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# Increasing levels of phytase in diets formulated with reduced available phosphorus content supplied to male and female broilers

Níveis crescentes de fitase em dietas formuladas com reduzido teor de fósforo disponível, fornecidas a frangos de corte machos e fêmeas

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#### ABSTRACT

The reduction of the available phosphorus (P)content in the broiler diet must be followed by the supplementation of adequate phytase. One experiment was conducted with broilers from 15 to 28 days of age to determine the level of necessary phytase when the mashed diet is formulated with 0.213% of P to enable the results of performance, tibia ash content, and calcium (Ca) utilization similar to those determined for birds fed with diet formulated to meet their nutritional requirements. Thus, 120 broilers were distributed in (4+1) x2 factorial arrangement corresponding to four deficient diets in  $_{\omega}P(0.213\%)$  supplemented with phytase (0; 750; 1,500 or 2,250FTU kg<sup>-1</sup>) plus one positive control diet without phytase (0.426% of  $_{\alpha}P$ ), supplied to male and female broilers. Males showed better performance and higher total retention. Decrease in the P content of the diet without phytase use worsened the performance, tibia ash content, and Ca retention; however, these parameters were improved gradually with the increase of the phytase level in the diet. Regardless of sex, using 2,250FTU kg<sup>-1</sup>, it is possible to reduce the  $_{ov}P$  to 0.213% without impairing performance, tibia ash content, "and Ca retention; in addition to reducing the  $_{total}P$  excretion in 56.75% and improving its utilization in 38.58%.

Key words: aviculture, bone ash, enzyme, metabolism, performance, phytate.

## RESUMO

A redução do teor de fósforo disponível  $(P_{disp})$  na dieta de frangos deve ser acompanhada de adequada suplementação da fitase. Um experimento foi conduzido com frangos de corte, no período de 15 a 28 dias de idade, para determinar o nível de fitase necessário quando a ração farelada é formulada com 0,213% de  $P_{disp}$  para permitir resultados de desempenho, teor de cinzas na tíbia e aproveitamento do cálcio (Ca) semelhantes ao determinado para aves alimentadas com uma dieta formulada para atender suas exigências nutricionais. Desse modo, 120 frangos foram distribuídos em esquema fatorial (4+1) x2, correspondendo a quatro rações deficientes em  $P_{disp}$  (0,213%) suplementadas com fitase (0; 750; 1.500 ou 2.250FTU kg<sup>-1</sup>) mais uma ração controle positivo sem fitase (0,426% de  $P_{disp}$ ), fornecidas a frangos machos e fêmeas. Os machos apresentaram melhor desempenho e maior retenção de  $P_{total}$  A redução do teor de  $P_{disp}$  da dieta sem o uso de fitase piorou o desempenho, o teor de cinzas ósseas e a retenção do Ca, todavia, esses parâmetros foram gradativamente melhorados com o aumento do nível de fitase na ração. Independente do sexo, ao utilizar 2.250FTU kg<sup>-1</sup>, é possível reduzir o teor de  $P_{disp}$  para 0,213% sem prejudicar o desempenho, teor de cinzas na tíbia e a retenção do Ca, além de reduzir a excreção do  $P_{total}$  em 56,75% e melhorar seu aproveitamento em 38,58%.

Palavras-chave: avicultura, cinza óssea, desempenho, enzima, fitato, metabolismo.

# **INTRODUCTION**

The main idea of using phytase in the diet for broilers is based on the reduction in the content of available phosphorus ( $_{av}P$ ) accompanied by the inclusion of this enzyme. With this, it is expected that the  $_{av}P$  content present in the diet, added to the content of phytate P ( $_{phy}P$ ) made available by the catalytic action of the phytase, meets the P requirement of the bird (HAN et al., 2009). The industry related to broiler nutrition, normally has recommended the phytase supplementation with an inclusion level between 500 and 1,000 units of phytase activity (FTU) kg<sup>-1</sup> of diet (BERTECHINI, 2012). According to JENDZA et al. (2006), 500FTU kg<sup>-1</sup> are equivalent to the addition of 0.12% inorganic P in the diet. SHAW et al. (2011), in

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turn, reported that it is possible to reduce P in the diet formulation in 0.16% using 1,000FTU kg<sup>-1</sup>. These values of equivalence represent good results; but with the constant progress of the techniques of commercial production of phytase, the price of the enzyme has decreased, arousing the interest of evaluating higher rates of inclusion of phytase so that the avP content in the broiler diet can be reduced even more resulting in lower cost with feeding birds and lower excretion of P in the environment (MENEGHETTI et al., 2011).

