	20.00	20.00	30.00	30.00
SBM 47.5	750	34.15	31.30	29.20	24.12
Canola oil	1100	2.45	2.06	2.51	2.96
Limestone (CaCO3)	75	1.31	1.38	1.21	1.13
Dicalcium phoshate 18P/21Ca	500	0.89	0.79	0.67	0.49
Sodium chloride	220	0.25	0.14	0.21	0.21
Sodium bicarbonate	450	0.11	0.10	0.10	0.10
Choline Cl 70%	3000	0.04	0.05	0.04	0.04
L-lysine HCl 78.4	1800	0.23	0.17	0.22	0.20
D,L-methionine	3400	0.36	0.32	0.31	0.28
L-threonine	2000	0.15	0.10	0.12	0.09
Xylanase	15000	0.01	0.01	0.01	0.01
[1]Phytase	20000	0.01	0.01	0.01	0.01
UNE vitamin premix	15000	0.09	0.09	0.08	0.08
UNE TM premix	4800	0.11	0.11	0.10	0.10
SDPP	8500	0.00	2.00	0.00	0.00
Nutrients					
AMEn kcal/kg		3000	3000	3075	3150
AMEn MJ/kg		12.55	12.55	12.87	13.18
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
d Arg		1.370	1.370	1.23	1.09
d Lys		1.280	1.280	1.15	1.02
d Met		0.648	0.608	0.59	0.53
d Cys		0.303	0.343	0.29	0.27
d M+C		0.950	0.950	0.87	0.80
d Trp		0.276	0.288	0.26	0.23
d Leu		1.666	1.725	1.62	1.49
d Ile		0.885	0.889	0.82	0.74
d Thr		0.860	0.860	0.77	0.68
d Val		0.971	1.014	0.91	0.83
d Gly		0.771	0.730	0.70	0.63
Calcium		0.96	0.96	0.86	0.78
Phosphorus avail		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
Chloride		0.25	0.20	0.23	0.22
Choline mg/kg		1699	1700	1600	1500
Linoleic		1.56	1.45	1.63	1.74

[1] Phytase = AXTRA PHY 10000 TPT (Dupont Inc) to provide 1000 FTU/kg feed.

Results and discussion

Performance

The effects of SDPP supplementation on growth performance during the starter phase are presented in Tables 3 and 4. Table 3 shows the performance results from day 0 to day 8 before inoculation with *Eimeria*. The average FCR of birds fed SDPP was 4.5 points lower (more efficient) (1.078 to 1.033 or 4.2%) than birds fed control feed ($P < 0.001$). Table 4 shows results from day 0 to day 10 after *Eimera* but before *Clostridium* inoculation. Birds inoculated with cocci oocysts on day 9 had higher weight gain ($P < 0.001$) and lower FCR ($P < 0.001$) than unchallenged birds. Birds fed SDPP had similar weight gain but lower FCR than controls ($P < 0.001$). No interactions were detected for weight gain, FCR or livability ($P > 0.05$) but there was a tendency ($P = 0.055$) for a challenge (cocci oocysts only) by SDPP interaction for feed intake indicating an increase in feed intake of birds fed SDPP only when inoculated with cocci for 1 day.

Table 3. Effects of SDPP on growth performance of broilers before necrotic enteritis challenge (0 to 8 days)

Treatments	Feed intake (g)	Weight gain (g)	FCR (g/g)	Livability (%)
Control	210	195	1.078[a]	99.4
SDPP (2%)	207	199	1.033[b]	99.2
SEM	2.28	1.97	0.008	0.43
P-value	0.287	0.218	< 0.001	0.676

