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Use of enzymes in diets with different percentages of added fat for broilers

[*Uso de enzimas em dietas com diferentes porcentagens de adição de gordura para frangos de corte*]

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ABSTRACT

We assessed the extent to which the removal of fat source, and consequently its compounds, such as linoleic acid, can affect the performance of broilers. We used 600 male Cobb 500 day old chicks. The birds were distributed in a completely randomized experimental design, with five treatments and six replicates of 20 birds each. The treatments were: (T1) diet - positive control (PC), which met the nutritional needs; (T2) diet – negative control (CN), a reduction of 100kcal/kg and low linoleic acid content; (T3): diet – negative control reformulated for low linoleic acid content and a set of Quantum phytase XT and Econase XT 25 (BAL + QFit-Eco), (T4): diet – negative control reformulated, with the percentage of linoleic acid adjusted to an intermediate value between the value of the diet and diet CP and CN to use a set of Quantum phytase XT and XT Econase 25 (IAL + QFit-Eco) and (T5): diet – negative control reformulated, with the percentage of linoleic acid adjusted to a value similar to that of the positive control diet and joint use of Quantum phytase XT and XT Econase 25 (AAL + QFit-Eco). The joint use of Quantum Phytase and Econase promoted improvement in the performance of broilers from 1 to 21 days. The greatest weight gain was obtained with diets containing percentages of total fat and linoleic acids. Dietary supplementation with enzymes resulted in higher levels of calcium in the tibia, whatever the percentage of linoleic studied.

Keywords: linoleic acid, meat quality, performance

RESUMO

Avaliou-se até que ponto a retirada de fonte de gordura e, consequentemente, de seus compostos, como o ácido linoleico, pode afetar o desempenho dos frangos de corte. Foram utilizados 600 pintos de um dia, machos da linhagem Cobb 500. As aves foram distribuídas num delineamento experimental inteiramente ao acaso, com cinco tratamentos e seis repetições de 20 aves cada. Os tratamentos foram: (T1) dieta-controle positivo (CP), que atendeu às necessidades nutricionais; (T2) dieta-controle negativo (CN), com redução de 100kcal de EM/kg e baixo teor de ácido linoleico; (T3): dieta- controle negativo reformulada, para baixo teor de ácido linoleico e com uso conjunto de Quantum fitase XT e Econase XT 25 (BAL+ Qfit-Eco); (T4): dieta-controle negativo reformulada, com porcentagem de ácido linoleico ajustada para um valor intermediário entre o valor da dieta CN e da dieta CP e com uso conjunto de Quantum fitase XT e Econase XT 25 (IAL + Qfit-Eco) e (T5): dieta-controle negativo reformulada, com porcentagem de ácido linoleico ajustada para um valor semelhante ao da dieta-controle positivo e com uso conjunto de Quantum fitase XT e Econase XT 25 (AAL+ Qfit-Eco). O uso conjunto de Quantum Fitase e Econase promoveu melhora no desempenho dos frangos de corte de um a 21 dias. O maior ganho de peso foi obtido com dietas que continham porcentagens de gorduras totais e de ácido linoleico. A suplementação com as enzimas resultou em maior teor de cálcio nas tíbias, independentemente da porcentagem de ácido linoleico estudada.

Palavras-chave: ácido linoleico, desempenho, qualidade de carne

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INTRODUCTION

The use of vegetable raw materials, mostly corn and soybean, in diets for broiler chickens are widely adopted by the poultry industry due to the high nutritional value, supply and cost benefits of these ingredients. However, these ingredients are of plant origin, usually with anti-nutritional factors that reduce the availability of minerals and reduce digestibility of nutrients in the diet (Bedford, 2011). Among the most important anti-nutritional factors we can highlight the non-starch polysaccharides (PNA) and phytic acid or phytate (Olukosi *et al*., 2007).

