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Performance, intestinal histomorphometry, and blood parameters of post-weaning piglets receiving different levels of soy protein concentrate in the diet

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ABSTRACT - Seventy-two barrows were used to evaluate the effects of the use of soy protein concentrate (SPC) in diets of post-weaning piglets on their performance, intestinal histomorphometry, and blood parameters. Piglets were weaned at 21 days of age and distributed in a randomized-block experimental design with four treatments (0, 3, 6, and 9% SPC), six replications, and three piglets per replication. Diets were formulated to meet the requirement of the piglets according to phases of 21 to 32, 33 to 42, and 43 to 66 days of age. Both feed and water were provided *ad libitum* during the entire experiment. The feed of the phase of 43 to 66 days was based on corn and soybean meal. In the period between 21 and 32 days of age, the use of SPC in the diet did not impact the daily weight gain (DWG) or the daily feed intake (DFI) of the piglets, whereas there was a linear effect on feed conversion (FC), which decreased as the levels of SPC in the feed were increased. In the period between 33 and 42 days of age, a linear effect was observed on FC, which increased as the level of SPC in the feed was increased. In this period, no effects of SPC were observed on DWG or DFI. Inclusion of SPC in the diets does not influence performance, intestinal histomorphometry, or blood parameters of piglets in the period between 21 and 66 days of age.

Key Words: food, performance, soybean, swine

Introduction

Weaning promotes a high level of stress to piglets. During this period, they go through several changes related to food, groupings, facilities, and environment. The nutritional management in this stage becomes very important and, if carried out inappropriately, may result in low performance. This is because piglets cease to ingest the milk from the sow and start to receive a new diet based on feed with a substantial difference in the composition of carbohydrates, proteins, and fats (Williams, 2003).

The soybean meal is one of the main sources of vegetable protein used in animal feeding. However, some antinutritional factors are present in its composition, such as allergenic proteins glycinin and ß-conglycinin and oligosaccharides (raffinose and stachyose), which trigger digestive disorders in young piglets, reducing their performance (Li et al., 1991a; Gdala et al., 1997). Based on this knowledge, several by-products have been produced from soybean, with the objective of reducing or even eliminating its antinutritional effects. Among those, Yang et al. (2007) mentioned processed soy products such as soy protein concentrate and soy protein isolate used in starter diets.

The SPC is a product obtained from a set of processes that involve oil extraction, the washing of defatted soy flakes with ethanol for the extraction of soluble carbohydrates, and finally the thermal treatment of inactivation of allergenic proteins (Turlington et al., 1990). As a result, the digestibility of soy is augmented and the changes in intestinal morphology are reduced, which results in an increase in the performance of piglets (Hancock et al., 1990a,b).

The introduction of this food in the diet of post-weaning piglets aims to reduce the level of soybean meal used in the formulation of the feed, reducing the antinutritional content present in the diet. However, Lenehan et al. (2007) noted that the protein solubility of this feed can impact the performance of the piglets. As the amount of SPC included in piglet diets is generally smaller than 10%, the improvement some authors have observed in the performance of early-weaned piglets that receive such diets, as mentioned by Zhang et al. (2013), is probably more closely related to the lower levels of antigenic proteins and antinutritional

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factors of SPC than to the slight increases observed in the digestibility of nutrients. Thus, the present study aimed to evaluate the levels of SPC in diets for post-weaning piglets on performance, intestinal histomorphometry, and some blood parameters.

Material and Methods

The experiment was carried out in Goiânia, GO, Brazil. The project was evaluated by the Ethics Committee on Animal Use (CEUA) of the Office of the Dean of Graduate Studies at the Federal University of Goiás (PRPPG/UFG) and approved under case no. 062/12.

The experimental shed was thoroughly cleaned and disinfected before the experiment was started. The nursery shed housed, in suspended cages, 72 barrows right after the weaning, at 21 days of age. The piglets were evaluated upon their arrival regarding umbilical and scrotum healing, hydration, and behavior.

