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Productive Performance, Intestinal Morphology and Carcass Yield of Broilers Fed Conventional and Alternative Diets Containing Commercial Enzymatic Complex

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Abstract: The present study aimed at evaluating the effect of adding a commercial enzymatic complex to conventional and alternative diets on the productive performance, intestinal morphology and carcass yield of broilers. 900 male broiler chicks were used, distributed according to a completely randomized design, with six treatments, with six repetitions each. Two control diets were formulated, one with conventional feedstuffs (T1) based on corn and soybean meal and another alternative (T4), containing corn, millet, meals of soybean, canola and sunflower. From these, other four diets were prepared with reduced nutritional levels of metabolizable energy, digestible amino acids, calcium and available phosphorus, without (T2 and T5) and with (T3 and T6) addition of the enzymatic complex. Enzyme supplementation and conventional ingredients propitiated better feed conversion. The broilers that received the alternative diet and reduced nutritional levels with added enzyme (T6) achieved productive performance, intestinal morphology and carcass yield similar to those fed conventional diets at regular nutritional levels (T1). The addition of enzymatic complex promoted better proliferative capacity of the crypts and villi height. The use alternative feedstuffs and nutritional levels reduction causes production performance loses and intestinal mucosa damages, but the inclusion of enzymes complex proportioned similar results to these feed conditions than conventional diets with regular nutritional density.

Key words: Carbohydrases, non-starch polysaccharides, fibrous brans

INTRODUCTION

The selection of ingredients for feeds is not based only in their nutritional quality. The price fluctuations lead to the search for replacement of traditional feedstuffs, aiming to minimize production costs (Campos *et al*., 2007). However, this replacement may be limited by the amount of ingredient, market availability, presence of anti-nutritional factors and bromatological composition (Farrell, 2005).

The presence of anti-nutritional factors and Non-Starch Polysaccharides (NSP) in the vegetable ingredients of the diet negatively affect intestinal health and performance of poultry and swine (Jia *et al*., 2009). The NSP are the main cell wall constituents of plant feedstuffs and cannot be digested by poults, due to their bonds nature, resistant to hydrolysis in the digestive tract (Ramos *et al*., 2007). Monogastric animals lack enzymes to digest cellulose, arabinoxylan, beta-glucans, pectins, among other NSP that make up the cell wall of some feeds (Fang *et al*., 2007; Ribeiro *et al*., 2011).

The difficulty in fiber digestion, besides reducing feed energy, may also impair the use of all other nutrients. This mainly occurs when the fiber type of the feed is soluble, i.e., has great capacity to absorb water, forming a gelatinous substance in the intestinal tract (Choct *et al*., 2010). This increased viscosity of the intestinal chime slows the passage of feed along the digestive tract, hampering the abundance and action of endogenous enzymes and interfering with the diffusion or transport of nutrients. Furthermore, the viscosity of digesta interferes with intestinal microflora and physiological functions of the intestine (Choct *et al*., 2004).

The addition of exogenous enzymes that hydrolyze the NSP of vegetable ingredients in the feeds for monogastric improves the availability of energy and use of nutrients, improving feed conversion ratio (Shirmohammad and Mehr, 2011). Other benefit of using these enzymes is the reduction of viscosity of intestinal digesta, that changes intestinal microflora and reduces the excreta moisture, improving the conditions of the litter (Oliveira and Moraes, 2007). With the viscosity reduction, there is increase in the diffusion rates of nutrients and endogenous enzymes, providing greater capacity of poultry to digest and absorb more nutrients (Lee *et al*., 2010).

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Diet supplementation with exogenous enzymes changes the intestinal microflora and decreases adverse effects of microbial fermentation in the small intestine (Choct *et al*., 1999; Bedford, 2000; Cowieson *et al*., 2006; Nian *et al*., 2011). The bacterial overgrowth promotes the dissociation of bile salts. This leads to decreased capacity of forming micelle by the process of digestion and absorption of lipids and as consequence, there is lower solubilization of dietary fats (Hofmann and Hagey, 2008).

Exogenous enzymes can reduce the competition for nutrients between the microflora, intestinal villous atrophy and enlargement of digestive organs (Viveiros *et al*., 1994). By-products of the arabinoxylan degradation present in the plant cell wall by the action of exogenous enzymes can be used in microbial fermentation in the cecum and in the production of short-chain fatty acids, which can be used by the animals as an energy source (Choct *et al*., 1996; Steenfeldt *et al*., 1998).

