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Immune Response of Broilers Fed Conventional and Alternative Diets Containing Multi-Enzyme Complex

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■**Keywords**

Carbohydrases, non-starch polysaccharides, fibrous brans.

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ABSTRACT

The present study aimed at evaluating the effect of adding a commercial multi-enzyme complex to conventional and alternative broiler diets on the immune response and occurrence of lesions in the intestinal mucosa. In total, 900 male broiler chicks were distributed according to a completely randomized design, with six treatments of six replicates each. Two control diets were formulated: one with conventional feedstuffs (T1), based on corn and soybean meal, and one with alternative feedstuffs (T4), containing corn, millet, and soybean, canola and sunflower meals. Based on these diets, other four were prepared with reduced metabolizable energy, digestible amino acids, calcium and available phosphorus levels and the addition (T3 and T6) or not (T2 and T5) of a multi-enzyme complex. Broilers fed diets based on conventional feedstuffs had higher levels of defense cells compared with those fed diets that included millet with canola and sunflower meals. On the other hand, the use of enzymes in conventional or alternative diets decreased the number of these cells in the ileal mucosa.

INTRODUCTION

The quality of feedstuffs for feed production can be highly variable, especially considering their nutritional composition (Maisonnier *et al.*, 2001). Antinutritional factors and non-starch polysaccharides (NSP) in plant ingredients that make up the diet may have negative effects on the gut health and performance of poultry and pigs (Farrell, 2005; Jia *et al.*, 2009). NSP are the main constituents of the cell wall of plant cells, but cannot be digested by poultry due to the nature of the chemical bonds that resist to hydrolysis in the digestive tract (Ramos *et al.*, 2007). Monogastric animals are not able to digest cellulose, arabinoxylans, beta-glucans, pectins, and other NSPs (Fang *et al.*, 2007).

The inability to digest fiber, in addition of reducing feed energy value, can impair the use of all other nutrients. This is especially the case of soluble fibers, which present high capacity to absorb water and gelatinize the ingesta in the intestinal tract (Choct *et al.*, 2010). This increased viscosity of the intestinal chime slows the passage of feed along the digestive tract, makes it difficult the dispersion and action of endogenous enzymes and negatively interferes with the diffusion or transport of nutrients by enterocytes (Choct *et al.*, 2004). Moreover, the utilization of dietary fats may be impaired due to the decrease in emulsification and conjugation of bile salts with these compounds (Campbell *et al.*, 1983).

In addition to digestion impairment caused by intestinal flow conditions induced by NSP, the presence of undigested fiber fragments negatively contributes to the inflammatory status of the enteric mucosa (Lentle & Janssen, 2008). The intake of NSP-rich ingredients promotes

epithelial apoptosis along crypts and villi, villus fusion and increase in number and cytoplasmic extension of goblet cells, making the mucus layer of the epithelial lining of the intestinal mucosa thicker (Lentle *et al.*, 2007). When injury occurs, there is a disruption of the intestinal dynamics that, in addition of reducing the amount of digested and absorbed substrate, also increases the metabolic cost for the recovery of the damaged tissue. Thus the regeneration of the intestinal mucosa is a costly and time-consuming process, which may take 96 hours that represents between 8 and 9% of the lifetime of a broiler (Macari *et al.*, 2002).

Diets with alternative plant feedstuffs, which are usually NSP-rich, also stimulate the replication of the microbial population in the intestinal lumen, elevating the fermentation rate and the risk of producing bacterial enterotoxins. The colonization by bacteria is favored by the increase in substrates that are not used by the animals and become available for the multiplication of microorganisms (Högberg & Lindberg, 2004). The shift in microbial populations can damage the mucosa and increase the infiltration of immune cells in the intestinal tract of poultry (Teirlynck *et al.*, 2009). Experiments have shown that some abrasive NSP, by breaking cells of the intestinal mucosa, stimulate the innate immune system and increase the proliferation of macrophages and monocytes at the site, resulting in cytokine production (Peng *et al.*, 1991; Zhang & Tizzard, 1996; Ross *et al.*, 2002). The nutritional and metabolic cost to support an immune response in broilers is high and inversely correlated with growth performance (Humphrey & Klasing, 2007).