The use of inclusion levels above of 1,000FTU kg<sup>-1</sup> of diet have been evaluated for the broilers of the commercial strain Cobb 500® (MANANGI & COON, 2008; MENEGHETTI et al., 2011; WALK et al., 2013). However, most of the recent researches have been conducted only with male broilers, necessitating further studies to evaluate whether there is an influence of the bird sex. Therefore, this study was conducted with male and female broilers Cobb 500<sup>®</sup>, in the period from 15 to 28 days of age, to determine the level of phytase that should be utilized when the diet is formulated with only 0.213% of  $_{av}$ P. For this, will be considered the phytase level which will allow to improve the P retention, in addition to maintain the performance, tibia ash content and utilization of the calcium (Ca) similar to determine for broilers fed with a positive control diet.

#### MATERIALS AND METHODS

One experiment was conducted in the Aviculture Sector of the Department of Animal Science of the Federal University of Lavras, Brazil. The experimental design was completely randomized, in a (4+1) x2 factorial arrangement, with four diets equally deficient in <sub>av</sub>P supplemented with increasing levels of 6-phytase synthesized by **Aspergillus** *oryzae* (0; 750; 1,500 and 2,250FTU kg<sup>-1</sup> of diet) plus one positive control diet without phytase, formulated according to the nutritional recommendations of ROSTAGNO et al. (2005), supplied to male and female broilers.

Each one of the five experimental diets was used in the mashed physical form, and was evaluated in three replications of four birds each, totaling 60 birds for each sex. Evaluating the effect of sex of birds is part of the objective of this research. Moreover, the use of male and female birds within a same cage (mixed housing) could increase the coefficient of variation of the experiment; thus, the sex was considered a factor within the factorial design to minimize the variation between the repetitions of the same treatment, promoting an experiment with a smaller mean square error.

Thus, 60 males and 60 females of chicks Cobb 500<sup>®</sup> were acquired at one day of age, sexed, and raised in a masonry shed up to 14 days of age, receiving basal diet formulated to meet their nutritional requirements (ROSTAGNO et al., 2005). On the 15th day of age, birds were weighed individually and transferred to cages (experimental units with dimensions 50x 50x50cm) in a metabolism room, so that the experimental units presented initial average weight of broilers similar to one another (0.465kg  $\pm$  0.002). The room was provided with constantly illumination (24 hours of light maintained by incandescent lamps of 100 watts) and the temperature was maintained between 22 and 26°C. Each cage was provided with one pressure-type drinker, one individual gutter-type feeder with edge, and one tray for the collection of excreta. Experimental diets (Table 1) and water were supplied ad libitum in the period from 15 to 28 days of age.

The total experimental period comprised 13 days, ten of which were for adaptation to the facilities and experimental diets, followed by three days (RODRIGUES et al., 2005) of total collection of excreta (SIBBALD & SLINGER, 1963). Feed intakes (FI) in the total period (from 15 to 28 days of age of the broilers) and in the collection period (from 25 to 28 days of age of the broilers) were calculated. Weight gain (WG) of the broilers was calculated in the total period and the feed conversion (FC) was calculated by dividing FI by WG.