Exogenous enzymes are most commercialized in form of enzymatic complexes made up by a mixture of several different enzymes activities, efficient in a wide range of substrates that compound the diets (Bedford and Schoulze, 1998). The enzymes with proved efficacy in feeds are xylanase, arabinoxylanase, beta-glucanase, cellulose, phytase (Odetallah *et al*., 2003; Santos *et al*., 2004; Brufau *et al*., 2006).

The role of the different enzymatic complexes available in the market can differ. This is due to specific factors inherent to the specific condition of enzymes production, such as: type and strain of the microorganisms, culture medium used, fermentation conditions and processing, process and quality control and others (Gilbert and Cooney, 2011). The enzymes should have specificity to the substrate present in the ingredients that made up the diet and the animal should be able to use the final product of the enzymatic action (for instance, fatty acid from the action of lipase supplementation) or get benefits of the dissociation of specific substrates (Bedford and Patridge, 2001). Besides that, the enzymes should remain stable during and after the processing of the feed until its consumption and in the intestinal environment where it will act (Gilbert and Cooney, 2011). The enzymatic supplement should be added in amounts proportional to the substrate that will interact (Thi, 2008). When these criteria are not met, the results can be controversial.

Another factor concerns the economic viability of using these enzymatic complexes. The economic analysis is essential, since the inclusion of additives can increase the costs in formulating the diets (Araujo *et al*., 2007). The use of enzymes has shown financial benefits to the extent that it is considered the possibility of greater use of nutrients, mainly when alternative feedstuffs are employed, with lower cost and lower digestibility (Strada *et al*., 2005; Costa *et al*., 2008).

The purpose of this study was to assess the effectiveness of a commercial enzymatic complex in conventional and alternative diets, with reduction of nutritional density, on the productive performance, intestinal morphometry and carcass yield of broilers.

MATERIALS AND METHODS

The experiment was conducted at the experimental poultry farm of the Federal University of Parana (UFPR), *Campus* Palotina, Paraná State, southern Brazil, with approval of the Ethical Conduct Committee in the Use of Animals in Experiments of the UFPR/Palotina, protocol number 13/2010 from March 14th, 2010.

900 male broiler chicks with one-day-old were used, of the Cobb Slow commercial lineage, from the same breeder flock. The broilers were distributed according to a completely randomized design, with six treatments and six replicates of 25 broilers each, totaling 36 experimental units.

Treatments were divided as follow: T1, conventional control diet, based on corn and soybean meal; T2, conventional diet with reduced nutritional density; T3, the same diet of the T2, but with inclusion of commercial enzymatic complex (Rovabio Max AP, Adisseo France SAS); T4, alternative control diet, with the same nutritional levels of T1, formulated with corn, millet, meals of soybean, canola and sunflower; T5, alternative diet with reduced nutritional density and T6, T5 diet with inclusion of the commercial enzymatic complex. For formulate the reduced nutritional density diets, were decreased 85 kcal/kg of metabolizable energy, 2% of digestible amino acids and 1.5 g/kg of both total calcium and available phosphorus, comparing to the nutritional levels of the control diets.

The feeds were formulated according to nutritional recommendations adopted by Brazilian agro-industries, being the feeding program made up by three stages: pre-initial (1-7 days), initial (8-21 days) and growth (22-42 days). The replacements of corn and soybean meal by alternative ingredients (T4, T5 and T6) followed the methodology: pre-initial stage, 75:25 ratio between corn and millet; only using soybean meal; initial stage: 50:50 between corn and millet; 75:12.5:12.5 between soybean meal, sunflower meal and canola meal and growth stage, 25:75 between corn and millet; 50:25:25 between soybean meal, sunflower meal and canola meal.

All the samples of ingredients from plant origin were previously analyzed in the Center for Nutrition Support of Adisseo Brazil Animal Nutrition Ltd., for determining the content of crude protein, fibers, crude fat, digestible amino acids, metabolizable energy and phytic phosphorus. These results enabled the formulation of the diets with higher precision. The composition of ingredients and nutritional levels of experimental rations for the different stages are listed in Table 1, 2 and 3.

