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Use of Soy Protein Concentrate in Pre-Starter and Starter Diets for Broilers

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ABSTRACT

Two experiments were carried out to evaluate the effect of using soy protein concentrate (SPC) in pre-starter and starter diets for broilers. In the first experiment, 600 male Cobb broilers, between one and 40 days of age, were distributed in a completely randomized design, with four treatments and six replications of 25 birds each. Treatments were ofered to broilers in the pre-starter and starter diets and consisted of inclusion of soy protein concentrate (0,3,6 and 9%) in diets. The parameters evaluated were: body weight gain, feed conversion ratio, consumption of ration, enzyme production in the pancreas, villus: crypt ratio, leukocyte count and immunoglobulin A (IgA) dosage. Aimed to determine the coefficient of nutrient metabolization of feeds, 144 male Cobb chicks were distributed, between 14 and 21 days of age, with four treatments and six replications of six birds per experimental unit. Treatments were the same as in the first experiment. The use of 3 and 9% of SPC did not affect weight gain, feed intake, feed conversion or viability of the poultry. The use of 6% of SPC provided an increase in trypsin activity, villi length and crypt depth; the coefficient of dry matter metabolization increased linearly; but no differences were observed in performance or immunological parameters.

INTRODUCTION

Some of the most relevant anti-nutritional factors (ANF) present in soybean meal that affects the development and performance of monogastrics include α-galactosides – raffinose, stachyose and verbascose – and antigenic factors – glycinin and β-conglycinin (Nunes *et al.*, 2001; Jankowski *et al.*, 2009). The presence of non-digestible glycoside substrate in the intestinal lumen involves changes in chyme viscosity, compromising the integrity of gut mucus and increasing the passage rate, reducing the efficiency of nutrient absorption (Góes & Ribeiro, 2002; Batal & Parsons, 2003). Enzymatic production and morphological structure of the digestive system of poultry, change significantly with age and frequency of feed intake (Souza *et al.*, 2005). Allergic factors cause hypersensitivity reactions, promoting a shortening of the intestinal villus and decreasing the ratio between the villi length and crypt depth (Thomaz *et al.,* 2011). The presence of ANF in the diets of broilers from one to 21 days of age implies a reduction in feed intake, impaired digestion and utilization of nutrients (Feng *et al.,* 2007; Kim *et al*., 2010).

Soy protein concentrate is an ingredient that may be used in poultry diets in replacement of soybean meal, as it contains certain benefits due to its processing: lower levels of oligosaccharides such as raffinose, stachyose and verbascose, higher crude protein, digestible and metabolizable energy compared to soybean meal. Scottá *et al.* (2013),

verified that the average true amino acids digestibility coefficient, of essential and non essential SPC for broilers, were respectively 95,21% e and 94,22% for the soy protein concentrate. Bansemer *et al.* (2015) verifyed that inclusion of SPC in fish diets, decreased globet cell numbers. This suggests SPC having an effect on reducing mucus production in the intestine.

This study was carried out to evaluate the effect of soy protein concentrate inclusion in pre-starter and starter diets of broilers on: performance, enzyme production in the pancreas, gut integrity, nutrient metabolization and immunological parameters.

MATERIALS AND METHODS

Animals and housing

The experimental protocol was submitted to the Ethics Committee for Use of Animals in Research (CEUA – Universidade Federal de Goiás) and approved under number 066/12.

Seven hundred forty-four (744) one-day-old male Cobb broilers were used in the two experiments.

For experiment 1, six hundred (600) one-day-old male Cobb broilers were distributed in a completely randomized design with four treatments (0, 3, 6 and 9% of soy protein concentrate – Table 1 e and 2), and six replications of 25 birds/experimental unit. At 22 days old, the broilers received the same diet, without soy protein concentrate (Table 3).

The birds were housed in a shed with 2.45 m headroom, inside 2.02 x 1.36 x 0.73 m boxes, provided with bed of rice husks (5 cm), tubular feeder and pendulum drinker. Environmental control consisted of heating hoods, negative ventilation system, nebulizer, side curtains, two thermometers, a moisture sealer and 23 hours of light/day. The temperature was recorded in the morning (8 a.m.) and mortality 2x/day (8 a.m. and 4 p.m.). In experiment 1 the variables studied were: body weight gain, feed intake, feed conversion ratio and viability, leukocyte count and immunoglobulin A (IgA) dosage, pancreas weight, amylase and trypsin activity, villi length and crypt depth of small intestine.

