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RESEARCH ARTICLE

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Non-phytate phosphorus requirement for male broilers subjected to two calcium supply regimens from 8 to 21 days of age under thermoneutral environment conditions

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Abstract

The purpose of this study was to determine the non-phytate phosphorus (nPP) requirement for male broiler subjected to two calcium supply regimens from 8 to 21 d of age, based on performance and bone mineralization. Birds were distributed in a complete randomized 4 × 2 factorial design with four nPP concentrations: 0.25, 0.35, 0.45 and 0.55%, and two Ca supply regimens: Ca fixed at 9.0 g/kg of diet (CaF) or varying together with the nPP concentrations tested keeping a fixed Ca:nPP ratio (CaV), with 8 battery cages/treatment and 9 birds/battery cage. Feed intake (FI), body weight gain (BWG) and Ca:P ratio deposited in the dry defatted tibia (TibCa:TibP) were quadraticaly increased by the increasing nPP concentrations, regardless of the Ca supply regimen adopted ($p<0.01$). The nPP levels tested also influenced FCR and TibAsh, which presented a quadratic and linear response when CaF and CaV diets were used, respectively, and TibP and TibCa, which presented a quadratic response with both Ca supply regimen, CaF and CaV diets. Also, CaV diets provided a 2.86 and 5.02% higher FCR ($p<0.05$) and TibCa ($p<0.01$), respectively, when compared to CaF diets. Therefore, nPP nutritional requirement for male broilers reared at thermoneutral conditions from 8 to 21 d of age that provided better performance and bone mineralization were, respectively, 0.480 and 0.459% with CaF and 0.550% with CaV diets, indicating that, under thermoneutral conditions, CaV diets negatively affected growth performance of broilers, while positively affecting bone mineralization especially when low nPP levels are applied.

Additional keywords: mineralization; nutritional requirement; performance; thermal comfort; tibia.

Abbreviations used: aP (available phosphorus); BGTH (black globe temperature and humidity); BWG (body weight gain); CaF (fixed calcium); CaV (varied calcium according to non-phytate phosphorus level); FCR (feed conversion ratio); FI (Feed intake); nPP (non-phytate phosphorus; TibAsh (tibia ash content); TibCa (tibia calcium content); TibP (tibia phosphorus content).

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Introduction

Phosphorus (P) is an important mineral required in poultry diets for normal growth and development (Ankra-Badu *et al.*, 2004). It constitutes the costliest mineral nutrient in animal nutrition and is the third most expensive component in non-ruminant diets, where the first two are energy and protein (Boling *et al.*, 2000).

Countless studies have been focused on the P requirement for poultry, not only due to its economic importance, but also due to its environmental impact. In many countries the concern is growing about the excess of this mineral in the soil due to soil and groundwater contamination (Runho *et al.*, 2001). The phosphorous, when leached to the fresh waters, stimulates the rapid growth of algae and cyanobacteria which, due to its high respiration rates, leads to hypoxia, impairing aquatic life (Silva *et al.*, 2008).

Beyond its economic and environmental importance, P is an essential element in the animal body for energy metabolism, synthesis of nucleic acids, and structure of cells membranes (Saraiva *et al.*, 2012). Along with calcium, it plays a major role in the development and maintenance of the skeletal system of the animal, being the second most abundant mineral in the body. Approximately 80% of all the P present in the body is found in the skeletal system and, when deficient, can cause rickets, retarded growth, and other skeletal deformities (Sethi *et al.*, 2008). Additionally, it serves as a reserve to be mobilized to fulfill functions in almost all metabolic processes, playing a vital role in almost every series of biochemical reactions in the body (Cromwell, 1989).

Calcium (Ca) and P are important for the development and maintenance of the skeletal system in poultry and deficiencies in amounts or improper ratios of these two minerals usually lead to greater incidences of leg abnormalities (Xie *et al.*, 2009).

Commonly the requirements of Ca or P are determined independently at a constant level of the other mineral (Ca or P). In fact, the requirements of Ca and P are interdependent. According to Rama Rao *et al.* (2003, 2006) and Xie *et al.* (2009) there is a significant interaction between dietary Ca and P on poultry nutrition and improper ratio of these two minerals can also depress growth performance.