The addition of exogenous enzymes to diets can used as a strategy to improve the utilization of NPS-rich ingredients (Kocher *et al.*, 2000; Montanhini *et al.*, 2012). Enzymes hydrolyze NSP from plant ingredients and improve the production efficiency of animals by increasing the digestion of low-quality products and reducing nutrient loss via feces (Kim *et al.*, 2005; Shirmohammad & Mehr, 2011). By calculating this reduction of losses with the better utilization of dietary nutrients, it is possible to decrease the dietary level of nutrients, making cheaper feed, without compromising the broiler performance (Meng *et al.*, 2005).

Another benefit of using these enzymes is the reduction of intestinal digesta viscosity by changing the intestinal microbiota and reducing the adverse effects of microbial fermentation in the small intestine. This effect can also reduce excreta moisture, which improves litter quality and increases nutrient diffusion rates (Oliveira & Moraes, 2007; Lee *et al.*, 2010). The degradation of NSP present in the plant cell wall by the action of exogenous enzymes increases the levels of substrate available for microbial fermentation in the cecum and the production of short chain fatty acids, which can be used by broilers as a source of energy (Choct *et al.*, 1996; Steenfeldt *et al.*, 1998).

Nevertheless, the addition of exogenous enzymes or enzyme complexes can sometimes render controversial results. The enzyme should be specific and supplied in an amount proportional to the substrate, but the determination of the amount of NSP present in feed hinders its inclusion (Rutherfurd *et al.*, 2007; Woyengo *et al.*, 2010). Furthermore, the enzyme should remain stable during and after feed processing until its consumption and in the intestinal environment where it will act (Odetallah *et al.*, 2002).

The present study aimed at evaluating the effect of adding a commercial multi-enzyme complex to diets made up by conventional and alternative plant ingredients, with reduced nutritional density, on the immune response and occurrence of intestinal injury in broilers.

MATERIAL AND METHODS

The experiment was conducted in the experimental poultry house of the Federal University of Paraná, campus of Palotina, Paraná State, Southern Brazil, with the approval of the Ethics Committee on the Use of Animals in Experimentation from the UFPR/Palotina, protocol #13/2010 of March 14, 2010.

Nine-hundred one-day-old male broiler chicks (Cobb Slow commercial line) from the same breeder flock were distributed according to a completely randomized design into six treatments with six replicates of 25 chicks each, comprising 36 experimental units.

Treatments were divided as follows: T1, conventional control diet based on corn and soybean meal; T2, conventional diet with reduced nutritional density; T3, T2 diet with the addition of a commercial multi-enzyme complex composed of carbohydrolases and phytase (Rovabio MAX AP, Adisseo France SAS); T4, alternative control diet with the same nutritional levels as T1, containing corn, millet, and soybean, canola and sunflower meals; T5, alternative diet with reduced nutritional density; and T6, T5 diet with the addition of the above-mentioned commercial multi-enzyme complex.

With the purpose of further discussing the results, the contrasts between the inclusion of the enzyme

complex (T3 and T6) in diets based on conventional and alternative ingredients and reduced nutritional density (T2 and T5), and the comparison between treatments with regular (T1 and T4) and reduced (T2 and T5) nutritional density were considered.

Feeds were formulated according to nutritional recommendations adopted by the Brazilian broiler industry (Rostagno, 2011), with a feeding schedule consisting in three phases: pre-starter (1-7 days), starter (8-21 days) and grower (22-42 days). The replacement of corn and soybean meal by alternative feedstuffs (T4, T5 and T6) followed this methodology: pre-starter phase, 75:25 ratio between corn and millet, in addition to soybean meal; starter phase, 50:50 between corn and millet, 75:12.5:12.5 between soybean, sunflower and canola meals; and grower phase, 25:75 between corn and millet, 50:25:25 between soybean, sunflower and canola meals.