One hundred forty-four (144) male Cobb broilers, 14 to 21 days of age, were distributed in a completely randomized design with four treatments, six replications of six birds/experimental unit. Treatments consisted of four levels of inclusion of soy protein concentrate (0, 3, 6 and 9%) in the diet. This group of 144 broilers

Table 1 – Composition of experimental diets of pre-starter phase (1-7 days old)

Ingredients	Soy protein concentrate (%)			
	0	3	6	9
Corn	55.192	57.235	59.279	61.322
Soybean meal 45%	38.215	33.690	29.164	24.639
Soy protein concentrat	0.000	3.000	6.000	9.000
Soybean oil	2.148	1.634	1.119	0.605
Dicalcium phosphate	1.907	1.920	1.933	1.947
Limestone	0.822	0.879	0.875	0.871
Salt	0.495	0.492	0.489	0.486
DL-methionine 99%	0.359	0.354	0.348	0.343
L-lysine HCl 78%	0.288	0.287	0.286	0.286
L-threonine 98%	0.114	0.110	0.107	0.103
Vitamin mineral premix	0.400	0.400	0.400	0.400
Total	100	100	100	100
Nutrient Composition				
Metabolizable energy (Mcal/Kg)	2,950	2,950	2,950	2,950
Crude Protein (%)	22.20	22.20	22.20	22.20
Phosphorus avaiable (%)	0.470	0.470	0.470	0.470
Calcium (%)	0.920	0.920	0.920	0.920
Sodium (%)	0.220	0.220	0.220	0.220
Glycine + Serine digestible (%)	1.8483	1.8465	1.8447	1.8429
Isoleucine digestible (%)	0.8772	0.8782	0.8791	0.8800
Lysine digestible (%)	1.3100	1.3100	1.3100	1.3100
Methionine + cystine digestible (%)	0.9440	0.9440	0.9440	0.9440
Threonine digestible (%)	0.8520	0.8520	0.8520	0.8520
Thriptophan digestible (%)	0.2471	0.2470	0.2469	0.2468

* Composition of Premix: Vit A 2000 Ui; Vit D3 600 Ui; Vit E 5000 Mg; Vit K3 450 Mg; Vit B1 500 Mg; Vit B2 1500 Mg; Vit B6 700 Mg; Vit B122500 Mg; Panthotenic Acid 3500 Mg; Niacin 9000 Mg; Folic Acid 250 Mg; Choline 80000 Mg; Cooper 2500 Mg; Iron 10000 Mg; Manganese 20000 Mg; Iodine 250 Mg; Zinc 18000 Mg; Selenium 750 Mg; Avilamicin 1500 Mg; Narasin/Nicarb.10000 Mg; Biotine 15 Mg; Benzoic Acid 60 G.

Table 2 – Composition of experimental diets of starter phase (8-21 days old)

Ingredients	Soy protein concentrate (%)			
	0	3	6	9
Corn	59.321	61.365	63.408	65.451
Soybean meal 45%	34.722	30.197	25.671	21.146
Soy protein concentrat	0.000	3.000	6.000	9.000
Soybean oil	2.116	1.601	1.087	0.572
Dicalcium phosphate	1.508	1.521	1.535	1.548
Limestone	0.895	0.892	0.888	0.884
Salt	0.468	0.465	0.462	0.459
DL-methionine 99%	0.287	0.282	0.276	0.271
L-lysine HCl 78%	0.218	0.217	0.217	0.216
L-threonine 98%	0.064	0.060	0.057	0.053
Vitamin mineral premix	0.400	0.400	0.400	0.400
Total	100	100	100	100
Nutrient Composition				
Metabolizable energy (Mcal/Kg)	3,0000	3,0000	3,0000	3,0000
Crude Protein (%)	20.8000	20.8000	20.8000	20.8000
Phosphorus avaiable (%)	0.3910	0.3910	0.3910	0.3910
Calcium (%)	0.8190	0.8190	0.8190	0.8190
Sodium (%)	0.2100	0.2100	0.2100	0.2100
Glycine + Serine digestible (%)	1.7344	1.7326	1.7308	1.7290
Isoleucine digestible (%)	0.8209	0.8218	0.8227	0.8237
Lysine digestible (%)	1.1740	1.1740	1.1740	1.1740
Methionine + cystine digestible (%)	0.8460	0.8460	0.8460	0.8460
Threonine digestible (%)	0.7630	0.7630	0.7630	0.7630
Thriptophan digestible (%)	0.2300	0.2299	0.2298	0.2297

