

## Phytase and amino acids for broilers from 36 to 42 days of age

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### ABSTRACT

The effects of the nutritional reduction of diets supplemented with phytase and amino acids provided to broilers from 36 to 42 days of age were evaluated. Five diets were evaluated in six replicates, totaling 750 and 90 birds in the assays of performance and metabolism, respectively. Control diet, without phytase, contained 18% of crude protein (CP), 0.386% of available phosphorus (aP) and 0.764% of calcium (Ca). The remaining diets (0.236% of aP and 0.464% of Ca) were supplemented with phytase and amino acids, with the CP reduced from 18 to 15%. The performance, yield and nutrient retention by the birds, besides of the energetic value (AMEn) and metabolizability of the diets were evaluated. There was no difference in the performance, yields of carcass and cuts, and in the AMEn. 16 and 15% of CP in the diet increased their metabolizability. The protein reduction improved N retention, but increased abdominal fat. Phytase increased the use of P. With the phytase and amino acids supplementation, the levels of CP, aP and Ca can be reduced to 15, 0.236 and 0.464%, respectively, without compromising on performance, yields of carcass and cuts and AMEn, besides improve the diet metabolizability and utilization of nitrogen and phosphorus.

**Key words:** carcass yield, electrolyte balance, ideal protein, metabolism, performance

### *Fitase e aminoácidos para frangos de corte de 36 a 42 dias de idade*

### RESUMO

Objetivou-se estudar os efeitos da redução nutricional de rações suplementadas com fitase e aminoácidos, fornecidas a frangos de corte de 36 a 42 dias de idade. Cinco dietas foram avaliadas em seis repetições, totalizando 750 e 90 aves nos ensaios de desempenho e metabolismo, respectivamente. A dieta controle, sem fitase, possuía 18% de proteína bruta (PB); 0,386% de fósforo disponível (Pdisp) e 0,764% de cálcio (Ca). As dietas restantes (0,236% de Pdisp e 0,464% de Ca) foram suplementadas com fitase e aminoácidos sendo a PB reduzida de 18 até 15%. Avaliaram-se desempenho, rendimento e retenção de nutrientes pelas aves, além do valor energético (EMAn) e metabolizabilidade das rações. Não houve diferença no desempenho, no rendimentos de carcaça nem cortes e na EMAn. 16 e 15% de PB na ração aumentaram sua metabolizabilidade. A redução proteica melhorou a retenção de N mas aumentou a gordura abdominal. A fitase elevou o aproveitamento do P. Com a suplementação de fitase e aminoácidos, os teores de PB, Pdisp e Ca podem ser reduzidos para 15; 0,236 e 0,464%, respectivamente, sem comprometer o desempenho, rendimentos de carcaça e cortes e a EMAn, além de melhorar a metabolizabilidade da ração e o aproveitamento do nitrogênio e fósforo.

**Palavras-chave:** rendimento de carcaça, balanço eletrolítico, proteína ideal, metabolismo, desempenho

## Introduction

The broiler production generates a great volume of excretas which are normally used as fertilizers. Excretas are rich in phosphorus (P) and nitrogen (N), therefore, their excessive use may compromise the retention ability of P and N by the soil, plants and edaphic microorganisms, unleashing the contamination of the hydric resources by the lixiviation of these nutrients (Fukayama et al., 2008). Thus, there is great interest in the optimization of the poultry production with environmental responsibility, by means of alternatives which may minimize the environmental impact of this activity (Bertechini, 2012).

An efficient alternative is based on the diets formulation with a higher precision in order to provide only what is required by the broilers and also on the improvement of the nutrient utilization by these animal, which results in a lower quantity of P and N excreted (Baker, 2009). Besides the environmental issue, the reduction of P and N rates in the broilers feed decreases the diet cost, improving the profitability of the production (Selle et al., 2009).

The reduction of the excreted P is possible by decreasing the inclusion of supplemental P source in the feed and also favoring the exploitation of the phytate P present in the ingredients of the diet. According to Rostagno et al. (2005), approximately two thirds of the P in the seeds and grains are in the form of phytate and its use requires the activity of the phytase enzyme which is not effectively produced by the monogastric animals (Han et al., 2009). Thus, microbial phytases are added to the broilers diet and studies (Silva et al., 2008; Gomide et al., 2011; Naves et al., 2014) demonstrate the benefits of its use.

