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Research Article
Comparative Effects of Spray-Dried Plasma and Bacitracin Methylene Disalicylate on Intestinal Development in Broiler Chicks

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Abstract

Background and Objectives: Spray-dried plasma (SDP) is a bioactive feed additive that frequently improves broiler growth performance and may therefore replace antibiotic growth promoters (AGPs). To improve our understanding of SDP mechanisms of action, a 2-week experiment was conducted to compare the potency of porcine SDP and bacitracin methylene disalicylate (BMD) antibiotic to stimulate intestinal development in neonate chicks. **Materials and Methods:** Day-old (288) Ross 708 broiler male chicks were obtained from a commercial hatchery and randomly assigned to 6 treatments in a completely randomized design. Treatments consisted of chicks given unmedicated corn-soybean meal basal diet containing no BMD or SDP (CX), at 0.055 g kg^{-1} diet (MX), SDP at 10 g kg^{-1} diet (SP1), SDP at 20 g kg^{-1} diet (SP2), SDP at 30 g kg^{-1} diet (SP3) and SDP at 40 g kg^{-1} diet (SP4). On day 3, 7 and 14 of experiment, intestinal maltase activity was determined. Villi morphometrics was also measured in the jejunum (d 7). **Results:** On day 7, all chicks that consumed SDP and BMD had lower villus: crypt ratio ($p = 0.0006$) and higher goblet cell density ($p < 0.0001$) compared to CX. Furthermore, on day 14, ileal maltase activity was higher for all chicks that consumed SDP (3.036-3.065 μg glucose/min/mL/μg protein, $p < 0.0001$) compared to CX (3.025 μg glucose/min/mL/μg protein). **Conclusion:** Like BMD antibiotic, dietary SDP at 30 or 40 g kg^{-1} diet improves feed conversion ratio in chicks in-part, by increasing ileal maltase activity, reducing intestinal villus/crypt ratio (indicative of ongoing villi renewal/regeneration) and increasing goblet cell density.

Key words: Intestinal development, spray-dried plasma, bacitracin methylene disalicylate, broiler chicks, maltase activity, goblet cell density, villi morphometry

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The intestine of the newly hatched chick is immature with respect to digestive and immune function until the about 2 weeks and growth during this early posthatch period is highly dependent on the development of the gastrointestinal tract[1-3]. Enhancing early intestinal maturation and digestive capacity in neonate poultry is crucial especially since digestion, absorption, assimilation and incorporation of nutrients into developing and growing tissues is directly dependent on intestinal functional capabilities[4-5]. It has been determined that chick growth during the first 7 days post-hatch represents about 20% of a broiler's life and is approximately equivalent to 10% of final market weight[6-7]. Furthermore, there is a positive correlation between early growth rate and market weight, uniformity of carcass weight and breast muscle growth[8].

At hatch, the weight of the small intestine of poultry increases at a very high rate with rapid proliferation and differentiation of enterocytes[9-10]. This rapid intestinal development is typified by significant increases in villus height and crypt depth, along with increases in the activities of brush-border enzymes that digest disaccharides (sucrase-isomaltase) and small peptides (aminopeptidase)[11]. These changes culminate in increased ability of the gut to digest and absorb nutrients, in addition to developing protective mechanisms against potentially colonizing exogenous pathogens[5,10-12].

Antibiotic growth promoters (AGPs) have been traditionally incorporated into broiler chick diets to enhance growth, feed efficiency and bird health[13]. However, the emergence of antibiotic-resistant bacterial strains and their transmission to humans have threatened food safety and public health[14-16], thereby forcing governmental legislation(s) that phase out their inclusion in poultry feed[17]. It has been proposed that alternatives to AGPs should exhibit some or all the following antibiotic mechanisms of action proposed to favor growth performance: (1) Reducing the incidence and severity of subclinical infections, (2) Reducing total microbial density and nutrient use in the gastrointestinal tract, (3) Improving absorption of nutrients because of thinning of the intestinal wall, (4) Reducing the production of growth-depressing/toxic bacterial metabolites and (5) Acting as immunomodulatory agents that modulate the shift of nutrients to metabolic functions[13,18-20].

