Field evaluation of feeding spray-dried plasma in the starter period on final performance and overall health of broilers

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ABSTRACT The effect of feeding spray-dried plasma (**SDP**) during the starter period was evaluated with a commercial broiler integrator on performance and overall health of broilers. The I See Inside (**ISI**) methodology assessing gut health in broilers was used as a tool to evaluate the impact of dietary interventions under commercial conditions. One hundred farms with approximately 1.1 million broilers were used at a Brazilian broiler integrator. Two groups of farms were fed either a control or an SDP diet containing 1% SDP, from 0 to 10 d of age. Diets were formulated to have similar nutritional density, containing zinc bacitracin and $CuSO_4$ from 0 to 28 d. After 10 d, both groups were fed common commercial diets. Performance data were analyzed together or by type of ventilation system: positive pressure or negative pressure. Birds were sent to market as they reached 3.05 kg; therefore, age at slaughter (**AS**) was evaluated as a dependent variable along with other performance measures. From the 100 farms in the trial, 35 (16 control and 19 SDP farms) were selected for the assessment of broilers health, biosecurity, and local management. For that, 6 broilers per farm at 14 ± 2 d of age were necropsied and ileum sampled for the ISI methodology evaluation. Biosecurity and management were also evaluated to obtain the influence of those parameters on animal health. SDP-fed birds demonstrated improved feed conversion ratio, reduced mortality, and 1 d less for AS ($P < 0.05$) vs. control group ($P < 0.05$) regardless of the type of ventilation. During necropsy, birds fed SDP showed lower coccidiosis and locomotor system lesions as the overall ISI score compared to controls. Histologic intestinal alterations were also lower in SDP-fed broilers ($P < 0.05$). In conclusion, feeding 1% SDP in the starter period to broilers resulted in improved performance and health under both good and bad management and biosecurity standards independent of the type of ventilation. Overall, there was good agreement between the ISI method and performance improvements observed.

Key words: ISI methodology, histology, gut, management, biosecurity

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INTRODUCTION

Spray-dried plasma (**SDP**) is a highly digestible protein ingredient rich in functional molecules, manufactured from animal blood collected from federally inspected slaughter facilities, and spray-dried to preserve the functionality of its components (Coffey and Cromwell, 2001). It is a diverse mixture of functional molecules consisting of immunoglobulins, albumin, fibrinogen, lipids, growth factors, enzymes, hormones, and other components that have biologic activity when orally fed independently of their nutritional value (Coffey and Cromwell et al., 2001; Bah et al., 2013).

The high digestibility of SDP allows fast amino acids absorption and lower protein fermentation in the gut, being able to improve the efficiency of the immune response, consequently restoring immune system homeostasis and maintaining the structural integrity of the intestinal barrier and stabilizing immune system activation (Humphrey and Klasing, 2004). Other studies have reported similar effects in broilers when added in the starter diets improving health and performance (Campbell et al., 2003, 2006; Henn et al., 2013).

Its bioactive components have been shown to modulate the immune response of the host providing improvements in body weight daily gain, feed efficiency, and animal survival in pig starter diets and calf milk

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replacers (Torrallardona et al., 2003; Bosi et al., 2004; Pierce et al., 2005). Although several studies (Pérez-Bosque et al., 2010, 2016; Maijó et al., 2012; Song et al., 2015) have reported modulation of the immune response in animals supplemented with SDP in the feed in different challenges and housing, it has not been determined which component in SDP elicits immunomodulation and reduced inflammation to improve the well-being of the animal. However, the diverse mixture of bioactive proteins or peptides is likely contributing to these beneficial effects (Campbell et al., 2019).

Campbell et al. (2019) reviewed the current understanding of the mode of action of SDP in the context of the published literature available in poultry and suggested that its immune modulatory effects are pivotal to understand SDP's functional properties. Inflammation is reduced by feeding SDP when induced either by stress or by pathogenic challenges, and regardless of whether the primary site affected is the gastrointestinal, respiratory, or the reproductive tracts. SDP reduces gut permeability and improves nutrient uptake and structural integrity during periods of stress (Pérez-Bosque et al., 2006). All these effects are likely and partially mediated by a reduction in the expression of pro-inflammatory cytokines, and by an elevation in the expression of anti-inflammatory cytokines. Data showing a reduction in lymphocyte activation and infiltration, lessening of edema, and changes in the gut microbiota have also been discussed by Campbell et al. (2019), suggesting that SDP could be improving intestinal health through multiple modes of action including directly influencing the immune inflammatory response and immunomodulation both locally and systemically according to these authors. All these changes may contribute to improved overall health and performance in chickens and may explain why the beneficial effects of the SDP seem to be more pronounced in animals under challenging conditions vs. those with no challenges (Campbell et al., 2008).

Under commercial broiler conditions, the presence of environmental challenges, pathogens, mycotoxins, heat or cold stress, antinutritional factors, high stocking density, etc., could compromise the intestinal barrier resulting in immune system activation, reduction of intestinal function (i.e., nutrient absorption), and lower performance (Johnson, 1997; Spurlock et al., 1997).

The I See Inside (**ISI**) methodology has been used in broilers to assess overall health, especially gut health by translating macroscopic and microscopic tissue alterations into numeric data. Due to the possible systemic effect of the SDP product, we used ISI macroscopic evaluation in all the animal systems. Different from traditional villi height and crypt depth measurement, ISI histology encompasses components of the inflammatory process such as immune cell infiltration and proliferative response at the villi (Kraieski et al., 2017; Belote et al., 2018, 2019). Thus, the use of ISI in the field may be a management tool to evaluate the effect of SDP and other diet changes in commercial production.

Table 1. Description of number of farms under type of ventilation system in different groups.

