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Dietary spray-dried plasma enhances the growth performance, villus:crypt ratio and cold-induced stress resistance in Nile tilapia (*Oreochromis niloticus*)

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ABSTRACT

Nutritional strategies can help fish manage stress, and functional feedstuffs are an interesting nutritional option. Therefore, this study evaluated the potential functional effect of spray-dried plasma (SDP) on Nile tilapia growth performance and the capacity of SDP to improve fish health under cold-induced stress (CIS). A total of 440 fish (12.64 ± 0.64 g) were randomly distributed into 40,250-L aquaria and fed five diets containing graded levels of SDP (0, 16.6, 33.2, 49.7 and 66.3 g kg^{-1}) for 60 days. The growth performance, villus height:crypt depth ratio, and hematological parameters were analyzed, and the same hematological parameters were then analyzed after 7 days of CIS. Based on the broken-line analysis of the FBW, SGR, RWG and FCR, the optimum dietary level supplementation of SDP was determined to be 49.70, 50.16, 51.83 and 41.83 g kg^{-1} diet, respectively. The crypt depth and villus:crypt ratio were positively affected by SDP supplementation. After CIS, hematocrit of fish fed 16.6 g kg^{-1} of dietary SDP was significantly lower than fish fed 66.3 g kg^{-1} level ($P < 0.05$). The supplementation level of dietary SDP and the CIS affected the leukocyte, lymphocyte and neutrophil counts. The monocyte count was affected by the dietary SDP supplementation level both before and after CIS ($P < 0.05$). Total plasma protein concentration in the fish fed 49.7 and 66.3 g kg^{-1} SDP were significantly higher than fed 16.6 g kg^{-1} SDP after CIS ($P < 0.05$), and CIS led to a higher Albumin:Globulin ratio ($P < 0.05$). Dietary SDP supplementation improved the growth performance, intestinal health, hematological profile and CIS resistance of the studied fish. Based on our results, we recommend a dietary supplementation level of 51.83 g kg^{-1} SDP for Nile tilapia.

1. Introduction

Spray-dried plasma (SDP) is a by-product of the slaughter industry and obtained from healthy porcine, bovine or poultry whole blood after anticoagulant treatment, erythrocyte elimination by centrifugation, and further dehydration using the spray-dry technique (van Dijk et al., 2001). SDP is a high-quality protein ingredient composed of high biological value proteins, such as immunoglobulins, albumin, fibrinogen, lipids, enzymes and transferrins (Campbell et al., 2008), and it is also rich in glutamic acid, glutamine, lysine, aspartate and threonine. Moreover, SDP is safe for use as a feed ingredient for different animal species, such as pigs (Ferreira et al., 2009; Frugé et al., 2009), poultry (Campbell et al., 2003; Campbell et al., 2004; Henn et al., 2013), cats (Rodriguez et al., 2016), rats (Pérez-Bosque et al., 2010) and fish (Gisbert et al., 2015).

Certain properties of SDP, such as its level of high-quality protein, are attributed to the spray-drying production process, which preserves the functional physicochemical and biological properties of the product (Luzier et al., 1995; Rodriguez et al., 2016). Moreover, SDP presents approximately 99% digestibility (Bureau et al., 1999; Bureau, 2006), an excellent amino acid profile and good palatability for animals primarily because of its glutamate content (Lawrence et al., 2004). Moreover, studies have shown an improvement in feed intake by piglets fed SDP in the nursery phase (Coffey and Cromwell, 2001, van Dijk et al., 2001, Ferreira et al., 2009, Rodriguez et al., 2016).

Studies on SDP dietary supplementation have shown improvements

Abbreviations: SDP, spray-dried plasma; CIS, cold-induced stress

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in the direct and indirect functional properties related to immunology, including a higher villus:crypt ratio and goblet cell number, and these changes results in better enzymatic activity, an effective immune barrier and enhanced antioxidant capacity (Gao et al., 2011; Gisbert et al., 2015). These functional proprieties may be attributable to the amino acid composition of SDP, including glutamine, glutamate and threonine, which are related to enterocyte nutrition, mucin production, energetic resources, nitrogenous base synthesis (nucleotides), mechanical barrier stimulation and innate immune response stimulation via increases in leucocytes and immunoglobulins (Pérez-Bosque et al., 2006; Campbell et al., 2010; Gao et al., 2011; Gisbert et al., 2015).

The aquafeed industry has been focusing on the development of functional feeds primarily because of the significant economic losses caused by productive stress and/or environmental challenges (Oliva-Teles, 2012; Barros et al., 2014). Although studies have shown the benefits of dietary SDP for terrestrial animals under different productive systems, to our knowledge, few reports have focused on its use in aquatic species, especially under stress conditions. Therefore, the aim of this study was to determine the optimum SDP dietary supplement level for the growth performance, villus:crypt ratio, and hematological profile of Nile tilapia under cold-induced stress.