Cages were of slatted plastic floor, with nipple drinkers with shell, PVC gutter feeders with a linear area of 0.90 m, and four holes. Incandescent 100 W lamps were used for heating. Temperature and humidity were measured daily by a thermometer and hygrometer installed in an empty cage in the center of the room, at the same height as the animals.

A completely randomized block design was adopted, with four treatments, six replications, and three animals per experimental unit, totaling 24 experimental units. The criteria adopted to form the blocks was the initial weight of the piglets. The pen was considered an experimental unit.

Water and feed were provided *ad libitum* for the piglets during the entire experimental period, starting at 21 and finishing at 66 days of age.

Treatments consisted of four levels of SPC (0, 3, 6, and 9) in the diets at phases of 21 to 32 and 33 to 42 days of age (Table 1). Between 43 and 66 days of age, the piglets received the same diet, based on corn and soybean meal, to evaluate the residual effect of the treatments applied in the previous phases (Table 2). A commercial SPC source was used, containing 60% crude protein, 5% fiber, 5% mineral matter, 2% crude fat, 10% moisture, 18% nitrogen-free extract, and 3 mg/g trypsin inhibitor activity.

Table 1 - Nutritional and centesimal composition of experimental diets in Phase I (21 to 32 days of age) and Phase 2 (32 to 42 days of age)

	21 to 32 days (% SPC)				32 to 42 days (% SPC)			
	0	3	6	9	0	3	6	9
Ingredient (kg/100 kg)								
Soy protein concentrate	0.00	3.00	6.00	9.00	0.00	3.00	6.00	9.00
Corn grain	50.50	52.27	54.05	55.84	51.24	53.03	54.82	56.60
Soybean meal (45% crude protein)	19.77	15.27	10.77	6.26	28.24	23.74	19.24	14.73
Dried whey	17.00	17.00	17.00	17.00	10.00	10.00	10.00	10.00
Blood plasma	5.00	5.00	5.00	5.00	3.00	3.00	3.00	3.00
Soybean oil	3.50	3.19	2.88	2.57	3.85	3.54	3.23	2.92
Dicalcium phosphate	1.72	1.74	1.75	1.76	1.68	1.69	1.71	1.72
Limestone	0.57	0.57	0.58	0.58	0.60	0.60	0.60	0.61
L-lysine HCl	0.91	0.91	0.90	0.90	0.56	0.55	0.55	0.55
DL-methionine	0.12	0.11	0.11	0.11	0.05	0.04	0.04	0.04
L-threonine	0.43	0.42	0.41	0.41	0.24	0.24	0.23	0.22
L-tryptophan	0.11	0.13	0.16	0.18	0.04	0.06	0.09	0.12
Vitamin supplement[1]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Mineral supplement[2]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Sodium chloride	0.01	0.01	0.01	0.01	0.13	0.13	0.13	0.13
Total	100	100	100	100	100	100	100	100
Calculated values[3]								
Metabolizable energy (Mcal/kg)	3,375	3,375	3,375	3,375	3,375	3,375	3,375	3,375
Crude protein (%)	20.00	20.00	20.00	20.00	21.00	21.00	21.00	21.00
Digestible lysine (%)	1.45	1.45	1.45	1.45	1.33	1.33	1.33	1.33
Digestible methionine (%)	0.406	0.406	0.406	0.406	0.372	0.372	0.372	0.372
Digestible threonine (%)	0.914	0.914	0.914	0.914	0.838	0.838	0.838	0.838
Digestible tryptophan (%)	0.261	0.261	0.261	0.261	0.239	0.239	0.239	0.239
Calcium (%)	0.850	0.850	0.850	0.850	0.825	0.825	0.825	0.825
Available phosphorus (%)	0.500	0.500	0.500	0.500	0.450	0.450	0.450	0.450
Sodium (%)	0.287	0.290	0.290	0.290	0.230	0.230	0.230	0.230

SPC - soy protein concentrate.