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

Reductions in feed intake, weight gain, livability and increased FCR of NE challenged birds relative to the uninfected birds were observed in subsequent weighings after inoculation with *Clostridium perfringens* on day 14. Table 5 shows results in the period from day 8 to day 16. Early feeding of SDPP had no effect on feed intake, weight gain, FCR or livability ($P > 0.05$) however birds challenged with NE showed a clear reduction in feed intake ($P < 0.001$), weight gain ($P < 0.001$) and livability ($P < 0.01$) and increased FCR ($P < 0.001$). Table 6 shows the performance results for the grower period from day 10 to day 24. Early feeding of SDPP had no effect on feed intake, weight gain, FCR or livability ($P > 0.05$). Birds challenged with NE showed a clear reduction in feed intake ($P < 0.001$), weight gain ($P < 0.001$), increased FCR ($P < 0.001$), and reduced livability ($P < 0.05$). No SDPP by NE interactions were detected ($P > 0.05$).

Table 4. Effects of SDPP on growth performance of broilers (0 to 10 days)

Interaction effect					
SDPP (%)	Challenge	Feed intake (g)	Weight gain (g)	FCR (g/g)	Livability (%)
0	No	330	307	1.077	99.4
	Yes	330	352	0.937	99.4
2	No	316	303	1.042	99.4
	Yes	331	358	0.908	97.8
SEM		3.70	3.71	0.006	0.68
Main effect					
SDPP (%)					
0		330	329	1.007[a]	99.4
2		324	331	0.975[b]	98.6
SEM		2.65	2.65	0.004	0.48
Challenge					
No		323	305[b]	1.059[a]	99.4
Yes		331	355[a]	0.922[b]	98.6
SEM		2.65	2.65	0.004	0.48
P-value					
SDPP		0.082	0.727	< 0.001	0.241
Challenge		0.053	< 0.001	< 0.001	0.219
SDPP × Challenge		0.055	0.215	0.636	0.241

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

Table 5. Effects of SDPP on growth performance of broilers during the period of necrotic enteritis challenge (8 to 16 days)

Interaction effect					
SDPP (%)	Challenge	Feed intake (g)	Weight gain (g)	FCR (g/g)	Livability (%)
0	No	552	445	1.241	98.9
	Yes	442	275	1.609	96.7
2	No	553	444	1.244	99.4
	Yes	442	275	1.614	92.7
SEM		7.51	5.70	0.027	1.55
Main effect					
SDPP (%)					
0		497	360	1.425	97.8
2		497	360	1.429	96.1
SEM		5.31	4.03	0.019	1.09
Challenge					
No		552[a]	445[a]	1.243[b]	99.2[a]
Yes		442[b]	275[b]	1.611[a]	94.7[b]
SEM		5.31	4.03	0.019	1.09
P-value					
SDPP		0.978	0.865	0.875	0.282
Challenge		<0.001	< 0.001	< 0.001	< 0.01
SDPP × Challenge		0.985	0.993	0.965	0.155

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

Table 6. Effects of SDPP on growth performance of broilers challenged with necrotic enteritis (10 to 24 days)

Interaction effect					
SDPP (%)	Challenge	Feed intake (g)	Weight gain (g)	FCR (g/g)	Livability (%)
0	No	1409	1015	1.391	98.9
	Yes	1121	700	1.587	96.7
2	No	1405	1021	1.376	98.9
	Yes	1136	725	1.566	93.8
SEM		14.7	11.74	0.013	1.52
Main effect					
SDPP (%)					
0		1265	857	1.489	97.8
2		1270	873	1.471	96.3
SEM		10.67	8.30	0.009	1.07
Challenge					
No		1407[a]	1018[a]	1.383[b]	98.9[a]
Yes		1128[b]	713[b]	1.576[a]	95.2[b]
SEM		10.67	8.30	0.009	1.07
P-value					
SDPP		0.727	0.183	0.186	0.345
Challenge		< 0.001	< 0.001	< 0.001	0.019
SDPP × Challenge		0.546	0.432	0.829	0.345

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

Table 7 shows the performance results from day 10 to day 29. Early feeding of SDPP had no effect on feed intake ($P > 0.05$) but weight gain tended to be higher ($P = 0.077$) and FCR tended to be lower ($P = 0.063$) compared to controls. The NE challenged birds showed a clear reduction in feed intake ($P < 0.001$), weight gain ($P < 0.001$), increased FCR ($P < 0.001$), and reduced livability ($P < 0.05$) relative to unchallenged birds. No SDPP by NE interactions were observed ($P > 0.05$) during this period.