2. Materials and methods

This research consisted of two studies. In Study I, Nile tilapia fingerlings were fed for 60 days with graded levels of SDP, and then the growth performance and villus:crypt ratio were evaluated. In Study II, fish were subjected to cold-induced stress (CIS) for seven days, and the putative protective effect of SDP on fish health, as measured by the hematological parameters, was evaluated by comparing their condition before and after stress.

2.1. Experimental diets (Study I and II)

Five practical diets were prepared with graded levels of SDP, ranging from 0 (control), 16.6, 33.2, 49.7 to 66.3 g kg^{-1}. The diets were formulated to contain 19 MJ kg^{-1} crude energy (CE) and 26.8% digestible protein (DP) according to Furuya (2010) and the National Research Council (NRC, 2011) (Table 1). All of the ingredients were ground and then mechanically mixed with water (32% of dry weight) and pelletized into 4 mm diameter pellets. The diets were dried overnight (55 °C) and stored at 4 °C until further use.

2.2. Growth performance (Study I)

A group of 1000 fish was obtained from a commercial fish farm (Piscicultura Fernandes, Palmital, SP, Brazil) and transferred to the AquaNutri Laboratory facilities (FMVZ, Unesp, Botucatu, SP, Brazil). The fish were stocked in 4500-L aquaria and acclimatized for two weeks.

Subsequently, 440 Nile tilapia with an initial body weight of 12.64 ± 0.64 g (mean ± SD) were randomly distributed in 40,250-L aquaria (11 fish/aquarium). The experimental design was completely randomized and consisted of five treatments and eight replicates.

The aquaria were supplied with 6 L min^{-1} of dechlorinated tap water passed through a biological filter and a heater. The water temperature (25.5 ± 1 °C), dissolved oxygen concentration (5.6 ± 0.15 mg L^{-1}) and pH level (5.8 ± 0.4) were monitored once a week with a YSI 556® multi-probe system (YSI Environmental, Yellow Spring, OH, USA), and the ammonia level (0.15 ± 0.09 mg L^{-1}) was determined using a commercial test kit (Alcon®, Camburiú, SC, Brazil). The fish were maintained on a 12:12-h light:dark photoperiod schedule. The accumulated waste was removed by syphoning weekly.

The fish were fed four times daily until apparent satiation between 0830 and 1730 h in a three hours interval for 60 days, and their feed intake was evaluated weekly. Then, the following growth performance parameters were calculated:

Final body weight (FBW) = final mean weight (g)

SGR = $(\ln(W_f) - (\ln(W_i)) \times 100/t$; where SGR = Specific growth rate (%body weight (BW)/day); W_t = average weight at day 60 (g); W_1 = average weight at day 1 (g); t = number of days

Relative weight gain (RWG) = (final weight − initial weight) × 100/initial weight

Feeding intake (FI) = dry feed intake (g/fish)

Feed conversion ratio (FCR) = FI/WG (feed: gain)

Visceral fat (VF) = visceral fat weight (g) × 100/fish weight (g)

Survival rate (SR) = (initial fish number − final fish number) /initial fish number × 100 (%)

Table 1
Ingredients, proximate composition and spray-dried plasma (SDP) concentration in the experimental diets.

Ingredients (g kg^{-1})	Spray-dried supplementation (g kg^{-1})				
	SDP0	SDP16.6	SDP33.2	SDP49.7	SDP66.3
Soybean meal[a]	560.9	535.3	509.8	484.4	458.8
Spray-dried plasma[b]	–	16.6	33.2	49.7	66.3
Corn	331.7	339.6	347.3	354.5	360.1
Wheat middlings	50	50	50	50	50
Soybean oil	19	20.5	22.1	24.2	28
DL-Methionine	2.2	2.2	2.3	2.3	2.4
Threonine	2.6	2.4	2.1	1.9	1.6
Dicalcium phosphate	20.6	20.4	20.2	20	19.8
BHT[c]	0.2	0.2	0.2	0.2	0.2
Premix vit/min[d]	6	6	6	6	6
Vitamin C[e]	0.5	0.5	0.5	0.5	0.5
NaCl	1	1	1	1	1
Choline chloride[f]	1.3	1.3	1.3	1.3	1.3
Antifungal[g]	4	4	4	4	4
Proximate analysis (% dry weight basis)					
Crude energy (MJ)	19	19	19	19	19
Crude protein (%)	29.9	30	30.2	32	31.4
Ether extract (%)	4	4.1	4.3	3.7	4.2
Ash (%)	6.1	6.1	6.2	6.3	6.3
Crude fiber (%)	4.7	4.5	4.5	4.1	4.2
Dry matter (%)	94.1	93.8	94	94.2	94.1

