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SUPPLEMENT ARTICLE

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The effect of dietary supplementation with globin and spray-dried porcine plasma on performance, digestibility and histomorphological traits in broiler chickens

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Abstract

This study evaluated the effects of globin and spray-dried porcine plasma (SDPP) on growth performance, digestibility, nitrogen retention, energy retention efficiency (ERE) and intestinal morphology of broiler chickens. A total of 336-day-old male broiler chickens were reared from 1 to 40 days of age and fed 3 diets (8 replicates/diet, 14 birds/replicate) during 3 feeding phases: starter (1–12 days), grower (12–25 days) and finisher (25–40 days). Isonitrogenous diets were formulated by replacing gluten protein isolate contained in the control diet (C diet) with 2% (starter) or 1% (grower and finisher) spray-dried porcine plasma in the plasma diet (SDPP diet). The globin diet (G diet) was obtained by adding globin on the top of C diet at a dose of 0.08% for the whole rearing period. Total tract apparent digestibility (aD), nitrogen retention and ERE were assessed during the three growing phases. At 12 and 40 days of age, one bird per pen was slaughtered to sample gut, liver, spleen and bursa of Fabricius for histomorphological investigations. The SDPP diet increased body weights of chickens at 12 (+60 g; $p < .001$), 25 (+101 g; $p < .001$) and 40 days (+130 g; $p = .018$) of age compared to C and G diets. Also SDPP improved crude protein aD (+9.7%) and ERE (+12.3%) during the starter phase ($p < .001$). Dietary globin and SDPP inclusion did not affect either the gut morphology or the histopathological findings in birds at 12 and 40 days of age, despite a numerical (+6.90% and +7.40% respectively) villus height improvement in the SDPP group. Overall, these results confirm that dietary supplementation with SDPP and, to a lesser extent, with globin can improve growth performance and dietary protein and energy utilization in broiler chickens without effect on gut functionality.

KEYWORDS

blood by-products, broiler chickens, digestibility, histomorphology, performance

1 | INTRODUCTION

Some protein sources can provide benefits in terms of improved animal health and productive performance, which can reduce the use of antimicrobials. Among these feedstuffs, processed blood by-products obtained from slaughterhouses, such as globin and spray-dried porcine plasma (SDPP), can be used in the diets for broiler chickens (Beski, Swick, & Iji, 2015a; Boyer et al., 2015; Dabbou et al., 2019).

The SDPP is considered a high quality protein source with functional properties (Henn et al., 2013); it contains approximately 78% crude protein (CP) with high levels of essential amino acids (AA), such as lysine, tryptophan and threonine (Stein, 1996). It has been found to increase the surface area of villi and thus intestinal absorptive function, which may improve feed efficiency and nutrient utilization (Thomson, Jones, & Eisen, 1995). In piglets, SDPP has been used as a source of highly digestible and palatable protein in starter diets, to increase protection against enteric infections and to enhance intestinal development over the stressful weaning period (Coffey & Cromwell, 2001; van Dijk, Everts, Nabuurs, Margry, & Beynen, 2001; Müller et al., 2018; Pérez-Bosque, Polo, & Torrallardona, 2016; Torrallardona, 2010). In poultry diets, SDPP improved nutrient digestibility (Campbell, Quigley, Russel, & Kidd, 2003) as well as growth performance of broilers and turkeys raised under bad sanitary conditions (Campbell, Quigley, & Russel, 2004; Campbell et al., 2003). Jamroz, Wiliczkiewicz, Orda, Kuryszko, and Stefaniak, (2012) also showed that SDPP was as effective as soybean meal in increasing the body weight of broilers and enhancing sodium and potassium retention.

Globin is produced by hydrolysis of porcine haemoglobin into haem and globin, and is considered as a protein-based emulsifier that acts in the duodenum and jejunum (Arnouts & Lippens, 2006; Dabbou et al., 2019). Emulsifiers act synergistically with natural bile salts in the animal's gut to favour fat digestion and absorption. They increase the active surface of fats, allowing the action of lipases that hydrolyze triglyceride molecules into fatty acids and monoglycerides, and favour the formation of micelles consisting of lipolysis products. Previous studies showed that the dietary supplementation with globin significantly improved feed conversion ratio (FCR) between 10 and 42 days of age (Arnouts & Lippens, 2006). Recently, also Dabbou et al. (2019) found that the addition of 0.05% globin to broiler chicken diets decreased FCR and increased fat digestibility, protein metabolism, protein efficiency ratio and net energy for production during the starter phase.