Table 1: Composition and nutritional levels of rations for the pre-initial stage

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
Enzymatic complex	-	-	0.10	-	-	0.10
Inert	-	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

*Supplied 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 rations for the initial stage

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
Enzymatic complex	-	-	0.10	-	-	0.10
Inert	-	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

*Supplied 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

The assessments of the results were divided into three sets of analysis: productive performance, morphometry of intestinal mucosa and carcass yield. The indices of productive performance (body weight, feed intake and feed conversion) were weekly registered.

Table 3: Composition and nutritional levels of rations for the growth stage

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
Enzymatic complex	-	-	0.10	-	-	0.10
Inert	-	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

*Supplied 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

For the morphometric study of intestinal villi, at 42 days age, two broilers per experimental unit, totaling twelve broilers per treatment, were sacrificed by cervical dislocation. After sacrifice, the small intestine was removed, for the obtainment of fragments with about 5 cm length of duodenum (from pylorus to the distal portion of the duodenal loop), of jejunum (from the distal portion of the duodenal loop to the Meckel's diverticulum) and of ileum (anterior portion to the cecum). These fragments were opened longitudinally on Styrofoam plates and washed with saline. The samples were fixed with Bouin's solution for 24 h for histological analysis, according to IAC (1995). Each fragment was subjected to semi-serial cuts of 5 μm thick and stained with hematoxylin and eosin. The images were captured by light microscopy (Image Pro-Plus, Version 5.2, Media Cybernetics). The height of the villi and the depth of the crypts were measured of each segment repetition.

To determine the carcass yield, at 32 and 42 days age, two broilers per experimental unit were randomly separated, identified and subjected to fasting for six hours and then slaughtered. It was considered the body weight, obtained individually before slaughter, the weight of the fresh eviscerated carcass (without the feet, head and abdominal fat) and the yield of prime cuts, from the whole breast (with skin and bones) and legs (thighs and drumsticks with bones and skin). The abdominal fat present around the cloaca, cloacae pouch, gizzard, proventriculus and adjacent abdominal muscles were removed, as described by Smith (1993) and also weighted.

Data were collected and tabulated individually for each experimental unit. The statistical analysis was performed using the procedure GLM of the SAS software (SAS, 1998).

RESULTS AND DISCUSSION

In the Table 4 are presented the results relative to productive performance of broilers fed distinct diets. For the period of 1 to 21 days, there was no interaction between the diet composition (conventional and alternative ingredients) and the enzyme inclusion. The addition of the enzymatic complex, regardless the ingredients, promoted the greatest weight gain and the best feed conversion ratio ($p<0.05$).

The effect of the enzymatic complex on feed conversion evidenced the action on the nutrient digestibility, improving the supply of metabolizable energy for the weight gain of broilers. There was an increase of more than 10% in weight gain of broilers supplemented with enzymatic complex. According to Campbell and Bedford (1992), in the initial phase of bird life, until around 21 days, is common to observe a greater efficiency of exogenous enzymes, mainly carbohydrases.

For Brenes *et al.* (1996), during the first weeks of life, the enzymatic activity and physiological development of

Table 4: Productive performance of broilers fed conventional or alternative diets, supplemented or not with enzymatic complex

	1-21 days		
Ingredients	Weight gain, g	Feed intake, g	Feed conversion, g:g
Conventional	849.70	1217.28	1.431
Alternative	870.91	1268.77	1.463
Enzymes			
Without addition	819.07[b]	1223.78	1.495[a]
With addition	901.55[a]	1262.27	1.401[b]
Analysis of variance			
Ingredients	0.0970	0.0580	0.4340
Enzymes	0.0010	0.1482	0.0128
Interaction	0.5682	0.0920	0.0731
	21-42 days		
Conventional	1564.36	3393.65	2.186
Alternative	1492.62	3473.87	2.331
Enzymes			
Without addition	1537.68	3429.81	2.235
With addition	1519.30	3437.71	2.268
Analysis of variance			
Ingredients	0.0581	0.1255	0.0002
Enzymes	0.6122	0.8765	0.3493
Interaction	0.5014	0.1546	0.0172*
	1-42 days		
Conventional	2608.73	4610.94	1.770
Alternative	2564.63	4742.64	1.853
Enzymes			
Without addition	2553.34	4653.59	1.823
With addition	2620.02	4699.99	1.796
Analysis of variance			
Ingredients	0.3512	0.0564	0.0006
Enzymes	0.1645	0.4839	0.1813
Interaction	0.3624	0.6614	0.0501*