The total collection of excreta was performed daily in the morning, so that the excreta of an experimental unit was collected in identified plastic bags and stored in freezer until the last day of collection, when the samples were homogenized and then 400 grams of each sample was pre-dried in an oven at 55°C during 72 hours. The dry matter, Ca and total P contents were determined in the excreta and diets according to methods of number 934.01, 935.13 and 965.17, respectively (AOAC, 2005). Balance and retention of the Ca and total P were calculated based on the dry matter. Retention coefficient of  $(_{Ret}C)$ of the nutrient was calculated by the equation <sub>Ref</sub>C (%) = [(nutrient intake - absolute excretion of thenutrient)/nutrient intake] x100. Moreover, at the end of the experiment, three birds per replication were slaughtered for the removal of the left tibia, which was subsequently striped off its flesh, dried at 105°C, scoured with ethyl ether and incinerated at 600°C, determining the ash content (method 942.05 of the AOAC, 2005).

Ingredient (%)	Positive control diet <sup>1</sup>	Negative control diet <sup>2</sup>	Negative control diet supplemented with phytase <sup>3</sup> 750/ 1,500/ 2,250					
Corn	59.620	60.486	60.486					
Soybean meal	33.529	33.371	33.371					
Soybean oil	3.161	2.868	2.868					
Salt	0.482	0.482	0.482					
L-lysine HCl 98%	0.167	0.170	0.170					
DL-methionine 99%	0.229	0.228	0.228					
L-threonine 98,5%	0.035	0.035	0.035					
Mineral premix <sup>4</sup>	0.050	0.050	0.050					
Vitamin premix <sup>5</sup>	0.040	0.040	0.040					
Choline chloride 60%	0.045	0.045	0.045					
Calcitic limestone	0.874	1.610	1.610					
Dicalcium phosphate	1.719	0.565	0.565					
Inert (kaolin)	0.0500	0.0500	0.0425/ 0.0350/ 0.0275					
6-phytase	0	0	0.0075/ 0.0150/ 0.0225					
	Calculated nutri	Calculated nutritional composition						
Metabolizable energy (kcal kg <sup>-1</sup> )	3,050	3,050	3,050					
Crude protein (%)	20.12	20.12	20.12					
Calcium (%)	0.855	0.855	0.855					
Total phosphorus (%)	0.639	0.426	0.426					
Available phosphorus (%)	0.426	0.213	0.213					
Phytate phosphorus (%)	0.213	0.213	0.213					
Activity of the 6-phytase <sup>3</sup>	0	0	750/ 1,500/ 2,250					

Table 1 - Composition and nutrient contents of the experimental diets on an as-fed basis.

<sup>1</sup>Diet formulated to meet the nutritional requirements of broilers of 15 to 28 days of age (ROSTAGNO et al., 2005). <sup>2</sup>Diet deficient in available phosphorus. <sup>3</sup>Enzymatic supplementation expressed in FTU kg<sup>-1</sup> of diet. <sup>4</sup>Provided per kg of diet: 55mg Zn; 48mg Fe; 10mg Cu; 78mg Mn; 0.70mg I; 0.18mg Se. <sup>5</sup>Provided per kg of diet: 4.8mg vit. B<sub>2</sub>; 8,000UI vit. A; 2,000UI vit. D<sub>3</sub>; 1.2mg vit. B<sub>1</sub>; 2.4mg vit B<sub>6</sub>; 10.8µg vit. B<sub>12</sub>; 0.024mg biotin; 0.64mg folic acid; 11.6mg pantothenic acid; 1.92mg vit. K<sub>3</sub>; 16.2UI vit. E.

The data were subjected to variance analysis utilizing the software SAS (2004). Models of regression (P<0.05) were utilized to evaluate the level of phytase supplementation. The significance of broiler sex was evaluated by the F test (P<0.05). Moreover, the positive control diet was compared with the other experimental diets by Dunnett's test (P<0.05).

# **RESULTS AND DISCUSSION**

There was no interaction (P>0.05) between the phytase level in the diet and the sex of birds for all performance parameters (Table 2). However, there was isolated effect of the sex (P<0.01), so that the males showed 10.26% bigger FI, 15.17% greater WG and 4.52% better FC, corroborating to MENDES et al. (2004), who reported that male broilers usually present better performance than females when reared under the same conditions.