The PNA cannot be digested by the birds due to the nature of their bonds being resistant to hydrolysis in the digestive tract. The difficulty in fiber digestion aside from the reduction in food energy can impair the use of all other nutrients. This is particularly true when the type of food is a soluble fiber, that is, has a great ability to absorb water and form a gel-like substance in the intestinal tract, causing an increase in digestion viscosity (Conte *et al*., 2003) and making it difficult, therefore, for the digestive enzymes, leading to the formation of excretion with high moisture content. The increased viscosity of digestion is usually related to the use of winter cereals such as wheat, barley and oats. Rations based on corn typically do not exhibit this increase in digestion viscosity due to the insoluble nature of the xylan and glucan present in the cell wall of cereal. The presence of insoluble fiber in the plant cell wall, however, acts as a barrier for endogenous enzymes, reducing their access to nutrients within cells.

Phytic acid has in its structure orthophosphates highly ionizable groups, forming insoluble complexes with cations such as calcium, phosphorus, zinc, copper, magnesium and iron in the gastrointestinal tract of birds reducing, therefore, the solubility and digestibility of nutrients (Tejedor *et al*., 2001, Wu *et al*., 2006).

The use of exogenous enzymes enables the degradation of these antinutritional compounds avoiding losses related to the presence of these compounds. Thus, the use of exogenous enzymes is presented as an effective option to improve the use of some nutrients and therefore enable the use of conventional and non-food diets, more flexible in helping to concomitantly increase the profitability of poultry production (Liu and Ru, 2010).

The use of carbohydrases enzymes such as xylanase in diets that have high amounts of PNA improves metabolizable energy and decreases the viscosity digestion, as has been shown in recent years (Windisch *et al*., 2008). Marsman *et al*. (1997) reported an improvement in ileal digestibility of nutrients in broilers fed a diet of corn and soybean meal supplemented with exogenous enzymes protease and carbohydrases. The exogenous phytase also inhibits the formation of binary complexes between proteins and phytic acid enabling an improvement in the use of amino acids (Selle and Ravindran, 2007).

The reduction of fat content of feed leads to reduced levels of linoleic acid, considered an essential fatty acid for broilers. This reduction in the amount of fat in the diet may reduce the absorption of soluble fat vitamins and accelerate the speed of passage of digestion through the intestinal tract.

Linoleic acid belongs to the Ω 6 family of fatty acids and once introduced in the diet, can be elongated and desaturated in the body of the birds for the production of other polyunsaturated fatty acids, precursors of eicosanoids that have many important hormonal functions (Hovenier *et al*., 2006). Its deficiency results in reduced growth rate, increase in water consumption, development of fatty liver and reduced resistance to disease (Balnave, 1970). Brenes and Roura (2010) commented in a literature review that the search for alternatives to suit the needs of today's market show that some foods have great potential to promote good husbandry results. According to these authors, the essential oils are part of this select group, as well as essential fatty acids such as conjugated linoleic acid.

The objective of this study was to evaluate the extent to which the removal of a fat source and the supplementation of phytase and xylanase, can affect the performance of broiler chickens during the period from 1 to 21 days old.

MATERIAL AND METHODS

The experiment was conducted in the Poultry Module of the Department of Animal Science, Agrarian Sciences Center, Federal University of Paraiba, Campus II, Cidade de Areia – PB.

A total of 600 one day old Cobb 500 strain male chicks were used. The birds were distributed in a completely randomized design with five treatments and six replicates of 20 birds each, and a trial period of 1 to 21 days.

The birds were weighed, standardized by initial weight and randomly distributed in metal plots with metal rails and floor covered with paper feeders and drinkers, each compartment being an experimental unit. The warming of the chicks was done through an electrical system with 60W incandescent bulbs per plot. The lighting program adopted during the trial period was continuous (24 hours of light = natural + artificial).

The treatments were: T1 – positive control (PC), meeting the nutritional needs defined by Rostagno *et al*., (2005); T2 – negative control (NC), with a reduction of 100kcal of EM/kg and low-fat (LF); T3 – reformulated negative control, containing low fat and the combined use of Quantum Phytase XT and Econase XT 25 (LF + QFit-Eco); T4 – negative control reformulated with the fat level adjusted to an intermediate value (IF) between the value of T1 and T2 diets and with the combined use of Quantum Phytase Econase XT and XT 25 (+ IF QFit-Eco); T5 – negative control reformulated with the fat level set to a value similar to the positive control feed (AG) and with the collective use of Quantum Phytase Econase XT and XT 25 (+ AG QFit-Eco). Diets with reduced energy content but with maintenance of fat were obtained by the inclusion of an inert material (kaolin) as described in Tables 1 and 2 below.