Based on the above-mentioned studies, the inclusion of SDPP and globin in broiler chickens has been found to affect growth performance, but the biological mechanisms underlying these effects have not been fully elucidated whereas an effect on the physiological intestinal development and morphology, and on the health status of the gut has been hypothesized. Therefore, the purpose of this study was to assess the effects of the dietary inclusion of SDPP and globin on growth performance, digestibility, nitrogen retention, energy retention efficiency, intestinal morphology and histological traits of broiler chickens throughout the starter, grower and finisher phases.

2 | MATERIALS AND METHODS

The trial was performed by the Department of Veterinary Sciences (DVS) of the University of Turin (Italy) in collaboration with a commercial poultry house located in Airasca (TO, Italy). The experimental protocol was approved by the Ethical Committee for Animal Experimentation of the DVS (Protocol no. 420078). All animals were handled in respect to the principles stated by the EC Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes.

2.1 | Birds and husbandry

A total of 336 one-day-old male broiler chicks (ROSS 308, Aviagen) were randomly allotted to 3 dietary treatments for a 40-days rearing cycle. Each dietary group consisted of 8 pens, as replicates, with 14 chicks per pen. The pens were 1.20 m wide × 1.20 m long, with rice hulls as litter. Feed and drinking water were distributed *ad libitum* in hanging feeders (1/pen) and automatic water dispensers (4/pen). The poultry house was equipped with automatic ventilation, infrared lamps and controlled light systems. The birds were individually identified using a wing mark, applied at their arrival. Twenty-three hours of light were provided during the first three days; afterwards, light hours were progressively reduced until a 18L:6D light program. All chicks were vaccinated against Infectious Bronchitis, Gumboro disease and Newcastle disease at the hatchery.

TABLE 1 The schedule of the feeding trial

	Starter phase			Grower phase			Finisher phase		
	C diet	G diet	SDPP diet	C diet	G diet	SDPP diet	C diet	G diet	SDPP diet
Ingredient (% as fed)									
GP	2.00	2.00	–	1.00	1.00	–	1.00	1.00	–
G	–	0.08	–	–	0.08	–	–	0.08	–
SDPP	–	–	2.00	–	–	1.00	–	–	1.00

Abbreviations: C, control; G, globin; GP, gluten protein; SDPP, spray-dried porcine plasma.

2.2 | Experimental diets

The experimental diets were prepared at the experimental facility of the Department of Agricultural, Forest and Food Science of the University of Turin. The ingredients were individually weighed and subsequently mixed in order to obtain crumble pelleted diets.

Three experimental diets were used, control (C diet), globin (G diet) and spray-dried porcine plasma (SDPP diet) (Tables 1 and 2). The SDPP diet was formulated by replacing the gluten protein isolate (lysine 1.83%; methionine 1.50%; threonine 2.40%; valine 4.30%; arginine 3.30%; glycine 2.90%; phenylalanine 4.73%; and cystine 2.02% on dry matter [DM] basis) in the C diet with spray-dried porcine plasma at a dose of 2% in the starter diet and 1% in the grower and finisher diets (SDPP, Actipro® 85PPS, Veos, Belgium; 94% DM; 13.1 MJ metabolizable energy (ME)/kg as is; 85% CP; 2% fat; lysine 4.04%; methionine 0.78%; threonine 3.10%; tyrosine 4.37%; valine 6.85%; arginine 3.83%; glycine 4.58%; phenylalanine 7.42%; and cystine 3.28% on DM basis) (Table 1). The G diet was obtained by topping the C diet with globin, at a dose of 0.08% (Globin, Actipro® 95PGS, Veos, Belgium; 93% DM; 14.4 MJ ME/kg as is; 92% CP;

TABLE 2 Ingredients of the experimental diets (% as fed)