Spray-dried plasma (SDP) is a blood-based palatable feed additive which contain functional proteins and essential nutrients that include biologically active peptides (defensins, transferrins), immunoglobulin, albumin, fibrinogen, lipids, growth factors, enzymes and other components that exert specific biological activities in the intestine in addition to their nutritional value[21-23]. Dietary incorporation of SDP has shown beneficial effects on the gastrointestinal health and growth performance of neonate animals, particularly piglets and dairy calves[22-25]. Similarly, a review of studies that investigated the impact of SDP on intestinal health and growth performance of broiler chickens indicated that incorporation of SDP at up to 40 g kg^{-1} (i.e. 4% level) of the diet during the first 21 days of life, often improved ($p < 0.05$) body weight gain and feed conversion[26]. The SDP-induced improvements in growth performance may be due to increases in intestinal digestive capacity. For instance, Beski *et al*.[27] observed increased activities ($p < 0.05$) of intestinal brush border enzymes (maltase, sucrase and alkaline phosphatase), increased villi length and crypt depth and reduced villi: crypt ratio in broilers fed diets supplemented with up to 20 g SDP kg^{-1} diet, compared to control birds. However, there is paucity of information regarding whether these beneficial mechanisms of SDP in the intestinal mucosa are comparable in magnitude or similar to those induced by AGPs.

This study compared the effect of porcine SDP supplementation (at 0, 10, 20, 30, or 40 g kg^{-1} diet) and bacitracin methylene disalicylate (BMD) antibiotic (at 0.055 g kg^{-1} diet) on intestinal development (i.e. intestinal maltase activity and villi morphometrics) in broiler chicks during the first 2 weeks of life.

MATERIALS AND METHODS

All the procedures used in this study were approved by the Institutional Animal Care and Use Committee (IACUC) of North Carolina Agricultural and Technical State University.

Experimental design, dietary treatments and animal husbandry: Day-old (288) Ross 708 broiler male chicks were obtained from a commercial hatchery and transported to the Poultry Research Unit and North Carolina Agricultural and Technical State University. Upon arrival, chicks were weighed and randomly assigned to 6 treatments in a completely randomized design. Treatment 1 (CX) consisted of chicks fed unmedicated corn-soybean meal (SBM) basal without porcine SDP supplementation. Treatment 2 (MX) consisted of chicks given unmedicated corn-SBM basal with bacitracin methylene disalicylate (BMD) added at 0.055 g kg^{-1} diet. Treatments 3 (SP1), 4 (SP2), 5 (SP3) and 6 (SP4) consisted of chicks given unmedicated corn-SBM basal with porcine SDP added at 10, 20, 30 and 40 g kg^{-1} diet, respectively. The porcine SDP used in this study was a kind gift from APC, Incorporated (Ankeny, IA).

Each treatment consisted of 4 replicate pens in battery cages (Alternative Design Manufacturing and amp Supply Inc., Siloam Springs, AR), with each pen containing 12 chicks. Each pen had a nipple drinker to supply fresh water and a feeder tray which was adjusted in height for reach according to the progressive growth of the chicks. Photoperiod consisted of continuous (23L:1D) lighting at 30 lux from placement to 7 day and 20L:4D lighting at 10 lux from 8-14 days according to breeder company recommendations[28].

Experimental diets were formulated to meet the recommendations of the National Research Council[29] and chickens were allowed *ad libitum* access to feed (crumbled) and water throughout the 14-day experiment. Proximate nutrient composition of experimental diets is presented in Table 1.

Monitoring chick growth performance: On day 7 and 14 of experiment, body weight, body weight gain and feed intake of chicks were recorded. From these data, feed conversion ratio (FCR) was calculated. Mortality was also recorded on daily basis throughout the experiment.