All samples of plant ingredients were previously analyzed at the Center for Nutrition Support of Adisseo Brazil Animal Nutrition Ltd. to determine crude protein, fiber, ether extract, digestible amino acids, metabolizable energy and phytic phosphorus contents. These analyses enabled higher accuracy in the formulation of diets. The composition of ingredients and nutritional levels of experimental diets for different phases are listed in Tables 1, 2 and 3.

At 42 days of age, blood was collected from two broilers per experimental unit (12 broilers/treatment) to evaluate the electrophoretic profile of immunoglobulin A (IgA). Serum samples were stored (-20ºC) and later subjected to vertical electrophoresis on polyacrylamide gel at 12% in the presence of reducing agent (SDS-PAGE). The electrophoretic run took approximately three hours, using 40 mA current in vertical vert (Loccus LCV). The obtained gel was stained with Coomassie Blue (Brilliant Blue R B 0630, Sigma), and distained with acetic acid at 10% (v/v) and kept in distilled water. Protein bands revealed were quantified by densitometry (Zenith Z-30 Turbo), comparing with a standard of gradient concentration of bovine serum albumin (Maciel *et al.*, 2007).

In order to evaluate the integrity of the intestinal mucosa, at 42 days of age, two broilers per experimental

Table 1 – Composition and nutritional levels of the pre-starter diets.

Ingredients	T1*	T2	T3	T4	T5	T6
Corn	46.79	49.40	49.40	38.86	40.45	40.45
Millet	-	-	-	9.74	10.14	10.14
Soybean meal	44.63	43.38	43.38	43.00	41.79	41.79
Soybean oil	3.79	2.82	2.82	3.56	3.19	3.19
Limestone	1.13	1.44	1.44	1.13	1.44	1.44
Dicalcium phosphate	1.97	1.14	1.14	1.97	1.15	1.15
Salt	0.53	0.54	0.54	0.54	0.55	0.55
L-Lysine 78%	0.18	0.19	0.19	0.21	0.22	0.22
DL-Methionine 99%	0.38	0.36	0.36	0.36	0.36	0.36
L-Threonine 98%	0.08	0.08	0.08	0.08	0.08	0.08
Multi-enzyme complex **	-	-	0.10	-	-	0.10
Inert material	-	0.10	-	-	0.10	-
Vitamin and mineral premix***	0.40	0.40	0.40	0.40	0.40	0.40
Metabolizable Energy (kcal/kg)	2.995	2.910	2.910	2.995	2.910	2.910
Crude Protein (%)	23.96	23.48	23.48	23.62	23.15	23.15
Crude Fiber (%)	3.26	3.15	3.15	3.43	3.32	3.32
Crude Fat (%)	5.97	5.10	5.10	5.85	5.55	5.55
Digestible Lysine (%)	1.26	1.23	1.23	1.26	1.23	1.23
Digestible Met + Cis (%)	0.91	0.89	0.89	0.91	0.89	0.89
Digestible Threonine (%)	0.81	0.79	0.79	0.81	0.79	0.79
Digestible Valine (%)	0.94	0.92	0.92	0.94	0.92	0.92
Total Calcium (%)	0.95	0.80	0.80	0.95	0.80	0.80
Available Phosphorus (%)	0.48	0.33	0.33	0.48	0.33	0.33

* T1, control diet with conventional feedstuffs; T2, T1 with nutritional reduction (metabolizable energy, digestible amino acids, phosphorus and calcium); T3, T2 with multi-enzyme complex; T4, control diet with alternative feedstuffs; T5, T4 with nutritional reduction; T6, T5 with multi-enzyme complex.