Type of ventilation	Spray-dried plasma in the feed: Yes	Spray-dried plasma in the feed: No	Total
Negative pressure	4	13	17
Positive pressure	50	33	83
Total	54	46	100

It was hypothesized that adding SDP in starter broiler diets could improve overall performance at the processing age in commercial broilers, and that the ISI methodology could be a management tool in the field to assess the impact of SDP on gut health.

MATERIALS AND METHODS

Animals, Experimental Design, and Housing

The study was conducted in a broiler integrator company in the State of São Paulo, Brazil. A total of 100 farms were used including 1,101,100 broilers housed under commercial conditions; thus, each farm was considered an epidemiological unit. The experiment followed a 2 × 2 factorial design (Table 1), considering starter diets with or without 1% SDP and 2 different house ventilation systems described as negative pressure (**NP**) and positive pressure (**PP**). The NP ventilation system used herein creates a partial vacuum and pulls air into the house evenly through all inlets, improving temperature uniformity within the barn. This system operates through fans and temperature sensors, while the barn walls have plastic canvas closed all the time. The PP ventilation system is composed of sidewalls and ridge vents that allow external conditions to control the inner environment. Fans are installed to regulate the internal temperature of the barn and air renewal to adjust the humidity, while the barn walls have plastic canvas opened by morning and closed by night depending on the weather and desired barn temperature.

Cobb500 Slow Feather broilers were raised in agreement with commercial management and sanitary practices in place at the integrator. Peanut shell, pine, rice husk, or wood shavings were used as litter material. Automatic feeders and nipple drinkers were used, and the birds were housed at a density between 10 and 12 birds/m^2.

Diet

The standard dietary program used consisted of 5 dietary phases with estimated intakes of 350, 700, 800, 2,000, and 1,700 g/bird for the pre-starter, starter, grower, finisher 1, and finisher 2 diets, respectively. Treatments consisted of starter experimental diets formulated with or without 1% SDP (AP 920, APC Brazil, São Paulo, Brazil) and offered from 0 to 10 d of age. The SDP and control formulations were isonutritional to avoid effects between treatments, being reformulated according to the nutritional profile of

Table 2. Description of the ingredients and calculated nutritional composition of diets.

	Starter	
Ingredients (kg/ton)	SDP	Control
Corn	568.69	554.66
Soybean oil	21.14	26.00
Soybean meal	326.29	344.29
Meat meal	52.00	52.57
SDP	10.000	0.000
Sodium chloride	4.199	4.286
Sodium sulphate	1.171	1.714
Limestone	2.714	2.000
Methionine MHA liquid	2.342	2.571
L-lysine 78.0%	2.128	2.400
DL-methionine 98.0%	1.428	1.429
L-threonine 98.0%	0.557	0.800
Choline 75% liquid	0.914	0.857
Premix[1]	6.701	6.701
Total	1,000	1,000
Crude protein (%)	22.50	22.50
Crude fat (%)	5.04	5.49
Crude fiber (%)	2.85	2.91
Moisture (%)	11.39	11.34

Composition of minimum (per kg): vitamin A, 8,818,342 IU; vitamin D3, 3,086,420 IU; vitamin E, 3,674 IU; vitamin B12, 130 mg; vitamin K, 1,177 mg; vitamin B2, 4,775 mg; pantothenic acid, 16,168 mg; vitamin B1, 2,350 mg; vitamin B3, 36,742 mg; vitamin B6, 5,732 mg; folic acid, 1.1,398 mg; choline, 104,460 mg; biotin, 441 mg. Minimum of Fe 12%; Cu 1.4%; I 800 ppm; Zn 12%; Mn 173.0 mg; Mg 12%.

Abbreviations: MHA, methionine hydroxy analog; SDP, spray-dried plasma.

[1]Premix mineral vitamin (4.737 g/ton); zinc bacitracin as growth promoter (15%/370 g/ton); mycotoxin adsorbent antioxidant (1.000 g/ton); phytase 500 FTU (0.065 g/ton), copper (II) sulfate ($CuSO_4$) (35%/0.800 g/ton).

the ingredients received weekly. The nutrient profile of SDP was accounted for in the formulation (Table 2). Nutrient levels were formulated to meet or exceed the bird's requirements (Cobb manual, 2018). The raw matter was analyzed weekly, and the analyses results of the feed were monitored following the method described in AOAC (2000); if there was a nutritional deviation above 10%, the feeds were reprocessed. However, in the experimental period, no nutritional deviation was identified.

Subsequent dietary phases were common diets fed to the 2 groups until slaughter age. All diets contained zinc bacitracin added as a growth promoter and 200 ppm of $CuSO_4$ from 0 to 28 d.

Performance

The following zootechnical parameters were evaluated: feed intake, ADG, feed conversion ratio (**FCR**), feed conversion ratio adjusted to 2.80 kg (**FCRA**), mortality (**MT**), age at slaughter (**AS**), and weight at processing. As birds were sent to the market upon achieving the desired weight (3.05 kg), the AS was evaluated as a dependent variable along with other performance measures in 100 farms.

ISI Methodology

Briefly, the ISI is a health evaluation methodology based on the quantification of selected pathologic

Table 3. ISI macroscopic parameters applied in necropsy.

System								
Locomotor		Respiratory		Gastrointestinal		Intestinal		Coccidiosis
Organ	Parameter	Organ	Parameter	Organ	Parameter	Organ	Parameter	Parameter
External and locomotor	Pododermatitis	Trachea	Tracheitis	Oral cavity	Oral mucosa lesion	Duodenum	Inflammation process at serosa or mucosa	*Eimeria acervulina*
	Foot pat pigmentation						Cell debris and thick mucus at mucosa	
				Liver	Reddish (congestion)	Jejunum	Necrosis	*Eimeria maxima*
	Femoral head necrosis				Yellowish		Inflammation process at serosa or mucosa	
	Tibial dyschondroplasia	Heart	Hydropericardium	Proventriculus	Proventriculitis		Cell debris and thick mucus at mucosa	
	Bone resistance			Gizzard	Erosion		Decreased muscular tonus	
				Pancreas	Hypertrophic	Ileum	Inflammation process at serosa or mucosa	*E. maxima*
	Muscle hemorrhage				Hypotrophic		Cell debris and thick mucus at mucosa	
		Air sacs	Airsacculitis	Yolk	Persistence		Undigested food and/or gas presence	
				Kidney	Hypertrophic	Cecum	Inflammation process at serosa or mucosa	*Eimeria tenella*
							Gas presence	

Table 4. Effect of adding SDP at 1% in the starter diet from 0 to 10 d of age on overall performance.