Similarly, an effective way to decrease the N excretion is to reduce the crude protein (CP) rate from the diet and use synthetic amino acids to meet the nutritional requirements of the broilers (Oliveira et al., 2012). However, the CP minimum level necessary to allow good results with broiler chickens from 36 to 42 days old is not well established yet. Moreover, Namroud et al. (2008) reported that there may be harm in the performance of broiler fed diet containing low rate of CP even when all essential amino acids are supplemented, which may be correlated to the insufficient provision of N for the synthesis of non-essential amino acids, to the alteration of the ionic balance of the diet caused by the reduction in the level of potassium or, even, to the unbalance between the amino acids.

Therefore, the present study was conducted aiming to evaluate the effects of diets with reduced levels of CP, available phosphorus and calcium (supplemented with phytase, essential amino acids and glycine and with the standardized electrolyte balance) over the performance, carcass yield and retention of N and P of broilers from 36 to 42 days old, besides of the determination of the metabolizability and energetic value of the diets.

## Material and Methods

A performance assay and another of metabolism were simultaneously conducted at the Poultry Farming Sector of the Animal Science Department of Federal University of Lavras

with the objective of evaluate the use of phytase and amino acids in the diet of Cobb-500® male broilers, in the period from 36 to 42 days old. All experimental procedures employed were approved by the Ethics Committee on Animal Use of this University.

In both assays, five diets based on corn and soybean meal were evaluated, being that one of them was denominated of control diet and was formulated without phytase according to the nutritional recommendations of Rostagno et al. (2005). The remaining four diets were supplemented with 80 g of phytase/ton of diet and formulated with reduction of available phosphorus (aP) and calcium (Ca) corresponding to 0.15 and 0.30 percentual points, in relation to the control diet, to maintain the ratio Ca:aP at 2:1. One of the four diets containing phytase provided the same rate of crude protein (CP) as the control diet (18% of CP) and for the three remaining diets, one percentual point of the CP associated to the use of synthetic amino acids was gradually reduced according to the nutritional requirements of the broilers (Rostagno et al., 2005) (Table 1).

The experimental diets were isoenergetic and formulated based on digestible amino acids, maintaining the ideal ratio of the amino acids with lysine. The proteic values of the amino acids were not considered in the formulation. The electrolyte balance of the diets was calculated by the equation described by Mongin (1981) and it was kept constant between the diets by use of potassium carbonate when necessary.

In the performance assay, a total of 750 one day old broiler chicks were raised up to 36 days old with diet based on corn and soybean meal, formulated to meet their nutritional requirements (Rostagno et al., 2005). At 36 days old, the birds were individually weighed, transferred to a masonry poultry house containing boxes of 3 m<sup>2</sup> each, and were distributed in a completely randomized experimental design, where there were six replications of 25 birds for each treatment. The initial average weight of the broilers was similar ( $2,170 \pm 3$  g) between the experimental units (or box).

Each box had the floor covered with wood shavings and contained a 100 watt incandescent lamp, one feeder and one drinker. The feed and water were provided for birds *ad libitum*. The illumination was constant (24 hours of light, between natural and artificial) and the temperatures of maximum and minimum recorded during the experimental period were of 25.8 °C (30 °C at the highest ) and 14.2 °C (12 °C at the lowest), respectively. The feed intake, weight gain and feed conversion of the birds in the period from 36 to 42 days old were evaluated. Moreover, at 42 days old, two broilers per box were slaughtered by cervical dislocation for the record of the carcass, breast, thigh and drumstick, and abdominal fat yields.

The metabolism assay was conducted in a completely randomized experimental design, with six replications of three birds. Therefore, 90 broilers at 36 days old were allocated in metabolic cages provided with a drinker, a feeder and a collection tray for excretas. The experimental period had duration of 7 days, where 4 days were of adaptation to the experimental diets and facilities, and 3 days of total excreta collection. The excretas were collected daily in the morning, packaged in plastic bags and stored in freezer until the end of the collection period.