Table 8 shows the cumulative results from day 0 to day 29. Early feeding of SDPP tended to increase weight gain ($P < 0.078$) and significantly reduced FCR (1.5 points from 1.413 to 1.398; $P < 0.01$) with no effect on feed intake or livability ($P > 0.05$). Table 9 shows intermediate performance results from day 16 to day 29. Early feeding of SDPP tended to increase weight gain ($P = 0.053$) and significantly decreased FCR ($P < 0.05$). Challenge with NE reduced feed intake ($P < 0.001$), and weight gain ($P < 0.001$) but had no effect on FCR or livability ($P > 0.05$). No SDPP by NE interactions were detected for this period ($P > 0.05$).

Table 10 shows the intermediate results from day 24 to day 29 after recovery from NE. Early feeding of SDPP had no effect on feed intake ($P > 0.05$) but increased weight gain ($P < 0.05$) and decreased FCR ($P < 0.05$). No mortality was detected during this period. No SDPP by NE interactions were detected. ($P > 0.05$). Table 11 shows the performance results for the finisher period from day 24 to day 35. Early feeding of SDPP had no effect on feed intake, weight gain,

FCR and no mortality were observed (P > 0.05). No SDPP by NE interactions were detected (P > 0.05) during this period. Birds challenged with NE had lower feed intake (P < 0.01) and lower weight gain (P < 0.01) but FCR was unchanged (P > 0.05) during this period.

Table 7. Effects of SDPP on growth performance of broilers challenged with necrotic enteritis (10 to 29 days)

Interaction effect					
SDPP (%)	Challenge	Feed intake (g)	Weight gain (g)	FCR (g/g)	Liveability (%)
0	No	2118	1474	1.437	98.9
	Yes	1799	1109	1.612	96.7
2	No	2123	1487	1.427	98.9
	Yes	1826	1146	1.593	93.8
SEM		20.78	13.48	0.007	1.52
Main effect					
SDPP (%)					
0		1959	1292	1.524	97.8
2		1974	1317	1.510	96.3
SEM		15.05	9.53	0.005	1.07
Challenge					
No		2120[a]	1481[a]	1.432[b]	98.9[a]
Yes		1813[b]	1128[b]	1.603[s]	95.2[b]
SEM		15.05	9.53	0.005	1.07
P-value					
SDPP		0.465	0.077	0.063	0.345
Challenge		< 0.001	< 0.001	< 0.001	0.019
SDPP × Challenge		0.605	0.387	0.512	0.345

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

Table 8. Effects of SDPP on growth performance of broilers challenged with necrotic enteritis (0 to 29 days)

Interaction effect					
SDPP (%)	Challenge	Feed intake (g)	Weight gain (g)	FCR (g/g)	Livability (%)
0	No	2447	1779	1.376	98.3
	Yes	2129	1461	1.451	96.1
2	No	2441	1791	1.362	98.3
	Yes	2157	1504	1.434	91.7
SEM		22.36	15.00	0.005	1.582
Main effect					
SDPP (%)					
0		2288	1620	1.413[a]	97.1
2		2299	1648	1.398[b]	95.0
SEM		15.81	10.61	0.004	1.119
Challenge					
No		2444[a]	1785[a]	1.369[b]	98.3[a]
Yes		2143[b]	1483[b]	1.442[a]	93.9[b]
SEM		15.81	10.61	0.004	1.119
P-value					
SDPP		0.648	0.078	< 0.01	0.171
Challenge		< 0.001	< 0.001	< 0.001	0.007
SDPP × Challenge		0.461	0.326	0.703	0.171

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

Table 9. Effects of SDPP on growth performance of broilers challenged with necrotic enteritis (16 to 29 days)