Periods on trial	Starter phase		Grower phase		Finisher phase	
Diets	C and G diets[a]	SDPP diet	C and G diets[a]	SDPP diet	C and G diets[a]	SDPP diet
Maize meal	43.085	43.085	35.000	35.000	35.000	35.000
Soybean meal (48% CP)	31.567	31.567	26.720	26.720	29.902	29.902
Wheat meal	15.000	15.000	27.536	27.536	23.571	23.571
Soybean oil	2.307	2.307	3.192	3.192	3.000	3.000
Extruded soybean	2.000	2.000	3.000	3.000	3.000	3.000
Gluten protein (GP)	2.000	–	1.000	–	1.000	–
Spray-dried porcine plasma (SDPP)	–	2.000	–	1.000	–	1.000
Dicalcium phosphate	1.273	1.273	0.915	0.915	1.973	1.973
Calcium carbonate	0.576	0.576	0.595	0.595	0.694	0.694
Trace mineral-vitamin premix[b]	0.500	0.500	0.493	0.493	0.634	0.634
L-Lysine HCl	0.479	0.479	0.400	0.400	0.282	0.282
DL-methionine[c]	0.335	0.335	0.338	0.338	0.191	0.191
Sodium chloride	0.237	0.237	0.250	0.250	0.180	0.180
L-Threonine	0.150	0.150	0.131	0.131	0.180	0.180
Sodium bicarbonate	0.131	0.131	0.100	0.100	0.100	0.100
Optifos 250 Bro[d]	0.100	0.100	0.100	0.100	0.100	0.100
Avizyme 1500x[e]	0.100	0.100	0.100	0.100	0.083	0.083
Choline	0.100	0.100	0.070	0.070	0.060	0.060
Maxiban[f]	0.060	0.060	0.060	0.060	0.050	0.050
AME (MJ/kg DM)	12.59	12.53	12.65	12.62	13.31	13.28
Total	100.00	100.00	100.00	100.00	100.00	100.00

Abbreviations: AME, apparent metabolisable energy; C, control; CP, crude protein; DM, dry matter; G, globin; GP, gluten protein; SDPP, spray-dried porcine plasma.

[a]G diet has the same ingredients of C diet, and globin (as Actipro® 95PGS) was added on top at the dose of 0.08%.

[b]Mineral–vitamin premix (per kg diet): vitamin A (retinyl acetate), 12,500 IU; vitamin D3 (cholecalciferol), 3,500 IU; vitamin E (DL-a-tocopheryl acetate), 40 mg; vitamin K (menadione sodium bisulphite), 2 mg; biotin, 0.20 mg; tiamin, 2 mg; riboflavin, 6 mg; pantothenate, 15.21 mg; niacin, 40 mg; choline, 750 mg; pyridoxin, 4 mg; folic acid, 0.75 mg; vitamin B12, 0.03 mg; Mn, 70 mg; Zn, 62.15 mg; Fe, 50 mg; Cu, 7 mg; I, 0.25 mg; Se, 0.25 mg.

[c]DL-methionine: analogous hydroxy methionine (Rhodimet AT88, Adisseo, Antony, France).

[d]Optifos 250 Bro (Huvepharma, Sofia, Bulgaria): Phytase (EC 3.1.3.26), 250 OTU/kg diet.

[e]Avizyme 1500 (Danisco Animal Nutrition, Marlborough, Wiltshire, UK): complex of endo 1–4-beta-xylanase (EC 3.2.1.8) (256 U/kg), subtilisine (Ec 3.4.21.62) (2,560 U/kg diet) and alpha-amylase (EC3.2.1.1), 1,472 U/kg diet.

[f]Maxiban (Elanco, Italy): anticoccidial composed of Nicarbazin 80,000 mg/kg and Narasin 80,000 mg/kg.

0.3% fat; lysine 5.34%; methionine 0.79%; threonine 3.70%; tyrosine 2.27%; valine 3.81%; arginine 3.62%; glycine 2.41%; phenylalanine 3.13%; and cystine 3.37% on DM basis). Both SDPP and globin supplementation doses were established according to the manufacturer's recommendations. All diets were formulated according to Aviagen (2014) specifications, identifying three different phases: starter (1–12 days), grower (12–25 days) and finisher (25–40 days) (Table 2 and 3) and were always provided *ad libitum*.

2.3 | Growth performance

The body weights (BW) of birds were recorded individually at their arrival and at the end of each feeding phase. Average daily feed intake (ADFI), average daily weight gain (ADG) and FCR were calculated for each experimental group within feeding phase and the entire rearing period. Feed intake and all other measurements were performed at pen level. All weighings were performed using electronic scales with an accuracy of 0.1g (Signum, Sartorius). During the trial, health was always good and only eight (2.4%) chickens died (two fed C diet, four fed G diet and two fed SDPP diet).

2.4 | Nutrient Digestibility

The digestibility trial was performed at the end of the starter phase using titanium dioxide (TiO_2; 5 g/kg diet) as an indigestible marker, which was added during the preparation of the experimental starter diet. The method of Kaczmarek, Bochenek, Samuelsson, and Rutkowski, (2015) was used to estimate the nutrient digestibility, with minor modifications reported by Dabbou et al. (2019). Briefly, excreta were collected for approximately 1 hr per day, for four consecutive days. After collection, the excreta of each pen were pooled and stored at −20°C, until freeze-drying and analyses. The apparent nutrient digestibility (aD) was calculated for CP and ether extract (EE). The uric acid (UA) content in the excreta samples was determined by a spectrophotometer (UNICAN UV–Vis Spectrometry, Helios Gamma, Fisher Scientific Ltd, Loughborough, UK) according to the Marquardt (1983) method. The CP amount of the excreta was corrected (CP corrected) for UA nitrogen as follows:

$$\text{CPcorrected} = \left([N]_{\text{Kjeldahl}} - [N]_{\text{uric acid}} \right) \times 6.25$$