**Table 1.** Percentual and nutritional composition of the experimental diets for broilers from 36 to 42 days of age

Ingredients (%)	Control <sup>2</sup>	Crude Protein Level <sup>1</sup>			
		18%	17%	16%	15%
Corn	66.453	68.609	71.297	73.566	75.897
Soybean meal	26.773	26.374	23.705	21.113	18.511
Soybean oil	3.404	2.677	2.329	2.121	1.892
Dicalcium phosphate	1.540	0.690	0.700	0.720	0.740
Calcitic limestone	0.620	0.430	0.450	0.450	0.460
Salt	0.450	0.450	0.450	0.450	0.450
L-lysine HCl 78%	0.230	0.235	0.315	0.395	0.475
DL-methionine	0.210	0.210	0.230	0.246	0.267
L-arginine	0.000	0.000	0.035	0.110	0.187
L-threonine	0.050	0.050	0.090	0.118	0.154
L-glycine + serine	0.000	0.000	0.020	0.114	0.210
L-valine	0.020	0.020	0.060	0.105	0.148
L-isoleucine	0.000	0.000	0.030	0.071	0.116
L-tryptophan	0.000	0.000	0.000	0.009	0.022
L-phenylalanine	0.000	0.000	0.000	0.000	0.003
Vitamin premix <sup>3</sup>	0.100	0.100	0.100	0.100	0.100
Mineral premix <sup>4</sup>	0.050	0.050	0.050	0.050	0.050
Choline chloride 60%	0.050	0.050	0.050	0.050	0.050
Salinomycin 12%	0.050	0.050	0.050	0.050	0.050
Phytase - Ronozyme <sup>®</sup> NP (M)	0.000	0.008	0.008	0.008	0.008
Potassium carbonate	0.000	0.000	0.075	0.148	0.220
Calculated nutritional composition					
Crude protein (%)	18.0	18.0	17.0	16.0	15.0
Metabolizable energy (kcal/kg)	3150	3150	3150	3150	3150
Available phosphorus (%)	0.386	0.236	0.236	0.236	0.236
Calcium (%)	0.764	0.464	0.464	0.464	0.464
Lysin (%)	1.019	1.017	1.017	1.017	1.017
Methionine + cystine (%)	0.736	0.739	0.738	0.732	0.732
Arginine (%)	1.116	1.111	1.069	1.067	1.068
Threonine (%)	0.664	0.664	0.668	0.661	0.661
Glycine + serine (%)	1.644	1.643	1.569	1.568	1.569
Valine (%)	0.784	0.784	0.781	0.783	0.783
Isoleucine (%)	0.701	0.700	0.684	0.681	0.681
Tryptophan	0.192	0.191	0.178	0.173	0.173
Phenylalanine (%)	0.831	0.830	0.783	0.736	0.688
Leucine (%)	1.540	1.549	1.488	1.426	1.364
Histidine (%)	0.469	0.470	0.446	0.422	0.399
Phenylalanine + tyrosine (%)	1.406	1.406	1.326	1.247	1.170
Sodium (%)	0.197	0.198	0.198	0.198	0.198
Potassium (%)	0.676	0.675	0.676	0.676	0.676
Chlorine (%)	0.315	0.316	0.316	0.316	0.315
Electrolyte balance (mEq/kg) <sup>5</sup>	170	170	170	170	170

<sup>1</sup>Diets supplemented with phytase and formulated with reduced levels of crude protein, available phosphorus, and calcium compared to the control diet.

<sup>2</sup>Diet without phytase and formulated to meet the nutritional requirements of broilers (36 to 42 days of age), according to the recommendations of Rostagno et al. (2005).

<sup>3</sup>Vitamin supplementation/kg of diet: vit.A: 12,500 UI; vit.D<sub>3</sub>: 5,760 UI; vit.E: 150 mg; vit. K<sub>3</sub>: 4 mg; vit. B<sub>1</sub>: 3 mg; vit. B<sub>2</sub>: 9 mg; vit. B<sub>6</sub>: 6 mg; vit. B<sub>12</sub>: 40 µg; biotine: 0,3 mg; folic acid: 2 mg; nicotinic acid: 80 mg; pantothenic acid: 18 mg.