Therefore, this study was designed to evaluate the impact of two Ca supply regimens on non-phytate phosphorus (nPP) requirement of male broilers from 8 to 21 d of age under thermoneutral conditions.

Material and methods

The protocol used in this study was reviewed and approved by the Animal Care and Use Committee of the Universidade Federal de Viçosa (MG, Brazil). The experiment was conducted at the Animal Bioclimatology Laboratory located at the Department of Animal Science, Agrarian Sciences Center, Universidade Federal de Viçosa, MG, Brazil to evaluate performance and bone mineralization.

Animal and housing

The 1-d-old male Cobb 500 × Cobb 500 broilers (slow feathering), used in these studies, were obtained from a commercial hatchery (Rio Brando Alimentos S/A, Pará de Minas, MG, Brazil) and raised in floor pens from hatch to 7 d and were fed a pre-starter diet that met or exceed Rostagno *et al.* (2005) recommendations for all nutrients.

On d 8, 576 birds with an initial body weight of 134.92 ± 0.32 g were transferred into environmental controlled chambers and allotted to one of the 64 metal battery cages (85.0 cm W × 85.0 cm D × 37.2 cm H), with mesh floor and supplied with hanging galvanized iron gutter type feeders and drinkers (83.0 cm L × 9.4 cm W × 5.5 cm D).

For the experimental period, the climatic chambers were set to remain with a constant air temperature of 29°C from 8 to 15 d of age and 27ºC from 16 to 21 d of age and relative air humidity between 55-65%, featuring a thermoneutral environment condition, according to Cobb Vantress (2012).

The environmental conditions inside the climatic chambers, represented by temperature and relative air humidity, were monitored twice a day (7 am and 6 pm) through thermometers of dry bulb and wet bulb, and black globe (Incoterm Industry of Thermometers Ltd., Porto Alegre, RS, Brazil) kept in the center of the room. Subsequently, these data were converted to the black globe temperature and humidity (BGTH) index, as proposed by Buffington *et al.* (1981). The lighting program adopted throughout the entire experimental period was continuous (24 h of artificial light).

Treatments and experimental design

On day 8, birds were assigned to eight treatments in a completely randomized 4 × 2 factorial arrangement with four nPP concentrations (0.25; 0.35; 0.45 and 0.55 %) and two Ca supply regimens, either at a fixed concentration (9.0 g/kg) (Table 1), regardless of nPP concentration, or at varying concentrations, to maintain a fixed Ca:nPP ratio (Table 2). A total of 8 replicate battery cages per treatment with 9 birds per battery cage were used. Weight of the birds was similar so that both pen to pen and within pen chick weight variation were minimized. The statistical model was as follows:

$$Yij = \mu + \alpha i + ßj + \gamma ij + \varepsilon ij,$$

where i = 1, 2, 3, 4; j = 1, 2, Y = mean of the experimental unit factor I and factor j; μ = constant inherent to all experimental units; αi = effect of level i of factor α; ßj = effect of level j of factor ß; γij = effect of interaction between factors α and ß; eij = error of the experimental unit factor i and j.

During the experimental period, diets were corn-soybean meal based and formulated to meet or exceed the bird's nutritional requirement, according to Rostagno *et al.* (2005) recommendations, except for nPP and in certain cases Ca, when diets varying Ca and

Table 1. Ingredient and chemical composition of experimental diets fed to broilers from 8 to 21 d of age.

