# AMINO ACID REDUCTION IN BROILER DIETS SUPPLEMENTED WITH PHYTASE AND ELABORATED ACCORDING TO DIFFERENT NUTRITIONAL PLANS

Redução de aminoácidos em rações para frangos de corte suplementadas com fitase e elaboradas de acordo com diferentes planos nutricionais

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#### ABSTRACT

The need for suitable diets in chickens, considering the ideal protein concept associated with phytase, is important in order to improve the productivity of birds. The aim of this study was to assess the need for the correction of amino acid levels in the diets of broilers, by using different combinations of crude protein (CP) supplemented with phytase. For performance, eight days old Cobb 875 broiler chicks, with initial body weights of  $155 \pm 1.4$ g were used. The birds were distributed in a completely randomised design with seven treatments (nutritional plans) and five replicates of 25 birds. For the metabolism trial, 300 birds were selected which were also distributed using the same design, but with five, four and three birds per experimental stage: 8-21 days, 22-35 days and 36-42 days. The nutritional plans consisted of different combinations of CP in diets with reduced calcium and phosphorus, supplemented with phytase and amino acids. The reduction of amino acids in diets with reduced levels of CP resulted in an increased intake, feed conversion and abdominal fat level, and decreased the yield of thigh + drumstick. The inclusion of phytase reduced the excretion of calcium and phosphorus, but increased nitrogen excretion when the CP was not reduced. As a result, this study concluded that the reduction of amino acids to 3.5%, supplemented with phytase and prepared according to a nutritional plan does not improve the performance and carcass characteristics of broilers, although it may reduce the emission of polluting elements by excreta.

Index terms: Carcass characteristics, ideal protein, nutrition, performance

#### RESUMO

A necessidade de dietas adequadas para frangos, considerando o conceito de proteína ideal associada à fitase, é importante para melhorar a produtividade das aves. Objetivou-se avaliar a necessidade de correção dos níveis de aminoácidos em rações para frangos de corte, formuladas em diferentes combinações de proteína bruta (PB) e suplementadas com fitase. Para o desempenho, foram utilizados 875 pintos de corte Cobb, com oito dias de idade e peso inicial de 155±1,4g. As aves foram distribuídas em delineamento inteiramente casualizado, com sete tratamentos (planos nutricionais) e cinco repetições de 25 aves cada. Para o ensaio de metabolismo, foram selecionadas 300 aves que foram também distribuídas no mesmo delineamento, porém com cinco, quatro e três aves por parcela experimental nas fases de 8 a 21, 22 a 35 e 36 a 42 dias. Os planos nutricionais consistiram em diferentes combinações de PB em rações com cálcio e fósforo reduzidos, suplementadas com fitase e aminoácidos. A redução de aminoácidos em dietas com teores reduzidos de PB aumentou o consumo, a conversão alimentar e a gordura abdominal e diminuiu o rendimento de coxa + sobrecoxa. A inclusão de fitase reduziu a excreção de cálcio e de fósforo, porém aumentou a de nitrogênio quando a PB não foi reduzida. Conclui-se que a redução de aminoácidos em 3,5% em rações para frangos de corte suplementadas com fitase e elaboradas segundo um plano nutricional não melhora o desempenho e as características de carcaça das aves, embora possa reduzir a emissão de elementos poluentes pelas excretas.

Termos para indexação: Características de carcaça, proteína ideal, nutrição, desempenho.

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#### INTRODUCTION

The high cost of feed in broiler production is a main factor that interferes directly on the profit margins. Currently, the use of some concepts in diet formulation can reduce costs by using less of some ingredients, such as soybean meal and dicalcium phosphate.

In this sense, the use of an ideal protein concept constitutes a reduction of the crude protein levels in diets through the use of industrial amino acids. This practice

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both reduces the cost and decreases the emission of polluting elements in the environment by excretion, without affecting the performance and carcass characteristics of birds (NAGATA et al., 2011a and b).

Conversely, the addition of exogenous enzymes allows for improved use of nutrients. An example is the formulation of diets using phytase, which allows for a reduction in dietary calcium and phosphorus levels and, at the same time, the loss of these elements in excrement (GOMIDE et al., 2011a). However, some studies have shown that the use of this enzyme in protein-reduced diets formulated according to the ideal protein concept have not had positive results (GOMIDE et al., 2011b). According to the authors, phytase is an enzyme that can influence the release of amino acids from dietary proteins during the digestive process. In this case, a correction in the levels of these nutrients in diets including the enzyme may be required.