<sup>4</sup>Mineral supplementation/kg of feed: Mn: 80 mg; Fe: 50 mg; Zn: 50 mg; Cu: 10 mg; Co: 1 mg; I: 1 mg; Se: 0.3 mg.

<sup>5</sup>Electrolyte balance calculated using the equation proposed by Mongin (1981), which relates the calculated concentrations of sodium, potassium, and chlorine ions.

After, the excretas were unfrozen, weighed, homogenized and dried in circulating air ovens at 60 °C for 72 hours. The dried samples (rations and excretas) were ground and used for the determination of dry matter, crude energy, nitrogen, and phosphorus according to methodologies of the AOAC (2005). The corrected metabolizable energy for the nitrogen balance (AMEn) of the diets was calculated according to Matterson et al. (1965). The metabolizability coefficient (MC) of the experimental feeds and the apparent retention coefficients (RC) of nitrogen and phosphorus were calculated based on the dry matter, using the following equation: MC or RC = [(g of intake nutrient – g of excreted nutrient)/g of intake nutrient]x100.

The analyses of variance of the data was conducted using the statistics software SISVAR developed by Ferreira (2010) and, when significant, the averages of the treatments were compared by the Scott-Knott test, at 5% of probability.

## Results and Discussion

The feed intake (FI), weight gain (WG) and feed conversion (FC) of the broilers were not affected ( $p>0.05$ ) by the evaluated diets (Table 2). Therefore, the birds fed diets containing reduced levels of crude protein (CP), calcium (Ca) and available phosphorus (aP), supplemented with phytase and amino acids, presented performance similar to the broilers fed the control diet.

These results indicate that the phytase contributed to the maintenance of the broiler performance when the aP and Ca levels of the diet were reduced to 0.236% and 0.464%, respectively. This can be explained by the phytase action over the phytate substrate present in the vegetable ingredients allowing that the phytate P, resulting from the enzymatic hydrolysis, could be used by the birds. Moreover, the phytase can improve the utilization of nutrients of the diet because the lower the degree of phytate phosphorylation, the smaller its ability to complex and render unavailable nutrients such as the Ca (Selle et al., 2009). Fukayama et al. (2008) also verified the beneficial effect of the phytase, because the nutritional reduction of the diet for to 2,940 kcal kg<sup>-1</sup>; 21.2% CP; 0.27% aP and 0.90% Ca worsened the FI, WG, and FC of broilers. However, when the feed was supplemented with phytase, the broiler performance was improved.

In a similar research using the concept of ideal protein in the diet formulation, Oliveira et al. (2010) verified that the CP can be reduced from 21.6 down to 17.6% without reducing the broiler performance in the period from 22 to 42 days old, even when raised in an environment stressful by heat. However, Vasconcellos et al. (2010) observed worsening in the performance of male broilers from 21 to 42 days old when CP level was reduced from 21 down to 15%, even supplementing the feed with essential amino acids in order to meet the nutritional requirements of the birds. Still according to Vasconcellos et al. (2010), it is probable that the requirements proteinous are altered or the ratios between the amino acids are modified when the CP levels are reduced.

In addition, an additional explanation for the fall in the performance may be due to the lack of supplementation of glycine, because according to Baker (2009), when the CP level in a diet based on corn and soybean meal is reduced from 23 to 16%, this amino acid becomes limiting for broilers. In the present study, the glycine was supplemented, which may seem to be important to avoid damages in the performance even in the ration with only 15% of CP.

Moreover, the performance may also be influenced by the electrolytic balance in the diet (Bertechini, 2012) and is important to highlight that the CP reduction, in general, is done decreasing the inclusion of soybean meal which is rich in potassium. Thus, the worsening of the broiler performance observed by Vasconcellos et al. (2010), may also be associated to the lack of correction of the electrolyte balance of the diets

**Table 2.** Performance and yields of carcass, cuts and abdominal fat of broilers fed diets with reduced nutrient levels, supplemented with phytase and amino acids