*Composition of Premix: Vit A 2000 Ui; Vit D3 600 Ui; Vit E 5000 Mg; Vit K3 450 Mg; Vit B1 500 Mg; Vit B2 1500 Mg; Vit B6 700 Mg; Vit B12 2500 Mg; Panthotenic Acid 3500 Mg; Niacia 9000 Mg; Folic Acid 250 Mg; Choline 80000 Mg; Cooper 2500 Mg; Iron 10000 Mg; Manganese 20000 Mg; Iodium 250 Mg; Zinc 18000 Mg; Selenium 750 Mg; Avilamicin 1500 Mg; Narasin/Nicarb.10000 Mg; Biotine 15 Mg; Benzoic Acid 60 G.

was housed and managed under the same condition of experiment 1 and received a basal diet until 14 days of age, without soy protein concentrate, according to recommendations of Rostagno *et al.* (2011). At 14 days of age, the birds were housed in metabolic cages and the experimental diets were administered (Table 2). In experiment 2 the coefficients of metabolizable dry matter, crude protein, ether extract and ash were calculated. The birds were housed in metabolic cages of 0.77 x 0.74 x 0.23 m in size, fitted with trays for excreta collection, food and water trough type; in a shed 2.00 m in height, provided with heating hoods, side curtains, two thermometers and 23 hours of light/day.

The average temperatures (minimum and maximum) recorded during the experimental period were 23 and 31°C in Experiment 1; 24 and 28°C in Experiment 2, respectively.

Diets and feeding

Treatments consisted of four inclusion levels of soy protein concentrate (0, 3, 6 and 9%) in pre-starter and starter diets.

The experimental diets were iso-nutrient and iso-energetic and were formulated according to Rostagno *et al*. (2011) recommendations, and followed a feeding program divided into pre-starter phase (1-7 days of age) and starter phase (8- 21 days of age) (Tables 1 and 2). The birds received the same diet from 22 to 40 days of age (Table 3). Feed and water were provided *ad libitum*.

Performance analysis

Body weight gain, feed intake, feed conversion ratio and viability of birds were calculated according to Sakomura & Rostagno (2007). Weights were taken on the 1st , 7^{th,} 21st and 40th days of the experiment.

Data collection

For leukocyte count and immunoglobulin A (IgA) dosage, samples were obtained from two birds per replication at 21 days of age. Blood samples were collected in a tube with heparin or without anticoagulante (respectively for WBC and IgA), from the femoral vein; for serum, the samples were centrifuged at 5000 rpm for three minutes and frozen at -20° C. The total leukocyte count followed the protocol set by Natt & Herrick (1952); the specific leukocyte count was obtained as per Garcia-Navarro (2005). The serum IgA

Table 3 – Composition of experimental diets of growing (22-34 days old) and finishing phase (35-40 days old)

Ingredients	22-34 d	35-40 d
Corn	61.921	67.057
Soybean meal 45%	31.519	27.224
Soybean oil	3.112	2.794
Dicalcium phosphate	1.273	1.070
Limestone	0.839	0.748
Salt	0.443	0.429
DL-methionine 99%	0.256	0.240
L-lysine HCl 78%	0.194	0.236
L-threonine 98%	0.043	0.053
Vitamin mineral premix	0.400	0.050
Total	100	100
Nutrient Composition		
Metabolizable energy (Mcal/Kg)	3,1000	3,1500
Crude Protein (%)	19.5000	18.0000
Phosphorus avaiable (%)	0.3420	0.2980
Calcium (%)	0.7320	0.6380
Sodium (%)	0.2000	0.1950
Lysine digestible (%)	1.0780	1.0100
Methionine + cystine digestible (%)	0.7870	0.7370
Threonine digestible (%)	0.7010	0.6560
Thriptophan digestible (%)	0.2137	0.1927

*Composition of Premix: Vit A 1750 Ui; Vit D3 500 Ui; Vit E 3000 Mg; Vit K3 375 Mg; Vit B1 400 Mg; Vit B2 1250 Mg; Vit B6 750 Mg; Vit B12 2500 Mg; Panthotenic Acid 3250 Mg; Niacin 8000 Mg; Folic Acid 250 Mg; Choline 80000 Mg; Copper 2500 Mg; Iron 10000 Mg; Manganese 20000 Mg; Iodium 250 Mg; Zinc 18000 Mg; Selrnium 75 Mg; Avilamicin 2000 Mg; Monensin 27500mg; Biotine 2500 Mg; Benzoic Acid 60 G.

levels were determined by immune turbidimetry (Cobas Mira Plus; Roche Diagnostic Systems, Mannheim, Germany) and using a commercial IgA kit (Turbiquest plus, Labtest®) under an absorbance wave length of 340nm.