To calculate the digestibility coefficients, the titanium dioxide contents of feed and freeze-dried excreta were measured by the colorimetric method described by Myers, Ludden, Nayigihugu, and Hess, (2004). The apparent digestibility (aD) of each nutrient (X) was calculated as follows:

$$\text{aD X}(\%) = \left(1 - \frac{[TiO_2]\ \text{diet} \times [\text{nutrient X}]\ \text{excreta}}{[TiO_2]\ \text{excreta} \times [\text{nutrient X}]\ \text{diet}} \right) \times 100$$

where [nutrient X]diet and [nutrient X]excreta are the measured contents of the nutrients (X) in the diets and excreta (g/kg), respectively, and $[TiO_2]$diet and $[TiO_2]$excreta are the measured contents of titanium dioxide in the diet and excreta (g/kg), respectively. Nitrogen retention was calculated using the digestibility formula and considering the excreta total CP.

2.5 | Slaughtering and sampling

A total of 56 chickens (8 birds at 1 day, 24 birds at 12 day and 24 birds at 40 day of age) were sacrificed by intravenous injection of pentobarbital sodium in the wing vein, and the whole body was stored to determine the whole-body energy. Further 48 chickens (24 birds at

TABLE 3 Analysed chemical composition of experimental diets during the rearing periods

	Starter phase			Grower phase			Finisher phase		
Diets	**C**	**G**	**SDPP**	**C**	**G**	**SDPP**	**C**	**G**	**SDPP**
DM, %	91.3	91.4	92.0	91.3	91.6	91.3	92.0	91.6	91.2
Ash, % DM	5.64	6.26	5.65	5.72	5.08	5.04	4.42	4.22	4.91
CP, % DM	23.7	25.0	24.0	23.2	23.4	24.0	21.2	20.1	21.2
EE, % DM	5.72	5.94	6.01	5.88	5.18	5.17	6.93	6.23	7.11
CF, % DM	2.25	2.21	2.05	2.65	2.63	2.57	2.72	2.56	2.57
NFE, % DM	62.4	60.4	61.8	62.2	63.2	62.5	64.4	66.5	63.6
Sugar + starch, % DM	45.3	44.4	43.4	44.5	46.0	46.9	46.7	50.4	46.2
GE, MJ/kg DM	18.96	18.94	18.81	18.81	18.75	18.81	18.97	19.09	19.16
Lysine, % DM	1.539	1.543	1.572	1.384	1.387	1.406	1.138	1.142	1.170
Methionine, % DM	0.694	0.694	0.674	0.674	0.674	0.667	0.510	0.510	0.507
Threonine, % DM	1.093	1.095	1.099	0.971	0.973	0.978	0.840	0.842	0.854

Abbreviations: C, control; CF, crude fibre; CP, crude protein; DM, dry matter; EE, ether extract; G, globin; GE, gross energy; NFE, nitrogen-free extract; SDPP, spray-dried porcine plasma.

12 day and 24 at 40 day of age) were slaughtered to sample organs for histomorphological investigations.

2.6 | Analyses of diets, excreta and whole body

Diets and freeze-dried excreta were ground through a 1-mm screen (Retsch ZM 200 Ultra Centrifugal Mill, Retsch). The DM content was determined by drying the samples at 103°C to constant weight. Ash content was determined by muffle furnace incineration (942.05), CP (N × 6.25) was measured with Kjeldahl method (2001.11), crude fibre (CF) was determined after sulphuric acid treatment (962.09 method) and EE by ether extraction (920.39) according to the AOAC (2005) standard procedures. The nitrogen-free extract (NFE) was calculated as follow: NFE% = [DM − (Ash% + CP% + EE% + CF%)], whereas the content of sugar + starch was measured polarimetrically (BOE, 2000). The gross energy (GE) content was determined using an adiabatic calorimetric bomb (C7000, IKA).

For the determination of amino acids contents in feed supplements (Globin, SDPP and gluten protein) and diets, the samples were hydrolysed with 6 N HCl for 22 hr at 112°C under nitrogen atmosphere. In the case of methionine and cystine, performic acid oxidation occurred prior to acid hydrolysis. The amino acids in hydrolysate were performed by HPLC (Waters) after post-column derivatization using the procedure indicated by Madrid et al. (2013).