[1] Vitamin supplement - quantity per kg of diet: 0.25 mg folic acid; 9.33 mg pantothenic acid; 7.98 mg Cu; 0.05 g Fe; 0.30 mg I; 14.90 mg Mn; 15.99 mg niacin; 0.36 mg Se; 22.00 mg tylosin; 3.19 IU vitamin A; 0.50 mg vitamin B1; 10.49 µg vitamin B12; 2.80 mg vitamin B2; 0.60 mg vitamin B6; 649 IU vitamin D3; 8.49 IU vitamin E; 0.99 mg vitamin K3; 0.08 g Zn.

[2] Mineral supplement - quantity per kg of diet: 0.05 g Cu; 0.29 g Fe; 1.80 mg I; 0.08 g Mn; 0.48 g Zn.

[3] According to Rostagno et al. (2011).

The diets followed the nutritional requirements for each raising stage recommended by Rostagno et al. (2011).

The performance variables studied were daily weight gain, daily feed intake, final weight, and feed conversion. Animals were weighted at 21, 32, 43, and 66 days of age, and so were the feed supplied and leftovers.

At 32 days of age, blood (5 mL) was collected from 24 animals, one per experimental unit. The collection was performed by a puncture in the jugular vein and the blood was transferred to a tube with ethylenediaminetetraacetic acid (EDTA) anticoagulant, homogenized and stored in a cooler box with recyclable ice until the moment of the analysis. After blood collection, these 24 piglets were euthanized, complying with the principles of animal welfare and humane slaughter to minimize any stress during the procedure. The piglets were stunned by electronarcosis followed by bloodletting, through incision of the blood vessels present in the region of the neck.

Three segments of approximately 2.0 cm each were collected from the small intestine. The segments were collected at 20 cm from the pylorus, in the median portion of the small intestine, and at 20 cm from the ileocecal junction. Fragments were opened by the mesenteric border, fixed in a formalin solution at 10%, and buffered in 7.2 pH, for a period of 24 h. After the fixation, they were transferred to a histological cassette and stored in 70% alcohol until the beginning of the material processing.

Table 2 - Nutritional and centesimal composition of experimental diet in the period of 43 to 66 days of age

Item	43 to 66 days (0% SPC)
Ingredient (kg/100 kg)	
Corn	68.61
Soybean meal (45% crude protein)	27.17
Soy oil	0.95
Dicalcium phosphate	1.39
Limestone	0.73
L-lysine HCl	0.30
DL-methionine	0.02
L-threonine	0.07
Vitamin supplement[1]	0.30
Mineral supplement[2]	0.05
Sodium chloride	0.40
Total	100
Calculated values[3]	
Metabolizable energy (Mcal/kg)	3,230
Crude protein (%)	18.13
Digestible lysine (%)	1.037
Digestible methionine (%)	0.290
Digestible threonine (%)	0.653
Digestible tryptophan (%)	0.190
Calcium (%)	0.733
Available phosphorus (%)	0.360
Sodium (%)	0.200

SPC - soy protein concentrate.
[1] Vitamin supplement - quantity per kg of diet: 0.25 mg folic acid; 9.33 mg pantothenic acid; 7.98 mg Cu; 0.05 g Fe; 0.30 mg I; 14.90 mg Mn; 15.99 mg niacin; 0.36 mg Se; 22.00 mg tylosin; 3.19 IU vitamin A; 0.50 mg vitamin B1; 10.49 µg vitamin B12; 2.80 mg vitamin B2; 0.60 mg vitamin B6; 649 IU vitamin D3; 8.49 IU vitamin E; 0.99 mg vitamin K3; 0.08 g Zn.
[2] Mineral supplement - Quantity per kg of diet: 0.05 g Cu; 0.29 g Fe; 1.80 mg I; 0.08 g Mn; 0.48 g Zn.
[3] According to Rostagno et al. (2011).

The procedures undertaken in the laboratory consisted of soaking the samples for one hour in increasing concentrations of alcohol (70, 85, 95% and absolute PA). The cassettes then went through three solutions of xylol for half an hour each, followed by three soakings in liquid paraffin before embedding in a block of paraffin wax. The blocks with the embedded material were kept frozen until the moment of the sections in the microtome. Sections were made with an approximate thickness of five micrometers, semi-seriated with a minimum interval of 50 µm between them.