These birds were euthanized by cervical dislocation for collection of the pancreas and small intestine.

After weighing, the pancreas was frozen in liquid nitrogen, homogenized on ice and the supernatant was extracted to measure total protein (Bradford, 1976), trypsin (Kunitz, 1947) and amylase content (CNPG amylase kit, Labtest®).

The pancreas and intestines were collected and weighing in analytical balance with three decimal places of accuracy. The pancreas was frozen in liquid nitrogen and homogenized on ice; the supernatant was extracted to measure total protein (Bradford, 1976), trypsin (Kunitz,1947) and amylase content (CNPG amylase kit, Labtest.). Enzyme assays were performed in the enzymology laboratory and Physiology of Digestion, the Institute of Biological Sciences (ICB II), Federal University of Goias.

The small intestine was cut between the proventriculus and the cecum-colic junction, and representative sample from its different anatomical regions was collected (duodenum – bounded by the contour of the pancreas, jejunum – near the Meckel diverticulum and ileum – near the cecum-colic junction). Tissue samples were fixed in 10% neutral buffered formalin for histological processing (Macari *et al.*, 2002), for 24 hours and then processed for paraffin embedding according to the routine protocols; 5 micron section were then stained with Harris haematoxylin and eosin, the slides mounted in Entellan. Microphotographs were collected using a magnification of 50x in a Leica DM2500 optic microscope, and retrospectively analyzed in order to obtain data corresponding to 30 readings of villus and crypts per intestinal segment.

Excreta collection

The birds were acquired from the same batch of experiment 1. By 14 days of age were managed according to the breed manual, and at 14 days of age were allocated in metabolic cages for experimental period. Both experiments occur simultaneously.

The experimental period was eight days – four days for adaptation to the cages and experimental diets, and four days to excreta collection.

The bromatological analysis of rations and excreta followed was proposed by Silva & Queiroz (2009). The coefficients of metabolizable dry matter, crude protein, ether extract and ash were calculated (Sakomura & Rostagno, 2007). Temperature was recorded in the morning (8 a.m.) and mortality 2x/day (8 a.m. and 4 p.m.).

Excreta were homogenized and a sample of 500g each replication collected and identified. Samples were pre-dried in a forced ventilation oven at 65°C for 72 hours. The air dried percentage of excreta was determined as a relation of the weight after and before drying. After this step, the samples were ground in a Wiley mill, and stored in labeled plastic bags. Analyzes of dry matter were held, nitrogen, lipids and ashes according to the methodology described in Silva & Queiroz (2009). In order to calculate the metabolizable coefficients of dry matter, crude protein, ether extract and ashes, the following equations were used:

CMDM= Dry matter intake (g) – Dry matter excreted (g)/ Dry matter intake (g) x 100

CMCP = Nitrogen intake (g) - Nitrogen excreted (g) / Nitrogen intake (g) x 100

CMEE = Ether extract intake (g) - Ether extract excreted (g) / Ether extract intake (g) x100

CM Ash% = ash intake (g) - ash excreted (g) / ash intake (g) x 100

Statistical analysis

The results were analyzed by ANOVA. Variables related to performance, gut integrity and enzymology had their means compared by the Scott-Knott test. The coefficients of metabolizability underwent polynomial regression. Hematological parameters were subjected to the Kruskal-Wallis test, with the difference between treatments assessed by Bonferroni test. We used R software (R Development Core Team, 2011). We adopted $\alpha = 0.05$.

RESULTS AND DISCUSSION

Inclusion of increased levels of SPC in pre-starter and starter diets did not affect the final body weight, body weight gain, feed intake, feed conversion ratio and viability of broilers (Table 4), suggesting that it did not influence broilers performance in any of the rearing periods surveyed (1 to 7, 1 to 21 and 1 to 40 days of age).