Currently, the use of nutritional plans for broilers has been considered, since the response to low protein diets at a certain stage of development in poultry can depend on the performance during the previous stage. Based on previous studies, Gomide et al. (2007) established nutritional plans for broilers and observed the need for adjustments in order to obtain positive results for both performance and carcass characteristics.

Therefore, the objective of this work was to verify the need for the correction of amino acid levels in diets for broilers that have been formulated with different combinations of protein (nutritional plans), and supplemented with phytase.

#### MATERIAL AND METHODS

Two experiments, based on metabolism and performance, were conducted at the Poultry Section of the Department of Animal Science (DZO) in the Federal

University of Lavras, in Lavras, Minas Gerais, Brazil, during the period of May-June 2010. Each experimental protocol was approved by the Bioethics Committee of Animal Care at the Federal University of Lavras.

In total, one thousand one hundred and seventy-five male eight day old Cobb broilers, with initial weights of  $155 \pm 1.4$ g were used. These were split into two groups, with 300 birds used in the metabolism trial and 875 in the performance trial.

In the metabolism trial, the animals were distributed in a completely randomised design with seven treatments (nutritional plans - NP) and five replicates. The experimental design involved testing five birds per experimental plot during days 8 to 21, four birds during days 22 to 35 and three birds during days 36 to 42. The total period of the experiment was 34 days.

The experimental plans used during the development of birds consisted of combinations of different levels of crude protein in diets containing added phytase but with reduced amounts of calcium, phosphorus and amino acids (Table 1). The diets were isocaloric and were mainly comprised of corn and soybean meal. The control diet was formulated to meet the minimal requirements for birds according to Rostagno et al. (2005) (Tables 2, 3 and 4), and the others contained different nutrient levels according to each nutritional plan. In diets containing phytase (750FTU/tonne - RONOZYME NP CT 10000FTU/g), calcium and phosphorus levels were reduced by 0.30% and 0.15%, respectively. In reduced-crude protein diets, synthetic amino acids were added to comprise at least 96.5% of the birds' requirements. In the metabolism trial, the excreta collection was performed once a day in the morning, for the last three days of each phase, according to the total excreta collection method described by Rodrigues et al. (2005).

Table 1 – Nutritional plans used at various stages of development of broilers.

	Phase (days)		
Nutritional plan (NP)	8 to 21	22 to 35	36 to 42
	Crude protein in diet (%)		
NP1 – (21 – 19 - 18%) of crude protein without phytase	21.0	19.0	18.0
NP2 - (21 - 19 - 18%) of crude protein with phytase	21.0	19.0	18.0
NP3 – (20 - 17 - 15%) of crude protein with phytase	20.0	17.0	15.0
NP4 – (19 - 17 - 16%) of crude protein with phytase	19.0	17.0	16.0
NP5 – (19 - 17 - 15%) of crude protein with phytase	19.0	17.0	15.0
NP6 – (19 - 16 - 15%) of crude protein with phytase	19.0	16.0	15.0
NP7 – (18 - 16 - 15%) of crude protein with phytase	18.0	16.0	15.0

Ciênc. agrotec., Lavras, v. 36, n. 2, p. 224 -232, mar./abr., 2012

Table 2 – Percentage and calculated composition of experimental diets used for broiler from 8 to 21 days of age.