The increase of the phytase level in the diet affected (P<0.05 and P<0.01, respectively), the FI and WG of the broilers in a quadratic manner,

estimating greater FI and WG with the use of 1,069 and 1,401FTU kg<sup>-1</sup> of diet, respectively. Already the FC of the broilers decreased (P<0.05) linearly with the increasing supplementation of phytase, with an improvement of up to 3.85% in this parameter with the use of 2,250FTU kg<sup>-1</sup> of diet. Similarly, FUKAYAMA et al. (2008) also verified quadratic effects for FI and WG and decreasing linear effect for FC in male broilers from 1 to 20 days of age fed with diets deficient in  $_{av}P(0.273\%)$  without phytase or supplemented with 500, 750 or 1,000FTU kg<sup>-1</sup> of diet. The quadratic effect of the phytase level in the diet on the FI and WG is probably due to the fact that, with lower inclusion levels of phytase, the broilers tend to consume more feed to compensate the reducing of the P in the feed, and thus they gain more weight. However, with the inclusion of higher phytase levels, there are a lot releasing of the phytate P from feed, which may cause a nutritional imbalance that reduces the FI and WG of the broilers.

Concerning the positive control diet, there was no difference (P>0.05) for FI; but, there was difference (P<0.05) for WG and FC. Broilers fed with

Diets	Feed Intake (kg bird <sup>-1</sup> )			Weight Gain (kg bird <sup>-1</sup> )				
	Males	Females	Mean <sup>1</sup>	Males	Females	Mean <sup>2</sup>		
0.213% <sub>av</sub> P + 0FTU kg <sup>-1</sup>	1.50	1.37	1.43	0.98	0.86	0.92*		
$0.213\% _{av}P + 750FTU kg^{-1}$	1.53	1.40	1.47	1.04	0.89	0.96		
0.213% <sub>av</sub> P + 1,500FTU kg <sup>-1</sup>	1.52	1.38	1.45	1.02	0.90	0.96		
0.213% <sub>av</sub> P + 2,250FTU kg <sup>-1</sup>	1.50	1.36	1.43	1.03	0.89	0.95		
Mean	1.52a	1.37b		1.03a	0.89b			
$0.426\%_{av}P + 0FTU \text{ kg}^{-1} (PC)$	1.53	1.37	1.45	1.07	0.92	0.99		
P-value								
Phytase level	P<0.05			P<0.01				
Sex		P<0.01			P<0.01			
Phytase level x Sex		P>0.05		P>0.05				
Coefficient of variation (%)		1.39			2.41			
	Fee	Feed Conversion (kg kg <sup>-1</sup> )			Tibia ash (g 100g <sup>-1</sup> DDM)			
Diets	Males Females Mean <sup>3</sup>			Mean of males and females <sup>4</sup>				
$0.213\% _{av}P + 0FTU kg^{-1}$	1.53	1.60	1.56*	48.14*				
$0.213\% _{av}P + 750FTU kg^{-1}$	1.48	1.57	1.52*	50.47*				
0.213% <sub>av</sub> P + 1,500FTU kg <sup>-1</sup>	1.49	1.53	1.51		50.67*			
$0.213\% _{av}P + 2,250FTU \text{ kg}^{-1}$	1.48	1.53	1.50	51.71				
Mean	1.48b	1.55a						
0.426% <sub>av</sub> P + 0FTU kg <sup>-1</sup> (PC)	1.43	1.50	1.46		52.13			
P-value								
Phytase level		P<0.05			P<0.01			
Sex		P<0.01			P>0.05			
Phytase level x Sex		P>0.05			P>0.05			
Coefficient of variation (%)		2.20		1.68				

Table 2 - Performance (in the period from 15 to 28 days of age) and tibia ash content (at 28 days of age) of broilers fed with diets deficient in available phosphorus (avP) supplemented with increasing levels of phytase.

DDM: defatted dry matter. PC: positive control diet. Means with different letters, in the row, differ by the F test (P<0.05). \*Differs from positive control diet by Dunnett's test (P<0.05).