[a] Soybean meal (45.93% crude protein, digestible protein coefficient 91.97%; 4210.15 kcal kg^{-1}, digestible energy coefficient 75.48%);
[b] AP920® Porcine Animal Plasma APC (an LGI Company product that contains 69.04% of crude protein, 94.15% of digestible protein coefficient and glutamic acid (11.7%), aspartic acid (7.9%), leucine (7.8%), lysine (6.8%), valine (5.3%), threonine (4.8%), serine and arginine (4.7%), phenylalanine (4.6%), alanine (4.2%), tyrosine (3.6%), glycine (3.0%), isoleucine (2.9%), histidine and cystine (2.8%), tryptophan (1.4%) and methionine (0.7%));
[c] Butylated hydroxytoluene, antioxidant;
[d] Mineral and vitamin supplement (kg^{-1}): vit. A = 1200,000 UI; vit. D3 = 200,000 UI; vit. E = 12,000 mg; vit. K3 = 2400 mg; vit. B1 = 4800 mg; vit. B2 = 4800 mg; vit. B6 = 4000 mg; vit. B12 = 4800 mg; folic acid = 1200 mg; pantothenate calcium = 12,000 mg; vit. C = 48,000 mg; biotin = 48 mg; nicotinic acid = 24,000 mg; Mn = 4000 mg; Zn = 6000 mg; I = 20 mg; Co = 2 mg and Se = 20 mg.
[e] Vitamin C Rovimix Stay-C® 35, DSM Nutritional Products, Switzerland.
[f] Choline chlorate, 60%.
[g] Fungitract-Dry® (propionic acid, acetic acid, formic acid and salts), Falcon Aditivos, Tectron.

2.3. Intestinal morphometric assays (Study I)

For the morphometric analyses, proximal intestine samples of 30

Table 2
Initial body weight (IBW), final body weight (FBW), specific growth rate (SGR), relative weight gain (RWG), feed intake (FI), feed conversion ratio (FCR), visceral fat (VF) and survival rate (SR) of Nile tilapia fed diets supplemented with graded levels of spray-dried plasma (SDP) for 60 days.

	SDP0	SDP16.6	SDP33.2	SDP49.7	SDP66.3	*P* value
IBW (g)	12.62 (± 0.15)	12.47 (± 0.22)	12.70 (± 0.35)	12.71 (± 0.23)	12.62 (± 0.35)	0.401
FBW (g)	76.25 (± 3.80^{b})	79.53 (± 2.78^{ab})	81.63 (± 4.14^{ab})	86.87 (± 8.06^{a})	86.14 (± 7.19^{a})	0.003
SGR	2.99 (± 0.07^{b})	3.09 (± 0.07^{ab})	3.11 (± 0.10^{ab})	3.20 (± 0.15^{a})	3.20 (± 0.17^{a})	0.010
RWG (%)	504.13 (± 27.41^{b})	537.94 (± 28.14^{ab})	549.19 (± 40.62^{ab})	583.75 (± 64.59^{a})	583.66 (± 66.62^{a})	0.010
FI (g)	80.25 (± 3.26^{b})	82.75 (± 1.88^{ab})	81.95 (± 1.92^{ab})	82.79 (± 2.94^{ab})	84.16 (± 3.24^{a})	0.047
FCR	1.18 (± 0.05^{a})	1.15 (± 0.03^{ab})	1.10 (± 0.08^{abc})	1.06 (± 0.09^{bc})	1.04 (± 0.05^{c})	0.032
VF (%)	1.09 (± 0.29)	1.39 (± 0.48)	1.08 (± 0.31)	1.56 (± 0.36)	1.35 (± 0.69)	0.316
SR (%)	100	100	98.86 (± 3.21)	97.73 (± 6.43)	100	0.533

Mean ± SD values (n = 8) in each row with different superscripts were significantly different ($P < 0.05$, Tukey's test). IBW = g/fish; FBW = g/fish; SGR = $(\ln(W_t) - (\ln(W_1)) \times 100 / t$; relative weight gain (RWG) = (final weight − initial weight) × 100 / initial weight (%); FI = dry feed intake (g/fish); FCR = FI/WG (feed:gain); VF = (visceral fat weight (g) × 100) / fish weight (g); SR = (initial fish number − final fish number) / initial fish number × 100 (%); SDP0 = control; SDP16.6 = 16.6 g kg^{-1}; SDP33.2 = 33.2 g kg^{-1}; SDP49.7 = 49.7 g kg^{-1}; and SDP66.3 = 66.3 g kg^{-1}.

fish (n = 6 per treatment) were fixed in 10% buffered formaldehyde, dehydrated through graded alcohol and then xylene, and finally embedded in paraffin. The paraffin blocks were serially cut at 4 μm using an automatic microtome (Leica, RM-2155, Wetzlar, Germany) and stained with hematoxylin and eosin (H & E) (Martoja and Martoja-Pierson, 1970) prior to examination under a microscope. Stained sections of the anterior intestine were assessed to determine the villus height and crypt depth (30 readings/parameter). The histological reading was performed using an optic microscope linked to the Leica image analyzer system (Image-Pro Plus version 4.5.0.27).

2.4. Stress and hematological analysis (Study II)

After a 60-day feeding period, blood was collected from eight anesthetized fish per treatment from different tanks to determine the hematological profiles. These data were designated “before CIS”. Then, a different group of fish was subjected to CIS for seven days, and the same hematological parameters were analyzed from six anesthetized fish per treatment. These data were designated “after CIS”. The data obtained before and after CIS were compared to analyze the capacity of the fish to manage stress after supplementation with the experimental diets.