Ingredient (%)	21% CP	21% CP	20% CP	19% CP	18% CP
Corn	56.82	56.82	62.12	64.80	66.27
Soybean meal	36.14	36.14	33.04	30.40	27.64
Soybean oil	2.300	2.300	1.700	1.408	1.940
Dicalcium phosphate	1.750	0.952	0.960	0.970	0.980
Limestone	0.985	0.675	0.690	0.700	0.710
Salt	0.492	0.492	0.491	0.491	0.490
L-lysine HCl (78%)	0.075	0.075	0.162	0.241	0.320
DL-methionine (99%)	0.228	0.228	0.210	0.230	0.254
L –threonine (98.5%)	0.025	0.025	0.030	0.065	0.100
L –arginine (99%)	-	-	-	-	0.048
L -glicine + Serine (99%)	-	-	-	0.068	0.160
L –isoleucine (99%)	-	-	-	-	0.007
L – valine (99%)	-	-	-	0.012	0.054
Potassium carbonate	-	-	0.070	0.142	0.215
Vitamin mix <sup>1</sup>	0.040	0.040	0.040	0.040	0.040
Mineral mix <sup>2</sup>	0.050	0.050	0.050	0.050	0.050
Choline chloride (60%)	0.050	0.050	0.050	0.050	0.050
Salinomicin (12%)	0.060	0.060	0.060	0.060	0.060
Zinc bacitracin (15%)	-	0.0075	0.0075	0.0075	0.0075
Phytase	0.025	0.025	0.025	0.025	0.025
Kaolin	0.675	2.000	0.237	0.317	0.315
Calculated composition					
Crude protein (%)	21.0	21.0	20.0	19.0	18.0
Metabolisable energy (kcal/kg)	3000	3000	3000	3000	3000
Calcium (%)	0.879	0.580	0.580	0.570	0.570
Available phosphorus (%)	0.440	0.290	0.290	0.290	0.290
Sodium (%)	0.214	0.214	0.214	0.214	0.214
Digestible lysine (%)	1.146	1.146	1.106	1.106	1.106
Digestible methionine+cystine (%)	0.814	0.814	0.786	0.786	0.786
Digestible threonine (%)	0.745	0.745	0.719	0.719	0.719
Digestible tryptophan (%)	0.236	0.236	0.220	0.210	0.200
Digestible arginine (%)	1.361	1.361	1.281	1.206	1.131
Digestible glicine + serine (%)	1.936	1.936	1.843	1.751	1.660
Electrolytic balance (mEq/kg) <sup>3</sup>	206	206	206	206	206

 $<sup>^1</sup>$  Supply per kg of product: 12,500,000 UI of vitamin A, 5,760,000 UI of vitamin D<sub>3</sub>, 150,000 mg of vitamin E, 4,000 mg of vitamin K<sub>3</sub>, 3,000 mg of vitamin B<sub>1</sub>, 9,000 mg of vitamin B<sub>2</sub>, 6,000 mg of vitamin B<sub>6</sub>, 40,000 mcg of vitamin B<sub>12</sub>, 300 mg of biotin, 2.000 mg of folic acid, 80,000 mg of nicotinic acid, 18,000 mg of pantothenic, 100,000 mg of vitamin C and 300 mg of selenium.

Ciênc. agrotec., Lavras, v. 36, n. 2, p. 224 -232, mar./abr., 2012

<sup>&</sup>lt;sup>2</sup> Supply per kg of product: 160,000 mg of manganese, 100,000 mg of iron, 100,000 mg of zinc, 20,000 mg of cupper, 2,000 mg of cobalt and 2,000 mg of iodine.

<sup>&</sup>lt;sup>3</sup> Calculated according to Mongin (1981): Na + K – Cl.

Table 3 – Percentage and calculated composition of experimental diets used for broiler from 22 to 35 days of age.

Ingredient (%)	19% CP	19% CP	17% CP	16% CP
Corn	60.6	60.6	68.4	70.7
Soybean meal	31.1	31.1	25.4	22.8
Soybean oil	3.960	3.960	2.450	2.210
Dicalcium phosphate	1.626	0.862	0.884	0.900
Limestone	0.941	0.642	0.659	0.665
Salt	0.470	0.470	0.469	0.469
L-lysine HCl (78%)	0.193	0.193	0.310	0.382
DL-methionine (99%)	0.229	0.229	0.241	0.258
L –threonine (98.5%)	0.045	0.045	0.091	0.124
L –tryptophan (98.5%)	-	-	-	0.005
L –arginine (99%)	-	-	0.029	0.099
L -glicine + Serine (99%)	-	-	0.030	0.116
L –isoleucine (99%)	-	-	0.029	0.069
L – valine (99%)	-	-	0.068	0.107
Potassium carbonate	-	-	0.146	0.219
Vitamin mix <sup>1</sup>	0.040	0.040	0.040	0.040
Mineral mix <sup>2</sup>	0.050	0.050	0.050	0.050
Choline chloride (60%)	0.040	0.040	0.040	0.040
Salinomicin (12%)	0.060	0.060	0.060	0.060
Zinc bacitracin (15%)	-	0.0075	0.0075	0.0075
Phytase	0.025	0.025	0.025	0.025
Kaolin	0.565	1.620	0.600	0.593
Calculated composition				
Crude protein (%)	19.0	19.0	17.0	16.0
Metabolisable energy (kcal/kg)	3100	3100	3100	3100
Calcium (%)	0.824	0.536	0.536	0.536
Available phosphorus (%)	0.411	0.267	0.267	0.267
Sodium (%)	0.205	0.205	0.205	0.205
Digestible lysine (%)	1.073	1.073	1.035	1.035
Digestible methionine+cystine (%)	0.773	0.773	0.746	0.746
Digestible threonine (%)	0.697	0.697	0.673	0.673
Digestible tryptophan (%)	0.206	0.206	0.199	0.199
Digestible arginine (%)	1.214	1.214	1.172	1.172
Digestible glicine + serine (%)	1.752	1.752	1.691	1.691
Electrolytic balance (mEq/kg) <sup>3</sup>	186	186	186	186