Experimental diets	Performance (36 to 42 days old)			Yields (%) (at 42 days of age)			
	FI (g/bird)	WG (g/bird)	FC (g/g)	Carcass	Breast	Thigh + drumstick	Abdominal fat
18% CP without phytase <sup>1</sup>	1,297	664	1.96	73.3	37.2	29.2	1.57 a
18% CP with phytase <sup>2</sup>	1,300	686	1.90	72.4	37.0	29.5	1.68 a
17% CP with phytase + AA <sup>2</sup>	1,308	652	2.01	73.2	37.4	29.7	1.92 b
16% CP with phytase + AA <sup>2</sup>	1,296	676	1.92	72.5	37.9	29.8	2.12 b
15% CP with phytase + AA <sup>2</sup>	1,296	650	2.00	73.2	38.0	29.2	1.94 b
Mean	1,299	666	1.96	72.9	37.5	29.5	
P value	p>0,05	p>0,05	p>0,05	p>0,05	p>0,05	p>0,05	p<0,05
Coefficient of variation (%)	2.89	4.77	3.50	1.58	2.82	2.75	14.90

Means followed by different letters in the column differ by Scott-Knott test ( $p < 0.05$ ).

FI = feed intake; WG = weight gain; FC = feed conversion; CP = crude protein; AA = amino acids.

<sup>1</sup>Control diet. Levels of available phosphorus and calcium = 0.386% e 0.764%, respectively. <sup>2</sup>Diets supplemented with amino acids and 80 g of phytase/ton of feed. Levels of available phosphorus and calcium = 0.236% and 0.464%, respectively.

and it is probable that the standardization of this factor in our study (170 mEq/kg) has positively influenced for a similar performance in all evaluated protein levels.

The broilers fed diets with reduced nutritional levels, supplemented with phytase and amino acids, presented carcass, breast, thigh and drumstick yields similar ( $p > 0.05$ ) to those that received the control diet (Table 2). On the other hand, a lower abdominal fat ( $p < 0.05$ ) was observed when the diet contained 18% of CP, independent of the level of aP and Ca, and higher deposition of fat when reducing the protein rate down to three percentual points which may be justified by the high caloric increment of the proteins (Nelson & Cox, 2011).

That means that when decreasing the CP level of the diet, less energy is required for the absorption, digestion and metabolism of the ingested proteins and, consequently, there will be greater amount of energy stored as body fat. In general, the protein reduction of the diet has showed increase in the rate of abdominal fat in broilers (Namroud et al., 2008; Gomide et al., 2012; Silva et al., 2012) which may damage its acceptability by the consumer. However, in general the reduction of the CP in the diet seems to present more positive points than negative, signaling the need to conduct new studies aiming to minimize the energy deviated to the synthesis of adipose tissue, either by the formulation of the diet more and more precise (close to the real requirement of the animal) and/or by the use of additives able to reduce the deposition of abdominal fat, for example, the chitooligosaccharides (Zhou et al., 2009).

The composition did not alter ( $p > 0.05$ ) the apparent metabolizable energy corrected by the nitrogen balance (AMEn) of the diets (Table 3), which disagrees with Silva et al. (2008) who verified an improvement of 162 kcal of AMEn/kg of feed when the protein rate of the diet was reduced from 19 to 15%.

The dry matter metabolizability coefficient (DMMC) was higher ( $p < 0.05$ ; Table 3) in the diets with 16 and 15% of CP, probably due to the higher supplementation of amino acids readily available and due to the lower rate of non starch polysaccharides (NSPs) in these diets due to the lower inclusion of soybean meal, because according to Abudabos (2010), the percentual increase of soybean meal in the diets reduces the digestibility of the dry matter and increases the viscosity of the intestinal digesta due to the presence of the NSPs in this feed.

The intake, excretion and retention coefficient of the nitrogen (N) and phosphorus were influenced ( $p < 0.05$ ) by the diet (Table 3). The birds fed diet containing 18% of CP presented higher intake and excretion of N and worse retention of this nutrient. The reduction of two or three percentual points in the CP, in relation to the diets with 18%, decreased the intake of N in 683 mg bird<sup>-1</sup> day<sup>-1</sup>. With 15% of CP in the diet, the N excretion was reduced in 520 mg bird<sup>-1</sup> day<sup>-1</sup>.