Quantum Phytase XT enzymes and Econase XT 25 were added to the feed in amounts of 200g/ton and 100 guton making up the expected addition in the final feed and 500FTU/kg 16000BXU/kg respectively. Feed samples of all treatments were collected to determine enzyme activity. All the rations analyzed showed values of enzymatic activity within the expected range for each treatment.

The birds received water and food ad libitum. The variables studied were: feed intake, weight gain, feed conversion, and analysis of the ash, calcium and bone phosphorus contents. Feed intake was calculated as the difference between the leftover and supplied in each experimental phase, considering the number of birds and mortality. Weight gain was calculated as the difference between the initial and final weights of each phase. Feed conversion was calculated by dividing the feed intake by weight gain, and the value was corrected for mortality in the evaluated phase. At 21 days old, four birds per treatment were sacrificed and the tibiotarsal was removed for further analysis of the mineral levels.

The results were submitted for variance analysis using the SAEG (Sistema..., 1999) program and SNK test at 5% probability in the average treatments.

RESULTS AND DISCUSSION

The data on the performance of animals in the complete phase of 1 to 21 days of age is presented in Table 3. The data for feed intake and weight gain was statistically different ($P<0.05$) between treatments. It was found that the combined use of phytase and xylanase promoted improvement in weight gain compared to the negative control diet, obtaining values similar to the positive control diet, and the best result was obtained with the diet containing high fat (HF + Phytase + Xylanase). Thus, it can be stated that supplementation with exogenous enzymes evaluated in this study improved bird performance when a minimum of fat was included in the diet, which probably improved the availability of energy for the processes of tissue synthesis, as well as digestibility of other nutrients such as amino acids, and protein synthesis used in the body, thus enabling a better performance of these animals.

Table 1. Percentage composition of experimental diets of the pre-initial phase

Ingredient	T1 (PC)	T2 (NC)	T3 (BG+ Qfit-Eco)	T4 (IG+ Qfit-Eco)	T5 (AG+ Qfit-Eco)
Corn	53.137	55.444	56.04	52.613	49.734
Soy bean meal	36.098	35.719	37.617	38.4	38.654
Soy oil	2.025	0.11	0.002	1.107	2.125
Meat and bone meal	7.236	7.213	4.51	4.535	4.572
Limestone	0.362	0.369	0.689	0.706	0.698
Salt	0.409	0.408	0.444	0.445	0.446
L-Lysine	0.196	0.203	0.188	0.17	0.17
DL-Methionine	0.312	0.309	0.288	0.29	0.296
L-Threonine	0.07	0.07	0.037	0.034	0.037
Coline chloride	0.07	0.07	0.07	0.07	0.07
Quantum Phytase XT[1]	0	0	0.02	0.02	0.02
Econase® XT 25 [2]	0	0	0.01	0.01	0.01
Antioxidant	0.01	0.01	0.01	0.01	0.01
Mineral mix[3]	0.05	0.05	0.05	0.05	0.05
Vitamin mix[4]	0.025	0.025	0.025	0.025	0.025
Inert	0	0	0	1.515	3.083
Total	100	100	100	100	100.00
Nutritional Composition					
Metabolizable energy, kcal/kg	2,980	2,880	2,980	2,980	2,980
Calculated crude protein. %	24.48	24.49	24.52	24.6	24.51
Analyzed Crude Protein. %	22.35	23.36	23.52	23.32	23.91
Crude fat. %	5.41	3.57	3.15	4.15	5.07
Analyzed Crude Fat. %	6.8	4.74	4.71	5.89	6.44
Linoleic acid. %	2.3	1.3	1.26	1.8	2.3
Crude fiber. %	2.77	2.79	2.9	2.88	2.84
NDF. %	11.64	11.87	12.21	11.9	11.59
Calcium. %	0.94	0.94	0.94	0.95	0.95
Analyzed Calcium. %	1.00	0.99	0.96	0.97	0.88
Available phosphorus. %	0.47	0.47	0.47	0.47	0.47
Total lysine. %	1.46	1.46	1.45	1.45	1.45
Digestible lysine. %	1.33	1.33	1.33	1.33	1.33
Digestible methionine + cystine. %	0.94	0.94	0.94	0.94	0.94
Digestible threonine. %	0.87	0.87	0.87	0.87	0.87
Digestible tryptophan. %	0.25	0.25	0.26	0.26	0.26
Digestible arginine. %	1.56	1.55	1.55	1.56	1.56
Digestible isoleucine. %	0.91	0.91	0.92	0.93	0.93
Digestible valine. %	1	1	1	1	1
Sodium. %	0.22	0.22	0.22	0.22	0.22