<sup>&</sup>lt;sup>1</sup> Supply per kg of product: 12,500,000 UI of vitamin A, 5,760,000 UI of vitamin D<sub>3</sub>, 150,000 mg of vitamin E, 4,000 mg of vitamin K<sub>3</sub>, 3,000 mg of vitamin B<sub>1</sub>, 9,000 mg of vitamin B<sub>2</sub>, 6,000 mg of vitamin B<sub>6</sub>, 40,000 mcg of vitamin B<sub>12</sub>, 300 mg of biotin, 2.000 mg of folic acid, 80,000 mg of nicotinic acid, 18,000 mg of pantothenic, 100,000 mg of vitamin C and 300 mg of selenium.

<sup>&</sup>lt;sup>2</sup> Supply per kg of product: 160,000 mg of manganese, 100,000 mg of iron, 100,000 mg of zinc, 20,000 mg of cupper, 2,000 mg of cobalt and 2,000 mg of iodine.

<sup>&</sup>lt;sup>3</sup> Calculated according to Mongin (1981): Na + K – Cl.

Table 4 – Percentage and calculated composition of experimental diets used for broiler from 36 to 42 days of age.

Ingredient (%)	18% CP	18% CP	16% CP	15% CF
Corn	63.4	63.4	71.6	74.0
Soybean meal	29.1	29.1	22.6	20.0
Soybean oil	4.0	4.0	2.5	2.24
Dicalcium phosphate	1.470	0.680	0.710	0.710
Limestone	0.870	0.570	0.600	0.600
Salt	0.440	0.440	0.440	0.441
L-lysine HCl (78%)	0.180	0.180	0.322	0.399
DL-methionine (99%)	0.202	0.202	0.220	0.242
L-threonine (98.5%)	0.034	0.034	0.091	0.127
L –arginine (99%)	-	-	0.050	0.125
L -glicine + Serine (99%)	-	-	0.039	0.132
L –isoleucine (99%)	-	-	0.037	0.081
L – valine (99%)	-	-	0.069	0.112
Potassium carbonate	-	-	0.169	0.241
Vitamin mix <sup>1</sup>	0.040	0.040	0.040	0.040
Mineral mix <sup>2</sup>	0.050	0.050	0.050	0.050
Choline chloride (60%)	0.020	0.020	0.020	0.020
Phytase	-	0.0075	0.0075	0.0075
Kaolin	0.23	1.32	0.46	0.43
Calculated composition				
Crude protein (%)	18.0	18.0	16.0	15.0
Metabolisable energy (kcal/kg)	3150	3150	3150	3150
Calcium (%)	0.764	0.460	0.460	0.463
Available phosphorus (%)	0.386	0.231	0.231	0.231
Sodium (%)	0.197	0.198	0.198	0.198
Digestible lysine (%)	1.017	1.017	0.981	0.981
Digestible methionine+cystine (%)	0.733	0.733	0.707	0.707
Digestible threonine (%)	0.660	0.660	0.637	0.637
Digestible tryptophan (%)	0.201	0.201	0.194	0.194
Digestible arginine (%)	1.159	1.159	1.118	1.118
Digestible glicine + serine (%)	1.687	1.687	1.628	1.628
Electrolytic balance (mEq/kg) <sup>3</sup>	178	178	178	178

 $<sup>^1</sup>$  Supply per kg of product: 12,500,000 UI of vitamin A, 5,760,000 UI of vitamin D $_3$ , 150,000 mg of vitamin E, 4,000 mg of vitamin K $_3$ , 3,000 mg of vitamin B $_1$ , 9,000 mg of vitamin B $_2$ , 6,000 mg of vitamin B $_6$ , 40,000 mcg of vitamin B $_{12}$ , 300 mg of biotin, 2.000 mg of folic acid, 80,000 mg of nicotinic acid, 18,000 mg of pantothenic, 100,000 mg of vitamin C and 300 mg of selenium.