The bodies of the chickens slaughtered at 0, 12 and 40 days of age were minced, homogenized and freeze-dried. Then, samples were used to determine the whole-body energy (GE_{bird}, MJ/bird) according to Fatufe, Timmler, and Rodehutscord, (2004) by adiabatic calorimetric bomb.

2.7 | Calculation of Energy Retention Efficiency

The difference between GE_{bird} at 12 and 0 days, at 40 and 12 days and at 40 and 0 days of age defined energy accretion (EA) during each phase (starter, grower and whole rearing periods). The GE intake was given by the product of FI and GE of diet during each growth phase. The energy retention efficiency (ERE, %) was calculated as the ratio between EA and GE intake during each growing phase, as reported by Dabbou et al. (2019), being dy and dx the final and first day of each period respectively.

$$\mathrm{ERE}\,(\%) = \frac{\mathrm{EA}}{\mathrm{GE_{intake}}} = \frac{\mathrm{GE_{bird\,dy}} - \mathrm{GE_{bird\,dx}}}{(\mathrm{FI}\,(\mathrm{dy} - \mathrm{dx})) * \mathrm{GE_{diet}}}$$

2.8 | Histomorphological investigations

A total of 48 birds were slaughtered at 12 and 40 days of age (8 animals per group per age and 1 animal per pen per age) and submitted to histomorphological investigations. Intestinal samples (approximately 5 cm in length) of duodenum (the duodenal loop), jejunum (the portion before the Meckel's diverticulum) and ileum (the tract before the ileocolic junction) were excised and flushed with 0.9% saline to remove the digesta. Samples of liver, spleen and bursa of Fabricius were also collected. Gut segments were fixed in Carnoy's solution for morphometric analysis, whereas the samples of other organs were fixed in a 10% buffered formalin solution for histopathological examination. Tissues were routinely processed, embedded in paraffin wax blocks, sectioned at 5 μm thickness, mounted on glass slides, stained with haematoxylin and eosin and examined by light microscopy. A total of three serial sections were prepared for each intestinal segment, and the same slide among the serial sections was considered for morphometric analyses. In particular, 10 well-oriented and intact villi and 10 crypts, selected from duodenum, jejunum and ileum, were measured per each bird (Qaisrani et al., 2014). The evaluated morphometric indices were the villus height (Vh, from the tip of the villus to the crypt), the crypt depth (Cd, from the base of the villus to the submucosa) and their ratio (Vh/Cd) (Laudadio et al., 2012). A total of three serial sections were prepared for each sampled organ too, with the most representative one per bird being considered for the histopathological examination. In particular, the following histopathological alterations were evaluated: hepatocyte degeneration and lymphoid tissue activation in the liver, white pulp hyperplasia or depletion in the spleen, and follicular depletion in the bursa of Fabricius. The observed histopathological alterations were assessed using a semi-quantitative scoring system as follows: absent (score = 0), mild (score = 1), moderate (score = 2) and severe (score = 3) as detailed by Biasato et al. (2017) and Dabbou et al. (2018). The slides were blinded evaluated by three different observers and the discordant cases were reviewed using a multi-head microscope until an unanimous consensus was reached.

2.9 | Statistical analysis

The statistical analysis of data was performed using the IBM SPSS software package (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, version 21.0. IBM Corp).

The experimental unit was the pen for growth performance and apparent nutrient digestibility and the bird for gut morphometric indices and organ histopathological traits. The assumption of equal variances was assessed by Levene's homogeneity of variance test. Growth performance, apparent nutrient digestibility, nitrogen retention and ERE data were tested by means of one-way ANOVA. The differences were tested using Duncan's new multiple range. Intestinal morphometric indices were analysed by fitting a general linear mixed model (GLMM). The GLMM allowed the morphometric indices (Vh, Cd and Vh/Cd, separately) to depend on three fixed factors (diet, intestinal segment and interaction between diet and intestinal segment) with the animal included as a random effect to account for repeated measurements on the same bird. The interactions between the levels of the fixed factors were evaluated by pairwise comparisons. The histopathological scores were analysed by means of the Kruskal–Wallis test (post

hoc test: Dunn's multiple comparison test). The results were expressed as means with pooled standard errors of the means (*SEM*). Significance was declared at $p \leq .05$.