** Rovabio MAX AP, Adisseo France SAS

*** Supplemented per kilogram of premix: biotin, 0.03 mg; choline, 385.55 mg; copper, 24.8 mg; folic acid, 0.69 mg; iodine, 3.31 mg; iron, 110.25 mg; manganese, 220.5 mg; niacin, 27.56 mg; pantothenic acid, 6.62 mg; selenium, 0.33 mg; thiamin 3.31 mg; vitamin A, 7,717.5 IU; vitamin B12B, 0.01 mg; vitamin B6, 1.38 mg; vitamin D3, 2,103.75 ICU; vitamin E, 16.54 IU; vitamin K, 0.83 mg; zinc, 220.5 mg. Monensin sodium, 110 g/ton; Bacitracin methylene disalicylate, 50 g/ton.

Table 2 – Composition and nutritional levels of the starter diets.

Ingredients	T1*	T2	T3	T4	T5	T6
Corn	50.32	53.26	53.26	28.83	31.26	31.26
Millet	-	-	-	14.38	15.60	15.60
Soybean meal	41.45	40.08	40.08	30.69	29.46	29.46
Sunflower meal	-	-	-	7.67	7.37	7.37
Canola meal	-	-	-	7.67	7.37	7.37
Soybean oil	4.05	3.00	3.00	6.58	5.28	5.28
Limestone	1.04	1.23	1.23	0.99	1.19	1.19
Dicalcium phosphate	1.73	0.90	0.90	1.61	0.80	0.80
Salt	0.46	0.47	0.47	0.46	0.46	0.46
L-Lysine 78%	0.16	0.17	0.17	0.32	0.32	0.32
DL-Methionine 99%	0.33	0.33	0.33	0.30	0.29	0.29
L-Threonine 98%	0.06	0.06	0.06	0.10	0.10	0.10
Multi-enzyme complex **	-	-	0.10	-	-	0.10
Inert material	-	0.10	-	-	0.10	-
Vitamin and Mineral Premix***	0.40	0.40	0.40	0.40	0.40	0.40
Metabolizable Energy (kcal/kg)	3.050	2.965	2.965	3.050	2.965	2.965
Crude Protein (%)	22.78	21.97	21.97	22.20	21.76	21.76
Crude Fiber (%)	3.08	3.06	3.06	5.13	5.07	5.07
Crude Fat (%)	6.33	5.37	5.37	7.77	6.23	6.23
Digestible Lysine (%)	1.18	1.16	1.16	1.18	1.16	1.16
Digestible Met + Cis (%)	0.86	0.84	0.84	0.86	0.84	0.84
Digestible Threonine (%)	0.76	0.74	0.74	0.76	0.74	0.74
Digestible Valine (%)	0.90	0.88	0.88	0.90	0.88	0.88
Total Calcium (%)	0.90	0.75	0.75	0.90	0.75	0.75
Available Phosphorus (%)	0.45	0.30	0.30	0.45	0.30	0.30

* T1, control diet with conventional feedstuffs; T2, T1 with nutritional reduction (metabolizable energy, digestible amino acids, phosphorus and calcium); T3, T2 with multi-enzyme complex; T4, control diet with alternative feedstuffs; T5, T4 with nutritional reduction; T6, T5 with multi-enzyme complex.

** Rovabio MAX AP, Adisseo France SAS

*** Supplemented per kilogram of premix: biotin, 0.03 mg; choline, 385.55 mg; copper, 24.8 mg; folic acid, 0.69 mg; iodine, 3.31 mg; iron, 110.25 mg; manganese, 220.5 mg; niacin, 27.56 mg; pantothenic acid, 6.62 mg; selenium, 0.33 mg; thiamin 3.31 mg; vitamin A, 7,717.5 IU; vitamin B12B, 0.01 mg; vitamin B6, 1.38 mg; vitamin D3, 2,103.75 ICU; vitamin E, 16.54 IU; vitamin K, 0.83 mg; zinc, 220.5 mg. Monensin sodium, 110 g/ton; Bacitracin methylene disalicylate, 50 g/ton.