	SDP			Control			
All farms	M	MD	SD	M	MD	SD	*P*-value
AS (d)	53	**53[b]**	2	54	**54[a]**	2	**0.016**
WP (g/bird)	3,081	3,100	209	3,115	3,090	136	0.614
ADG (g/bird/d)	58.9	58.7	3.2	58.7	58.3	2.5	0.810
FCRA (g/g)	1.922	1.910	0.124	1.944	1.940	0.107	0.163
FCR (g/g)	2.002	1.978	0.102	2.033	2.050	0.093	0.077
MT (%)	3.8	3.7	1.2	4.1	3.7	1.0	0.174

The numbers in bold have a statistically significant difference ($P < 0.05$) between them.

[ab] Means with different letters in the same column are significantly different in Mann-Whitney U test ($P < 0.05$).

Abbreviations: AS, age at slaughter; FCR, feed conversion ratio; FCRA, feed conversion ratio adjusted to 2.8 kg; M, mean; MD, median; MT, mortality; SDP, spray-dried plasma; WP, weight at processing.

parameters present in a predetermined list of organs and/or tissues. This methodology was first described by Kraieski et al. (2017), whereby a predefined and fixed impact factor (**IF**) value, ranging from 1 to 3, is attributed to each macroscopic (necropsy) and microscopic (histology) parameter evaluated in each organ or tissue. The IF values reflect the level of impairment that each alteration causes to the selected organ or tissue's functionality based on previous knowledge from the literature and research. For example, necrosis has an IF of 3 since that alteration causes complete loss of tissue functionality. In addition, during the evaluation, the observer assigns a score value (**S**) ranging from 0 to 3 to describe the extent of the observed alteration (score 0 = no alteration; score 1 = alteration reaches up to 25% of extension or intensity in relation to the normal organ; score 2 = alteration reaches up to 50% of extension or intensity in relation to the normal organ; and score 3 = alteration is greater than 50% of extension or intensity relative to the normal organ). The final provided value is the ISI total score which is obtained by the formula $\text{ISI} = \sum (\text{IF} * \text{S})$, where the IF is multiplied by the assigned score and the values obtained from each parameter are summed up. The ISI results are presented as mean scores, with a higher value indicating greater alterations observed. For example, necrosis has an IF = 3. If the observer assigns the maximum alteration score (S = 3) to this parameter, then the final score for necrosis alteration will be 9 (IF * S or 3 * 3 = 9).

A total of 35 farms (16 controls and 19 SDP) randomly selected from the 100 farms previously mentioned and representing 526,011 broilers were used. However, the farm was considered the experimental unit and not the birds.

The farm subgroups were stratified in a random sampling model by region and date of housing. A total of 6 birds per farm aged 14 ± 2 d were sampled and euthanized by cervical dislocation (210 birds in total) and used for both macroscopic and microscopic evaluations.

This value was based on a study by Johnson and Reid (1970), where they report that a total of 5 to 10 birds from each house would be reasonable for macroscopic evaluation, with at least 5 birds being used to evaluate gross lesions per experimental unit. The birds that were euthanized must represent the uniformity of the flock. Therefore, if the flock has over 80% uniformity, for example, from 6 birds that are selected, 4 birds should be on average, 1 bird should be above average, and 1 bird below average.

Table 5. Effect of adding SDP at 1% in the starter diet from 0 to 10 d of age on overall performance in birds raised in farms with different ventilation systems.

	SDP			Control			
Type of ventilation	M	MD	SD	M	MD	SD	*P*-value
Negative pressure farms							
AS (d)	52	**52[b]**	0	53	**53[a]**	1	**0.023**
WP (g/bird)	3,240	3,240	92	3,130	3,180	191	0.296
ADG (g/bird/d)	62.3	62.3	1.7	60.2	60.9	1.8	0.202
FCRA (g/g)	1.805	**1.805[b]**	0.075	1.883	**1.900[a]**	0.061	**0.023**
FCR (g/g)	1.932	1.932	0.054	1.977	1.994	0.058	0.130
MT (%)	2.5	**2.5[b]**	0.6	3.4	**3.3[a]**	0.4	**0.045**
Positive pressure farms							
AS (d)	53	**53[b]**	2	54	**54[a]**	2	**0.033**
WP (g/bird)	3,068	3,090	211	3,078	3,070	84	0.812
ADG (g/bird/d)	58.6	58.7	3.1	57.5	57.2	1.9	0.172
FCRA (g/g)	1.932	**1.930[b]**	0.123	1.981	**2.020[a]**	0.111	**0.042**
FCR (g/g)	2.008	**1.984[b]**	0.103	2.059	**2.102[a]**	0.101	**0.013**
MT (%)	3.9	**3.9[b]**	1.2	4.5	**4.4[a]**	1.1	**0.018**

The numbers in bold have a statistically significant difference ($P < 0.05$) between them.

[ab] Means with different letters in the same column are significantly different in Mann-Whitney U test ($P < 0.05$).

Abbreviations: AS, age at slaughter; FCR, feed conversion ratio; FCRA, feed conversion ratio adjusted to 2.8 kg; M, mean; MD, median; MT, mortality; SDP, spray-dried plasma; WP, weight at processing.