3 | RESULTS

3.1 | Growth performance

The dietary treatment increased BW at 12, 25 and 40 days of age ($p < .001$; $p < .001$ and $p = .018$, respectively) in chickens fed the SDPP diet compared to the other groups (Table 4). During the starter period, the greatest ADG (28.4 g/day *versus* 22.4 and 23.6 g/day; $p < .001$) and ADFI (33.1 g/day versus 30.5 and 31.5 g/day; $p = .031$) were found in chickens fed the SDPP diet compared to those fed the C and G ones respectively. Moreover, a lower FCR was found in chickens fed SDPP diet compared to birds fed the C diet (1.16 versus 1.37, respectively; $p < .001$). During the entire rearing cycle, ADG was higher and FCR was lower in the SDPP group compared to the C and G ones.

TABLE 4 Effects of dietary globin and SDPP on growth performance of broiler chickens ($n = 8$)

	C diet	G diet	SDPP diet	*SEM*	*p*-value
Starter phase (1–12 days)					
BW at 1 day, g	48.6	48.8	48.7	0.11	.651
BW at 12 days, g	295[b]	309[b]	362[a]	6.70	<.001
ADG, g/day	22.4[b]	23.6[b]	28.4[a]	0.61	<.001
ADFI, g/day	30.5[b]	31.5[b]	33.1[a]	0.42	.031
FCR, g/g	1.37[a]	1.33[a]	1.16[b]	0.022	<.001
Grower phase (12–25 days)					
BW at 25 days, g	1144[b]	1181[b]	1264[a]	15.31	.001
ADG, g/day	61.2	62.4	64.6	0.76	.176
ADFI, g/day	89.9	93.0	94.4	1.00	.180
FCR, g/g	1.47	1.49	1.46	0.017	.786
Finisher phase (25–40 days)					
BW at 40 days, g	2416[b]	2430[b]	2553[a]	22.69	.018
ADG, g/day	83.9	82.4	85.9	0.81	.210
ADFI, g/day	148.2	150.7	149.0	1.43	.789
FCR, g/g	1.77	1.83	1.73	0.017	.075
Whole period (1–40 days)					
ADG, g/day	59.0[b]	59.1[b]	62.6[a]	0.58	.010
ADFI, g/day	95.6	97.8	98.1	0.75	.336
FCR, g/g	1.62[a]	1.65[a]	1.57[b]	0.011	.001

Note: The means with different superscript letters (a, b) within the same row differ significantly ($p < .05$).

Abbreviations: ADFI, average daily feed intake; ADG, average daily weight gain; BW, body weight; C, control; FCR, feed conversion ratio; G, globin; SDPP, spray-dried porcine plasma.

TABLE 5 Effect of dietary globin and SDPP on apparent nutrient digestibility (aD) and nitrogen retention of broiler starter feed ($n = 8$)

	C diet	G diet	SDPP diet	*SEM*	*p*-value
aD_{CP}	87.1[b]	95.6[a]	95.5[a]	0.91	<.001
aD_{EE}	95.1	95.4	95.8	0.20	.462
N retention	68.1[c]	73.7[a]	70.3[b]	0.607	<.001

Note: The means with different superscript letters (a, b, c) within the same row differ significantly ($p < .05$).

Abbreviations: C, control; CP, crude protein; EE, ether extract; G, globin; *N* retention, nitrogen retention; SDPP, spray-dried porcine plasma.

3.2 | Nutrient digestibility

No significant difference was observed among dietary groups for the digestibility of EE. The digestibility of CP was higher in chickens fed the G and SDPP diets (95.6% and 95.5%, respectively) compared to the C diet (87.1%) ($p < .001$; Table 5). Nitrogen retention was highest in the G diet (73.7%), followed by the SDPP diet (70.3%) and significantly lowest in the C diet (68.1%) ($p < .001$).

3.3 | Energy retention efficiency

During the starter phase, the ERE was higher ($p = .004$) in chickens fed the SDPP diet compared to those with the C and G diets (36.04 *versus* 32.43 and 31.67%, respectively; Table 6). Nevertheless, no significant difference among treatments was observed for ERE during the grower–finisher phase (12–40 days of age) and during the overall rearing period (1–40 days of age).

3.4 | Histomorphological investigations

The dietary inclusion of either SDPP or globin did not influence ($p > .05$) the gut morphometric indices of birds slaughtered at 12 days or 40 days of age, despite a numerical (+6.90% and 7.40%) villus height improvement in SDPP group at 12 and 40 days of age, respectively (Table 7).

Histopathological alterations were observed in all organs regardless from the dietary treatment or the slaughtering age. The dietary

TABLE 6 Effects of dietary globin and SDPP on ERE (%) of broiler chickens ($n = 8$)

	C diet	G diet	SDPP diet	*SEM*	*p*-value
1–12 days of age	32.43[b]	31.67[b]	36.04[a]	0.618	.004
12–40 days of age	33.66	33.83	33.51	0.392	.951
1–40 days of age	33.54	33.60	33.80	0.357	.958

Note: The means with different superscript letters (a, b) within the same row differ significantly ($p < .05$).