Collection of intestinal tissue for downstream analysis: Intestinal tissue sections were collected on day 3, 7 and 14 for the determination of maltase activity and villi morphometric analysis. On each day, two chicks were randomly taken from each pen (totaling 8 chickens per treatment) and euthanized by CO_2 asphyxiation. Thereafter, the small intestine of each chick was aseptically excised and placed on ice. Next, approximately 2 cm long tissue sections were taken from the mid-portion of duodenum (from the gizzard to the point of entry of the pancreo-biliary ducts), jejunum (from the pancreo-biliary ducts to the yolk stalk) and ileum (from yolk stalk to the ileo-cecal junction). The tissue from each segment was flushed with 0.9% saline (9 g of NaCl L^{-1} deionized water), placed in a sterile cryogenic vial and immediately snap frozen in liquid nitrogen (N2). Samples were then stored at -80°C until it was time to analyze them for maltase activity.

Additionally, another 2 cm long tissue section was taken on day 7 from each bird euthanized. Each tissue section was fixed by careful immersion in10% neutral buffered formalin (Fisher Scientific, Pittsburgh, PA) until time for tissue processing and examination of villi morphology.

Table 1: Composition of experimental diets[1] (% "as is")

Ingredients	Control diet	BMD diet[1]	SDP1 diet[1]	SDP2 diet[1]	SDP3 diet[1]	SDP4 dite[1]
Corn	50.59	50.590	52.07	53.54	55.02	56.50
Soybean meal	40.67	40.670	38.72	36.78	34.83	32.89
Spray-dried plasma (SDP, AP920)	0.00	0.000	1.00	2.00	3.00	4.00
Poultry fat	4.53	4.530	4.10	3.68	3.26	2.83
Limestone	1.37	1.370	1.39	1.42	1.45	1.47
Mono-dicalcium phosphate	1.51	1.510	1.47	1.43	1.39	1.35
Salt NaCl	0.24	0.240	0.21	0.19	0.16	0.13
Soda bicarbonate	0.16	0.160	0.12	0.08	0.04	0.00
L-Lysine HCl 98%	0.17	0.170	0.16	0.14	0.13	0.11
DL-Methionine 99.0%	0.34	0.340	0.33	0.33	0.32	0.31
L-Threonine 98.5%	0.10	0.100	0.09	0.08	0.07	0.06
NCSU poultry vitamin premix[2]	0.05	0.050	0.05	0.05	0.05	0.05
NCSU poultry mineral premix[3]	0.20	0.200	0.20	0.20	0.20	0.20
Bacitracin (antibiotic, g kg^{-1})	----	0.055	----	----		
Choline chloride 60%	0.07	0.070	0.10	0.10	0.10	0.10
Analyzed nutrient composition[4]						
Metabolizable energy (kcal kg^{-1})	3146.00	3181.00	3150.00	3111.00	3150.00	3111.00
Crude protein (%)	23.71	24.86	23.79	23.96	23.86	24.39
Crude fat (%)	6.17	6.31	5.84	5.42	5.32	5.09
Crude fiber (%)	2.30	2.30	2.30	2.20	2.20	2.60
Ash (%)	5.76	5.85	5.76	5.61	5.49	5.49
Calculated nutrient composition						
Total sulfur amino acids (%)	1.04	1.04	1.06	1.06	1.06	1.06
Lysine (%)	1.42	1.42	1.43	1.43	1.43	1.43
Calcium (%)	0.96	0.96	0.95	0.95	0.95	0.95
Available phosphorus (%)	0.48	0.48	0.48	0.48	0.48	0.48

[1]Diets used in this study included the following: (1) Unmedicated corn-soybean meal (SBM) basal without SDP (Control diet), (2) Unmedicated corn-SBM basal into which bacitracin methylene disalicylate (BMD) was added at 0.055 g kg^{-1} diet (BMD diet) and (3) SP1, SP2, SP3 and SP4 diets in which SDP was incorporated into unmedicated corn-SBM basal at 1% (10 g kg^{-1} diet), 2% (20 g kg^{-1} diet), 3% (30 g kg^{-1} diet) and 4% (40 g kg^{-1} diet), respectively. [2]Vitamin Premix, supplied per kilogram of diet; Vitamin A: 6,600 IU, Vitamin D: 1,980 IU, Vitamin E: 33 IU, Vitamin B12: 0.02 mg, Biotin: 0.13 mg, Menadione: 1.98 mg, Thiamine: 1.98 mg, Riboflavin: 6.60 mg, d-Pantothenic acid: 11.0 mg, Vitamin B6: 3.96 mg, Niacin: 55.0 mg, Folic acid: 1.1 mg. [3]Mineral Premix, supplied per kilogram of diet; Manganese (Mn): 60 mg, Zinc (Zn): 60 mg, Iron (Fe): 40 mg, Copper (Cu): 5 mg, Iodine (I): 1.2 mg, Cobalt (Co): 0.5 mg. [4]Experimental diets were analyzed for proximate nutrient composition by Eurofins Scientific Inc. Nutrient Analysis Center, 2200 Rittenhouse Street, Suite 150, Des Moines, IA 50321