unit (12 broilers/treatment) were killed by cervical dislocation. The small intestine was removed and fragments measuring approximately 5 cm long were taken from the duodenum, jejunum and ileum. Each fragment was semi-serially sectioned (5 µm thick) and then stained with hematoxylin and eosin (HE). Stained slides allowed assessing microscopic injuries and the scoring (0, 1, 2 or 3) of the lesions of the epithelium and lamina propria of the mucosa, presence of edema, hemorrhage, polymorphonuclear neutrophils and dilated lymphatics vessels.

In order to quantify goblet cells, slides with ileum fragments were stained with HE and Alcian Blue, and subsequently marked by the immunohistochemical technique with Anti-CD3 primary antibody (CD3 Dako 1:750). We quantified ten microscopic fields/slide under light microscopy (Olympus America Inc., NY), with 100x magnification for each fragment.

Statistical analysis of data was undertaken by means of the GLM procedure of SAS. Since the data did not present normal distribution, data relative to microscopic lesions of the intestinal mucosa were analyzed by the Kruskal-Wallis non-parametric test (SAS, 1998).

RESULTS AND DISCUSSION

The feeding of diets containing conventional or alternative feedstuffs, added or not with enzymes, had no influence on the percentage of microscopic lesions in the mucosa in none of the intestinal segments examined (Tables 4 and 5). On the other hand, there was a higher incidence of lesions for alternative ingredients, regardless the nutritional density. These lesions, located in the luminal epithelium and lamina propria of the mucosa, occurred more often in the jejunum and ileum. The alternative feedstuffs used, besides being

Table 3 – Composition and nutritional levels of the grower diets.

Ingredients	T1*	T2	T3	T4	T5	T6
Corn	55.35	58.32	58.32	22.72	24.63	24.63
Millet	-	-	-	22.56	24.43	24.43
Soybean meal	35.71	34.35	34.35	21.16	20.36	20.36
Sunflower meal	-	-	-	10.58	10.18	10.18
Canola meal	-	-	-	10.58	10.18	10.18
Soybean oil	5.17	4.19	4.19	8.77	7.16	7.16
Limestone	0.95	1.14	1.14	0.88	1.08	1.08
Dicalcium phosphate	1.52	0.69	0.69	1.34	0.52	0.52
Salt	0.39	0.40	0.40	0.37	0.39	0.39
L-Lysine 78%	0.16	0.17	0.17	0.36	0.36	0.36
DL-Methionine 99%	0.29	0.29	0.29	0.23	0.23	0.23
L-Threonine 98%	0.06	0.06	0.06	0.10	0.10	0.10
Multi-enzyme complex**	-	-	0.10	-	-	0.10
Inert material	-	0.10	-	-	0.10	-
Vitamin and Mineral Premix***	0.40	0.40	0.40	0.40	0.40	0.40
Metabolizable Energy (kcal/kg)	3.175	3.090	3.090	3.175	3.090	3.090
Crude Protein (%)	20.62	20.21	20.21	19.69	19.30	19.30
Crude Fiber (%)	2.89	2.88	2.88	5.80	5.74	5.74
Crude Fat (%)	7.57	6.70	6.70	10.08	8.62	8.62
Digestible Lysine (%)	1.05	1.03	1.03	1.05	1.03	1.03
Digestible Met + Cis (%)	0.78	0.76	0.76	0.78	0.76	0.76
Digestible Threonine (%)	0.68	0.67	0.67	0.68	0.67	0.67
Digestible Valine (%)	0.81	0.79	0.79	0.81	0.79	0.79
Total Calcium (%)	0.80	0.65	0.65	0.80	0.65	0.65
Available Phosphorus (%)	0.40	0.25	0.25	0.40	0.25	0.25

* T1, control diet with conventional feedstuffs; T2, T1 with nutritional reduction (metabolizable energy, digestible amino acids, phosphorus and calcium); T3, T2 with multi-complex enzyme; T4, control diet with alternative feedstuffs; T5, T4 with nutritional reduction; T6, T5 with multi-enzyme complex.