<sup>1</sup>Feed intake =  $-0.00002259x^2 + 0.04832x + 1433.3250$  (R<sup>2</sup>=0.82). <sup>2</sup>Weight gain =  $-0.00002430x^2 + 0.06809x + 919.65$  (R<sup>2</sup>=0.92). <sup>3</sup>Feed conversion = -0.00002520x + 1.55305 (R<sup>2</sup>=0.84). <sup>4</sup>Tibia ash = 0.00146x + 48.61169 (R<sup>2</sup>=0.87).

diet containing 0.213% <sub>av</sub>P without phytase presented worsening of 7.75% in WG, indicating that this diet did not supply P at an amount enough for the adequate development of the birds, but when phytase was utilized at levels as from 750FTU kg<sup>-1</sup>, the <sub>phy</sub>P present in the vegetable ingredients of the diet was hydrolyzed by the enzyme at amount enough to prevent damage in the WG of the birds. However, only the diets deficient in <sub>av</sub>P containing 1,500 or 2,250FTU kg<sup>-1</sup> promoted FC similar to the observed for broilers from the positive control group. Nevertheless, the recommendation of the phytase level in the diet formulation should also consider an adequate bone mineralization (SAKOMURA & ROSTAGNO, 2007).

There was no interaction (P>0.05) between the phytase levels and the bird sex, nor isolate effect (P>0.05) from sex for the tibia ash content (Table 2). However, the increase of the phytase level improvement linearly (P<0.01) this bone parameter. These results corroborate to SANTOS et al. (2008) and HAN et al. (2009) who also observed improvement in the bone mineralization of broiler fed diets deficient in P, but supplemented with phytase.

Furthermore, it was observed that only using 2,250FTU kg<sup>-1</sup> of diet, the deposition of mineral matter in the tibia was similar to that determined for the broilers from the positive control group. Contrarily, FUKAYAMA et al. (2008) verified that 750FTU kg<sup>-1</sup> was enough to prevent reduction in bone mineralization; however, the decrease of the <sub>av</sub>P content in this diet was of only 36%, which is inferior to the value evaluated in this research (50% of requirement). Besides, in the experiment of FUKAYAMA et al. (2008), diets contained 5.5% of defatted rice bran, increasing the content of <sub>phy</sub>P in the diet, which might have increased the catalytic efficiency of phytase because there was more substrate to be catalyzed.

There was no interaction (P>0.05) between the level of phytase and sex for the balance and retention of Ca (Table 3). However, males

consumed and excreted (P<0.01) more Ca, which can be explained by the greater FI that they presented (Table 2), associated with the fact of that the sex did not affect (P>0.05) the utilization of this mineral (Table 3). Similarly to what was verified for FI, the phytase level affected of quadratic manner (P<0.01) the Ca intake, with the highest value estimated for the diet with 1,222FTU kg<sup>-1</sup>. The Ca excretion was reduced linearly (P<0.01) in 14.13% with the use of 2,250FTU kg<sup>-1</sup> of diet, which is related to the linear improvement (P<0.01) in the Ca utilization promoted by the increasing levels of enzyme in the diet.

The broilers feed diet without phytase, formulated with reduced content of  $_{av}P$ , presented

Table 3 - Balance and retention coefficient of calcium and total phosphorus (in dry matter) of broilers, in the period from 25 to 28 days of age, fed diets deficient in available phosphorus (<sub>av</sub>P) supplemented with increasing levels of phytase.