Ingredient (g/kg)	Non-phytate phosphorus levels, %							
	CaF[(1)]				CaV[(2)]			
	0.25	**0.35**	**0.45**	**0.55**	**0.25**	**0.35**	**0.45**	**0.55**
Ground corn (7.8% CP)	487.4	487.4	487.4	487.4	487.4	487.4	487.4	487.4
Soybean meal (45% CP)	414.7	414.7	414.7	414.7	414.7	414.7	414.7	414.7
Soybean oil	51.2	51.2	51.2	51.2	51.2	51.2	51.2	51.2
Dicalcium phosphate	7.4	12.8	18.2	23.6	7.4	12.8	18.2	23.6
Ground limestone	15.7	12.3	8.8	5.4	5.4	7.1	8.9	10.6
Silica sand	11.6	9.7	7.7	5.8	22.0	14.8	7.7	0.5
Salt	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
DL–Methionine (990 g /kg)	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3
L-Lysine HCl (785 g/kg)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
L-Threonine	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
Mineral supplement[(3)]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin supplement[(4)]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Coccidiostats[(5)]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Choline chloride (600 g/kg)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Growth promoter[(6)]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Antioxidant[(7)]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Total	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0
Formulated (analyzed)[(8)] nutrient content (g/kg)[(9)]								
ME, Mcal/kg	3,050	3,050	3,050	3,050	3,050	3,050	3,050	3,050
CP	230.0	230.0	230.0	230.0	230.0	230.0	230.0	230.0
Digestible Met + cist	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4
Digestible Lysine	11.9	11.9	11.9	11.9	11.9	11.9	11.9	11.9
Digestible Threonine	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8
Digestible Tryptophan	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6
Sodium	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2
Calcium	9.0 (8.9)	9.0 (8.3)	9.0 (8.5)	9.0 (9.4)	5.0 (4.6)	7.0 (6.1)	9.0 (8.9)	11.0 (10.4)
Total Phosphorus	4.7 (4.9)	5.7 (6.2)	6.7 (6.8)	7.7 (8.1)	4.7 (4.9)	5.7 (6.0)	6.7 (7.0)	7.7 (8.2)
Phytic Acid	2.2 (2.1)	2.2 (2.1)	2.2 (2.0)	2.2 (2.0)	2.2 (2.1)	2.2 (2.1)	2.2 (2.0)	2.2 (2.0)
Non-phytate Phosphorus[(10)]	2.5 (2.8)	3.5 (4.1)	4.5 (4.8)	5.5 (6.1)	2.5 (2.8)	3.5 (3.9)	4.5 (5.0)	5.5 (6.2)
Ca:nPP ratio	3.6:1 (3.2:1)	2.6:1 (2.0:1)	2.0:1 (1.8:1)	1.6:1 (1.5:1)	2.0:1 (1.6:1)	2.0:1 (1.6:1)	2.0:1 (1.8:1)	2.0:1 (1.7:1)

[(1)]Ca fixed at 9.0 g per kg of diet, independently of the nPP concentration. [(2)]Ca varying together with nPP to keep a fixed Ca:nPP ratio. [(3)]Provided per kg of the complete diet: 75 mg of Mn (as $MnSO_4$), 70 mg of Zn (as $ZnSO_4$), 50 mg of Fe (as $FeSO_4$), 8 mg of Cu (as Cu SO_4), and 0.75 mg of I (as $Ca(IO_3)_2$). [(4)]Provided per kg of the complete diet: retinyl acetate, 5600 IU; cholecalciferol, 1200 IU; DL-α-tocopheryl acetate, 10 IU; thiamin, 1.55 mg; riboflavin, 4.00 mg; pyridoxine, 2.08 mg; pantothenic acid, 10.40 mg; menadione sodium bisulphite, 1.20 mg; folic acid, 0,65 mg; niacin, 28,00 mg; cyanocobalamin, 8 µg; selenium, 300 µg; and antioxidant, 0,50 mg. [(5)]Coxistac® 12% Granular (Phibro Animal Health Corporation, Guarulhos, São Paulo, Brazil). Supplied per kg of diet: Salinomycin, 60 mg; Calcium carbonate, 0.40g. [(6)]SURMAX 100 (Elanco Saúde Animal, Greenfield, Indiana, EUA). Supplied per kg of diet: Avilamicin, 10 mg. [(7)]Butyl hydroxy toluene (EMFAL Especialidades Químicas, Betim, Minas Gerais, Brazil). Supplied per kg of diet: Butylated hydroxytoluene, 99 mg (as $C_{15}H_{24}O$). [(8)]Analyzed in triplicate. [(9)]According to Rostagno *et al.* (2005). [(10)]Determined nPP concentration based on analyzed P minus analyzed phytate P.

Table 2. Growth performance of male broilers fed diets containing different non-phytate phosphorus (nPP) concentrations, keeping or not the Ca:nPP ratio of diets, under thermoneutral conditions.