** Rovabio MAX AP, Adisseo France SAS

*** Supplemented per kilogram of premix: biotin, 0.03 mg; choline, 385.55 mg; copper, 24.8 mg; folic acid, 0.69 mg; iodine, 3.31 mg; iron, 110.25 mg; manganese, 220.5 mg; niacin, 27.56 mg; pantothenic acid, 6.62 mg; selenium, 0.33 mg; thiamin 3.31 mg; vitamin A, 7,717.5 IU; vitamin B12B, 0.01 mg; vitamin B6, 1.38 mg; vitamin D3, 2,103.75 ICU; vitamin E, 16.54 IU; vitamin K, 0.83 mg; zinc, 220.5 mg. Monensin sodium, 110 g/ton; Bacitracin methylene disalicylate, 50 g/ton.

more abrasive, are richer in non-digestible fiber for poultry, becoming thus substrate for the development of microorganisms at the end of the small intestine. The use of non-digestible substrates by bacteria causes adverse effects on the intestinal mucosa, in addition of villus atrophy in the intestinal mucosa.

The digestive system is naturally in permanent contact with agents and substances from the external environment. In addition of its primary role and importance in digestion and absorption of nutrients, the gastrointestinal mucosa represents a gateway to dietary antigens and non-pathogenic bacterial

Table 4 – Percentage of microscopic lesion scores in intestinal segments of 42-d-old broilers fed conventional or alternative diets, supplemented or not with an multi-enzyme complex.

	Duodenum			Jejunum			Ileum		
	Lesion 1*	Lesion 2	Lesion 3	Lesion 1	Lesion 2	Lesion 3	Lesion 1	Lesion 2	Lesion 3
Treatments									
Conventional ingredients									
With enzyme	27.78	33.33	8.33	12.12	21.21	12.12	11.11	13.89	5.55
Without enzyme	19.44	25.00	2.78	24.24	30.31	15.15	8.33	27.78	2.77
Alternative ingredients									
With enzyme	5.55	19.44	0.00	39.39	30.30	6.06	27.78	36.11	13.89
Without enzyme	24.24	33.33	9.09	22.22	18.52	18.18	11.11	22.22	8.33
CV, %	48.33	53.28	34.53	49.84	52.84	50.38	43.94	50.96	40.45
p - value	0.0596	0.4803	0.1960	0.0669	0.5554	0.8240	0.1670	0.1192	0.3605

* 1. Lesion of the epithelium and lamina propria of the mucosa, 2. Presence of edema, hemorrhage, polymorphonuclear neutrophils, 3. Dilated lymphatics vessels.

Table 5 – Percentage of microscopic lesion scores in intestinal segments of 42-d-old broilers fed conventional or alternative diets with normal or reduced nutritional density.

	Duodenum			Jejunum			Ileum		
	Lesion 1*	Lesion 2	Lesion 3	Lesion 1	Lesion 2	Lesion 3	Lesion 1	Lesion 2	Lesion
Treatments									
Conventional ingredients									
Normal density	8.33ab	27.77	2.78	21.21ab	24.24ab	9.09	5.55b	27.78	2.78
Reduced density	27.78a	33.33	8.33	12.12b	12.21b	12.12	11.11ab	13.90	5.55
Alternative ingredients									
Normal density	27.78a	33.33	8.33	27.27ab	12.12b	6.06	27.78a	38.89	5.55
Reduced density	5.55b	19.44	0.00	39.39a	30.30a	18.18	27.78a	36.11	13.89
CV, %	45.27	50.56	37.87	48.80	52.10	46.67	47.52	50.20	38.81
p - value	0.0073	0.3585	0.2218	0.0493	0.4476	0.5038	0.0335	0.0651	0.3209