[1] Exogenous phytase;[2] exogenous xilanase;[3]content/kg of product: Mn – 160g; Fe – 100g; Zn – 100g; Cu - 20g; Co – 2g; I – 2g. Carrier q.s.p. . 1.000g.;[4]content/kg of product: vit. A - 12.000.000 UI (IU); vit. D_3 - 3.600.000UI (IU); vit. B_1 – 2.5g; vit. B_2 – 8g; vit. B_6 – 3g; Panthotenic acid – 12g; Biotine – 0.2g; vit. K – 3g; Folic acid – 3.5g; Nicotinic acid – 40g; vit. B_{12} – 20mg; Se – 0.13g; Carrier q.s.p. – 1000g.

Table 2. Percentage composition of experimental diets in the initial phase

Ingredient	T1 (CP)	T2 (CN)	T3 (BG+ Qfit-Eco)	T4 (IG+ Qfit-Eco)	T5 (AG+ Qfit-Eco)
Corn	61.814	64.121	64.527	61.862	58.888
Soybean meal	27.902	27.522	29.589	29.982	30.380
Soy oil	1.990	0.075	0.000	0.908	1.928
Meat and bone meal	6.845	6.823	4.116	4.144	4.177
Limestone	0.362	0.369	0.689	0.723	0.715
Sodium chloride	0.399	0.398	0.435	0.435	0.436
L-Lysine	0.220	0.227	0.207	0.200	0.200
DL-Methionine	0.249	0.246	0.224	0.228	0.233
L-Threonine	0.063	0.063	0.028	0.029	0.030
Coline chloride	0.070	0.070	0.070	0.070	0.070
Quantum FC 500[1]	0.000	0.000	0.020	0.020	0.020
Econase XT 25 FC[2]	0.000	0.000	0.010	0.010	0.010
Antioxidant	0.010	0.010	0.010	0.010	0.010
Mineral mix[3]	0.050	0.050	0.050	0.050	0.050
Vitamin mix[4]	0.025	0.025	0.025	0.025	0.025
Inert	0.000	0.000	0.000	1.304	2.829
Total	100.00	100.000	100.000	100.00	100.00
Nutritional composition					
Metabolizable energy, kcal/kg	3,070	2,970	3,070	3,070	3,070
Calculated crude protein. %	21.216	21.221	21.307	21.283	21.246
Analyzed Crude Protein. %	20.97	20.89	20.19	20.66	22.25
Crude fat. %	5.494	3.658	3.271	4.093	5.015
Analyzed Crude Fat. %	7.10	5.10	4.40	5.90	6.60
Linoleic acid. %	2.379	1.384	1.355	1.800	2.300
Crude fiber. %	2.516	2.539	2.650	2.622	2.588
NDF. %	11.537	11.766	12.109	11.839	11.531
Calcium. %	0.884	0.884	0.884	0.900	0.900
Analyzed Calcium. %	1.0	0.94	0.64	0.97	1.01
Available phosphorus. %	0.442	0.442	0.442	0.442	0.442
Total lysine. %	1.255	1.255	1.249	1.249	1.254
Digestible lysine. %	1.146	1.146	1.146	1.146	1.151
Digestible methionine + cystine. %	0.814	0.814	0.814	0.814	0.814
Digestible threonine. %	0.745	0.745	0.745	0.745	0.745
Digestible tryptophan. %	0.208	0.207	0.214	0.215	0.215
Digestible arginine. %	1.310	1.305	1.302	1.306	1.310
Digestible isoleucine. %	0.767	0.765	0.781	0.783	0.783
Digestible valine. %	0.860	0.860	0.863	0.862	0.860
Sodium. %	0.214	0.214	0.214	0.214	0.214