There was an improvement in the N utilization by the broilers due to the protein reduction in the diet. However, the diets with 15, 16, and 17% of CP presented similar retention coefficients. The phytase did not improve the N use in the diets with 18% of CP and it is probable that it did not influence

**Table 3.** Energetic value and metabolizability coefficient of feeds, and balance/retention of nitrogen and phosphorus of broilers fed diets with reduced nutrient levels, supplemented with phytase and amino acids (from 36 to 42 days of age)

Experimental diets	AMEn (kcal/kg)	DMMC (%)	Nutrient intake		Excreted nutrient		Coefficient of retention (%)	
			(mg bird <sup>-1</sup> day <sup>-1</sup> )				N	P
			N	P	N	P		
18% CP without phytase <sup>1</sup>	3,252	78.08 b	5,696 a	1,276 a	1,899 a	710 a	66.63 b	44.24 b
18% CP with phytase <sup>2</sup>	3,251	77.45 b	5,617 a	867 b	1,827 a	439 b	67.46 b	49.31 a
17% CP with phytase + AA <sup>2</sup>	3,230	76.14 b	5,364 b	896 b	1,628 b	444 b	69.61 a	50.27 a
16% CP with phytase + AA <sup>2</sup>	3,292	79.50 a	5,077 c	880 b	1,553 b	424 b	69.48 a	51.68 a
15% CP with phytase + AA <sup>2</sup>	3,367	80.93 a	4,871 c	833 b	1,343 c	400 b	72.26 a	51.83 a
Mean	3,278							
P value	p>0,05	p<0,05	p<0,05	p<0,05	p<0,05	p<0,05	p<0,05	p<0,05
Coefficient of variation (%)	2.76	3.00	4.34	6.47	8.04	9.80	3.63	9.83

Means followed by different letters in the column differ by Scott-Knott test ( $p < 0.05$ ).

CP = crude protein; AA = amino acids; AMEn = apparent metabolizable energy corrected by the nitrogen balance; DMMC = dry matter metabolizability coefficient; N = nitrogen; P = phosphorus.

<sup>1</sup>Control diet. Levels of available phosphorus and calcium = 0.386% e 0.764%, respectively. <sup>2</sup>Diets supplemented with amino acids and 80 g of phytase/ton of feed. Levels of available phosphorus and calcium = 0.236% and 0.464%, respectively.



this parameter in the remaining evaluated diets. Although the phytase effect in increasing the P utilization of the diet is quite reported in the literature (Selle et al., 2009; Gomide et al., 2011; Santos 2011a,b), its effect on the amino acids, proteins and others nitrogenized compounds is not well established yet, where more works are necessary in this research area.

Independent of the CP level, the aP reduction of 0.386% (control diet) to 0.236% and the addition of phytase resulted in lower ( $p<0.05$ ) intake and excretion of P, besides improving ( $p<0.05$ ) its utilization by the broilers in up to 7.59 percentual points. The phytase contribution for the increase of the P use is due to the action of this enzyme in the release of phosphate groups present in the molecule of phytate enabling the phytate P to be exploited by the birds, reducing consequently, its excretion. This was demonstrated by Naves et al. (2014), who quantified the concentration of phytate phosphorus in both the feed and the excreta from broilers and estimated a retention coefficient of phytate phosphorus up to 87.85% when the diet contained 1,950 FTU/kg of diet. The knowledge of the positive effect of the phytase in to reduce the P excretion into the environment and increase of phytate P utilization constitutes an important alternative for the poultry production, reducing the environmental impact of this activity and the costs with feeding (Bertechini, 2012).

## Conclusions

The supplementation of the diet with amino acids (essential and glycine) and phytase associated to the correction of the electrolyte balance for 170 mEq kg<sup>-1</sup> allows the reduction of crude protein, available phosphorus and calcium to 15, 0.236, and 0.464%, respectively, without worsening the performance and yield of carcass, and cuts of broiler chickens, in the period from 36 to 42 days old.

Moreover, there are an improvement in the dry matter metabolizability coefficient and in the utilization of the nitrogen and phosphorus by the birds. However, the abdominal fat is increased in 23.6%.

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