2.4.1. Hematological analysis

Fish were randomly collected and anesthetized with benzocaine solution (67 mg L^{-1}), and blood was collected from the caudal vein using a tuberculin syringe rinsed with anti-coagulant (3% EDTA, Vtec, Quimica Fina Ltda, Duque de Caxias, RJ, Brazil). Red blood cell (RBC) and leukocyte (Leuk) counts were determined by dilution and enumeration using a hemocytometer. Leuk differentiation was performed using blood extension stained with May-Grunwald-Giemsa-Wright stain according to Jain (1986). Differential counting was performed under a microscope at 100 × in immersion oil. To establish the percentage of each cellular component, 200 cells were counted on each extension. Hemoglobin (Hb) was determined by the cyanomethemoglobin colorimetric method using a commercial kit (Gold Analisa Diagnostica, Belo Horizonte, MG, Brazil) according to Collier (1944). The hematocrit (Htc) percentage was determined using the microhematocrit method described by Goldenfard et al. (1971). The total plasma protein (TPP) level was measured using a manual Goldberg refractometer (Model SPR – N, Atago CO LTD, Japan) after the blood was centrifuged at 5000 rpm for 15 min. The mean corpuscular volume [MCV = (Htc × 10) / erythrocytes] and the mean corpuscular hemoglobin concentration [MCHC = (Hb × 100) / Ht] were calculated according to Wintrobe (1934). The albumin concentration (ALB) was determined by the bromocresol method using the commercial kit Albumina Analisa Diagnostica® for colorimetric determination. The albumin:globulin ratio (A:G) was determined using the ALB and TPP values [Globulin = TPP − ALB; A:G = ALB:Globulin].

2.4.2. Cold-induced stress (CIS)

After blood sampling for the hematological profile determination, a different group was transferred to the challenge room and subjected to CIS for seven days. The water temperature was gradually reduced from 26 to 16 °C (2 °C/day). The challenge room contained 30 40-L aquaria with individual filters, aeration and heaters. Sixty fish were randomly stocked at a density of two fish per aquarium (six/treatment). The fish were fed the same experimental diet as in Study I. After seven days, the same initial hematological parameters were evaluated.

2.5. Statistical analysis

The growth performance data and the villus:crypt values were analyzed via a one-way ANOVA ($P < 0.05$), and Tukey's method was used for multiple comparisons (Zar, 2009). Points of maximum final body weight, relative weight gain and optimum feed conversion ratio were determined by the broken-line analysis method described by Zeitoun et al. (1976). The hematological profile was analyzed via Tukey's test or the Mann-Whitney comparison test, depending on whether the data were normally distributed. The statistical package Minitab version 16 (Minitab® v. 15, Minitab Inc., State College, PA, USA) was used for the statistical analysis.

2.6. Ethics statement

All experimental procedures were approved by the Animal Ethics Committee of the Veterinary and Animal Science College, Sao Paulo State University (protocol no. 134/2013 – CEUA).