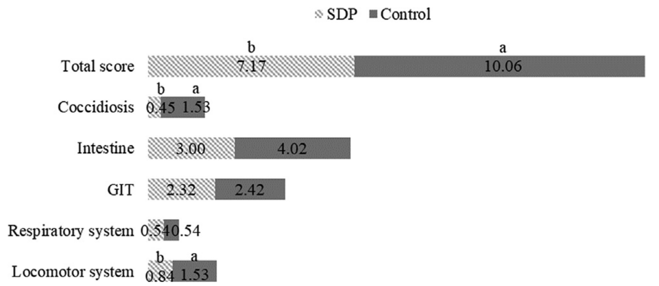

Figure 1. Necropsy findings in broilers raised in farms fed SDP or a control starter diet by the ISI methodology described in Table 3. Different letters (a, b) in the same column indicate a significant difference ($P < 0.05$). Abbreviations: GIT, gastrointestinal tract; ISI, I See Inside; SDP, spray-dried plasma.

ISI Macroscopic Evaluation (Necropsy)

After euthanasia, the carcass was systematically evaluated to identify the overall health status of the broiler. The ISI macroscopic evaluation is categorized by systems as locomotor, respiratory, gastrointestinal organs, intestine, and coccidiosis lesions. Each of these systems was evaluated using parameters described in Table 3. The final ISI score is calculated as the sum of all parameters evaluated. Therefore, the higher the final ISI total score, the worse the intestinal health of the animal. This system can also be assessed by a software in www.isiinstitute.com.

ISI Microscopic Evaluation (Histology)

For the histological evaluation, samples of ileum were collected and immediately fixed in Davidson's solution (100 mL glacial acetic acid, 300 mL 95% ethyl alcohol, 200 mL 10% neutral buffered formalin, and 300 mL distilled water) for at least 24 h. Samples were then dehydrated, infiltrated, and embedded in paraffin following common histological routine. Blocks were cut in 5 μM sections and stained with hematoxylin and eosin associated with Alcian blue for goblet cell staining (Rapp and Wurster, 1978).

For histologic ileal evaluation, 1 slide per bird containing 20 intestinal villi was analyzed under 10× magnification (using 20× and 40× magnification to confirm alterations) under an optical microscope (Eclipse E200, Nikon, Sao Paulo, Brazil).

The ISI histology parameters evaluated included lamina propria thickness, epithelial thickness, enterocytes proliferation, inflammatory cell infiltration in the epithelium, inflammatory cell infiltration in the lamina propria, goblet cells proliferation, congestion, and presence of *Eimeria* spp. oocysts. The final ISI score is calculated as the sum of all parameters evaluated. The higher the final ISI total score, the worse the intestinal health of the animal (Kraieski et al., 2017; Belote et al., 2018, 2019).

Biosecurity and Management

Biosecurity and local management practices were evaluated to obtain the influence of those parameters on animal health. During each visit, in the 35 farms used for the ISI evaluation, farms were classified according to their biosecurity standards as good or bad based on the following observations: presence of a farm gate, a functional vehicle disinfection system at the farm's gate, farm's appearance (organized and clean), presence of appropriate fencing, and presence of barn seals. If more than half of these items were in place, biosecurity was regarded as good or otherwise considered bad. Management practices were also evaluated and regarded as good or bad based on the following observations: quality of drinking water (clarity/water flow [mL/1 min/presence of leaks]), nipple height, ambience (temperature, ventilation), feed's physical appearance, feeder height, and litter quality (compaction and moisture). If more

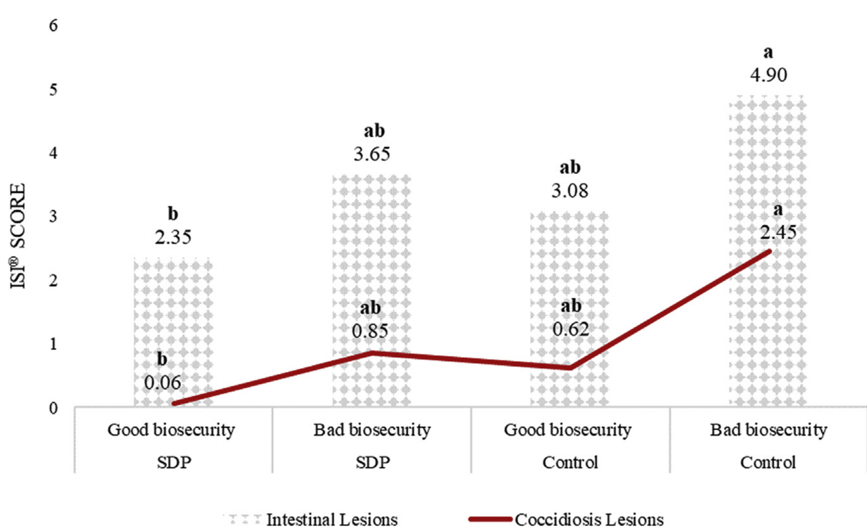

Figure 2. ISI scores observed during necropsy relative to intestinal lesions and coccidiosis lesions in broilers fed a starter diet with or without SDP and housed in farms with good or bad biosecurity standards. Different letters (a, b) in the same column indicate a significant difference ($P < 0.05$) in ISI scores as described in Table 3. Abbreviations: ISI, I See Inside; SDP, spray-dried plasma.