Abbreviations: C, control; ERE, energy retention efficiency; G, globin; SDPP, spray-dried porcine plasma.

TABLE 7 Effects of dietary globin and SDPP on intestinal morphometric indices in the broiler chickens in relation to the diet and the intestinal segment at 12 and 40 days of age (n = 8)

	Diet (D)			Intestinal segment (IS)				*p*-value		
	C	G	SDPP	Duodenum	Jejunum	Ileum	*SEM*	D	IS	D × IS
12 days of age										
Vh, mm	1.16	1.19	1.24	1.74[a]	0.99[b]	0.86[b]	0.080	.782	<.001	.359
Cd, mm	0.15	0.15	0.14	0.16[a]	0.14[b]	0.14[b]	0.010	.902	.023	.731
Vh/Cd	7.93	8.27	9.00	11.23[a]	7.52[b]	6.45[b]	0.710	.637	<.001	.768
40 days of age										
Vh, mm	1.48	1.55	1.59	2.19[a]	1.43[b]	0.99[c]	0.100	.768	<.001	.804
Cd, mm	0.17	0.17	0.19	0.23[a]	0.16[b]	0.14[b]	0.010	.587	<.001	.380
Vh/Cd	8.73	9.00	8.51	10.20[a]	9.08[a]	6.96[b]	0.540	.833	<.001	.474

Note: The means with different superscript letters (a, b and c) within the same row per fixed effect (i.e. diet, intestinal segment) differ significantly (p < .05).

Abbreviations: C, control; Cd, crypt depth; G, globin; SDPP, spray-dried porcine plasma; Vh, villus height; Vh/Cd, villus height to crypt depth ratio.

inclusion of either SDPP or globin did not affect the severity of the histopathological alterations (p > .05, Table 8). In details, the liver showed multifocal to diffuse, mild-to-severe vacuolar degeneration of the hepatocytes, as well as focal to multifocal, mild-to-moderate, interstitial and/or periportal lymphoid tissue activation. Multifocal, mild-to-severe white pulp hyperplasia or depletion was observed in the spleen. Finally, the bursa of Fabricius showed multifocal to diffuse, mild-to-severe follicular depletion, with or without intrafollicular cysts, fibrous tissue deposition and/or follicular atrophy.

4 | DISCUSSION

Processed protein sources, such as soybean protein isolate or gluten protein isolate, SDPP and globin, are valuable protein sources that can be used in poultry diets as high-nutritive feedstuffs (Peisker, 2001; Torrallardona, 2010). SDPP was found to positively influence poultry performance, digestibility and health during stressful production conditions (Campbell et al., 2003, 2004; Campbell, Russell, Crenshaw, & Koehnk, 2006). Therefore, the aim of this study was to assess the intestinal mechanisms, namely digestive efficiency and gut morphology, involved in the improvement of broiler performance fed diets supplemented with SPDD or globin.

In the current study, supplementation with globin did not lead to improved growth performance, unlike observations of previous studies (Arnouts & Lippens, 2006; Dabbou et al., 2019). Together with improved performance, Dabbou et al. (2019) found higher aD_{EE} and nitrogen retention but similar aD_{CP} in the globin (0.05%) group compared to the control group during the starter period. Also, Upadhaya, Lee, Jung, and Kim (2018) observed a higher aD_{EE} but

TABLE 8 Histopathological scores[a] of organs of the broiler chickens (n = 8)

	C diet	G diet	SDPP diet	*SEM*	*p*-value
12 days of age					
Liver					
Degenerative changes	0.25	1.00	0.56	0.162	.197
Lymphoid tissue activation	0.75	0.63	0.63	0.098	.836
Spleen	0.25	0.38	0.25	0.127	.532
Bursa of Fabricius	1.06	1.00	1.00	0.136	.944
40 days of age					
Liver					
Degenerative changes	1.88	1.75	1.31	0.187	.294
Lymphoid tissue activation	1.75	1.25	1.13	0.118	.068
Spleen	0.25	1.38	0.25	0.207	.186
Bursa of Fabricius	1.75	2.25	1.44	0.180	.112

Abbreviations: C, control; G, globin; SDPP, spray-dried porcine plasma.