3. Results

3.1. Study I

The FBW, SGR, RWG, FI and FCR were affected by SDP

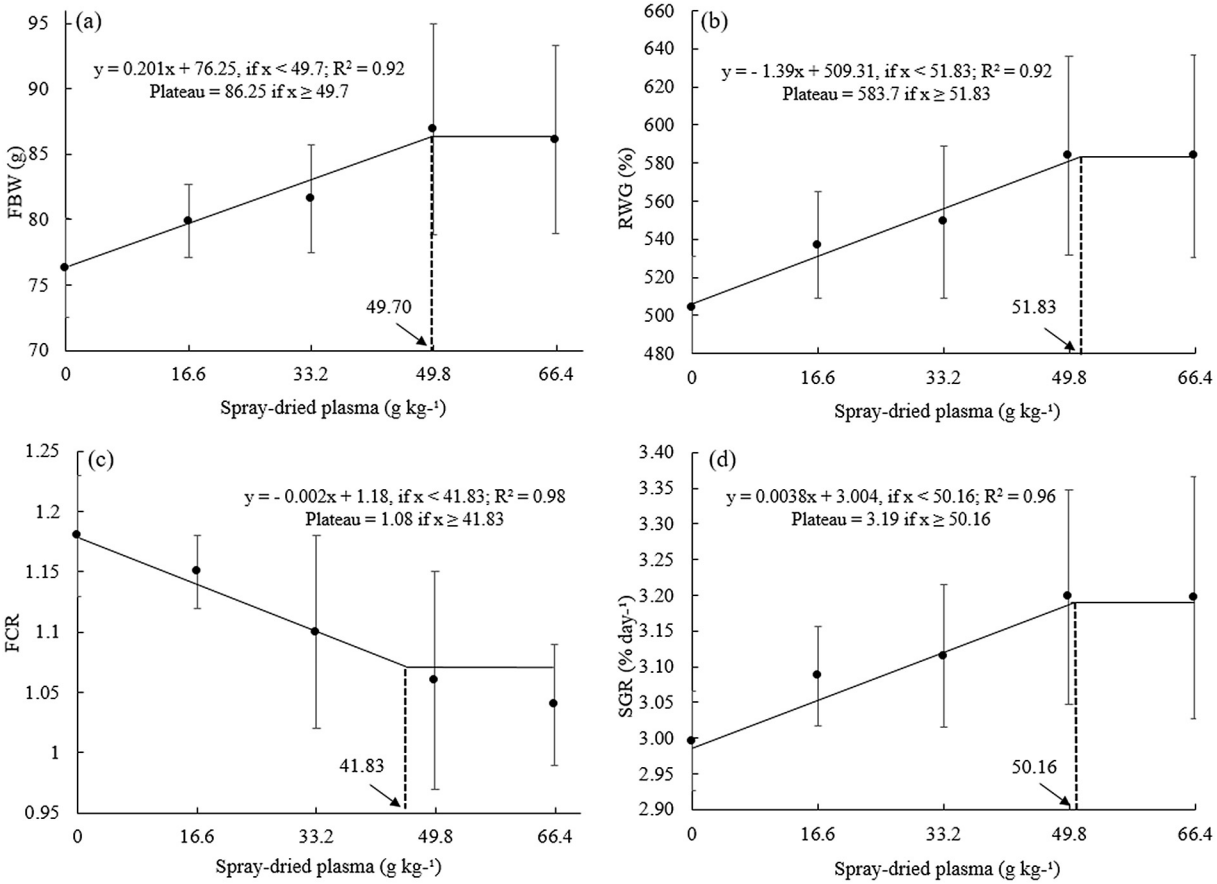

Fig. 1. Final body weight, FBW (a), relative weight gain, RWG (b), feed conversion ratio, FCR (c), specific growth rate, SGR (d) of Nile tilapia fed for 60 days with graded levels of spray-dried plasma (SDP). Values are means of 11 fish per tank and eight tanks per treatment.

supplementation (Table 2). The dietary supplementation levels of 49.7 and 66.3 g kg^{-1} SDP produced the highest FBW, SGR and RWG ($P < 0.05$) compared with the control diet. The highest dietary SDP supplementation level produced the highest FI compared with the control diet ($P < 0.05$), although the value did not differ from that of other supplementation levels. The optimal FCR was achieved with 66.3 g kg^{-1} SDP, however there were no significant difference among 33.2, 49.7 and 66.3 g kg^{-1} SDP. Based on the broken-line analysis of the FBW, SGR, RWG and FCR, the optimum dietary level supplementation of SDP was determined to be 49.70, 50.16, 51.83 and 41.83 g kg^{-1} diet, respectively (Fig. 1).

Dietary SDP supplementation levels of 33.2 g kg^{-1} diet and above produced a lower crypt depth ($P < 0.01$) compared with that of the control diet (Table 3). The highest V:C ratio was achieved with 49.7 g kg^{-1} of dietary SDP supplementation compared with that of the control and 16.6 g kg^{-1} diets (Table 3).

Table 3
Effect of dietary spray-dried plasma (SDP) supplementation on the intestine morphology of Nile tilapia after a 60-day feeding trial.

Treatment	Villus height (μm)	Crypt depth (μm)	Villus:crypt
SDP0	558.94 ± 66.57	63.37 ± 4.48^{a}	8.84 ± 1.04^{b}
SDP16.6	486.97 ± 99.56	55.20 ± 7.88^{ab}	8.82 ± 1.29^{b}
SDP33.2	508.67 ± 189.03	52.13 ± 6.77^{b}	9.57 ± 2.67^{ab}
SDP49.7	561.65 ± 66.51	48.94 ± 1.40^{b}	11.50 ± 1.54^{a}
SDP66.3	537.26 ± 76.22	49.92 ± 4.30^{b}	10.74 ± 0.94^{ab}
P value	0.721	0.001	0.029

Mean ± SD values (n = 12) in each column with different superscripts were significantly different $P < 0.05$ (Tukey's test). Crypt depth = 63.14 − 5.13x + 0.47x^2 ($r^2 = 0.98$); villus:crypt = 8.87 + 0.34x ($r^2 = 0.74$); SDP0 = control; SDP16.6 = 16.6 g kg^{-1}; SDP33.2 = 33.2 g kg^{-1}; SDP49.7 = 49.7 g kg^{-1}; and SDP66.3 = 66.3 g kg^{-1}.

3.2. Study II

The hematological analysis (Table 4) showed that after CIS, the lowest Htc was observed for the fish fed 16.6 g kg^{-1} SDP compared with the fish fed 66.3 g kg^{-1} SDP ($P < 0.05$). A comparison of the values before and after CIS revealed that fish fed 16.6 g kg^{-1} SDP showed lower MCV values after stress ($P < 0.01$) and fish fed 49.7 g kg^{-1} SDP showed higher MCHC values after stress ($P < 0.01$).