Analyses of feed and excreta were performed at the Laboratory of Animal Research of the DZO/UFLA. The analyses of calcium and phosphorus concentrations in both diet and excreta were performed, respectively, using

a titration method with potassium permanganate and colourimetry, following the methodology presented by Silva and Queiroz (2002). For nitrogen, the Kjeldahl method (AOAC, 1990) was used.

<sup>&</sup>lt;sup>2</sup> Supply per kg of product: 160,000 mg of manganese, 100,000 mg of iron, 100,000 mg of zinc, 20,000 mg of cupper, 2,000 mg of cobalt and 2,000 mg of iodine.

 $<sup>^3</sup>$  Calculated according to Mongin (1981): Na + K – Cl.

For the performance study, the same experimental design was used. Each plot consisted of 25 birds. At the end of the experimental period, feed intake, weight gain and feed conversion were evaluated. Feed intake was calculated by the difference between the food supplied and the scraps of each plot, divided by the average number of live birds. In the event of some mortality during the experimental period, diet at the feeder was weighed and the number of birds was registered. Weight gain was determined by comparing the difference between weight at the beginning and weight at the end of the experiment. Feed conversion was obtained by comparing feed intake to weight gain.

At 42 days of age, two birds from each plot (those closest to the average weight of the plot) were slaughtered after fasting for 12 hours. They were then plucked and eviscerated, and the carcasses were weighed, without heads, feet and abdominal fat. The carcass yield was calculated in relation to live weight before slaughter, cut yields and abdominal fat relative to carcass weight.

All data were submitted to ANOVA and means were compared using the Scott-Knott test, with a significance of 5%. All statistical analysis was performed using the computational SAEG - Statistical Analysis Systems, version 9.0.

## RESULTS AND DISCUSSION

The weight gain of birds was not influenced by nutritional plans (P>0.05) (Table 5). The phytase reduced the feed intake (P<0.05) only in no-reduced crude protein diets (PN2), resulting in lower (P<0.05) feed:gain compared

to others phytase-diets. Compared to control, PN2 was similar (P>0.05).

The results show that the reduction of crude protein in diets with phytase is not beneficial to the performance of birds. According to Diambra and McCartney (1995), the reduction of crude protein stimulates the feed intake in attempt to supply some of the deficient amino acids, which was observed in the present study, even correcting most of the amino acids. In this case, it is assumed that the phytase did not influence the release of non-essential amino acids from dietary protein, resulting in higher feed:gain of the broilers.

Another possible explanation is the reduction of crude protein in the initial phase (8 to 21 days of age) of the birds. Studies show that the reduction of crude protein at this stage of development can compromise the growing of birds in the later stages. Gomide et al. (2007) concluded that it is possible to reduce the protein level of diets in the middle growth phase (22-42 days of age) to 16%, since the diet for initial phase (8-21 days of age) is formulated with the recommended nutritional levels. These results confirm that the nutritional balance in the early stages of developing of broilers is important, especially the amino acids balance, suggesting studies of nutrients digestibility in birds fed diets supplemented with phytase.

How about carcass yield and characteristics of some cuts, there was no effect (P>0.05) of different nutritional plans on the carcass and chest yield (Table 6). The thigh + drumstick yields were higher (P<0.05) in broilers fed diets containing phytase with no reduced crude protein diets. The reduction of crude protein from 20 to 19 or 18% in the

Table 5 – Performance of broilers from 8 to 42 days of age submitted to different nutritional plans composed of diets formulated with the ideal protein concept, plus phytase addition, with low levels of calcium, phosphorus and amino acids.