Variable	nPP concentration (%)				Ca supply regimen	Average	*p*-value			Regression[(4)]	SEM[(5)]
	0.25	0.35	0.45	0.55			nPP	Ca	nPP × Ca		
FI, g[(1)]	891	924	940	926	CaF	920	0.005	0.942	0.734	Q**	8.82
					CaV	920					
BWG, g[(2)]	598	653	678	671	CaF	660	< 0.001	0.082	0.509	Q**	10.71
					CaV	640					
FCR[(3)]	1.44	1.38	1.37	1.39	CaF	1.40[b]	< 0.001	0.004	0.031	Q*	0.01
	1.54	1.45	1.41	1.37	CaV	1.45[a]				L**	0.01

[(1)]FI: feed intake, as-fed basis. [(2)]BWG: body weight gain. [(3)]FCR: feed conversion rate, based on live bird days within each period. [(4)]*Q*: quadratic effect; *L*: linear effect. Means followed by different letters for each variable between the Ca supply regimens employed differ by the F test ($p < 0.05$). *$p < 0.05$; **$p < 0.01$. [(5)]SEM: standard error of the mean; n = 8.

nPP concentrations, in order to maintain a fixed Ca:nPP ratios (CaV), were used. The nPP content of feed ingredients was calculated as 33% of total P (Rostagno *et al.*, 2005).

The levels of dicalcium phosphate, limestone and inert material (silica sand) were adjusted to obtain the desired nPP and Ca levels, then experimental diets were analysed in triplicate for Ca by atomic absorption spectrophotometry (method 4.8.03; AOAC, 2000), and total P by the colorimetric method (method 3.4.11; AOAC, 2000). Phytate P (PP) content of the basal diet was analyzed according to the ferric precipitation method as described by Ellis *et al.* (1977). All experimental diets were isoenergetic, isoproteic and isoaminoacidic. Animals were fed *ad libitum* and had free access to water throughout the experimental period.

Procedures

Pen weights were recorded at the beginning (8 d of age) and at the end (21 d of age) of the experimental period. Dead birds were weighed and removed from the pens twice daily and dead weights were used to correct feed conversion ratio (FCR). At the end of the experimental period (21 d), all birds and feeders were weighed to determine feed intake (FI), body weight gain (BWG), and FCR for the entire experimental period (8-21 d). Also, at 21 d of age, two broiler chickens from each battery cage, closest to the cage average were selected, fasted for 12 h, and then slaughtered by cervical dislocation to remove the right tibia. The right tibia was removed, forming a "pool" of two samples per replicate, and soft tissue and cartilaginous caps were removed. Samples were then pre-dried at 65°C in a drying oven for 72 h to reduce the moisture content below 15% enabling mechanical processing and sample conservation (Detmann *et al.*, 2012).

Once dried, tibias were defatted with petroleum ether using Soxhlet apparatus for 4 h. Dry-defatted tibias were then placed in a 105°C drying oven for 24 h and ashed at 600°C for 8 h. Ashed tibias were analyzed in triplicate for Ca by atomic absorption spectrophotometry (method 4.8.03; AOAC, 2000) and for P by the colorimetric method (method 3.4.11; AOAC, 2000). The amount of phosphorus (TibP), calcium (TibCa) and ash (TibAsh) were expressed as g/kg of dry defatted tibias.

Statistical analyses

Data were analyzed using the GLM procedure of JMP PRO 10 (SAS Inst, 2012). The effects included in the model were: nPP level, Ca supply regimen and the interaction between those factors (nPP and Ca supply regimen). The results for all treatments were presented or not according to the interaction significance and the estimated nPP requirement for male broilers from 8 to 21 d of age were established by means of linear and quadratic regression model as the best fit obtained for each variable. Tukey's adjustment test was applied to the means of the Ca supply regimen factor in order to determine *p*-values. Significance was declared at $p<0.05$.

Results

During the experimental period, air temperature inside the facilities was kept at 29.08 ± 0.70°C and 27.28 ± 0.94°C and relative air humidity at 62.35 ± 4.07% and 61.32 ± 4.73 %, corresponding to a calculated BGTH index of 78.4 ± 0.66 and 76.3 ± 0.91, respectively, for the periods from 8 to 15 and 16 to 21 d of age.

The results of performance and bone mineralization of birds kept in thermoneutral environment conditions and fed diets with different levels of nPP, keeping or not a fixed Ca:nPP ratio, are shown in Tables 2 and 3, respectively.

Table 3. Bone mineralization of male broilers fed diets containing different non-phytate phosphorus (nPP) concentrations, keeping or not the Ca:nPP ratio of diets, under thermoneutral conditions.