The Leuk, lymphocyte (Lymph) and neutrophil (Neutr) values were affected by the dietary SDP supplementation level and CIS (Table 5). The monocyte number was affected by the dietary SDP supplementation level both before and after CIS ($P < 0.05$) (Table 5). The highest TPP concentration was observed in fish fed 49.7 and 66.3 g kg^{-1} SDP compared with fish fed 16.6 g kg^{-1} SDP after CIS ($P < 0.05$) (Table 6). All dietary SDP supplementation levels except 33.2 g kg^{-1} SDP produced higher ALB concentrations after CIS ($P < 0.05$) (Table 6). The Glob concentration showed an impact of dietary SDP supplementation after CIS ($P < 0.05$), and the lowest Glob concentration was observed in fish fed 16.6 and 33.2 g kg^{-1} SDP ($P < 0.05$) (Table 6). A comparison of the values before and after CIS revealed that only fish fed 33.2 g kg^{-1} SDP showed lower Glob concentrations after CIS ($P < 0.01$) (Table 6), and a higher A:G ratio was also observed after CIS ($P < 0.05$) (Table 6).

4. Discussion

The benefits of dietary SDP supplementation have been widely demonstrated for early-weaned pigs (van Dijk et al., 2001; Touchette et al., 2002; Bosi et al., 2004; Hernández et al., 2010; Frank et al., 2011;

Table 4
Erythrocyte (Eryt), hemoglobin (Hb), hematocrit (Htc), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) of Nile tilapia fed experimental diets before (Study I) and after (Study II) cold-induced stress.

SDP g kg^{-1}	Eryt (10^6 µL^{-1})			Hb (g dL^{-1})			Htc (%)			MCV (fL)			MCHC (%)		
	Before	After	P^{**}	Before	After	P^{**}	Before	After	P^{**}	Before	After	P^{**}	Before	After	P^{**}
0	1.84 (1.85)	1.75 (1.78)	0.518	6.92 (6.79)	6.51 (6.55)	0.401	29.69 (28.2)	26.42^{ab} (26.5)	0.106	162.36 (158)	150.66 (147)	0.137	23.29 (23.7)	24.61 (23.7)	0.605
16.6	1.80 (1.80)	1.72 (1.72)	0.477	6.41 (6.52)	6.03 (5.79)	0.220	27.81 (27.2^{A})	23.50^{b} (23.2^{B})	0.033	155.64 (149^{A})	136.34 (138^{B})	0.017	23.10 (24.0)	25.59 (25.8)	0.061
33.2	1.80 (1.85)	1.81 (1.93)	0.651	6.57 (6.63)	6.66 (7.16)	0.846	28.44 (28.7)	26.75^{ab} (26.5)	0.477	159.18 (154)	150.53 (161)	0.846	23.01 (24.0)	24.98 (24.8)	0.081
49.7	2.00 (2.02)	1.82 (1.79)	0.333	6.99 (7.25)	7.22 (7.44)	0.477	29.19 (29.5)	28.08^{ab} (28.2)	0.796	146.51 (149)	154.07 (153)	0.106	23.94 (23.8^{B})	25.78 (25.3^{A})	0.017
66.3	1.87 (1.89)	2.04 (2.08)	0.106	6.94 (6.72)	7.50 (7.36)	0.137	28.88 (28.7)	30.92^{a} (31.2)	0.196	154.88 (152)	152.69 (154)	0.948	24.04 (24.7)	24.27 (24.4)	1.000
P^{*}	0.291	0.190		0.559	0.099		0.715	0.019		0.218	0.238		0.639	0.643	

* Mean values (n = 8) in each column with different lowercase superscripts were significantly different ($P < 0.05$) between treatments (Tukey's test).
** Medians (n = 6) in each row with different uppercase superscripts were significantly different ($P < 0.05$) between periods (Mann-Whitney test).

Crenshaw et al., 2013). However, few studies have focused on this functional feedstuff in other animals, such as fish (Gisbert et al., 2015) and cats (Rodriguez et al., 2016). Although the mechanisms by which SDP improves growth performance have not been totally elucidated (Touchette et al., 2002; Tran et al., 2014), the majority of studies have observed that enhanced growth performance (Ermer et al., 1994; Bosi et al., 2004; Diehl, 2004; Pierce et al., 2005; Hernández et al., 2010; Gisbert et al., 2015) and intestinal protection (Bosi et al., 2004; Campbell et al., 2010; Tran et al., 2014; Gisbert et al., 2015) are associated with higher feed intake. In our study, these benefits were observed for the growth performance parameters, such as the FBW, SGR, RWG and FI, as well as for the intestinal functions as demonstrated by decreases in the crypt depth and increases in the villus:crypt ratio.