Mean values in the column followed by distinct minuscule letters are significantly different ($p<0.05$)

Table 5: Unfolding of the interaction between ingredients and the addition or not of enzymes on the feed conversion in the period from 21-42 days and from 1-42 days

	Feed conversion 21-42 days, g:g		
Ingredients	Without addition	With addition	Effect
Conventional	2.203	2.147[b]	0.3727
Alternative	2.389[B]	2.268[aA]	0.0039
Effect	0.3084	0.0001	
	Feed conversion 1-42 days, g:g		
Conventional	1.804[A]	1.736[bB]	0.0498
Alternative	1.842	1.856[a]	0.6035
Effect	0.2103	0.0011	

Mean values in the row followed by different capital letters are significantly different ($p<0.05$). Mean values in the column followed by distinct minuscule letters are significantly different ($p<0.05$)

poultry are not completely consolidated and therefore the digestibility is significantly lower in birds up to three weeks old. Negative effects of non-starch polysaccharides are much more evident at this age. Boguhn and Rodehutscord (2010) also observed effect of the enzymes in diets of turkeys only at initial phase. The authors attributed the decrease in enzymes effect in the growth/slaughter phase to the gradual adaptation that occurs in the intestinal environment to diets based on grains rich in NSP.

For the period between 21 and 42 days, a significant interaction was detected between ingredients and use of enzymes for feed conversion. By unfolding the interaction, we observed that the supplementation of enzymes did not change the feed conversion of broilers fed with conventional rations. On the other hand, when alternative ingredients were included in the diets, the feed conversion was better with the inclusion of enzyme (Table 5). In the composition of diets at this stage, the replacement of corn and soybean meal by alternative ingredients was greater at initial phase. The ratio between corn and millet increased from 50:50 to 25:75 between soybean meal, sunflower meal and canola meal from 75:12.5:12.5 to 50:25:25. In this way, the results clearly show that the replacement of half the corn and soybean meal by alternative ingredients does not affect the feed conversion of the broilers, if the diets are supplemented with this enzymatic complex.

In the evaluation of the entire experimental period, 1-42 days, there was significant interaction between the diet ingredients and enzyme addition. Unfolding the interaction, we could observe that the enzyme inclusion improved the feed conversion of broilers from 1-42 days of age in the presence of conventional ingredients (Table 5).

Steenfeldt *et al.* (1998) consider that the degradation of arabinoxylans present in the cell wall of grains in diets supplemented with enzymes increases the amount of material available for microbial fermentation in the cecum. This increase leads to the production of short chain fatty acids that constitute an extra energy source for the maintenance and growth of broilers, as well as stimulate the development of intestinal mucosa and proliferation of microbiota (Yang *et al.*, 2009). The fact that the feed conversion had been positively influenced by the addition of enzymes in all studied stages can be explained by this effect on the fermentation of substrates and release of extra energy.

The performance of broilers fed alternative ingredients supplemented with enzymes was similar to that observed with the use of conventional diets without enzymes, allowing the nutritionist include in the formulation alternative or seasonal ingredients with this enzymatic complex, reducing the costs and maintaining the performance of the broilers.

Comparing the diets based on conventional and alternative ingredients with regular or reduced nutritional density, from 1-21 days of age, we verified that the weight gain of the broilers, regardless type of ingredient, was greater for the diets with regular nutritional density ($p<0.05$). Also, there was a better feed conversion for those animals that received diets based on conventional ingredients and diets with regular nutritional density ($p<0.05$).

Considering the total period (1-42 days), the broilers that were given conventional diets presented better feed conversion ($p<0.05$) than those fed alternative ingredients. Besides that, regardless of ingredient type, the reduction in nutritional levels of diets worsened the weight gain and feed conversion of broilers compared to those that received diets with nutritional levels within the requirements. Comparing these results with those in Table 5, there is a great potential of using enzymes as a tool to keep broilers performance, both in diets with reduced nutritional levels and those elaborated with alternative ingredients.