	C	Calcium intake (g bird <sup>-1</sup> )		Calcium excretion (g bird <sup>-1</sup> )				
Diets	Males	Females	Mean <sup>1</sup>	Males	Females	Mean <sup>2</sup>		
$0.213\% _{av}P + 0FTU kg^{-1}$	3.71	3.25	3.48*	2.28	1.99	2.14*		
$0.213\% _{av}P + 750FTU kg^{-1}$	3.87	3.41	3.64	2.20	1.99	2.09*		
$0.213\% _{av}P + 1,500FTU kg^{-1}$	3.88	3.44	3.66	2.08	1.86	1.97		
0.213% <sub>av</sub> P + 2,250FTU kg <sup>-1</sup>	3.75	3.32	3.53*	1.95	1.72	1.84		
Mean	3.82a	3.39b		2.08a	1.88b			
$0.426\%_{av}P + 0FTU kg^{-1} (PC)$	3.87	3.54	3.71	1.87	1.82	1.85		
P-value								
Phytase level	P<0.01			P<0.01				
Sex	P<0.01			P<0.01				
Phytase level x Sex	P>0.05			P>0.05				
Coefficient of variation (%)	2.42			4.42				
	Calciu	Calcium retention coefficient $(\%)^3$			Total phosphorus intake (g bird <sup>-1</sup> )			
Diets	Me	Mean of males and females			Females	Mean <sup>4</sup>		
0.213% <sub>av</sub> P + 0FTU kg <sup>-1</sup>		38.38*			1.65	1.77*		
$0.213\% _{av}P + 750FTU kg^{-1}$		42.49*		1.99	1.79	1.89*		
$0.213\% _{av}P + 1,500FTU kg^{-1}$		46.15*			1.74	1.84*		
0.213% <sub>av</sub> P + 2,250FTU kg <sup>-1</sup>		47.72		1.86	1.62	1.74*		
Mean				2.12a	1.89b			
0.426% <sub>av</sub> P + 0FTU kg <sup>-1</sup> (PC)		50.11		2.91	2.66	2.78		
P-value								
Phytase level	P<0.01			P<0.05				
Sex		P>0.05			P<0.01			
Phytase level x Sex	P>0.05			P>0.05				
Coefficient of variation (%)	5.42			4.29				
	Total phosphorus excretion (g bird <sup>-1</sup> )Total phosphorus retention coefficient (c							
Diets	Males	Females	Mean <sup>5</sup>	Males	Females	Mean <sup>6</sup>		
0.213% <sub>av</sub> P + 0FTU kg <sup>-1</sup>	0.87	0.79	0.83*	54.46	52.04	53.25*		
$0.213\% _{av}P + 750FTU kg^{-1}$	0.75	0.75	0.75*	63.82	58.41	61.12*		
$0.213\% _{av}P + 1,500FTU \text{ kg}^{-1}$	0.76	0.70	0.73*	60.67	58.97	59.82*		
$0.213\% {}_{av}P + 2,250FTU {}_{kg}^{-1}$	0.67	0.63	0.65*	63.73	63.48	63.61*		
Mean	0.91a	0.87b		58.11a	55.37b			
$0.426\%_{av}P + 0FTU \text{ kg}^{-1} (PC)$	1.52	1.49	1.50	47.84	43.95	45.90		
P-value								
Phytase level	P<0.01			P<0.01				
Sex	P<0.01			P<0.01				
Phytase level x Sex	P>0.05			P>0.05				
Coefficient of variation (%)	3.77			2.76				

Means with different letters, in the row, differ by the F test (P<0.05). PC: positive control diet. \*Differs from positive control diet by Dunnett's test (P<0.05). <sup>1</sup>Calcium intake =  $(-1.29556 \times 10^{-7})x^2 + 0.00031672x + 3.47654$  (R<sup>2</sup> = 1.0). <sup>2</sup>Calcium excretion = -0.00013711x + 2.16317 (R<sup>2</sup>=0.96). <sup>3</sup>Calcium retention coefficient = 0.00422x + 38.929 (R<sup>2</sup> = 0.97). <sup>4</sup>Total phosphorus intake =  $(-9.48148 \times 10^{-8})x^2 + 0.00019431x + 1.77815$  (R<sup>2</sup>=0.94). <sup>5</sup>Total phosphorus excretion = -0.00007276x + 0.82110 (R<sup>2</sup> = 0.96). <sup>6</sup>Total phosphorus retention coefficient = -0.00007276x + 53.9625 (R<sup>2</sup>=0.83).