An increase in feed intake has been attributed to the good palatability of SDP (Ermer et al., 1994), which has an excellent amino acid profile (Bureau et al., 1999) and high levels of glutamic acid (11.7%, Table 1) and is recognized as a palatability enhancer (Shamushaki et al., 2007; Li et al., 2009). Pierce et al. (2005) also attributed higher feed intake to the IgG fraction present in SDP as observed in early-weaned pigs. Moreover, Gisbert et al. (2015) studied the health benefits of SDP in gilthead sea bream and observed an expansion of the goblet cell population, which can enhance the immune barrier and improve the commensal intestinal microbiota. According to the literature, improvements to the intestinal villus may result in higher absorptive functions as well as better nutrient utilization (Grinstead et al., 2000; van Dijk et al., 2001; Lawrence et al., 2004). An increased villus height and villus height:crypt depth ratio are directly correlated with increased epithelial turnover (Fan et al., 1997), whereas decreases in the villus height and deeper crypts are correlated with poor nutrient absorption and reduced performance (Xu et al., 2003). While we did not analyze the goblet cell population in our study, the reduction in crypt depth and a better villus:crypt ratio were associated with increased dietary SDP supplementation levels.

Moreover, improvements in the growth performance can also be explained by the high biological value of SDP (Bureau et al., 1999; Campbell et al., 2004; Campbell et al., 2008). As previously stated, SDP is rich in many amino acids, such as glutamic acid, aspartic acid, lysine and threonine. Glutamic acid is essential to the antioxidant system and acts as a free radical scavenger. Additionally, glutamic acid acts as a glutamine precursor and may provide energy to the villus structure, thus contributing to intestinal protection. Aspartic acid plays major roles in gene expression, protein synthesis and lymphocyte proliferation. Similar to other proteinogenic amino acids, lysine primarily serves as one of the 20 types of building blocks for the synthesis of body proteins and peptides, and it is also a substrate for the synthesis of carnitine. Threonine is responsible for mucin synthesis and essential for maintaining intestinal integrity and function (Wu, 2013). Despite the positive effects on Nile tilapia performance observed with SDP supplementation, additional parameters should be further analyzed to better understand the function of SDP as a dietary supplement.

In the present study, SDP was used as a functional protein ingredient in the diet of Nile tilapia and analyzed to determine whether it improved the growth performance, enhanced the resistance to CIS and/or maintained healthy conditions after CIS as measured by hematological parameters. According to Hrubec and Smith (2010), fish hematological profiles can be used as a tool for analyzing the health status of the

Table 5
Total leukocyte (Leuk), lymphocyte (Lymph), neutrophil (Neutr) and monocyte (Mon) counts of Nile tilapia fed experimental diets before (Study I) and after (Study II) cold-induced stress.

SDP g kg^{-1}	Leuk (µL^{-1})			Lymph (µL^{-1})			Neutr (µL^{-1})			Mon (µL^{-1})		
	Before	After	P^{**}	Before	After	P^{**}	Before	After	P^{**}	Before	After	P^{**}
0	59,129^{b} (57,635^{A})	42,411^{b} (38,799^{B})	0.045	55,432^{b} (54,028^{A})	35,541 (30,157^{B})	0.033	230 (199^{B})	2949^{ab} (2931^{A})	0.002	3466^{b} (4056)	3919^{b} (3795)	0.846
16.6	69,361^{b} (72,249^{A})	44,455^{ab} (45,220^{B})	0.045	64,389^{ab} (67,721^{A})	37,169 (39,278^{B})	0.045	87 (0^{B})	2863^{b} (2676^{A})	0.002	4576^{ab} (4957)	4422^{b} (3034)	0.651
33.2	71,642^{ab} (71630)	63,462^{ab} (60675)	0.333	68,074^{ab} (67,475^{A})	49,481 (49,047^{B})	0.045	55 (0^{B})	6794^{a} (5953^{A})	0.002	3512^{b} (3214)	7186^{ab} (6812)	0.061
49.7	83,553^{ab} (89,063^{A})	50,529^{ab} (51,170^{B})	0.033	74,992^{ab} (78,608^{A})	40,140 (39,978^{B})	0.024	244 (203^{B})	5686^{ab} (4456^{A})	0.002	8317^{a} (10021)	4653^{ab} (4435)	0.081
66.3	97,622^{a} (99,227^{A})	68,863^{a} (65,436^{B})	0.017	90,338^{a} (91,615^{A})	49,135 (50,200^{B})	0.003	100 (0^{B})	5517^{ab} (5513^{A})	0.002	7223^{ab} (6848)	9717^{a} (8806)	0.401
P^{*}	0.014	0.017		0.026	0.274		0.270	0.021		0.003	0.013	

* Mean values (n = 8) in each column with different lowercase superscripts were significantly different ($P < 0.05$) between treatments (Tukey's test).
** Medians (n = 6) in each row with different uppercase superscripts were significantly different ($P < 0.05$) between periods (Mann-Whitney test).

Table 6
Total plasma protein concentration (TPP), albumin (ALB), globulin (Glob), and albumin:globulin ratio (A:G) of Nile tilapia fed experimental diets before (Study I) and after (Study II) cold-induced stress.