Toledo *et al.* (2007) evaluated the effect of exogenous enzymes (xylanases, beta-glucanase and cellulases) added to diets based on corn and soybean meal with different nutritional densities (low density and standard) and observed that diets with low nutritional density supplemented with enzymes presented performance similar to the broilers that received diet with standard density. Sunflower seeds have around 27.6% of NSP, assuming the oil extraction at 40%, the amount of NSP in the sunflower meal can reach 46% (Irish and Balnave, 1993; Dusterhoft *et al.*, 1997). Despite this high quantity, the supplementation of diets with high levels of sunflower meal and added with several commercial enzymes have no negative effects on the performance and carcass yield of broilers (Sherif *et al.*, 1995; Mushtaq *et al.*, 2009; Raza *et al.*, 2009). Kocher *et al.* (2000) evaluated the influence of adding two commercial enzymatic products on the nutritional values of canola meal and sunflower meal. These authors verified a reduction in NSP concentration in the jejunum and also a better protein digestibility in the ileum when the enzyme was added.

Although corn and soybean meal do not present high levels of NSP and promote low viscosity in the digesta, the enzymatic supplementation resulted in an increase in the availability of starch and protein, respectively (Abudabos, 2010; Cao *et al.*, 2010; Zanella *et al.*, 1999). The metabolizable energy of soybean meal for broilers is considered low when compared to the crude protein. This occurs due to the low digestibility of the fraction of carbohydrates, such as the oligosaccharides, stachyose and raffinose (Parsons *et al.*, 2000).

Meng *et al.* (2005) evaluated the effect of adding carbohydrases in nutritional values of corn, soybean meal, canola meal and peas for broilers from 5 to 18 days of age in diets formulated to meet the NRC requirements (except for energy and CP that corresponded to 95 and 92% of the requirements) (NRC, 1994). The enzymatic addition had no effect on feed intake and weight gain, but improved the feed conversion of broilers that received diet based on corn and protein of animal origin. The addition of enzymes had significant effect on the NSP digestibility, on the value of metabolizable energy and on ileal digestibility of starch, in broilers that received diets based on corn. An increase was registered in ileal concentration of soluble NSP and decrease of the insoluble, with enzymatic addition in all diets. Despite the improvement of NSP digestibility, there was an increase in metabolizable energy only for the diets based on corn and corn-soybean, probably more adequate in terms of substrates for the enzyme complex.

Rutherford *et al.* (2007) conducted a metabolic essay aiming to test the effects of a commercial combination of the enzymes xylanase, alpha-amylase and beta-glucanase on the metabolizable energy and true ileal digestibility of amino acids in broilers receiving diets based on corn and soybean, containing meal of wheat and canola. The content of metabolizable energy of diets supplemented with enzymes was significantly higher (2.3%) than diets not supplemented. The ileal digestibility of amino acids was also higher for the diets supplemented with enzymes. There was an increase in the apparent ileal digestibility of nitrogen in 7% with the

Table 6: Histomorphometry of intestinal mucosa of broilers fed conventional or alternative diets, supplemented or not with enzymatic complex

	Duodenum			Jejunum			Ileum		
Ingredients	Villus (µm)	Crypt (µm)	V:C	Villus (µm)	Crypt (µm)	V:C	Villus (µm)	Crypt (µm)	V:C
Conventional	658.76	110.87	6.05[a]	343.46	44.85	7.72	257.30	71.44	3.64[b]
Alternative	582.98	127.45	4.76[b]	314.27	43.79	7.26	286.18	65.80	4.33[a]
Enzymes									
Without addition	591.84[b]	119.11	5.20	328.55	44.94	7.29	254.49[b]	68.20	3.77[b]
With addition	653.24[a]	118.80	5.61	330.58	43.75	7.71	294.59[a]	69.00	4.28[a]
Analysis of variance									
Ingredients	0.0595	0.1219	0.0030	0.2072	0.6161	0.3928	0.0595	0.1219	0.0002
Enzymes	0.0154	0.8540	0.1477	0.9736	0.6075	0.4281	0.0154	0.8540	0.0038
Interaction	0.5147	0.5194	0.0052	1.1784	0.3222	0.5122	0.5147	0.5194	0.7001

Mean values in the column followed by distinct minuscule letters are significantly different ($p<0.05$)

addition of enzymes. Nevertheless, no difference was observed between the treatments for the endogenous loss of lysine.

In the study performed by Pack *et al.* (1998), with rations containing 65% of sorghum and 17% of soybean, or 62% of sorghum and 13% of soybean and from 5 to 10% of canola meal and added of multi-enzymatic complex, showed improvement in weight gain, feed intake and feed conversion of broilers with age from 8 to 21 days. Still, these authors highlight that the reduction in the density of energy and amino acids of the diets based on meal of soybean and corn, containing multi-enzymatic complex, does not undermine the performance of broilers.