Slides were colored by the hematoxylin-eosin technique (Luna, 1968) and subjected to evaluation in a light microscope (Leica DM2500) with 50x magnification (objective of 5x) to capture the images of the segments. From the images, 25 intact villi were measured in each segment with the help of ImageJ software, i.e., the villus height and crypt depth of the same villus.

Total erythrocytes, platelets, and leukocytes were counted in a Mindray® equipment model BC 2800 VET, compact automatic hematology analyzer. For the differential count of leukocytes, a blood film was prepared for each blood sample. In a small beaker, 100 drops of Giempa (Doles®) were diluted in 100 mL of distilled water. This solution was distributed across the slides to color them. After 25 min, the slides were washed in running water and dried in air. After the preparation, the blood films were taken to a light microscope of 1000x magnification (optical of 100x), where the 100 leukocytes were counted, 50 in the lower border and 50 in the upper border of the film. Based on these relative values, the absolute values were obtained, by multiplying the relative value by the total number of leukocytes of the sample.

The statistical analyses were performed on software R (R Development Core Team, 2011). All variables were subjected to analysis of variance and regression analysis; a statistical significance level of 5% was adopted ($\alpha = 0.05$). Data were tested for two regression models (linear and quadratic). Models in which the regression coefficients were significant were selected.

Results and Discussion

The introduction of SPC in the piglet diets in the period from 21 to 42 days of age did not influence the performance variables of the piglets (Table 3), agreeing with the results of Bertol et al. (2000), who evaluated the inclusion of different levels of SPC in diets for piglets in the nursery phase. The soy protein concentrate was expected to provide a reduction of the allergenic effect and an increase in the performance of the piglets, as described by Li et al. (1991b). One of the possible explanations for the non-improvement of the performance of the piglets between 21 and 42 days of age in the present work, after the introduction of the SPC, might be the variation during the process of obtainment of the feed. This is in agreement with Lenehan et al. (2007), who evaluated two SPC of distinct origins at two levels of inclusion and concluded that one of the SPC (58.86% of protein solubility in potassium hydroxide) did not improve the performance of the animals when compared with the diet with the protein source based on soybean meal (80.06% of protein solubility in potassium hydroxide). According to Urbaityte et al. (2009), this variation could be both due to the origin, i.e., the process of obtainment of the SPC, and due to the form of presentation of the product, with finely or coarsely ground flakes.

When analyzing the phases of 21 to 32 and 33 to 42 days (Table 3) separately, a negative linear effect (P = 0.033) was observed in feed/gain in the phase of 21 to 32 days of age, allowing us to associate these results with the presence of antigenic protein in the diet, probably due to a greater inclusion of soybean meal for the levels 0% and 3% of SPC, which led to a discreet reduction in the utilization of nutrients in the period from 21 to 32 days of age. A positive linear effect (P = 0.041) was observed in feed/gain in the phase of 33 to 42 days of age as the levels of SPC were increased in the piglet diets. The results of this work were similar to those obtained by Li et al. (1991a), who demonstrated that the allergenic proteins resulted in an initial reduction of performance, but, after the immune adaptation of the piglet to these proteins, performance returned to normal values.

In the same lines, Sohn et al. (1994) reported better performance of piglets during the two initial post-weaning weeks with the use of SPC in substitution of soybean meal, but between the third and fifth experimental week, with the supply of a single feed for all treatments, there was no difference in the performance of piglets. In a work published by Lenehan et al. (2007), in which the effects of the introduction of SPC in the diet of piglets were evaluated,

Table 3 - Effects of inclusion of soy protein concentrate (SPC) on performance variables of piglets in the periods of 21-32, 33-42, 21-42, 43-66, and 21-66 days of age