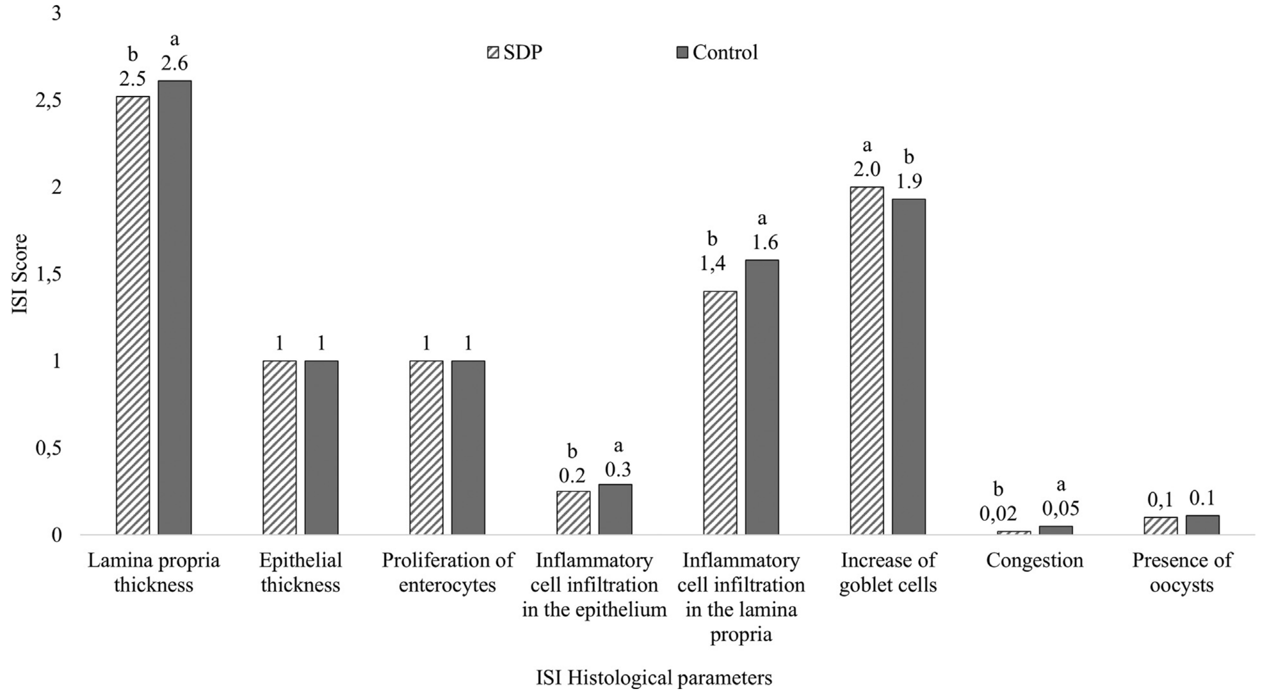

Figure 3. Parameters of the ISI methodology of ileum evaluation between control farms and SDP-fed farms. Different letters (a, b) indicate a significant difference ($P < 0.01$). Abbreviations: ISI, I See Inside; SDP, spray-dried plasma.

than half of these parameters were regarded as acceptable, the farm was considered to have good management, or otherwise considered bad.

Statistical Analysis

Data on AS, ADG, weight at processing, FCR, FCRA, and MT were provided by the broiler integrator. Complete descriptive statistics of all variables in general is included in the Supplementary Material (mean, median, SD, minimum, maximum, and skewness calculation). Mean, median, SD, and interquartile range by group are presented in Tables 4 and 5. The Shapiro-Wilk test

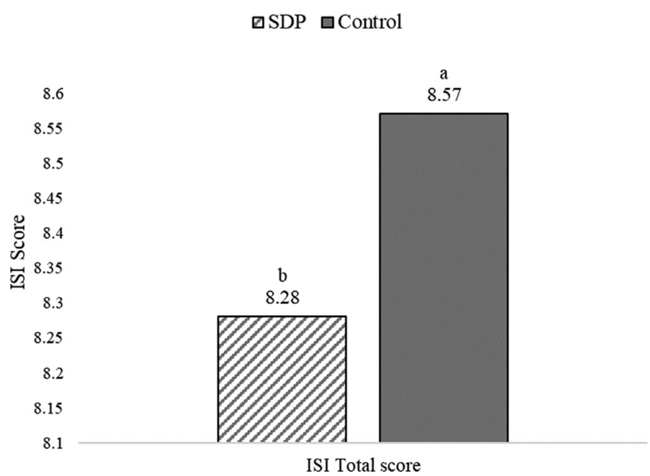

Figure 4. ISI total score, Σ(IF*S), of ileum evaluation between control farms and SDP-fed farms. Different letters (a, b) indicate a significant difference ($P < 0.01$). Abbreviations: ISI, I See Inside; SDP, spray-dried plasma.

was used to evaluate the adherence of data to a normal distribution, which revealed that all the 6 performance variables tested were asymmetric to normal distribution (P-values in Supplementary Material). Because of this, a mean test (t test, e.g.) cannot be used to compare groups and the Mann-Whitney U test (nonparametric approach) was used to verify differences between farms fed with or without SDP for each variable separated by the type of housing. All performance parameters were analyzed using SPSS 20.0 (IBM, 2011) software (IBM Corp., Armonk, NY).

The Shapiro-Wilk normality test was used for macroscopic, microscopic (histology), biosecurity, management, and factorial analyses. Parametric data were subjected to ANOVA and Tukey's test was used to establish differences among means. Conversely, the Kruskal-Wallis test at 5% probability was used for nonparametric data. All data were analyzed using the statistical software Statistix 10 (Analytical Software, Tallahassee, FL). Statistical significance was considered for $P < 0.05$ and tendency for $P < 0.10$.

RESULTS

The results of the interaction between diet and performance are presented in clusters by type of ventilation in Tables 4 and 5. Independent of the ventilation system, feeding SDP reduced the AS by 1 d compared to the untreated group of farms ($P < 0.05$; Table 4) and had a tendency to reduce FCR ($P = 0.077$; Table 4). Farms with NP presented better results of FCR than PP farms, showing a reduction of 0.07 points (mean) in farms that received SDP, and 0.08 points (mean) in control farms among NP and PP, respectively (Table 5).