SDP g kg^{-1}	TPP (g dL^{-1})			ALB (g dL^{-1})			Glob (g dL^{-1})			A:G		
	Before	After	P^{**}	Before	After	P^{**}	Before	After	P^{**}	Before	After	P^{**}
0	2.81 (2.82)	2.92^{ab} (2.90)	0.744	0.49 (0.48^{B})	0.67 (0.66^{A})	0.028	2.31 (2.37)	2.25^{abc} (2.30)	0.796	0.21 (0.23^{B})	0.30 (0.29^{A})	0.039
16.6	2.83 (3.00)	2.53^{b} (2.60)	0.106	0.54 (0.53^{B})	0.76 (0.79^{A})	0.023	2.28 (2.26)	1.77^{c} (1.88)	0.057	0.24 (0.23^{B})	0.44 (0.44^{A})	0.023
33.2	3,16 (2.92)	2.72^{ab} (2.70)	0.106	0.54 (0.45)	0.78 (0.80)	0.121	2.63 (2.41^{A})	1.94^{bc} (1.97^{B})	0.011	0.22 (0.19^{B})	0.41 (0.42^{A})	0.017
49.7	2.75 (2.75)	3.28^{a} (3.05)	0.053	0.47 (0.41^{B})	0.93 (0.90^{A})	0.008	2.29 (2.33)	2.35^{ab} (2.33)	0.698	0.21 (0.17^{B})	0.39 (0^{A})	0.012
66.3	2.79 (2.82)	3.17^{a} (3.20)	0.053	0.48 (0.49^{B})	0.78 (0.76^{A})	0.006	2.31 (2.37)	2.39^{a} (2.46)	0.518	0.21 (0.20^{B})	0.33 (0.31^{A})	0.017
P^{*}	0.205	0.005		0.913	0.433		0.348	0.004		0.919	0.305	

* Mean values (n = 8) in each column with different lowercase superscripts were significantly different ($P < 0.05$) between treatments (Tukey's test).
** Medians (n = 6) in each row with different uppercase superscripts were significantly different ($P < 0.05$) between periods (Mann-Whitney test).

animal and may vary because of stress, disease, nutritional conditions, seasonal cycle, gender and gonadal maturation stage.

The erythrogram analysis showed certain alterations in the hematological profile. The values before and after CIS were within the normal range for healthy Nile tilapia (Falcon et al., 2007; Barros et al., 2009; Hrubec and Smith, 2010), and this likely represents an important result, as fish were able to maintain RBC production even after CIS.

As previously stated, SDP is rich in glutamic acid, which is important for erythrocyte maturation because it is a source of folic acid (Kaneko et al., 1997; Barros et al., 2009), and it is a precursor of glutamine and glutathione (Wu, 2013) that displays antioxidant properties (Gisbert et al., 2015). Therefore, we believe that fish were able to maintain RBC production, even under stress, because they were provided with appropriate nutritional support.

As a tropical fish, the Nile tilapia is well adapted to warm water but sensitive to low temperatures (Zerai et al., 2010). Temperatures below the normal tolerance range are considered a stress factor that might impair fish resistance as observed by Falcon et al. (2007). The leukopenia, lymphopenia and neutrophilia observed in our study after CIS were previously reported by Falcon et al. (2007), Falcon et al. (2008), Barros et al. (2015a), Barros et al. (2015b) and Guimarães et al. (2016). The reduction in the Leuk and Lymph counts could be attributed to an increase in the cortisol level induced by stress (Barton and Iwama, 1991). However, we observed an increase in the Neutr count, which is essential because these immune cells are responsible for the engulfment and destruction of invading microorganisms (Tizard, 2002; Feldman et al., 2010). An increase was also observed in the number of monocytes in the fish fed 49.7 g SDP kg $^{-1}$ before CIS and 66.3 g SDP kg $^{-1}$ after CIS compared with the fish that were not fed SDP. Overall, we may conclude that SDP-supplemented fish were better able to manage CIS. Based on these results, further studies should be performed to address the effect of SDP on the number and activity of white blood cells after bacterial challenge to better understand the putative effect of SDP on the immune system and Nile tilapia resistance.

The total plasma protein values and the A:G ratio did not show trends related to SDP supplementation. Although a decrease in the A:G ratio after CIS was expected, the results showed an increase in this ratio. These findings are inconsistent with previously reported findings on fish resistance and the results of Barros et al. (2015a) for Nile tilapia challenged by *Aeromonas hydrophila*.

Dietary SDP supplementation improved the growth performance, intestinal health, hematological profile and CIS resistance in Nile tilapia. However, further studies should be performed to better understand the mechanisms underlying the effect of SDP on the growth and health of Nile tilapia. To our knowledge, this is the first study addressing the utilization of SDP as a functional protein source in the diet of Nile tilapia, which supports the importance of the results obtained in this study. Based on our findings, we recommend a dietary supplementation level of 51.83 g kg^{-1} SDP.

Conflict of interest

The authors declare that there are no conflicts of interest.

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