Fischer *et al.* (2002) evaluated the performance of broilers, from 1 to 35 days of age, using an enzymatic complex based on proteases, amylases and cellulases on diets containing corn and soybean meal and observed no improvement on performance. On the other hand, Torres *et al.* (2003), examining the effect of the enzymatic complex (amylase, protease and xylanase), verified an increase in weight gain and improvement in feed conversion in the age from 1 to 28 days. Brito *et al.* (2006) used the same enzymes in conventional diets, but with addition of extruded soybean and also observed increased weight gain and improvement in feed conversion in the stage from 1 to 21 days of age, with respective improvements of 3.8% and 4.4%.

In the Table 6-8 is shown the result relative to evaluation of histomorphometry of intestinal mucosa of the broilers. The inclusion of enzymatic complex increased significantly ($p<0.05$) the height of the villi of duodenal mucosa. For the villus:crypt ratio, a significant interaction was recorded between the treatments ($p<0.05$). By unfolding the interaction, we observed that, when the enzymatic complex is added to a conventional diet, based on corn and soybean, there is no change in the villus:crypt ratio, compared to a diet based on alternative ingredients. On the other hand, when these ingredients are included, this ratio decreases with enzyme inclusion (Table 6). The inclusion of enzymatic complex ensured similarity of the villus:crypt ratio between conventional

Table 7: Unfolding of the interaction between ingredients and the addition or not of enzymes on the villus:crypt ratio of duodenal mucosa of broilers

Ingredients	Without enzyme	With enzyme	Effect
Conventional	6.33[a]	5.76	0.1738
Alternative	4.07[bB]	5.45[A]	0.0190
Effect	0.0001	0.5202	

Mean values in the row followed by different capital letters are significantly different ($p<0.05$). Mean values in the column followed by distinct minuscule letters are significantly different ($p<0.05$)

and alternative diets, this latter usually more aggressive to mucosa, due to its higher NSP content.

The addition of enzymes to alternative diets increased the villus:crypt ratio of duodenal mucosa, showing that the enzyme operates on the non-starch polysaccharides present in plant ingredients, ensuring a regular proliferative capacity of the mucosa. The greater height of the villi of the broiler's duodenal mucosa that received enzymes, in comparison to those of broilers supplemented with the additive, can be related to a regeneration process and consequently, to a better absorption.

In agreement with Dowling *et al.* (2002), after the aggression suffered by the mucosa, by anti-nutritional factors or by the action of pathogens, the remaining epithelium becomes hyperplastic, with greater villus height and crypt depth. It is noteworthy that, in this case, the increase in villus height may correspond to an increase in the number of cells that are not the absorptive ones, such as enterocytes, but at the expense of the increase of goblet cells. The increase of these cells can be attributed to a physiological response to anti-nutritional factors present in cereal grains (Montagne *et al.*, 2003).

The corn is relatively free of fibrous NSP, the main anti-nutritional factors present in most cereals (Ward *et al.*, 2008). Soy has in its composition around 20% of NSP, with virtually no digestibility (Rodrigues *et al.*, 2003). Even not considered viscous grains, the insoluble components of the NSP in the corn and soybeans can harm the intestinal mucosa. The difficulty in specifying

Table 8: Histomorphometry of intestinal mucosa of broilers fed conventional or alternative diets, with regular or reduced nutritional density

	Duodenum			Jejunum			Ileum		
Ingredients	Villus (µm)	Crypt (µm)	V:C	Villus (µm)	Crypt (µm)	V:C	Villus (µm)	Crypt (µm)	V:C
Conventional	659.98	114.52	5.77	344.59	46.35	7.46	266.97	63.57	4.19[a]
Alternative	632.09	125.69	5.24	308.83	44.06	7.04	256.57	69.03	3.75[b]
Density									
Regular	634.93	122.36	5.33	323.48	45.35	7.19	269.24	64.48	4.17[a]
Reduced	653.24	118.80	5.61	328.55	44.94	7.29	254.49	68.20	3.77[b]
Analysis of variance									
Ingredients	0.4897	0.1012	0.1562	0.0783	0.2867	0.2394	0.4897	0.1012	0.0076
Density	0.3525	0.2311	0.5908	0.8021	0.8710	0.7542	0.3525	0.2311	0.0144
Interaction	0.5783	0.6015	0.5588	0.2634	0.6062	0.3579	0.5783	0.6015	0.3173