No effect of SPC inclusion on broiler performance in any period studied (1 to 7, 1 to 21 and 1 to 40 days of age) was observed. Reduction in anti-nutritional factors does not appear to be the determining factor for improved performance. The results disagreed with Trindade Neto *et al.* (2007), who reported low feed intake associated with the presence of trypsin inhibitors, and with Thomas *et al.* (2011) who associated reduced growth with digestive disorders resulting from transient hypersensitivity reaction caused by glycinin and β-conglycinin allergenic proteins.

Siugzdaite *et al.* (2008) found significant improvements in the performance of piglets with 10% inclusion of SPC in the weaning diet; Lenehan *et al.* (2007), with 14-21% inclusion of SPC; Bertol *et al.* (2001) with 50% replacement of soybean meal by SPC in the nursery phase of pigs diet. These reports suggest that levels of inclusion of SPC proposed in the present study may not have been sufficient to demonstrate improvements in performance.

The inclusion of 3 to 9 % of SPC in the pre-starter and starter diets showed no decrease in pancreatic weight (Table 5), although higher activities of trypsin and amylase were recorded with the use of 3, 6 and 9% of SPC, respectively.

Li *et al.* (1991a and 1991b) hypothesized that the reduction in anti-nutritional factors, provided by the use of SPC, deletes their antigenic power, minimizing the transient hypersensitivity and improving growth. These reports, combined with the results obtained herein, corroborate the theory that the levels proposed in the study were not sufficient to demonstrate improvements in performance.

Diets without SPC resulted in increased small intestine weight and higher villus, crypt ratio in the jejunum (Table 6). The treatments with 6 and 9%

Table 4 – Performance of broilers from one to 40 days, fed increasing levels of SPC in the pre-starter and starter diets (Mean ±SEM)*

Variables	Soy protein concentrate (%)				p Value		CV (%)
	0	3	6	9	Linear	LF	
	Pre-initial phase (1 - 7 days)						
Initial body weight (g)	44.17±0.1304	44.13±0.1304	44.23±0.1304	44.20±0.1304	0.7352	0.9012	0.72
Final body weight (g)	145.11 ±1.9838	146.62±1.9838	144.86±1.9838	142.17±1.9838	0.2473	0.5605	3.36
Body weight gain (g)	100.94±2.0233	102.49±2.0233	100.62±2.0233	97.97±2.0233	0.2477	0.5686	4.93
Feed intake (g)	113.33±2.7702	111.55±2.7702	114.57±2.7702	114.17±2.7702	0.6609	0.7803	5.98
Feed conversion ratio	1.122±0.0249	1.091±0.0249	1.139±0.0249	1.166±0.0249	0.1243	0.3548	5.40
Viability (%)	100.00±0.3333	99.33±0.3333	100.00±0.3333	100.00±0.3333	0.6595	0.2697	0.82
	Initial phase (1 - 21 days)						
Final body weight (g)	782.28±13.8551	772.72±13.8551	746.30±13.8551	756.61±13.8551	0.1107	0.5423	4.44
Body weight gain (g)	738.11±13.8525	728.59±13.8525	702.07±13.8525	712.41±13.8525	0.1100	0.5403	4.71
Feed intake (g)	1073.99±16.8165	1063.81±16.8165	1032.90±16.8165	1057.73±16.8165	0.3018	0.3655	3.90
Feed conversion ratio	1.457±0.0216	1.460±0.0216	1.475±0.0216	1.485±0.0216	0.3149	0.9754	3.60
Viability (%)	99.33±0.7601	98.67±0.7601	98.67±0.7601	99.33±0.7601	1.0000	0.6856	1.88
	Total phase (1 - 40 days)						
Final body weight (g)	2490.77±33.6955	2548.73±33.6955	2472.44±33.6955	2482.8±33.6955	0.5141	0.2884	3.30
Body weight gain (g)	2446.60±33.6512	2504.60±33.6512	2428.21±33.6512	2438.64±33.6512	0.5127	0.2869	3.36
Feed intake (g)	4201.79±54.9045	4289.57±54.9045	4148.01±54.9045	4215.41±54.9045	0.6861	0.2247	3.19
Feed conversion ratio	1.717±0.0125	1.713±0.0125	1.708±0.0125	1.729±0.0125	0.5988	0.5552	1.78
Viability (%)	98.00±1.1055	95.33±1.1055	98.67±1.1055	98.00±1.1055	0.5079	0.1114	2.78

LF = lack of fit; CV = coefficient of variation, SEM = standard error of mean

*mean of 6 replicates, with 25 broilers/replicate.