Interaction effect					
SDPP (%)	Challenge	Feed intake (g)	Weight gain (g)	FCR (g/g)	Livability (%)
0	No	1688	1139	1.482	100
	Yes	1476	993	1.477	100
2	No	1685	1151	1.465	99.3
	Yes	1498	1029	1.456	100
SEM		15.83	11.79	0.009	0.35
Main effect					
SDPP (%)					
0		1582	1066	1.479[a]	100
2		1591	1090	1.460[b]	99.7
SEM		11.19	8.33	0.006	0.25
Challenge					
No		1687[a]	1145[a]	1.474	99.7
Yes		1487[b]	1011[b]	1.466	100
SEM		11.19	8.33	0.006	0.25
P-value					
SDPP		0.567	0.053	0.049	0.323
Challenge		< 0.001	< 0.001	0.446	0.323
SDPP × Challenge		0.449	0.309	0.859	0.323

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

Table 10. Effects of SDPP on growth performance of broilers challenged with necrotic enteritis (24 to 29 days)

Interaction effect					
SDPP (%)	Challenge	Feed intake (g)	Weight gain (g)	FCR (g/g)	Liveability (%)
0	No	713	451	1.581	
	Yes	678	409	1.659	
2	No	718	466	1.543	
	Yes	690	421	1.641	
SEM		8.19	6.08	0.012	
Main effect					
SDPP (%)					
0		695	430[b]	1.620[a]	
2		704	443[a]	1.592[b]	
SEM		5.79	4.30	0.008	
Challenge					
No		715[a]	458[a]	1.562[b]	
Yes		684[b]	415[b]	1.650[a]	
SEM		5.79	4.30	0.008	
P-value					
SDPP		0.303	0.038	0.026	
Challenge		< 0.001	< 0.001	< 0.001	
SDPP × Challenge		0.673	0.812	0.403	

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

Table 11. Effects of SDPP on growth performance of broilers challenged with necrotic enteritis (24 to 35 days)

Interaction effect					
SDPP (%)	Challenge	Feed intake (g)	Weight gain (g)	FCR (g/g)	Livability (%)
0	No	1795	1056	1.674	100
	Yes	1722	1013	1.700	100
2	No	1824	1073	1.702	100
	Yes	1743	1022	1.707	100
SEM		22.47	16.91	0.012	0.0
Main effect					
SDPP (%)					
0		1759	1035	1.687	100
2		1783	1047	1.704	100
SEM		15.89	11.96	0.008	0.0
Challenge					
No		1810[a]	1064[a]	1.688	100
Yes		1732[b]	1018[b]	1.704	100
SEM		15.89	11.96	0.008	
P-value					
SDPP		0.289	0.466	0.165	1.000
Challenge		< 0.01	< 0.01	0.206	1.000
SDPP × Challenge		0.849	0.809	0.369	1.000

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

The results for entire experiment from day 0 to day 35 are shown in Table 12. Early feeding of SDPP had no effect on feed intake, weight gain, FCR or livability ($P > 0.05$). Birds challenged with NE has lower feed intake ($P < 0.001$, lower weight gain ($P < 0.001$) higher FCR ($P < 0.001$ and lower livability ($P < 0.01$) than unchallenged birds.

The reason for the loss in significance for SDPP from day 0 to 29 to day 0 to 35 is possibly due to the removal of birds from pens on day 29 resulting in higher variability. This was required by the Animal Ethics Committee as conditions in the pens looked crowded to an inspection team on day 29, although the experiment followed the approved protocol and stocking density recommendations.