Variable	SPC in the diet (%)				P-value		CV (%)
	0	3	6	9	Linear	Quadratic	
			Pre-starter I (21 to 32 days of age)				
Weight gain (kg/d)	0.255	0.275	0.328	0.292	0.106	0.206	16.95
Feed intake (kg/d)	0.348	0.391	0.393	0.376	0.386	0.196	13.39
Feed/gain[1] (g/g)	1.49	1.44	1.20	1.30	0.033	0.344	12.6
			Pre-starter II (33 to 42 days of age)				
Weight gain (kg/d)	0.506	0.479	0.499	0.454	0.365	0.788	15.26
Feed intake (kg/d)	0.654	0.675	0.706	0.662	0.745	0.406	12.83
Feed/gain[2] (g/g)	1.28	1.41	1.44	1.46	0.041	0.331	8.91
			Pre-starter (21 to 42 days of age)				
Initial weight (kg)	6.922	6.94	6.937	6.886	0.284	0.141	0.79
Final weight (kg)	14.449	15.354	14.754	14.483	0.759	0.208	6.94
Weight gain (kg/d)	0.383	0.388	0.388	0.349	0.321	0.325	13.92
Feed intake (kg/d)	0.498	0.533	0.524	0.486	0.729	0.187	12.32
Feed/gain (g/g)	1.30	1.39	1.37	1.39	0.16	0.452	6.73
			Starter (43 to 66 days of age)[3]				
Weight gain (kg/d)	0.691	0.717	0.730	0.719	0.349	0.433	7.77
Feed intake (kg/d)	1.265	1.236	1.213	1.27	0.970	0.291	7.75
Feed/gain (g/g)	1.84	1.73	1.65	1.76	0,295	0,095	8.66
			Nursery (21 to 66 days of age)				
Initial weight (kg)	6.922	6.94	6.937	6.886	0.284	0.141	0.79
Final weight (kg)	31.620	33.275	33.583	32.462	0.519	0.169	7.19
Weight gain (kg/d)	0.537	0.571	0.578	0.553	0.536	0.153	8.42
Feed intake (kg/d)	0.911	0.923	0.907	0.922	0.911	0.964	8.41
Feed/gain (g/g)	1.70	1.62	1.56	1.66	0.38	0.053	6.49

CV - coefficient of variation.
[1] Linear effect: W = 0.03, $y = 1.5515 - 0.0763x$, $R^2 = 0.54$.
[2] Linear effect: W = 0.04, $y = 1.2734 + 0.0501x$, $R^2 = 0.82$.
[3] All piglets were fed the same diet from 43 to 66 days of age.

a linear effect was observed in feed/gain between zero and 14 days after the weaning.

During the phase 43 to 66 days of age, a single type of diet was provided to the animals to analyze the residual effect of the SPC supply in the phases 21 to 42 days of age. No statistical difference was noted (Table 3); therefore, there was no residual effect of SPC on performance in this second part of the experiment, which agrees with Sohn et al. (1994), Lenehan et al. (2007), and Oliveira et al. (2012).

A different result regarding residual effect was obtained by Bertol et al. (2001), who evaluated substitution of soybean meal for texturized soy protein (TSP), which was used by the authors to evaluate the effects of the reduction of the levels of glycinine and ß-conglycinine in the diet on piglet performance. They associated the residual effect of the initial phase (15 to 35 days) to the increased feed intake provided by the substitution of soybean meal for TSP in the pre-starter diet (zero to 14 days post-weaning).

In the evaluation of the entire experimental period (21 to 66 days of age) (Table 3), no effect of the inclusion of SPC was observed on the performance variables. Lenehan et al. (2007), on the other hand, observed a linear effect on feed/gain during the entire experimental period, as the soybean meal had been completely substituted for one of the studied sources of SPC. In the work of Bertol et al. (2001), however, no change was observed in performance variables, corroborating the data of the present work.

Despite the absence of effect of SPC inclusion on the performance of the piglets between 21 and 66 days of age, Zhang et al. (2013) stated that the extraction of oligosaccharide from the soybean flakes during the processing of SPC improves the digestibility of the nutrients of this food, although due to the small content of SPC normally used, the best results observed may be more closely related to a reduction of the antinutritional factors of these SPC.