Table 5 – Weight and enzymology of the pancreas of broilers at 21 days of age, fed increasing levels of soy protein concentrate in the pre-starter and starter diets (Mean ±SEM)

Variables	Soy protein concentrate (%)				p Value	CV (%)
	0	3	6	9		
Weight of pancreas (g)	2.467±0.1249	2.358±0.1249	2.433±0.1249	2.567±0.1249	0.7006	12.46
Total protein (mg/mL)	2830.08±381.978	4049.37±381.978	3908.64±381.978	3630.86±381.978	0.1413	25.96
Trypsin activity (UT/g)*	5.74 ±0.4705b	8.27±0.4705a	8.32±0.4705a	6.31±0.4705b	0.0011	16.09
Amylase activity (mg/mL)*	6110.68 ±1304.987b	8457.77±1304.987b	4405.95±1304.98b	13489.61±1304.987a	0.0061	39.39

*Different letters in the line indicate differences between treatments by the Scott-Knott test.

UT = trypsin units; CV = coefficient of variation.

*mean of 6 replicates, with 2 broilers/replicate.

SEM = standard error of mean

Table 6 – Weight and intestinal histomorphometry of broilers from 21 days of age, fed increasing levels of soy protein concentrate in the pre-starter and starter diets (Mean ±SEM)

Variables		Soy protein concentrate (%)				p Value	CV (%)
		0	3	6	9		
Small intestine weight		57.9000±1.4089[a]	50.9083±1.4089[b]	51.7583±1.4089[b]	48.8417±1.4089[b]	0.0013	6.59
Duodenum	Length villi	957.27±47.5510[b]	969.51±52.0895[b]	1803.90±47.5510[a]	1924.10±47.5510[a]	0.0010	8.13
	Depth crypt	203.89±20.8778[b]	200.76±22.8704[b]	402.35±20.8778[a]	444.69±20.8778[a]	0.0010	16.09
	Villus:Crypt	5.277±0.2699	4.977±0.2956	4.93±0.2699	4.208±0.2699	0.0674	13.65
Jejunum	Length villi	658.68 ±58.3792[b]	671.39±58.3792[b]	1284.35±58.3792[a]	1357.15±58.3792[a]	0.0010	14.40
	Depth crypt	137.54±16.6565[b]	152.99±16.6565[b]	332. ±16.6565[a]	332.92±16.6565[a]	0.0010	17.07
	Villus:Crypt	4.993±0.1741[a]	4.503±0.1741[b]	3.995±0.1741[b]	4.187±0.1741[b]	0.0036	9.65
Ileum	Length villi	570.12±64.5633[b]	581.04±64.5633[b]	1096.25±64.5633[a]	1181.05±64.5633[a]	0.0010	18.45
	Depth crypt	132.76±16.2917[b]	151.42±16.2917[b]	284.12±16.2917[a]	283.01±16.2917[a]	0.0010	18.75
	Villus:Crypt	4.475±0.1447	4.029±0.1447	3.923±0.1447	4.252±0.1447	0.0624	8.50

*Different letters in the line indicate differences between treatments.

CV = coefficient of variation.

SEM = standard error of mean

inclusion of SPC provided greater villi length and crypt depth in the duodenum, jejunum and ileum, indicating greater absorption area which resulted in better utilization of nutrients in the diets.

There are very few reports on the effects of glycinin and β-conglycinin on the mucous secretion and moisture in the excreta of birds, which does not make it possible to discard them as predisposing factors to inflammatory reactions observed in broilers between seven and ten days of age (Ortiz, 2009).

Cortés (2012) tested the effect of a mono competent protease on anti-nutritional factors of soybean, and a reduction in the presence of these factors was proven. A linear relationship was observed for villi length and a cubic relationship to crypt depth, with increased surface area for absorption and villus: crypt ratio at 14 days of age. This author drew attention to the fact that the reduction of the stimulus on the pancreatic secretion and increasing the absorptive surface of the intestine brought about a shift in nutrient utilization for performance and carcass yield with lower levels of fat.

The use of 3 to 9% SPC did not affect leukocyte count and the dosage of immunoglobulin A in blood (Table 7). Based on these results, it can be inferred that the reduction of allergenic dietary factors was not enough, which explains the lack of difference in performance and in pancreatic weight, despite increased CMDM.