Nutritional Plan (PN)*	Feed intake (g/bird)	Weight gain (g/bird)	Feed:gain
NP1 – (21 – 19 - 18%) of crude protein without phytase	4,694 b	2,852	1.64 b
NP2 – (21 - 19 - 18%) of crude protein with phytase	4,558 c	2,803	1.62 b
NP3 – (20 - 17 - 15%) of crude protein with phytase	4,855 a	2,814	1.72 a
NP4 – (19 - 17 - 16%) of crude protein with phytase	4,750 b	2,788	1.70 a
NP5 - (19 - 17 - 15%) of crude protein with phytase	4,902 a	2,832	1.73 a
NP6 – (19 - 16 - 15%) of crude protein with phytase	4,835 a	2,789	1.73 a
NP7 – (18 - 16 - 15%) of crude protein with phytase	4,900 a	2,852	1.72 a
Coefficient of variation (%)	2.20	1.60	1.67

<sup>\*</sup> Levels of crude protein, with amino acid correction, according to the development phase of broilers: 8 to 21; 22 to 35 and 36 to 42 days of age. In phytase diets, levels of calcium, phosphorus and amino acid were reduced.

a,b Means followed by different letters differ by Scott-Knott test (P<0.05)

initial phase increased (P<0.05) the abdominal fat of the birds.

The appropriate amino acid balance in the diet is essential for the deposition of protein in muscles. In this study, the higher thigh + drumstick yield suggested higher muscle protein synthesis, although it was not observed on the carcass or on the chest of the birds. Compared to control, the higher thigh + drumstick yield observed in broilers fed PN2 diets can be related to lower feed intake, probably due to lower energy intake and fat deposition on the carcass.

Again, the crude protein levels on the initial phase of development of the broilers can be related at this results. According to Sklan and Noy (2004), the amino acid deposition in the carcass is greater at 14 days of age of the broilers. In this respect, the reduction of non-essential amino acids caused by the reduction of crude protein may have influenced the development of the birds in later phases.

With regard to abdominal fat, Gomide et al. (2011b) observed an increase in broilers fed diets with reduced crude protein. According to authors, the smaller caloric increment in diets containing crystalline amino acids requires a smaller amount of energy to metabolise the excess amino acids. In this case, the excess energy can be directed to fat deposition.

How about the balance of some nutrients, the inclusion of phytase in low-nutrient diets reduced (P<0.05) the absolute excretion of calcium and phosphorus, with more positive results in nutritional plan 3 and 7 for calcium and 2, 4, 6 and 7 for phosphorus (Table 7). The use of phytase increased (P<0.05) the coefficient of retention of these minerals compared to control. The higher values of

coefficient of retention of phosphorus and calcium were obtained with nutritional plans 6 and 7, respectively.

Several studies had shown that phytase is efficient in releasing the phytate phosphorus, and other elements associated with phytic acid like calcium (CARDOSO JÚNIOR et al., 2010; GOMIDE et al. 2011a). Thus, the reduction of these elements in phytase diets decreased their excretion, favouring their retention. Considering the nutritional plan that resulted in better performance and carcass yield (PN2), a 64.5% reduction in calcium excretion and 38% reduction of phosphorus were observed in comparison to the control. This corresponded to an increase of 39.2% and 15.2% for the retention of these elements, respectively, similar at the results obtained by other authors (ALVARENGA et al., 2011).

With regards to nitrogen balance, reducing the crude protein caused a reduction in the emission of this element by excreta (P<0.05), how observed in nutritional plans 3, 4, 5, 6 and 7. This also increased (P<0.05) the coefficient of retention of this element, as already expected. For each percentage unit of reduction in crude protein in phytase-diets, there was reduction of 6.8% for nitrogen excretion by broilers, similar to Gomide et al. (2011a) that found values of 5.5 and 6.0% in the growing and retirement phase of broilers receiving phytase diets. However, in relation to the performance and some carcass characteristics, the reduction of crude protein in phytase diets was not positive, suggesting more studies with digestibility of nutrients when this enzyme is associated to crystalline amino acids.

Table 6 – Carcass and cut yield of broilers from 8 to 42 days of age submitted to different nutritional plans composed of diets formulated with the ideal protein concept, plus phytase addition, with low levels of calcium, phosphorus and amino acids.