Variable	nPP concentration (%)				Ca supply regimen	Average	*p*-value			Regression(5)	SEM(6)
	0.25	0.35	0.45	0.55			nPP	Ca	nPP × Ca		
TibP, g/kg(1)	61.24	74.44	80.95	78.00	CaF	73.66	< 0.001	< 0.001	< 0.001	Q**	0.93
	69.35	76.35	80.98	78.70	CaV	76.34				Q**	0.85
TibCa, g/kg(2)	129.75	153.50	151.00	151.00	CaF	146.31[b]	< 0.001	< 0.001	0.020	Q**	1.57
	145.50	160.38	152.88	155.88	CaV	153.66[a]				Q**	2.42
TibAsh, %(3)	37.98	45.04	46.57	45.44	CaF	43.76	< 0.001	0.710	< 0.001	Q**	0.43
	40.23	43.36	45.69	46.24	CaV	43.88				L**	0.44
TibCa: TibP(4)	2.11	2.08	1.88	1.96	CaF	2.00	< 0.001	0.410	0.790	Q*	0.02
					CaV	2.02					

(1)TibP: amount of P in the dry defated tibia. (2)TibCa: amount of Ca in the dry defated tibia. (3)TibAsh: amount of ash in the dry defated tibia. (4)Ca:P ratio in the dry defated tibia. (5)*Q*: quadratic effect; *L*: linear effect. Means followed by different letters for each variable between the Ca supply regimens employed differ by the F test ($p < 0.05$). *$p < 0.05$; **$p < 0.01$. (6)SEM: standard error of the mean; n=8.

No nPP × Ca supply regimen interaction ($p>0.05$) was found on FI, BWG and TibCa:TibP data; however, an interaction effect ($p<0.05$) was observed on FCR, TibP, TibCa and TibAsh (Table 4).

The nPP levels tested significantly affected ($p<0.01$) FI and BWG of broilers regardless of the Ca supply regimen adopted, which increased quadraticaly to the estimated level of 0.453 and 0.480% nPP for FI and BWG, respectively (Table 2).

When CaF diets were used, nPP levels significantly influenced ($p<0.01$) FCR, which increased quadratically to the estimated levels of 0.438% nPP. However, when

Table 4. Non-phytate phosphorus (nPP) requirement of male broilers from 8 to 21 d of age, keeping or not the Ca:nPP ratio, under thermoneutral conditions.

Variable	Equation	*p*-value	R^2	nPP(5)
Non-significant nPP × Ca interaction				
Performance parameters				
Feed intake (FI)	$Yij = 698.0 + 1061\ nPP – 1172\ nPP^2$	0.001	0.21	0.453
Body weight gain (BWG)	$Yij = 326.2 + 1473\ nPP – 1536\ nPP^2$	0.006	0.36	0.480
Bone mineralization				
TibCa:TibP ratio deposited(1)	$Yij = 2.666 – 2.816\ nPP + 2.713\ nPP^2$	0.045	0.36	0.519
Significant nPP × Ca interaction				
Ca supply regimen: CaF				
Performance parameters				
Feed conversion rate (FCR)	$Yij = 1.769 – 1.833\ nPP + 2.093\ nPP^2$	0.012	0.30	0.438
Bone characteristics				
TibP(2)	$Yij = - 8.617 + 379.8\ nPP – 403.7\ nPP^2$	< 0.001	0.89	0.470
TibCa(3)	$Yij = 34.23 + 536.2\ nPP – 593.8\ nPP^2$	< 0.001	0.73	0.452
TibAsh(4)	$Yij = 3.991 + 187.7\ nPP – 204.7\ nPP^2$	< 0.001	0.88	0.459
Ca supply regimen: CaV				
Performance parameters				
Feed conversion rate (FCR)	$Yij = 1.672 – 0.5723\ nPP$	< 0.001	0.48	-
Bone mineralization				
TibP	$Yij = 29.07 + 218.2\ nPP – 231.9\ nPP^2$	< 0.001	0.76	0.471
TibCa	$Yij = 100.4 + 261.1\ nPP – 296.9\ nPP^2$	0.054	0.20	0.440
TibAsh	$Yij = 35.74 + 20.35\ nPP$	< 0.001	0.74	-

(1)Ca: P ratio in the dry defated tibia. (2)TibP: amount of P in the dry defated tibia. (3)TibCa: amount of Ca in the dry defated tibia. (4)TibAsh: amount of ash in the dry defated tibia. (5)Requirement of dietetic nPP (%).

the CaV diet was provided, FCR increased linearly ($p<0.01$).