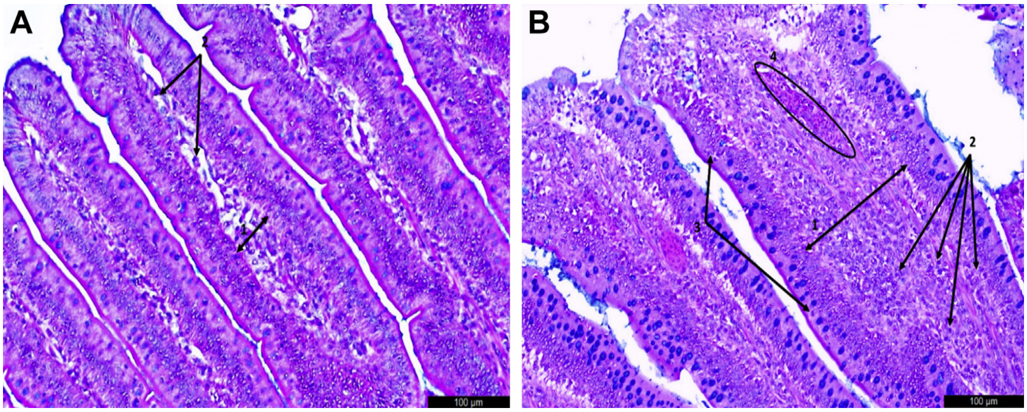

Figure 5. Photomicrography of ileum sections of broilers stained with hematoxylin and eosin. Alcian blue was used to stain goblet cells. (A) 1, Low ISI score of lamina propria thickness; 2, low ISI score of inflammatory cells infiltration in the lamina propria in the farms fed with SDP (200×). (B) 1, High ISI score of lamina propria thickness; 2, high ISI score of inflammatory cells infiltration in the lamina propria; 3, inflammatory cells infiltration in the epithelium; 4, high ISI score of congestion in birds of the control group (200×). Abbreviations: ISI, I See Inside; SDP, spray-dried plasma.

Feeding SDP in the starter diet yielded improvements in FCRA and MT at the end of the production cycle in both NP and PP farms ($P < 0.05$) vs. the control birds; a reduction of 0.08 and 0.05 points (mean) in FCRA and 0.8 and 0.5% in overall MT were observed when broilers were raised in NP and PP farms, respectively.

Results of the ISI macroscopic evaluation (Figure 1) indicate that farms fed SDP had ($P < 0.05$) 45% less alterations in the locomotor systems, 70% less coccidiosis-associated lesions in the gut, and a reduction of 28% in the ISI total score. The other parameters were not significantly different.

Higher ($P < 0.05$) ISI values for intestinal scores and coccidia scores during necropsy were observed in birds housed under bad vs. good biosecurity conditions, and in birds fed the control diets vs. SDP (Figure 2). It was observed that birds under bad biosecurity fed SDP showed the same ($P < 0.05$) results compared with birds of the control group under good biosecurity, while birds under good biosecurity fed SDP presented better ($P < 0.05$) results compared with birds of the control group under bad biosecurity.

Ileal histologic evaluation pointed out less pathologic alterations in birds fed SDP in comparison to those fed a control starter diet. The product promoted lower scores of lamina propria thickness, inflammatory cell infiltration in the epithelium and lamina propria, and congestion ($P < 0.05$). Collectively, the reduced scores in these parameters resulted in a lower ISI total score recorded in SDP-treated birds, even though the product resulted in an increased score of goblet cell proliferation in these animals (Figures 3–5).

A factorial analysis of ileal histology (Table 6) showed that birds fed SDP had lesser total pathologic alterations independently of whether biosecurity measures were classified as good or bad; thus, higher ISI scores for lamina propria thickness, inflammatory cell infiltration in the epithelium and the lamina propria, greater congestion, but lesser goblet cells were observed in birds fed a starter diet without SDP ($P < 0.05$). Birds from farms under bad biosecurity showed greater total scores and presence of oocysts vs. those under good biosecurity, and lower congestion was scored in this group when birds were fed a control diet. Birds with the lowest ($P < 0.05$) total scores were those that were fed SDP and kept under good biosecurity measures.

The interaction between SDP feeding and farm management on ileal histologic alteration scores is detailed in Table 7. Feeding SDP lowered the total ISI scores independent of whether the birds were under good or bad management practices ($P < 0.05$). Feeding SDP lowered lamina propria thickness, inflammatory cell infiltration in both epithelium and lamina propria, and congestion ($P < 0.05$), but increased goblet cell scores. Farms classified as having bad management showed lower total ISI scores, lamina propria thickness, and presence of oocysts particularly when SDP was fed.

Table 6. Ileal histologic ISI scores as affected by feeding SDP and biosecurity standards at the farm in broilers.

SDP in feed	Biosecurity standards	Lamina propria thickness	Epithelial thickness	Proliferation of enterocytes	Inflammatory cell infiltration in the epithelium	Inflammatory cell infiltration in lamina propria	Increase of goblet cells	Congestion	Presence of oocysts	Total score
Yes	Good	2.47^{b}	1.00	1.00	0.23^{b}	1.27^{c}	1.99^{a}	0.02^{b}	0.00^{b}	8.00^{c}
Yes	Bad	2.49^{b}	0.99	0.99	0.24^{a,b}	1.45^{b}	2.00^{a}	0.01^{b}	0.13^{a}	8.35^{b}
Control	Good	2.68^{a}	0.98	0.98	0.29^{a}	1.64^{a}	1.85^{b}	0.09^{a}	0.00^{b}	8.54^{a,b}
Control	Bad	2.80^{a}	1.01	1.01	0.28^{a,b}	1.67^{a}	1.85^{b}	0.008^{b}	0.17^{a}	8.82^{a}
P-value		0.05	0.09	0.09	0.05	0.05	0.05	<0.001	0.05	<0.001

[a–c]Different letters in the same column indicate a significant difference ($P < 0.05$).
Abbreviations: ISI, I See Inside; SDP, spray-dried plasma.