In Brazil there is paucity of data on reference levels for hematological and biochemical values in broiler chickens, showing the importance of studies that include such evaluations in several experimental situations (Minafra, 2010). Furthermore, the method commonly used, immune histochemistry, enables the determination of dimeric IgA and observation of leukocytes in slide, contributing to greater accuracy in the discussion about the relevance of antigenic factors in catabolic processes associated with the immune response.

Table 7 – Leukocyte count (total and specific) and dosage of immunoglobulin A in broilers at 21 days of age, fed increasing levels of Soy protein concentrate in the pre-starter and starter diets (Mean ±SEM)

Variables	Soy protein concentrate (%)				p Value	CV (%)
	0	3	6	9		
Total leukocyte	9891.67±665.9135 (9725.00)	8510.00±729.4716 (8950.00)	8441.67±665.9135 (8962.50)	8962.50±665.9135 (8625.00)	0.6949	18.18
Eosinophils	0.92±0.3241 (0.50)	0.30±0.3551 (0.33)	0.4±0.32412 (0.25)	0.39±0.3241 (0.17)	0.8553	154.31
Lymphocytes	52.37±6.8496 (57.33)	47.03±7.5034 (45.25)	47.67±6.8496 (55.25)	43.50±6.8496 (45.00)	0.5929	35.37
IgA dosage	20.49±2.547 (20.52)	16.39±2.547 (16.68)	14.70±2.547 (14.02)	13.24±2.547 (13.80)	0.3865	38.49

*Values in parentheses represent the median
CV = coefficient of variation.
SEM = standard error of mean

In the present study the coefficient of metabolization of dry matter increased linearly and the coefficient of metabolization of ash decreased linearly according to higher SPC inclusion in diets (Table 8). According to Batal & Parsons (2003), the increase in the use of nutrients in SPC is due to the removal of soluble non-starch oligosaccharides.

Soluble non-starch oligosaccharides negatively interfere with the absorption of minerals (Arruda, 2003). Although SPC has a higher concentration of minerals due to processing (Miranda, 2012), the metabolization of nutrients decrease in the content of α-galactosides, the excretion of minerals (Fischer *et al.*, 2002) causing CMA increase - contradicting the results.

The coefficients of metabolization of crude protein and ether extract were not affected by using SPC. Pinheiro *et al.* (2008), comparing the metabolization of different soybean subproducts also found no significant differences for the CMCP and CMEE diets low in fiber. Methods that allow the exclusion of nitrogen from uric acid in the calculation of the CMCP and to enable inclusion of the percentage of complex lost minerals with organic matter in the calculation of CMA, contribute to a greater accuracy in the analysis of results.

In conclusion, the inclusion of 6% of soy protein concentrate increased trypsin activity, the villi length and crypt depth in the small intestine, suggesting an improvement in the process of digestion and nutrient absorption, yet insufficient to show an increase in performance parameters. Similar to what occurs with other species (pigs, cattle and fish), studies using SPC for broilers from one to 21 days of age should be encouraged. The use of soy protein concentrate does not affect broiler performance and can be used in pre-starter and starter diets until 9%.

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Table 8 – Coefficients of metabolization of dry matter, crude protein, ether extract and ash of the experimental diets (Mean ±SEM)

Variables	Soy protein concentrate (%)				P Value		CV (%)
	0	3	6	9	linear	LF	
CMDM (%)[1]	74.32±0.5366	74.53±0.5366	76.11±0.5366	76.41±0.5366	0.0038	0.5505	1.74
CMCP (%)	67.64±1.1418	67.39±1.1418	68.82±1.1418	68.40±1.1418	0.4681	0.7813	4.03
CMEE (%)	87.67±0.8583	86.00±0.8583	87.85±0.8583	88.82±0.8583	0.1823	18.4600	2.40
CMA (%)[2]	91.17±0.7989	89.38±0.7989	89.48±0.7989	87.04±0.7989	0.0026	0.4433	2.19

[1]y = 74.1640 + 0.0262x (R^2 = 0.89); [2]y = 91.1139 – 0.0409x (R^2 = 0.86).
LF = lack of fit; CV = coefficient of variation, CMDM = Coefficients of metabolization of dry matter, CMCP = Coefficients of metabolization of crude protein, CMEE = Coefficients of metabolization ether extract, CMA= Coefficients of metabolization of ash.
*mean of 6 replicates, with 6 broilers/replicate
SEM = standard error of mean

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