Determination of intestinal maltase activity: Frozen duodenal, jejunal and ileac tissue samples were thawed on ice. Next, 30 mg of each sample was weighed and homogenized in 15 mL 0.9% saline for 30 sec using a Polytron® PT 2500 E (Kinematica AG, Switzerland). The homogenized samples were then centrifuged at high speed (400× g force) for 20 min at 4°C. Supernatant was collected for each sample and subjected to total protein and maltaseactivity assays. Protein content was measured colorimetricallyaccording to the procedures in a commercial Bicinchoninic acid assay (BCA) kit (Thermo Scientific Inc., Waltham, MA). Bovine serum albumin was used as the standard protein from which a standard curve was derived. In this assay, proteins in each sample reduced Cu^{+2} to Cu^{+1} in an alkaline solution (the biuret reaction). The Cu^{+1} and BCA then formeda purple-colored complex that is read at 562 nm usinga microplate reader (AccuSkanGo, Thermo Fisher Scientific, Finland). Protein concentration of each sample was then determined in reference to the standard curve and expressed as µg protein mL^{-1}.

Maltase enzyme activity was determined according to the procedures of Dahlqvist[30] that was modified by Black *et al.*[31]. Homogenates (supernatant) were incubated with 5mM concentration of maltose [D-(+)-Maltose monohydrate] at 37°C for 15 min (Sigma-Aldrich, MO), after which tris-glucose oxidase reagent (TGO; Sigma-Aldrich, MO) was added. Thereafter, maltose was added to sample blanks, after which and all assay mixtures were incubated for an additional 30 min at 37°C for orange color development. Absorbance were read at 420 nm immediately after incubation and enzyme activity calculated from the glucose standard curve. The activity of maltase enzyme was expressed as µg glucose/min/1 mL $µg^{-1}$ protein.

Morphometric assessment of jejunal epithelium: Formalin-fixed jejunal tissues were embedded in paraffin and 5-micron sections placed on glass slides and stained with hematoxylin and eosin stain (HE). Ten well-orientated villi images were captured from each prepared histology slide using the Nikon Eclipse Ci light microscope with high resolution Canon camera (Nikon Instruments Inc., USA). The NIS elements imaging software was then used to measure the villus height (from the tip of the villus to the villus-crypt junction), villus width (length across the villus in the middle) and crypt depth (from the villus-crypt junction to the base of crypt joining the tunica muscularis) from ten well-oriented villi per bird. Villus width was measured as the average of width at one-third and two-thirds of the villus. Furthermore, the number of goblet cells (GC) were counted in each villus and cell density was calculated as number of GC per mm^2 villus area.

Statistical analyses: Data were subjected to one-way ANOVA[32], with dietary treatments (CX, MX, SP1, SP2, SP3 and SP4) as independent variables, while all data obtained on growth performance, intestinal maltase activity and jejunal morphometric evaluation were regarded as dependent variables. Significant differences among means were determined using the Tukey option of the general linear model (GLM) procedure as a post hoc test. Statements of statistical significance were based upon $p < 0.05$. Data were presented as Means±SEM.

RESULTS AND DISCUSSION

This study investigated the comparative potency of porcine SDP and BMD (antibiotic) to enhance early intestinal development in broiler chicks in a 14-day battery trial. The antibiotic, BMD, is active against Gram-positive bacteria by inhibiting cell wall synthesis and it is one of the most common growth promoters incorporated into commercial poultry diets[13,33]. The experiment conducted evaluated the effect of dietary SDP supplementation (at 0, 10, 20, 30, or 40 g kg^{-1} diet) and BMD supplementation at 0.055 g kg^{-1} diet (i.e. standard concentration allowed in poultry diets) on intestinal maltase activity, villi functional morphometrics and chick growth performance.