Table 7. Ileal histologic ISI scores as affected by feeding SDP and management conditions at the farm in broilers.

SDP in feed	Management	Lamina propria thickness	Epithelial thickness	Proliferation of enterocytes	Inflammatory cell infiltration in the epithelium	Inflammatory cell infiltration in the lamina propria	Increase of goblet cells	Congestion	Presence of oocysts	Total score
Yes	Good	2.60^{b}	0.99	0.99	$0.25^{a,b}$	1.41^{b}	2.01^{a}	0.01^{b}	0.13^{a}	8.43^{b}
Yes	Bad	2.36^{c}	1.00	1.00	0.22^{b}	1.31^{b}	1.98^{a}	$0.02^{a,b}$	0.00^{b}	7.92^{c}
Control	Good	2.83^{a}	0.98	0.98	0.30^{a}	1.74^{a}	1.85^{b}	$0.05^{a,b}$	$0.06^{a,b}$	8.82^{a}
Control	Bad	$2.64^{a,b}$	1.01	1.01	$0.28^{a,b}$	1.57^{a}	1.86^{b}	0.06^{a}	$0.10^{a,b}$	$8.54^{a,b}$
P-value		0.05	0.41	0.41	0.05	0.05	0.05	0.05	0.006	<0.001

[a–c]Different letters in the same column indicate a significant difference ($P < 0.05$).
Abbreviations: ISI, I See Inside; SDP, spray-dried plasma.

Data analysis exploring the 3-way interaction among SDP feeding, biosecurity, and management practices (Table 8) for ileal histologic alteration scores showed that feeding SDP lowered scores of inflammatory cell infiltration in the lamina propria, independent of having good or bad management and biosecurity. The lowest ISI total scores were observed in birds fed SDP and housed under bad management and biosecurity compared with other combinations without SDP in the diet.

DISCUSSION

The current trial reports data from approximately 1.1 million broilers housed in 100 commercial farms located in the same region, housed at similar time, and fed a commercial starter diet with (54 farms) or without (46 farms) 1% SDP. To the best of our knowledge, this is the largest field broiler trial evaluating the effect of feeding SDP in the starter diet on commercial performance and health, along with the first evaluation of the ISI scoring system used and validated in a large commercial broiler flock. This large number of farms (farm as experimental unit) allowed us to test the effect of SDP and to use the ISI scores, under a wide variety of situations such as different housing systems, and good or bad management and/or biosecurity measures.

Nutritional strategies that support the immune system, promote intestinal integrity and functionality, and increase tolerance to stress and disease challenges are of interest to the poultry industry. Additionally, early nutrition has been recognized as an opportunity to further advance nutritional practices and improve overall performance and health status of commercial broiler flocks (Noy and Uni, 2010). In the current trial, 1% SDP was fed for the first 10 d of life while performance benefits and a reduction in MT were reported at the end of the production cycle at 53 and 54 d of age Tables 4 and 5). Total intake of SDP per bird in those 10 d was calculated to be approximately 3 g per broiler.

The performance of broilers was better in NP vs. PP farms indicating that NP provided more favorable conditions for growth rather than PP, and that feeding 1% SDP improved the performance of birds housed in both types of ventilation at about the same magnitude (Tables 4 and 5) Feeding SDP to broilers in the first few days of life improved performance such as weight gain and feed efficiency at the end of the production cycle in healthy flocks (Beski et al., 2015, 2016a). Walters et al. (2019) fed 0 or 2% SDP to coccidia-vaccinated broilers for 10 d of life, and 0 or 50 ppm of bacitracin methylene disalicylate (**BMD**) from 0 to 41 d of age in a factorial 2 × 2 design. These authors reported that birds showed improved performance at 41 d when fed either BMD or SDP. Furthermore, the birds fed BMD demonstrated improved performance when SDP was offered suggesting that the effect of SDP was additive and independent of BMD. In the current experiment, no coccidia vaccine was used but all diets contained zinc bacitracin as a growth promoter and improvements in response to feeding SDP were observed in growth as evidenced by the reduction in AS by 1 d while maintaining similar BW (Table 5) and by an improvement in feed efficiency of about 4.5 and 5% for birds under NP and PP barns, respectively.

Campbell et al. (2008) suggested that the benefits of feeding SDP could be more pronounced when fed to birds with higher pathogen exposure; however, in the current study, the performance improvements and reductions in MT attributed to feeding SDP were similar in PP and NP farms vs. controls. Likewise, significantly less pathologic alterations were recorded in birds fed SDP vs. the control diet after a holistic necropsy evaluation (Table 3), particularly in those associated with coccidiosis and the locomotor system ($P < 0.05$; Figure 1). However, greater reductions in macroscopic intestinal lesions associated or not associated to coccidiosis were found in farms with bad vs. good biosecurity standards in response to SDP (Figure 2). For locomotor systems, the parameter that showed less frequency of alterations was bone resistance, presenting 9.6% of normal tissue (score 0), 7.1% less mild lesions (score 1), 1.6% less moderate lesions (score 2), and no serious lesion (score 3) compared to the control group. Similar to this report, Jiang et al. (2000) found an increase in bone density in swine, when feeding SDP.

The improvement of locomotor systems could demonstrate the importance of intestinal health in relation to the absorption of nutrients that act on bone structure and maintenance. This alteration could reinforce the theory described by Campbell et al. (2019), about the systematic action of the product.