The inclusion of SPC in the piglet diets did not have any effect on their intestinal histomorphometry (Table 4). These results are in accordance with those observed by Yang et al. (2007), but different from those observed by Li et al. (1991a,b). Bertol et al. (2000), however, observed a reduction of crypt depth as the level of SPC was increased in the diet in substitution of soybean meal, but there were no differences in relation to villus height.

In reviewing the factors that influence the structure of the small intestine, Pluske et al. (1996) described that the atrophy of intestinal villi after weaning is a consequence of the increased rate of loss of enterocytes. This atrophy has a direct relationship with the digestive and absorptive capacity of the small intestine, having as predisposing factors the withdrawal of sow milk and the change of diet, the maladaptation to the stress of weaning, and the sanitary challenge through which the piglet is subjected. Li et al. (1991a,b) associated the reduction of villus height and the consequent increase in crypt depth with the allergenic proteins of soy. The inflammatory process generated by glycinine and ß-conglycinine, which result in this villous atrophy, is described in detail by Sun et al. (2008) and Hao et al. (2009), respectively. With the absence of effects of the SPC inclusion in the diets on intestinal histomorphology, it is suggested that it may have occurred, or even occurred at a much lower intensity than what is described by the above authors – an inflammatory process induced by the allergenic proteins of soy due to the previous adaptation of the piglets during the lactation period. Friesen et al. (1993) demonstrated that the earlier the contact of piglets with the allergenic proteins of soy, the earlier they will adapt to them and the better their subsequent performance. These authors

Table 4 - Effects of inclusion of soy protein concentrate (SPC) in the diet on intestinal histomorphometry of piglets at 32 days of age

Variable	SPC in the diet (%)				P-value		CV (%)
	0	3	6	9	Linear	Quadratic	
			Duodenum				
Villus height (µm)	344	361	378	375	0.41	0.73	17.78
Crypt depth (µm)	466	47	472	469	0.91	0.90	12.63
Villus height/crypt depth	0.758	0.774	0.813	0.804	0.58	0.86	19.73
			Jejunum				
Villus height (µm)	278	35	347	342	0.07	0.10	14.56
Crypt depth (µm)	389	345	361	363	0.49	0.25	11.48
Villus height/crypt depth	0.722	1.032	0.969	0.954	0.12	0.08	20.51
			Ileum				
Villus height (µm)	272	323	270	284	0.81	0.25	11.89
Crypt depth (µm)	278	288	276	276	0.76	0.68	9.19
Villus height/crypt depth	0.997	1.13	0.988	1.031	0.91	0.57	16.53

CV - coefficient of variation.

Table 5 - Effects of inclusion of soy protein concentrate (SPC) in the diets on some blood cells of piglets at 32 days of age

Variable	SPC in the diet (%)				P-value		CV (%)
	0	3	6	9	Linear	Quadratic	
Leukocytes ($\times 10^3$/mm^3)	14.92	14.38	12.13	14.03	0.44	0.4	20.62
Lymphocytes ($\times 10^3$/mm^3)	7.90	8.52	8.38	7.82	0.94	0.6	27.34
Lymphocytes (%)	50.20	61.20	67.05	55.05	0.77	0.16	21.78
Eosinophils ($\times 10^3$/mm^3)	0.329	0.199	0.312	0.242	0.62	0.66	48.89
Eosinophils (%)	2.20	1.40	2.64	1.73	0.94	0.92	55.7

CV - coefficient of variation.

did not analyze the villi and the crypts of the intestinal mucosa of the piglets; however, by making a connection between adaptation to these proteins and changes in the intestinal mucosa, it can be inferred that the sooner the contact with them, the earlier the recovery of that mucosa.

There was no effect of the SPC inclusion on the counts of eosinophils, lymphocytes, and leukocytes in the blood samples of the piglets (Table 5) and, according to the description in Meyer and Harvey (2004), cell counts are within the patterns of count of these cells for the swine species.

Conclusions

Inclusion of soy protein concentrate in piglet diets does not influence performance, intestinal histomorphometry, or leukocyte, lymphocyte, and eosinophil counts of these animals in the period from 21 to 66 days of life.

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