* 1. Lesion of the epithelium and lamina propria of the mucosa, 2. Presence of edema, hemorrhage, polymorphonuclear neutrophils, 3. Dilated lymphatics vessels.

microflora, which are a strong source of disturbance for the immune activity in the organism (Berg, 1999). Therefore, the intestinal mucosa should have adequate structural morphophysiological characteristics. The absorption process is dependent on transport mechanisms that occur in the membrane of epithelial cells of the mucosa, and thus their integrity is essential, since this is the route of entry of nutrients used for broiler development.

The amount of cells positive for CD3 (CD3+) in the mucosa of the ileum was significantly different ($p<0.05$), when conventional and alternative feedstuffs are compared (Tables 6 and 7). Broilers fed diets based on corn and soybeans presented higher amounts of these defense cells than those fed the diets that included fibrous ingredients.

Table 6 – Quantification of $CD3^+$ cells and goblet cell counts in the mucosa of the ileum of broilers, and serum electrophoretic profile of immunoglobulin A (IgA) of broilers fed conventional or alternative diets supplemented or not with an multi-enzyme complex.

Treatments	Ileal mucosa		Serum
	CD3+	Goblet cells	IgA (g/dL)
Diets			
Conventional ingredients	94.95[a]	51.28[a]	0.103
Alternative ingredients	92.14b	39.41b	0.108
Enzyme			
With enzyme inclusion	94.57[a]	43.20	0.105
With no enzyme inclusion	92.52b	47.97	0.106
CV, %	2.63	19.09	36.66
p - values			
Ingredients	0.0111	0.0031	0.9325
Enzyme	0.0500	0.2388	0.2659
Interaction	0.5730	0.2477	0.8713

Means of a same factor followed by different letters indicate significant effect ($p<0.05$) of treatments by the Fisher LSD test. No interactions between the factors tested were observed ($p>0.05$).

The inclusion of enzymes in conventional or alternative diets reduced ($p<0.05$) the number of CD3 in the mucosa of the ileum. These cells, counted using immunohistochemical analysis, are supposed to be T lymphocytes, because T lymphocytes present molecules that are expressed on their surface, called cell determinants (CD). CD3 is expressed by all lymphocytes at their origin and remains present throughout the life of cells, being a surface antigen common of T lymphocytes (Barua & Yoshimura, 2004). CD3 is the best marker for total T lymphocyte count, because it is a molecular complex associated with specific receptors of T lymphocytes and is found in all subpopulations of mature T cells.

Table 7 – Quantification of $CD3^+$ cells and count of goblet cells in the mucosa of the ileum of broilers and serum electrophoretic profile of immunoglobulin A (IgA) of broilers fed conventional or alternative diets, and with normal or reduced nutritional density.

	Ileal mucosa		Serum
Treatments	CD3	Goblet	IgA (g/dL)
Diets			
Conventional ingredients	97.66[a]	48.87[a]	0.112
Alternative ingredients	93.19b	41.65b	0.108
Nutritional density			
Normal	96.28	47.32	0.105
Reduced	94.57	43.20	0.114
CV, %	3.94	19.16	35.93
P-values			
Ingredients	0.0087	0.0500	0.8518
Nutritional density	0.2773	0.2575	0.2147
Interaction	0.1618	0.9019	0.6857

Means of a same factor followed by different letters indicate significant effect ($p<0.05$) of treatments by the Fisher LSD test. No interactions between the factors tested were observed ($p>0.05$).