	Yield (%)			
Nutritional plan (NP)	carcass	chest	thigh+ drumstick	abdominal fat
NP1 - (21 - 19 - 18%) of crude protein without phytase	74.57	37.61	28.01 b	1.45 b
NP2 – (21 - 19 - 18%) of crude protein with phytase	74.32	37.68	29.49 a	1.35 b
NP3 – (20 - 17 - 15%) of crude protein with phytase	76.12	39.06	27.31 b	1.57 b
<i>NP4</i> – (19 - 17 - 16%) of crude protein with phytase	76.02	39.52	27.61 b	1.78 a
<i>NP5</i> – (19 - 17 - 15%) of crude protein with phytase	75.80	38.80	27.87 b	1.74 a
<i>NP6</i> – (19 - 16 - 15%) of crude protein with phytase	75.16	38.59	28.09 b	2.01 a
NP7 – (18 - 16 - 15%) of crude protein with phytase	75.40	38.30	28.30 b	1.90 a
Coefficient of variation (%)	1.96	3.10	3.09	20.51

<sup>\*</sup> Levels of crude protein, with amino acid correction, according to the development phase of broilers: 8 to 21; 22 to 35 and 36 to 42 days of age. In phytase diets, levels of calcium, phosphorus and amino acid were reduced.

a,b Means followed by different letters differ by Scott-Knott test (P<0.05)

Table 7 – Calcium, phosphorus and nitrogen balance in broilers from 8 to 42 days of age submitted to different nutritional plans composed of diets formulated with the ideal protein concept, plus phytase addition, with low levels of calcium, phosphorus and amino acids.

Nutritional plan*	Feed intake (g/bird)	Excretion (g/bird)	Coefficient of retention (%)
r		- Calcium -	` '
NP1 - (21 - 19 - 18%) of crude protein without phytase	43.47 a	19.57 a	54.98 g
NP2 – (21 - 19 - 18) of crude protein with phytase	29.65 b	6.95 d	76.55 c
NP3 – (20 - 17 - 15) of crude protein with phytase	29.43 c	6.48 e	77.95 b
NP4 – (19 - 17 - 16) of crude protein with phytase	28.99 c	6.86 d	76.31 d
NP5 – (19 - 17 - 15) of crude protein with phytase	30.20 b	8.19 b	72.88 e
NP6 – (19 - 16 - 15) of crude protein with phytase	28.95 c	7.90 c	72.70 f
NP7 – (18 - 16 - 15) of crude protein with phytase	29.89 b	6.48 e	78.32 a
Coefficient of variation (%)	2.15	2.03	0.12
		- Phosphorus -	
NP1 - (21 - 19 - 18%) of crude protein without phytase	34.88 a	15.99 a	54.16 f
NP2 - (21 - 19 - 18) of crude protein with phytase	26.30 c	9.92 c	62.37 e
NP3 – (20 - 17 - 15) of crude protein with phytase	27.40 b	10.26 b	62.54 d
NP4 – (19 - 17 - 16) of crude protein with phytase	26.96 c	10.01 c	62.85 c
NP5 - (19 - 17 - 15) of crude protein with phytase	27.69 b	10.28 b	62.86 c
NP6 - (19 - 16 - 15) of crude protein with phytase	27.93 b	9.75 c	65.11 a
NP7 – (18 - 16 - 15) of crude protein with phytase	28.35 b	9.94 c	64.93 b
Coefficient of variation (%)	2.16	2.16	0.04
		- Nitrogen -	
NP1 - (21 - 19 - 18%) of crude protein without phytase	150 a	47.19 b	68.48 e
NP2 – (21 - 19 - 18) of crude protein with phytase	144 b	48.83 a	66.19 f
NP3 – (20 - 17 - 15) of crude protein with phytase	143 b	42.45 c	70.24 d
NP4 – (19 - 17 - 16) of crude protein with phytase	139 с	40.55 d	70.89 c
NP5 - (19 - 17 - 15) of crude protein with phytase	142 b	41.54 c	70.86 c
NP6 - (19 - 16 - 15) of crude protein with phytase	138 c	38.86 e	71.83 a
NP7 – (18 - 16 - 15) of crude protein with phytase	135 с	38.81 e	71.35 b
Coefficient of variation (%)	2.17	2.19	0.04

In addition, considering phytase diets without reducing crude protein, it was observed that this enzyme increased the nitrogen excretion (47.2 vs. 48.8% comparing control to NP2). Again, this result may be associated with the effect of the enzyme on the release of amino acids from dietary protein.

The results of this study show the need to adjust the nutrient levels for broilers in diets containing phytase. They confirm that it is important to keep the protein requirements consistent in the early stages of development of the birds, and only adjust the crude protein levels in relation to amino acids in later phases. Further studies should therefore be conducted to determine the best amino acid balance required in phytase-diets for broilers.

## CONCLUSION

The crude protein and amino acid levels should not be reduced in diets containing phytase. The inclusion of the enzyme in broiler diets improves the performance and yield of cuts.

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