Table 12. Effects of SDPP on growth performance of broilers challenged with necrotic enteritis (0 to 35 days)

Interaction effect					
SDPP (%)	Challenge	Feed intake (g)	Weight gain (g)	FCR (g/g)	Liveability (%)
0	No	3533	2362	1.487	98.3
	Yes	3171	2066	1.535	96.1
2	No	3547	2398	1.479	98.3
	Yes	3202	2105	1.521	91.7
SEM		33.8	24.7	0.007	1.582
Main effect					
SDPP (%)					
0		3352	2214	1.511	97.1
2		3375	2252	1.500	95.0
SEM		23.9	17.7	0.005	1.119
Challenge					
No		3540[a]	2380[a]	1.483[b]	98.3[a]
Yes		3186[b]	2086[b]	1.528[a]	93.9[b]
SEM		23.9	17.7	0.005	1.119
P-value					
SDPP		0.511	0.135	0.132	0.171
Challenge		< 0.001	< 0.001	< 0.001	0.007
SDPP × Challenge		0.789	0.950	0.704	0.171

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

Oocysts, lesion scores, gut leakage, and gut morphology

The effects of dietary treatments on *Eimeria* oocyst counts are shown in Table 13. Sporulated oocysts were given by gavage to birds in the NE treatments on day 9. *Eimeria* challenge as a predisposing factor for NE was effective as oocysts were present in excreta both on day 14 and 16 in only the birds infected with the oocysts ($P < 0.05$). No oocysts were found in the excreta on day 11 (i.e. 2 days after *Eimeria* challenge, unpresented data). No effect of SDPP was observed on oocyst shedding and there no interactions were detected between oocyst gavage (NE) and SDPP on either day ($P > 0.05$). The results showed that *Eimeria* (vaccine strain) as a predisposing factor for NE was effective.

Table 13. Effects of dietary SDPP supplementation on *Eimeria* oocyst counts (log_{10}) in the excreta of broilers at different time points post challenge

Interaction effects		Days of age	
SDPP (%)	Challenge	14	16
0	No	0	0
	Yes	5.57	4.73
2	No	0	0
	Yes	5.67	4.87
SEM		0.086	0.075
Main effect			
SDPP (%)			
0		2.78	2.36
2		2.84	2.43
SEM		0.061	0.053
Challenge			
No		0.00[b]	0.00[b]
Yes		5.62[a]	4.80[a]
SEM		0.061	0.053
P-value			
SDPP		0.533	0.350
Challenge		< 0.001	< 0.001
SDPP × Challenge		0.533	0.350

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

As expected, the results in Table 14 indicate that NE challenged birds had higher lesion scores in duodenum, jejunum, and ileum than the unchallenged birds. This further indicates the success of the NE challenge model. Dietary supplementation of SDPP did not improve lesion scores in the gut (P > 0.05).

Table 14. Effects of SDPP on lesion scores (day 16) in broilers challenged with necrotic enteritis

	Lesion score		
Treatments	Duodenum	Jejunum	Ileum
No Challenge	0.00[b]	0.00[b]	0.00[b]
No challenge + SDPP	0.00[b]	0.03[b]	0.00[b]
Challenge	0.19[a]	0.75[a]	0.33[a]
Challenge + SDPP	0.18[a]	0.72[a]	0.31[a]
SEM	0.09	0.08	0.07
P-value	0.019	< 0.001	< 0.001

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

Table 15. Effects of dietary SDPP supplementation on blood serum FITC-d concentrations of broilers at 16 days of age

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

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

Table 15 shows the effect of FITCd gavage on blood serum FITCd levels 1.5 hr post gavage. Challenged birds had higher FITC-d concentrations in blood serum than unchallenged birds ($P < 0.001$) indicating the effectiveness of the challenge on increasing FITCd leakage from the gut to serum. An NE × SDPP interaction was observed ($P < 0.01$) indicating no effect of SDPP in unchallenged birds but a reduction in FITCd transfer from gut to serum in NE infected birds on day 16 as a result of early feeding of SDPP from 0 to 10 days. This is strong evidence that the effect of early feeding of SDPP persists after it is no longer in the diet.