Growth performance: There were no differences among treatments in body weight (BW), body weight gain (BWG) and feed intake (FI) of chicks throughout the experiment (Table 2). However, differences were observed in feed conversion ratio (FCR). On day 7, chicks in SP3 treatment had superior FCR (0.997; p = 0.0446) compared to chicks in SP4 treatment (1.105) but not were statistically different from the FCR values obtained for CX, MX, SP1 and SP2 treatments. Interestingly, by day 14 of experiment, FCR was similar for chicks in MX (1.170), SP3 (1.147) and SP4 (1.166). In addition, the FCR values for SP3 and SP4 were superior (p = 0.0004) to those for CX, SP1 and SP2 treatments. The cumulative growth performance data showed that only SP3 and to a lesser degree SP4, had superior FCR (p = 0.0481) compared to CX. However, the FCR values for SP3 and SP4 remained comparable to that of MX treatment. In a 28-day battery cage trial, Jamroz *et al.*[34] similarly reported that incorporation of SDP at 2 and 4% level of broiler chick diets improved BW and FCR at day 14. The present study agrees with the findings of Campbell *et al.*[26] who stated that dietary SDP frequently enhance growth performance in neonatal poultry.

Table 2: Effect of SDP supplementation on growth performance of broiler chicks

	Parameters measured[2]			
Treatments[1]	Body weight (BW, kg bird^{-1})[3]	Body weight gain (BWG, kg bird^{-1})	Feed intake (FI, kg bird^{-1})	FCR[4] (kg kg^{-1})
Day 7				
CX	0.1750	0.1370	0.1420	1.0450^{ab}
MX	0.1680	0.1270	0.1340	1.0550^{ab}
SP1	0.1700	0.1300	0.1370	1.0550^{ab}
SP2	0.1700	0.1300	0.1410	1.0770^{ab}
SP3	0.1700	0.1320	0.1310	0.9970^{b}
SP4	0.1700	0.1300	0.1420	1.1050^{a}
SEM	0.0040	0.0040	0.0050	0.0210
p-value	0.8966	0.7006	0.4672	0.0446
Day 14				
CX	0.4550	0.3120	0.3980	1.2760^{a}
MX	0.4500	0.2880	0.3520	1.2210^{ab}
SP1	0.4480	0.2780	0.3550	1.2760^{a}
SP2	0.4550	0.2820	0.3600	1.2780^{a}
SP3	0.4720	0.3050	0.3500	1.1470^{b}
SP4	0.4650	0.3000	0.3500	1.1660^{b}
SEM	0.0120	0.0090	0.0150	0.0300
p-value	0.7177	0.0830	0.1457	0.0004
Cummulative[5]				
CX	0.4550	0.4490	0.5400	1.2030^{ab}
MX	0.4500	0.4150	0.4860	1.1700^{abc}
SP1	0.4480	0.4080	0.4920	1.2060^{abc}
SP2	0.4550	0.4120	0.5010	1.2170^{a}
SP3	0.4720	0.4370	0.4810	1.1000^{c}
SP4	0.4650	0.4300	0.4920	1.1430^{bc}
SEM	0.0120	0.0130	0.0210	0.0220
p-value	0.7177	0.1366	0.3125	0.0481

[a-c]Mean values bearing different superscript letters within a column are significantly different ($p < 0.05$). [1]Treatment CX consisted of chicks fed unmedicated corn-soybean meal (SBM) basal without SDP; Treatment MX consisted of chicks given unmedicated corn-SBM basal into which bacitracin methylene disalicylate (BMD) was added at 0.055 g kg^{-1} diet; Treatments SP1, SP2, SP3 and SP4 consisted of chicks given unmedicated corn-SBM basal with SDP added at 10, 20, 30 and 40 g kg^{-1} diet, respectively. [2]Values represent the mean of 4 replicate pens per treatment. [3]Values are based only on weight of live birds. [4]FCR: Feed conversion ratio calculated as feed-to-gain ratio and adjusted for mortality by including the gains of dead birds in the calculations. [5]Cummulative: Growth performance data from day 1-14