[1]Exogenous phytase;[2]exogenous xilanase;[3]Content/kg of product: Mn – 160g; Fe – 100g; Zn – 100g; Cu - 20g; Co – 2g; I – 2g. Carrier q.s.p. 1.000g.;[4]Content/kg of product: vit. A - 12.000.000 UI (IU); vit. D_3 - 3.600.000 UI (IU); vit. B_1 – 2.5g; vit. B_2 – 8g; vit. B_6 – 3g; Panthotenic acid – 12g; Biotine – 0.2g; vit. K – 3g; Folic acid – 3.5g; Nicotinic acid – 40g; vit. B_{12} – 20mg; Se – 0.13g; Carrier q.s.p. – 1000g.

Table 3. Effect of using exogenous enzymes and the level of dietary fat on feed intake (FI), weight gain (WG) and feed conversion (FC) of broiler chicks during the period from 1 to 21 days old

Treatment	FI (g/bird)	WG (g/bird)	FC (g/g)
CP[1]	1275.2a	933.5ab	1.366
CN[2]	1236.4b	902.0b	1.371
BG[3] + Phytase[6]- Xylanase[7]	1248.0b	919.2ab	1.358
IG[4] + Phytase[6]- Xylanase[7]	1302.2a	938.8ab	1.387
AG[5] + Phytase[6]- Xylanase[7]	1288.6a	950.9a	1.355
P	<0.05	<0.05	>0.05
Average	1270.1	928.9	1.368
CV	1.63	2.59	2.05

Means followed by different letters in the same column differ by the SNK test at 5% probability; [1]PC = Positive Control; [2]NC = Negative Control; [3] Low-fat; [4] intermediate-fat; [5] high-fat; [6] Quantum Phytase: 200g/t; [7] Econase: 100g/t

Studying the influence of enzymes on broiler performance, Torres *et al.* (2003) found that lower levels of protein and energy, supplemented with exogenous enzymes, provided a similar performance to broilers fed with diets in levels considered normal, showing that the inclusion of enzymes in the diets allowed a reduction of nutrient levels in the diet without affecting performance by reducing production costs. On the other hand, the highest level of linoleic acid in the diet as well as total fat, resulted in greater weight gain, probably due to the effects of extra-calorie fats, increasing the digestibility of nutrients as a whole and the importance of linoleic acid as a precursor of many eicosanoids, with important metabolic functions in the bird's body.

According to Conte *et al*. (2003), carbohydrases enzymes hydrolyze non-starch polysaccharides in the cell wall, exposing the internal contents and allowing enzymatic digestion to occur increasing the use of energy and other nutrients such as amino acids and minerals present in these foods. Thus, the combination of carbohydrases with exogenous phytase could therefore potentiate the action of phytase, since phytic acid would become accessible to enzymatic action (Olukosi *et al*., 2007).

Bertechini (2006) observed that only certain specific functions are performed by lipids with extra-caloric value, which would result from a combination of factors such as providing soluble fat vitamins, essential fatty acids and increasing low-calorie energy, favoring absorption and use of calcium, vitamins and fatty acids, improving digestibility of amino acids and carbohydrates in the diet, among others. According to Watkins (1991), linoleic acid influences the fluidity, the activity of receptors and enzyme function of cell membranes. Most of the metabolic effects attributed to linoleic acid may be related to the biosynthesis of eicosanoids, which affect numerous biochemical reactions and physiological processes.