Histologic scoring of ileal samples indicated an overall reduction in alterations in observations related to inflammation such as inflammatory cell infiltration, congestion, and lamina propria thickness suggesting a

Table 8. Ileal histologic ISI scores as affected by feeding SDP, biosecurity, and management conditions at the farm in broilers.

SDP in feed	Biosecurity	Management	Lamina propria thickness	Epithelial thickness	Proliferation of enterocytes	Inflammatory cell infiltration in the epithelium	Inflammatory cell infiltration in the lamina propria	Increase of goblet cells	Congestion	Presence of oocysts	Total score
Yes	Good	Good	$2.56^{c,d}$	$1.00^{a,b}$	1.00	0.21	1.20^{d}	2.02^{a}	0.01	0.00^{b}	$8.04^{b,c}$
Yes	Good	Bad	2.37^{d}	$1.00^{a,b}$	1.00	0.24	$1.34^{c,d}$	1.95^{b}	0.04	0.00^{b}	7.96^{c}
Yes	Bad	Good	$2.63^{b,c}$	$0.98^{a,b}$	0.98	0.29	$1.62^{a,b}$	$1.99^{a,b}$	0.01	0.27^{a}	8.81^{a}
Yes	Bad	Bad	2.35^{d}	$1.01^{a,b}$	1.01	0.20	$1.28^{c,d}$	$2.01^{a,b}$	0.00	0.00^{b}	7.88^{c}
Control	Good	Good	$2.52^{c,d}$	0.96^{b}	0.96	0.30	$1.61^{a,b}$	$2.00^{a,b}$	0.10	0.00^{b}	$8.47^{a,b}$
Control	Good	Bad	$2.84^{a,b}$	$1.00^{a,b}$	1.00	0.29	$1.67^{a,b}$	1.71^{c}	0.09	0.00^{b}	8.62^{a}
Control	Bad	Good	3.15^{a}	$1.00^{a,b}$	1.00	0.30	1.88^{a}	1.70^{c}	0.00	$0.12^{a,b}$	9.16^{a}
Control	Bad	Bad	$2.45^{c,d}$	1.02^{a}	1.02	0.26	$1.47^{b,c}$	$2.01^{a,b}$	0.01	0.21^{a}	8.47^{a}
P-value			0.00	0.05	0.26	0.24	0.00	0.00	0.16	0.006	0.00

[a–d]Different letters in the same column indicate a significant difference ($P < 0.05$).

Abbreviations: ISI, I See Inside; SDP, spray-dried plasma.

healthier gut in birds fed SDP (Figure 3). During the inflammation process, innate immune cells (macrophages, granulocytes, dendritic cells) are found diffusely in the epithelium and lamina propria (Keestra et al., 2013; Kogut et al., 2018). The increase of lamina propria thickness and inflammatory cells infiltration were described by Belote et al. (2018) as good parameters to compare intestinal health between different treatments. These parameters have a strong correlation with zootechnical performance, which means, the higher the score in these parameters, the worse the animal's performance results.

Coordinate activation of inflammation is part of the nonspecific immune response that occurs in reaction to any type of bodily damage caused by several factors (Ferrero-Miliani et al., 2007). The signs of inflammation are characterized by congestion due to increased blood flow and vasodilatation, cells recruited from the blood, and inflammatory mediator levels in resident tissue cells (Ferrero-Miliani et al., 2007; Chen et al., 2018).

In general, these observations were similar irrespective of the biosecurity and management standards as defined herein (Tables 6–8), suggesting that feeding SDP can benefit broilers in a wide variety of conditions. As discussed by Pérez-Bosque et al. (2016) and Campbell et al. (2019), gut health benefits measured as improved barrier function, mucosa permeability, and integrity are frequently associated with feeding SDP to animals in many disease and stress experimental models. Likewise, greater resistance to disease has been reported in broilers under a natural outbreak of necrotic enteritis (Campbell et al., 2004a), challenged with *Salmonella sofia* (Beski et al., 2016b), with high MT where *Escherichia coli* and *Streptococcus* were isolated (Gonzalez-Esquerra et al., 2019), and subjected to heat stress, and in turkeys challenged with *Pasteurella multocida* (Campbell et al., 2004b) when fed SDP. Collectively, these observations suggest that feeding SDP causes an unspecific and systemic effect.

Belote et al. (2019) observed that the intestinal mucosal alterations scored and analyzed using the ISI methodology correlate well with the final performance of broilers at 28 d of age. The former findings indicate the importance of gut health at that age for optimal performance later in life. In the current report, the ISI macroscopic and histologic evaluations were performed in 14 ± 2 day-old-broilers and both were able to indicate health benefits from feeding SDP in commercial broilers correlating well with performance observations at market age. Likewise, higher ISI scores were found in farms with bad biosecurity, which is a well-known factor that impacts performance. Those observations indicate that this technique can be a useful tool to uncover factors that can impact early health, and therefore the final performance, of broilers under commercial conditions.

CONCLUSION

Feeding SDP in the starter diet reduced MT and improved growth and feed efficiency at a similar rate in commercial broilers housed in either NP or PP barns, with PP being less favorable to broilers performance than NP. During necropsy procedures, irrespective of having good or bad biosecurity standards, feeding SDP to broilers resulted in better overall health with reduced coccidia lesions, and other pathologic alterations in the small intestine and cecum, and less pathologies in the locomotor system. Additionally, SDP-fed birds under good or bad management practices had lower histopathologic alterations in the ileum. Collectively, these observations suggest that feeding SDP in the first days of life can provide benefits to commercial broilers under a wide variety of circumstances. Additionally, there was good agreement between necropsy and histologic ISI scores obtained in commercial broilers and their final performance suggesting that this method could be a useful tool to evaluate the impact of nutritional strategies in the health and performance of broilers under field conditions or even in experimental tests.

DISCLOSURES

The authors declare that there is no conflict of interest in this research.

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.psj.2021.101080.

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