Table 3: Effect of SDP supplementation on intestinal maltase activity (μg glucose min^{-1} mL^{-1} μg^{-1} protein)

	Duodenum			Jejunum			Ileum		
Treatments[1]	Day 3	Day 7	Day 14	Day 3	Day 7	Day 14	Day 3	Day 7	Day 14
CX	0.7390	0.5620	0.3930	2.5620	2.2950^{b}	0.4300^{a}	0.7420	3.0160^{c}	3.0250^{c}
MX	0.7400	0.5620	0.3930	2.5760	2.2930^{b}	0.4250^{a}	0.7500	3.0420^{ab}	3.0650^{a}
SP1	0.7420	0.5630	0.3880	2.5680	2.2910^{b}	0.3590^{b}	0.7430	3.0460^{a}	3.0360^{b}
SP2	0.7430	0.5630	0.3900	2.5550	2.5230^{a}	0.3580^{b}	0.7450	3.0190^{c}	3.0580^{a}
SP3	0.7400	0.5650	0.3830	2.5750	2.2370^{c}	0.3590^{b}	0.7450	3.0460^{a}	3.0650^{a}
SP4	0.7320	0.5630	0.3930	2.5730	2.2590^{cb}	0.3620^{b}	0.7540	3.0360^{b}	3.0450^{b}
SEM	0.0030	0.0010	0.0040	0.0160	0.0150	0.0020	0.0030	0.0030	0.0040
p-value	0.2771	0.3689	0.3281	0.9173	<0.0001	<0.0001	0.0789	<0.0001	<0.0001

[a-c]Mean values bearing different superscript letters within a column are significantly different ($p < 0.05$). [1]Treatment CX consisted of chicks fed unmedicated corn-soybean meal (SBM) basal without SDP; Treatment MX consisted of chicks given unmedicated corn-SBM basal into which bacitracin methylene disalicylate (BMD) was added at 0.055 g kg^{-1} diet; Treatments SP1, SP2, SP3 and SP4 consisted of chicks given unmedicated corn-SBM basal with SDP added at 10, 20, 30 and 40 g kg^{-1} diet, respectively

Intestinal maltase activity: Measurement of intestinal maltase activity is an established marker of intestinal maturation and digestive capability[11,35]. In this study, comparison of intestinal segments on day 3, 7 and 14 revealed that duodenum had the lowest level of maltase activity compared to jejunum and ileum (Table 3). The present study agrees with the findings of previous studies which reported that intestinal maltase activity is highest in the distal jejunum and proximal ileum of chicks[36-38]. This is expected because the jejunum and to a lesser extent the ileum are the main sites for glucose absorption in the intestine[38]. Developmental trends in maltase activity was also evaluated. In this study, maltase

Table 4: Effect of SDP on jejunal villi morphometric indices (day 7)

Treatments[1]	Villus height (μm)	Villus width (μm)	Crypt depth (μm)	Villus: crypt ratio	Density of goblet cells[2]
CX	381.2400	145.1200	57.9900	6.9500[a]	151.0000[c]
MX	385.3400	139.0000	82.0600	4.8400[b]	239.0000[a]
SPI	405.1000	152.1600	85.7000	5.0000[b]	181.0000[b]
SP2	349.8600	177.2100	76.0600	4.8900[b]	193.0000[b]
SP3	376.9000	164.8900	87.0100	4.5900[b]	242.0000[a]
SP4	377.9400	174.7500	74.5700	5.3300[b]	201.0000[b]
SEM	41.8800	14.4000	10.2200	0.2640	0.0090
p-value	0.9812	0.4150	0.5208	0.0006	<0.0001

[a-c]Means bearing different superscripts within the same column differ significantly (p <0.05). Number of observations per mean n = 16. [1]Treatment CX consisted of chicks fed unmedicated corn-soybean meal (SBM) basal without SDP; Treatment MX consisted of chicks given unmedicated corn-SBM basal into which bacitracin methylene disalicylate (BMD) was added at 0.055 g kg^{-1} diet, Treatments SP1, SP2, SP3 and SP4 consisted of chicks given unmedicated corn-SBM basal with SDP added at 10, 20, 30 and 40 g kg^{-1} diet, respectively. [2]Density of goblet cells: No. of goblet cells per mm^2 area of villi

activity was highest on day 3 and lowest on day 14 in the duodenum and jejunum (Table 3). However, the reverse was the case in the ileum with maltase activity being lowest on day 3 and increasing thereafter. A similar trend was observed by Uni *et al.*[9] for maltase activity in the duodenum, jejunum and ileum of broiler chicks from hatch (day-old) to 12 days of age.