lower Ca utilization (38.38%) compared with the broilers fed with the positive control diet (50.11%). According to TAMIM et al. (2004), the Ca of the diet can bind to phytate, forming calcium phytate, or to <sup>inorg</sup>P, forming calcium phosphates such as  $Ca_3(PO_4)_2$ . Thus, it is likely that the increase in the  $Ca_{av}$ P ratio from 2:1 (positive control diet) to 4:1 (negative control diet) worsened the Ca absorption due to a greater rate of complexation of Ca with the  $_{av}P$ , since the Ca: $_{phv}P$ ratio in the diets was kept the same (4:1). Moreover, limestone tends to increase the pH of the feed, especially during its passage through the intestine, favoring the formation of calcium phytate (SELLE et al., 2009). Thus, in the present study, an increase in the formation of calcium phytate might have occurred in the broilers fed diet containing 0.213% P, because this diet has 84.2% more limestone than the positive control diet. Therefore, the increase in the formation rate of these insoluble complexes in the digestive tract of the broilers fed the negative control diet, without phytase, resulted in greater excretion of Ca followed by worsening of its coefficient of utilization.

Although the decrease in the  $_{av}P$  content of the diet has impaired the Ca absorption, this parameter was improved by increasing the phytase level in the diet, indicating that the more phytase is utilized greater will be its catalytic action on the phytic acid, thus reducing the power of complexation of its products from catalysis (myo-inositol phosphates with five or fewer phosphate groups) and thus allowing more free Ca to be utilized by the bird (SELLE et al., 2009). With the use of 2,250FTU kg<sup>-1</sup> of diet, the retention coefficient of Ca was similar to that determined for broilers fed with the positive control diet.

There was no interaction (P>0.05) between the level of phytase and sex for the balance and retention of <sub>total</sub>P (Table 3). However, males presented greater (P<0.01) intake, excretion and retention coefficient of <sub>total</sub>P, corroborating to SCHOULTEN et al. (2002) who also verified that male broilers makes better use of P. Also following the trend verified to FI (Table 2), the <sub>total</sub>P intake was influenced of quadratic manner (P<0.05) by the phytase level in the diet, with the greatest <sub>total</sub>P intake estimated for the diet containing 1,025FTU kg<sup>-1</sup> (Table 3). Besides, as expected, the diets formulated with 0.213% <sub>av</sub>P promoted less <sub>total</sub>P intake than the positive control diet, according to Dunnett's test.

The decrease in the  $_{av}P$  content of the diet from 0.426% to 0.213% (without the phytase supplementation) reduced the excretion of  $_{total}P$  in 45.04%; however, this parameter improved (P<0.05) even more by the increase in the phytase level in the

diet, indicating that the greater supplementation of this enzyme promotes greater hydrolysis of phytate that results in the increase of the <sub>phy</sub>P utilization which, in turn, results in the lower excretion of <sub>total</sub>P. Thus, the use of 2,250FTU kg<sup>-1</sup> of diet decreases the <sub>total</sub>P excretion in 56.75% when compared with what was observed for birds from the positive control group.

The utilization of  $_{total}P$  was influenced (P<0.01) by the phytase level in the diet (Table 3), with the highest retention coefficient estimated for the diet containing 2,224FTU kg<sup>-1</sup> of diet. This improvement in the retention of  $_{total}P$  can be explained by the action of phytase on the phytate of the diet, as reported by several authors (HAN et al., 2009; SANTOS et al., 2011; GOMIDE et al., 2012), who verified gradual increase in the utilization of  $_{phy}P$  in the function of the level of phytase in the diet.

#### CONCLUSION

Males presented greater feed intake, and better weight gain, feed conversion and total phosphorus utilization. Regardless of sex, when 2,250FTU kg<sup>-1</sup> of diet formulated for broilers in the period from 15 to 28 days of age are used, it is possible to reduce the available phosphorus content from 0.426% to 0.213% without impairing performance, the tibia ash content and the retention coefficient of calcium, in addition to reducing the total phosphorus excretion in 56.75% and improving its utilization by birds in 38.58%.

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## BIOETHICS AND BIOSSECURITY COMMITTEE APROVAL

The present study was approved by Committee of Ethics in the Use of Animals (CEUA) of the Federal University of Lavras, under protocol number 004/11.

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