Mean values in the column followed by distinct minuscule letters are significantly different ($p<0.05$)

exactly the effect of enzymes interaction, added to the difficulty of determining the amount of NSP present in feeds, the results of enzymatic supplementation in these diets can be controversial (Bedford and Schoulze, 1998). Moreover, since the inclusion of enzymes was tested only in diets with low nutritional density, possibly the excess of energy, obtained with the use of enzymes, had not allowed meeting the demand of the mucosa. The intestinal tissues represent only about 5% of body weight, but consume between 15 and 30% of all supply of O_2 and proteins of the organism and 20% of the gross energy consumed, due to high renewal rate and intense metabolic activity of cells (Muramatsu *et al.*, 1994; Gaskins, 2001).

One important criterion for including enzymes is that should have specificity with the substrate present in the ingredient that composes the diet (Thi, 2008). In this way, the enzymatic complex in diets containing millet, canola meal and sunflower meal does not have effect and thus it can be attributed the lack of specificity of the enzymes activities that make up the complex on the components of these grains, despite the improvement observed in the feed conversion between diets with alternative ingredients and reduced nutritional density and diets with enzymes.

In the morphometry assessment of jejunum mucosa, there was no significant effect of ingredients and enzymes ($p>0.05$). Meanwhile, a significant effect of adding enzymes in the diets regardless the type of ingredient used on the villus height ($p<0.05$). Despite the value of the statistical test be very close to probability significance, it is possible to verify that the villi height of the ileum mucosa of the broilers fed diets based on alternative ingredients was lower in comparison to the villi height of broilers receiving conventional diets (Table 6).

The increase in the height of villi of the ileum mucosa obtained with the enzyme addition can be due to the reduction in the amount of substrate available for proliferation of undesirable microflora that causes adverse effects to intestinal mucosa, besides the dissociation of bile salts that lead to the reduction of fat digestion and the villi atrophy. As previously reported, the feed conversion was improved with the use of enzymes and the lower microbial activity at ileum level assumes that there was substrate degradation by enzymes, with posterior fermentation in the cecum and production of energy via short-chain fatty acids.

There was significant effect ($p<0.05$) of the ingredients and density of rations only on the villus:crypt ratio of the ileum (Table 8). The formulation of diets with conventional ingredients improved the ratio, as well as the use of diets with regular nutritional density, showing that alternative ingredients and the diets with reduced nutritional levels in the absence of enzymes have reduced the proliferative capacity.

In the Table 9 are listed the results regarding to the carcass yield at 32 and 42 days of age of broilers fed diets based on conventional or alternative ingredients and reduced nutritional density, added or not with enzymatic complex. There was no significant interaction between diets and enzymes, or between diets and nutritional density for the yield of carcass or cuts ($p>0.05$). Also, no isolated effect was observed for any source of variation, except for percentage of fat deposited in the broilers carcass. The addition of enzymatic complex, regardless of ingredients present in the diets, reduced significantly ($p<0.05$) the abdominal fat percentage at 32 days.

The positive effect of enzymatic supplementation on the reduction of abdominal fat clearly shows that the supplementation of enzymatic complexes can make available more nutrients for muscle synthesis, without the need to deaminate the amino acids to be used as energy source. In this phase, there is the maximum rate of muscle protein deposition and the unbalance in the profile of amino acids required is reflected in the accumulation of fat in the carcass of the broilers (Summers *et al.*, 1992). On the other hand, it is known that adopting diets with nutritional levels above the required also reflects negatively on the abdominal fat accumulation (Delezie *et al.*, 2010). In the present experiment, despite the nutritional levels of the control diet based or not on conventional ingredients are within

Table 9: Carcass yield of broilers fed conventional or alternative diets, supplemented or not with enzymatic complex