Differences in maltase activity among treatments were observed only in the jejunum and ileum on day 7 and 14 (Table 3). On day 7, SP2 had higher maltase activity ($p < 0.0001$) in the jejunum compared to all other treatments. Similarly, Beski *et al.*[27] reported that dietary SDP improved intestinal maltase activity in 24-d old chicks. In the ileum, SP3 and SP4 had higher maltase activity ($p < 0.0001$) compared to CX, with other treatments in-between. On day 14, in the jejunum, the CX (0.430) and MX (0.425) had higher maltase activity ($p < 0.0001$) than all SPD treatments (0.358-0.362). However, in the ileum, maltase activity was higher for all chicks that consumed SDP (3.036 to 3.065; $p < 0.0001$) compared to CX (3.025). Thus, SDP induced higher maltase activity in the ileum of chicks at day 7 and 14 of experiment. This in turn possibly enhanced glucose absorption and subsequently culminated in improved FCR for SP3 and SP4 chicks to levels comparable to MX chicks.

Jejunal villi morphometrics: On day 7 of experiment, there were no differences in the villus height, villus width and crypt depth among treatments (Table 4). However, villus: crypt ratio was lower ($p < 0.05$) in all SDP treatments and MX, compared to CX. Similarly, Beski *et al.*[27] reported that broiler chicks given porcine SDP-containing wheat- or maize-based diets had lower villi: crypt ratio ($p < 0.05$) compared to the control at 24 days of age. In addition, these chicks had significantly longer villi and deeper crypts. The mechanistic implications of the villi: crypt ratio in the small intestine lies in the established fact that higher villus/crypt ratio is indicative of an increases in villi height and absorptive surface area for absorption, while a lower villus/crypt ratio is indicative of the presence of deeper crypts which allows greater renewal of the villi[39]. Accordingly, the low villus/crypt ratio observed in this study indicates that like dietary BMD antibiotic in the diet given to MX chicks, dietary SDP stimulated villi renewal in the intestine of SP1, SP2, SP3 and SP4 chicks.

Dietary supplementation of SDP increased goblet cell density in this study (Table 4). Specifically, on day 7, like MX treatment, all SP treatments had higher goblet cell numbers ($p < 0.05$) compared to CX. However, only SP3 (242 cells/mm^2 villi area) showed similar potency to MX (239 cells/mm^2 villi area) in increasing goblet cell numbers. Mucin secreted by goblet cells is the main component of the protective gastrointestinal mucosal layer, which is the first line of defense that protects the intestinal epithelium against damage and infections by pathogenic bacteria[5,40,41].

CONCLUSION

Results from this study indicated that similar to BMD antibiotic, dietary supplementation of porcine SDP in broiler chick diets at 30 or 40 g kg^{-1} improved FCR, increased ileal maltase activity on day 7 and 14, reduced villus/crypt ratio (indicative of ongoing villi renewal/regeneration) and increased jejunal goblet cell density compared to CX treatment ($p < 0.05$). It was concluded that SDP in broiler chick starter diets at 30 or 40 g kg^{-1} had similar efficacy to BMD (at 0.055 g kg^{-1} diet) in enhancing early intestinal development. Further investigation is needed to determine possible growth-promoting interaction(s) of SDP with intestinal microbiota in the intestine of poultry.

SIGNIFICANCE STATEMENT

This study discovered the dietary concentration of SDP that was comparable to BMD antibiotic in enhancing intestinal development in young chicks. This study will encourage

poultry producers to consider supplementing the diets for neonate poultry with SDP, to boost intestinal digestive function and early growth.

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