Soybeans have a significant amount NSP in the form of pectins, hemicelluloses and oligosaccharides (stachyose and raffinose). Out of these NSP, 20% have null digestibility, and 2% are xylans (Cleophas *et al.*, 1995). The tissue defense triggered against an infection basically consists of three mechanisms, which frequently act according to a chronological sequence. This protective mechanism consists of non-induced innate defense, induced innate defense and adaptive immune response. The cellular immune response in the intestinal mucosa is mainly composed of T-CD3 and T-CD4 lymphocytes.

Corroborating this hypothesis, we verified an increase ($p<0.05$) in the number of goblet cells in conventional diets. These cells are responsible for the production of a mucous layer mainly consisting of glycoproteins, known as mucins, which contain a long polysaccharide portion that makes them hydrophilic and viscous. The functions of mucins include protection of the brush border membrane from chemicals and from the abrasive effects of the digesta. They act as a barrier against microorganisms, because the natural intestinal flora and immunoglobulins are included in the mucus layer, and influence the transport between the luminal content and the brush border membrane (Snyder & Walker, 1987; Uni *et al.*, 2003).

The hyperplasia and/or hypertrophy of goblet cells in the small intestine are considered as a response to some type of aggression, aiming at maintaining mucosal integrity. Several studies (Mitjavila *et al.*, 1977; Ortiz *et al.*, 1994; Oliveira *et al.*, 2000; Uni *et al.*, 2003) have reported this effect in response to antinutritional factors of feedstuffs or delay in supplying feed after hatching, for instance.

Teirlynck *et al.* (2009) mentioned that the inclusion of ingredients such as wheat and rye in the diet for broilers – thus containing high levels of NSP – in comparison with a diet based on corn, induces the fusion of intestinal villi, infiltration of T lymphocytes, hyperplasia and hypertrophy of goblet cells, apoptosis of epithelial cells in the mucosa, and shifts in the microbiota. In the present experiment, the supplementation of enzymes or the reduction of the nutritional density of diets, independently of the feedstuffs used, did not influence the number of goblet cells.

Mucin production may increase in the presence of biologically-active proteins, aiming at protecting the intestinal epithelium. However, mucin significantly contributs to the endogenous loss in chickens (Montagne *et al.*, 2000). This hypothesis is supported by the fact that amino acids that compose the intestinal mucin are excreted in response to the addition of enzymes (Cowieson *et al.*, 2006).

Serum IgA immunoglobulin electrophoretic profile was not affected by the ingredients used in the diets, supplemented or not with enzyme complex, and with reduction or not of the nutritional density. The immune stimulation of the mucosa results in the production of IgA antibodies that block the receptors and reduce the number of pathogenic bacteria in the intestinal lumen (Jin *et al.*, 1997). Despite the increase in lymphocytes observed, as shown by the marker CD3, no IgA increase was observed in the serum of broilers.

IgA is especially produced by B-cells located on the intestinal lamina propria and has a key role in the maintenance of homeostasis of the intestinal mucosa. Dendritic cells that present the antigens from commensal bacteria of the intestinal mucosal surface induce the production of IgA by B-cells through mechanisms dependent or not of T cells. Notwithstanding it is well documented that IgA production is induced by bacterial colonization, the kinetics and duration of this response, as well as possible ways of inducing it have not yet been fully elucidated (Tezuka *et al.*, 2011).

Jackson *et al.* (2003) reported that in chickens infected with *Eimeria sp* and *Clostridium perfringens* the addition of β-mananase resulted in the reduction of the severity of the challenge generated by these microorganisms, and this effect was observed not only as increased body weight, but also as a reduction of intestinal lesions. However, Mushtaq *et al.* (2007) added glucanase and xylanase in diets based on canola meal, and did not record any benefits in terms of performance, nutrient digestibility, immune response, or carcass characteristics of broilers.

CONCLUSIONS

The use of alternative plant feedstuffs in broiler diets promoted an increase in intestinal lesions and enhanced cellular immune response. However, this undesirable gut health condition can be mitigated to a certain degree, as found with the use of conventional corn and soybean meal diets, by using a multi-enzyme complex.

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