Table 16 shows the effect of NE challenge and early feeding of SDPP on gut morphology measured on day 16. No SDPP by NE interactions were detected for any parameter measured ($P > 0.05$). There was no effect of early feeding of SDPP on gut morphology ($P > 0.05$). Challenge with NE however reduced villi height ($P < 0.001$), increased crypt depth ($P < 0.001$) and reduced the villi height: crypt depth ratio ($P < 0.001$). Villus width was not affected by NE challenge ($P > 0.05$) but villus surface area was greatly reduced by NE challenge ($P < 0.001$). These results show that infection with cocci followed by *Clostridium perfringens* has a marked negative effect on gut morphology.

Table 16. Effects of SDPP on jejunal morphology (day 16) in broilers challenged with necrotic enteritis

Interaction effect		Measurement (μm)				
SDPP (%)	Challenge	Villus height	Crypt depth	Villus width	Villus height/ crypt depth	Villus surface area[1]
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[1]		3.76	0.97	0.82	0.29	401.2
Main effect						
SDPP (%)						
0		98.9	19.4	20.4	6.0	7026.8
2		104.7	19.8	20.4	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						
SDPP		0.134	0.665	0.959	0.177	0.361
Challenge		< 0.001	< 0.001	0.307	< 0.001	< 0.001
SDPP × 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).
[1]villus surface area= 2π(villus width/2)(villus height+(villus width/2))

Table 17. shows the effects of dietary treatments on carcass yield on day 35 and internal organ weights (liver, spleen, bursa of Fabricius) on day 16 and day 35. Early feeding of SDPP supplementation had no effect on the relative weights of breast, thigh or drumstick ($P > 0.05$) but increased the relative weight of bursa both on 16 and 35 days. Challenge with NE however increased the relative weight of bursa on day 16 but not on day 35. This shows that birds were immunologically challenged on day 16 but completely recovered from NE on day 35. The bursa is a lymphoid organ and enlargement well after cessation of feeding of SDPP indicates long-term immune benefits.

Table 18 shows the effects of NE challenge and early dietary SDPP supplementation on blood immunological parameters on day 16. Challenge with NE increased the levels of IgA, IgM, and alpha-1-acid glycoprotein levels in the serum on day 16 ($P < 005$). Early feeding of SDPP decreased the level of alpha-1-acid glycoprotein in serum on day 16 ($P < 0.05$). Alpha-1-acid glycoprotein is an acute-phase protein that is increased during inflammation. Interleukin analysis (IL4, 6 and 10) were completed. Test kits were unavailable from IL10 ELISA Cusabio, Wuhan, China due to Covid 19 requiring lower detection limit kits from Ray Biotech, Norcross, Georgia, USA to be used. Cytokine IL6 was successfully analyzed and found to be reduced in serum collected on day 16 from birds fed 0.2% SDPP from 0 to 10 days. This indicated reduced inflammation as a result of the early feeding of SDPP from day 0 to day 10. No SDPP by NE interactions were detected ($P > 0.05$) for blood immunological parameters on day 16.

Table 17. Effects of SDPP on carcass and organ weights (g/kg live BW) of male broiler chickens at 16 and 35 days of age

		Day 16			Day 35					
SDPP (%) Treatment effects	Challenge	Liver	Bursa	Spleen	Liver	Bursa	Spleen	Breast	Thigh and drumstick	Abdom fat pad
0	No	29.41	2.076	0.683	22.11	1.30	0.806	191.69	200.42	9.58
	Yes	28.67	2.320	0.699	23.24	1.41	0.782	176.36	205.97	10.51
2	No	28.60	2.345	0.651	21.27	1.67	0.785	194.23	197.65	10.15
	Yes	28.41	2.416	0.714	22.49	1.56	0.762	181.07	204.71	9.34
SEM[1]		0.4828	0.0715	0.0342	0.867	0.120	0.0294	2.507	2.005	0.417
Main effects										
SDPP (%)										
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										
SDPP		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
SDPP × Challenge		0.572	0.232	0.511	0.961	0.366	0.967	0.668	0.707	0.042

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

Table 18. Effects of dietary SDPP on blood immunological parameters of broilers challenged with necrotic enteritis