	32 days			
	Carcass	Legs	Breast	Fat
Ingredients	(%)			
Conventional	74.52	27.51	33.96	0.97
Alternative	74.57	27.12	34.78	0.99
Enzymes				
Without addition	74.34	27.54	34.89	1.16[a]
With addition	74.75	27.10	33.85	0.78[b]
Analysis of variance				
Ingredients	0.9190	0.3846	0.2139	0.9452
Enzymes	0.4715	0.3229	0.1219	0.0309
Interaction	0.1268	0.2535	0.1437	0.5820
	42 days			
Conventional	75.47	27.55	35.70	1.40[a]
Alternative	74.78	27.83	35.91	1.03[b]
Enzymes				
Without addition	74.61	27.75	35.47	1.28
With addition	75.64	27.63	36.13	1.16
Analysis of variance				
Ingredients	0.2479	0.5068	0.7544	0.0205
Enzymes	0.0865	0.7513	0.3149	0.4375
Interaction	0.4005	0.9918	0.2744	0.4997

Mean values in the column followed by distinct minuscule letters are significantly different (p<0.05)

Table 10: Carcass yield of broilers fed conventional or alternative diets, with regular or reduced nutritional density

	32 days			
	Carcass	Legs	Breast	Fat
Ingredients	(%)			
Conventional	74.92	27.32	33.31	0.99
Alternative	74.11	27.70	34.44	1.02
Density				
Regular	74.69	27.48	32.86b	0.84[b]
Reduced	74.34	27.54	34.89[a]	1.16[a]
Analysis of variance				
Ingredients	0.1648	0.4809	0.1021	0.8127
Enzymes	0.5390	0.9146	0.0046	0.0481
Interaction	0.7733	0.6349	0.3252	0.6581
	42 days			
Conventional	75.56[a]	27.44	35.00[b]	1.69[a]
Alternative	73.90[b]	28.07	36.29[a]	1.29[b]
Density				
Regular	74.85	27.76	35.82	1.69[a]
Reduced	74.61	27.75	35.47	1.28[b]
Analysis of variance				
Ingredients	0.0500	0.1188	0.0369	0.0179
Enzymes	0.7649	0.9842	0.5574	0.0152
Interaction	0.5841	0.3628	0.5529	0.8336

Mean values in the column followed by distinct minuscule letters are significantly different (p<0.05)

the requirements for commercial rearing conditions, they were excessive for experimental conditions, higher in ambience and rearing density, since the higher fat accumulation in carcass.

It was still observed that the reduction in nutritional levels, regardless the type of ingredient and the addition or not of enzymes, significantly reduced ($p<0.05$) the fat deposition in the carcass and increased the yield of chicken breast at 32 days of age (Table 10). At 42 days of age, there was a higher yield of the carcass and a lower yield of breast for the broilers fed with conventional diets in comparison to diets with alternative ingredients. On the other hand, it was observed that both the use of alternative ingredients and the reduction in nutritional density in the diets decreased the carcass fat ($p<0.05$). The supply of diets elaborated with nutritional levels above the requirements can imply in a greater deposition of fat (Delezie *et al.*, 2010). High levels of NSP, as found in the meals of canola and sunflower, 18 and 23%, respectively, interfere with intestinal lipid absorption and cause a reduction in the deposition of abdominal fat (Francesch *et al.*, 1994).

The addition of exogenous enzymes to broilers feeds, in general, resulted in a better feed conversion and morphometric evaluations of the mucosa, pointing out a direct action on substrate that would not be digested by endogenous enzymes, which resulted in fermentation and production of energy via short-chain fatty acids. The addition of enzymes in diets based on corn and soybean meal, ingredients most used in feeds, was more effective, indicating an important potential for improving the use of energy and other nutrients of these ingredients. The inclusion of enzymes complex in feeds formulated with alternative ingredients requires further studies aiming the concentrations and actual availability of nutrients by the enzyme for the different ingredients. The use of several types of alternative ingredients can also influence the effect of enzymes and in this way, the ideal combination of enzymes should also be sought after under these conditions.

The use of exogenous enzymes can improve the nutritional value of feeds and allow a greater flexibility in formulating diets, reducing costs and ensuring the productive and slaughter performance of broilers, as well as being an important tool to encourage the use of alternative vegetable ingredients. Understanding the mechanism, mode of action, possibilities of use, cost-benefit and ingredients recovery and the nutritional matrix by the use of enzymatic complexes will assist the decision-making process for the use of these additives.

Conclusion: The use of enzymes complex can be a tool additive to recover the production performance loses and intestinal mucosa damages caused by alternative feedstuffs inclusion and nutritional levels reduction, guaranteeing similar results than conventional diets with regular nutritional density.

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