In general, the NRC (1994) and Rostagno *et al*. (2005) recommended 1.0% of linoleic acid for chickens in the initial phase. However, Zornig *et al*. (2001) stated that an excellent feed conversion and growth rate can be achieved by broilers fed with diets with less than 0.20% of linoleic acid in the diet, since it contains adequate levels of total lipids and energy. The discrepancy in recommendations may be due to the pressure of the growing poultry industry, particularly in the stocking density, and in return the search for the behavior of these birds, coupled with the need for alternatives to the use of antibiotics, or additives that serve as growth promoters for the broilers. For this new generation of food in this category we include essential fatty acids such as linoleic acid (Brenes and Roura, 2010).

The average values of the levels of ash, phosphorus and calcium in the tibias of the broilers at 21 days of age are presented in Table 4. The levels of total phosphorus were not affected by treatments (P>0.05). However, the experimental treatments provided a statistically significant difference (P<0.05) for ash and calcium in tibiotarsal of chickens at 21 days old.

Table 4. Exogenous enzymes and the level of dietary fat content in ash, phosphorus and calcium in the tibiotarsal of broilers at 21 days (*in natural matter*)

Treatment	Ash (%)	Phosphorus (%)	Calcium (%)
CP[1]	20.8ab	15.85	8.4b
CN[2]	20.8ab	15.80	9.1b
BG[3] + Phytase[6]- Xylanase[7]	21.7ab	15.12	11.3a
IG[4] + Phytase[6]- Xylanase[7]	20.3b	17.24	10.9a
AG[5] + Phytase[6]- Xylanase[7]	22.5a	15.02	11.4a
P	<0.05	>0.05	<0.05
Average	21.2	15.81	10.2
CV	5.72	8.56	4.62

Means followed by different letters in the same column differ by the SNK test at 5% probability; [1]PC = Positive Control; [2]NC = Negative Control; [3] Low Linoleic Acid; [4] Intermediate Linoleic Acid; [5] High Linoleic Acid; [6]Quantum Phytase: 200g/t; [7] Econase: 100g/t.

Enzyme supplementation resulted in higher calcium content ($P<0.05$) in the tibiotarsal, regardless of the level of linoleic acid diets studied in comparison to PC and NC. According to Selle and Ravindran (2007), the phytate molecule is a polyanion with the potential to chelate positively charged nutrients (Na^+, Mg^{++}, K^+, Ca^{++} and Zn^{++}, etc.), which characterizes its antinutritional property, compromising the use of protein, energy, calcium and trace minerals. Thus, the use of phytase enzyme in combination with xylanase, by allowing an increase in the hydrolysis of phytic acid may have increased the availability of calcium for animal metabolism (Wu *et al*., 2006), allowing a greater deposition of this mineral in the bones of chickens at 21 days old.

There was no difference ($P>0.05$) between the calcium and phosphorus levels between linoleic acid studied, except ash content, indicating that the lower level of linoleic acid is sufficient for healthy bones of chickens kept for court. According to Bernardino (2009), lipids play an important role in the production and regulation of eicosanoids. These, in turn, are derived from linoleic acid and regulate bone formation and remodeling. Liu and Denbow (2001) reported that the polyunsaturated fatty acids are important for the regulation of mineralization and bone remodeling by serving as substrates for the synthesis of eicosanoids such as prostaglandins and leukotrienes, which are involved in the local regulation of bone growth and development. Thus, with linoleic acid supplementation in the diets of the birds, there was a better balance between fatty acids, which possibly promoted greater regulation of eicosanoids, thus contributing for better bone mineralization.

Phytase supplementation in the diet of broilers can promote, according to Liu and Ru (2010) a lower flow of amino acids in the ileum of chickens, reducing endogenous losses and also with the degradation of phytate, allowing greater use of the manganese content, iron and potassium by birds. The effects of this practice is that the smallest loss of endogenous amino acids and better use of minerals can promote better bone mineral deposition of the animals, and Table 4 confirms this statement made by Liu and Ru (2010).

CONCLUSION

The combined use of Quantum Phytase XT and Econase XT 25 showed better production performance and a greater bone mineralization in broilers at 21 days old, regardless of the level of linoleic acid in the diet. During the feed formulation for broilers in the initial phase the inclusion of a minimum amount of fat is important to ensure that the performance of birds will be maintained as expected.

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