Interaction effect							
SDPP (%)	Challenge	IgA (µg/mL)	IgM (µg/mL)	IgG (µg/mL)	Alpha 1 acid glycoprotein (µg/mL)	Ovotransferrin (µg/mL)	Interleukin 6 (ng/ml)
0	No	142.2	159.5	1223.1	190.5	1345.8	0.244
	Yes	214.3	171.9	1167.3	227.8	1362.7	0.231
2	No	144.0	142.2	1112.5	171.4	1312.9	0.143
	Yes	204.3	194.8	1111.9	197.8	1479.9	0.194
SEM		18.62	19.07	117.2	12.25	88.61	
Main effect							
SDPP (%)							
0		178.3	165.7	1195	209.1[a]	1354.2	0.237[a]
2		174.2	168.5	1112	184.6[b]	1396.4	0.169[b]
SEM		13.17	13.48	82.89	8.66	61.80	
Challenge							
No		143.1[b]	150.8	1168	180.9[b]	1329.4	0.189
Yes		209.3[a]	183.4	1140	212.8[a]	1421.3	0.212
SEM		13.17	13.48	82.89	8.66	61.8	
P-value							
Porcine plasma		0.826	0.886	0.483	0.051	0.633	0.015
Challenge		< 0.001	0.095	0.811	0.013	0.301	0.239
SDPP × Challenge		0.753	0.297	0.815	0.661	0.397	0.494

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

Table 19 shows an economic analysis of NE challenge and early feeding of SDPP. Challenge with NE increased the cost to produce a kg of live chicken by over 7.4% (P< 0.05) indicating the magnitude of economic losses associated with this disease. The analysis indicates that SDPP would need to be priced between AUD 4 and 5 per kg to break even.

Table 19. Effect of SDPP cost and NE challenge on feed cost per kg live weight at various SDPP prices (0 to 29 days)

Trt means		Price of SDPP AUD/kg					
SDPP (%)	Challenge	4.00	5.00	6.00	7.00	8.00	9.00
0	No	0.663	0.663	0.663	0.663	0.663	0.663
	Yes	0.712	0.712	0.712	0.712	0.712	0.712
2	No	0.662	0.665	0.669	0.672	0.676	0.679
	Yes	0.710	0.714	0.719	0.723	0.728	0.732
Main effects							
SDPP (%)							
0		0.687	0.687	0.687	0.687	0.687	0.687
2		0.686	0.690	0.694	0.698	0.702	0.706
Challenge							
No		0.662	0.664	0.666	0.668	0.669	0.671
Yes		0.711	0.713	0.715	0.718	0.720	0.722
P-value							
Plasma		0.890	0.795	0.512	0.295	0.153	.071
Challenge		0.001	0.001	0.001	0.001	0.001	0.001
Plasma×Challenge		0.950	0.987	0.976	0.939	0.902	0.866

Implications

Early feeding of SDPP at 2% of the starter feed from day 0 to day 10 decreased overall FCR to day 29. Early feeding of SDPP resulted in larger relative bursa weights on day 16 that persisting to day 35, reduced leakage of FITC-d from the gut into blood on day 16 and increased alpha-1 glycoprotein in blood on day 16. This confirms the mode of action of the product is related to the immune system. Increased leakage of FITC-d across the gut on day 16 as a result of damage by NE infection was effectively reduced by early feeding of SDPP from day 0 to day 16. This indicates the beneficial effects of SDPP persist after cessation of feeding. While SDPP was demonstrated to have a positive effect on performance, with the prevailing

ingredient prices used its price would need to be reduced from AUD 8.50 to below AUD 5.00 to be economical. The situation may be improved by using higher levels for shorter periods.

Recommendations

Further work should examine shorter periods of inclusion perhaps at higher doses to reduce cost. Because the NE challenge increased the cost to produce a kg of live weight by 7.4% future NE studies should include economic evaluations.

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Media and Publications

None as yet

Intellectual Property Arising

None as yet.

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