[a]The data are expressed as the mean of the scores (0 = absence of alterations; 1 = mild alterations; 2 = moderate alterations; and 3 = severe alterations).

a similar aD_{CP} in broilers fed a diet supplemented with a commercial blend of emulsifiers (0.05 up to 0.10% supplementation). On the contrary, we observed that globin supplementation did not affect aD_{EE} but enhanced aD_{CP} and nitrogen retention during the starter period. However, this improvement was not associated with higher energy retention efficiency. Neither gut morphology nor the development and the severity of the organ histopathological findings were influenced by globin supplementation, and to our knowledge, no previous studies are available for any comparison. Nevertheless, the absence of a difference in histopathological scores among birds fed different diets reliably rules out a role of this feed additive in the occurrence of the observed alterations. Probably, the very good bird's performance, as revealed by the low mortality and high body weight, could be associated with the lack of the potential positive effects of globin, previously reported by Dabbou et al. (2019).

On the contrary, dietary SDPP inclusion positively influenced the growth performance of birds, especially BW, consistently with previous results (Campbell et al., 2006). In fact, higher BW and improved FCR during the starter phase (1–21 days of age) were reported in broilers fed 0.5% to 1.5% SDPP (Henn et al., 2013), 2% SDPP (Walters, Jasek, Campbell, Coufal, & Lee, 2019) and up to 4% SDPP (Jamroz et al., 2012). Other studies also showed that the enhancement of growth performance persisted from 28 days to 41 days (Walters et al., 2019) or during the whole growth period (Beski, Swick, & Iji, 2015b, 2015c). These positive results could be associated with the high digestibility of SDPP that likely contributed to improve chickens performance (Beski, Swick, & Iji, 2015b; Torrallardona, Conde, Badiola, Polo, & Brufau, 2003). In the present study, higher protein digestibility and nitrogen retention in birds fed SDPP, as compared with C group, reflect a lower nitrogen excretion in SDPP group during the starter period. Currently, there is no literature concerning the effects of SDPP on the total tract apparent digestibility coefficients of broiler chickens, which could support differences in performance. However, no effects of SDPP supplementation in broiler diets were previously reported on ileal digestibility of DM, CP and GE (Beski et al., 2015b; Jamroz et al., 2012), despite weak differences in digestibility of EE (Jamroz et al., 2012). No effects of different inclusion levels of SDPP have been reported for the total apparent digestibility coefficient of CP in weanling pigs by Sun, Ma, Li, and Ji, (2009), whereas other authors found higher DM and CP digestibility for SDPP compared to other protein sources in young pigs (Pendergraft, Hancock, Hines, Mills, & Burnham, 1993).

Energy retention efficiency provides a further indication of how efficiently broiler chickens utilize dietary energy. The higher ERE observed in the SDPP group during the starter phase, compared to the C and G groups, suggests a more efficient energy utilization in the former group compared to the latter ones. This could be potentially attributed to the higher nitrogen retention associated with SDPP supplementation.

On the other hand, in the present study, SDPP supplementation did not affect the gut morphology of the broiler chickens nor the development or the severity of the organ histopathological traits. However, a numerical villus height improvement in SDPP group was observed at 12 and 40 days of age. The magnitude of this increment (+6.90% and +7.40% respectively) is similar or even higher than that reported by other authors (Beski, Swick, & Iji, 2015c; Jamroz et al., 2011, 2012; King et al., 2005) when comparing similar age, intestinal segment and SDPP inclusion level. The lack of statistical significance in our study could be associated with a high variability among the collected samples. Thus, we may hypothesize that the improved growth performance in chickens fed SDPP diets could also be related to changes in gut morphology, even if this observation needs further investigation to be confirmed.

Finally, regardless of dietary treatment, we confirmed that the chickens' duodenum had greater development in relation to the other gut segments. Indeed, the identification of a proximodistal decreasing gradient of the morphometric indices from the duodenum to the ileum has previously been demonstrated to represent the physiological gut development (Forder, Howarth, Tivey, & Hughes, 2007; Iji, Saki, & Tivey, 2001; de Verdal et al., 2010).

5 | CONCLUSIONS

Our findings and literature data report an improvement of growth performance, total tract aD of protein, nitrogen retention and ERE during the starter period in broilers fed SDPP and to a lower extent, globin diets. Nevertheless, improved performance and digestibility traits could not be explained by significant changes at the level of gut morphology, even if promising results have been observed. Under the normal rearing conditions of this experiment, SDPP has greatest potential as a feed ingredient for broiler chickens during the starter period, with positive impact on production performance and without negative effects on gut health.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

ANIMAL WELFARE STATEMENT

The experimental protocol was approved by the Ethical Committee for Animal Experimentation of the DVS (Protocol n. 420078). All animals were handled in respect to the principles stated by the EC Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes.

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