The nPP levels influenced FCR of birds, which improved ($p<0.05$) quadraticaly to the estimated level of 0.438% nPP, when CaF diets were used, and linearly ($p<0.01$) with CaV diets. Also, animals fed CaV diets had an increased ($p<0.05$) average FCR by 2.86%, when compared with the animals fed CaF diets.

The nPP levels tested influenced ($p<0.05$) TibP and TibCa content, which increased quadraticaly up to the estimated level of, respectively, 0.470% nPP for both Ca regimen tested and 0.452 and 0.440%, respectively, with CaF and CaV diets. As for TibAsh content, it was significant affected ($p<0.05$) by the nPP levels tested, which increased quadraticaly up to the estimated nPP level of 0.459% with CaF diets and linearly with the use of CaV diets. Also, the average TibP of broilers did not differ ($p>0.05$) between the groups of birds fed the CaF and CaV diets, whereas the same was not observed for the average TibCa, where a significant difference was observed ($p<0.01$) among animals fed the CaF and CaV diets, as the CaV diet provided an average TibCa 5.02% higher ($p<0.01$) when compared to the CaF diet.

Regardless of the Ca supply regimen adopted the nPP levels tested had a significant effect ($p<0.01$) on TibCa:TibP, which decreased ($p<0.05$) up to the estimated level of 0.519.

Discussion

According to the Roncbi (2004) and Cobb Vantress (2012), air temperatures ranging from 26 to 29°C with relative air humidity of 60% characterize an optimal environmental condition for broiler chickens from 8 to 21 d. In addition, according to Valério *et al.* (2003) and Lana *et al.* (2005), BGTH index values ranging from 74 to 83 are suitable for this category. Therefore, based on this information, we can infer that in this study all birds were kept in a thermoneutral environment condition.

Similar FI response has also been reported by Runho *et al.* (2001), Persia & Saylor (2006), Kill *et al.* (2008), Puppo *et al.* (2008) and Maia *et al.* (2009), who also found that available phosphorus (aP) levels influence voluntary FI of broilers fed diets with fixed Ca, independently of sex and strain of birds. On the contrary, when working with broilers, keeping a fixed 2:1 Ca to aP ratio, Yan *et al.* (2000), Bünzen *et al.* (2008) and Mello *et al.* (2012) failed to observe any aP effect on FI.

According to Rama Rao *et al.* (2006), the negative effect of low aP concentration on the voluntary FI of broilers is dependent on the Ca level used. Also, Qian *et al.* (1997) stated that the wider the Ca:aP ratio, the lowest is the FI of broilers. Several authors (Kill *et al.*, 2008; Maia *et al.*, 2009; Cardoso *et al.*, 2010) identified that the highest FI for broiler chickens were obtained with diets in which the Ca:aP ratio were, respectively, 2:1, 1,96:1 and 2,12:1 while working with fixed Ca concentrations and increasing aP levels.

The estimated nPP levels that provided the best BWG results of broilers did not differ ($p>0.05$) between groups of birds fed CaF and CaV diets. According to Tamin *et al.* (2004), birds nPP requirement is influenced by the Ca level of the diet, due to its possible interaction with phytic phosphorus, negatively influencing the digestibility of both.

The nPP level that provided best BWG result in this study (0.480%) was superior to those obtained by Brugalli *et al.* (1999) and Maia *et al.* (2009), 0.45% and 0.46%, respectively, for broilers from 8 to 21 d of age fed CaF diets, and Mello *et al.* (2012), corresponding to 0.19%, for broilers from 11 to 21 d of age fed CaV diets. Rostagno *et al.* (2011) proposed the level of 0.401% nPP as the requirement for broilers from 8 to 21 d for best performance. The low Ca level (8.41 g/kg), when compared to the present study (8.99 g/kg), proposed by those authors is one of the factors that would justify the lower nPP requirement value proposed.

Similarly to our results, Brugalli *et al.* (1999), Runho *et al.* (2001), and Maia *et al.* (2009) also found a positive influence of the aP levels on FCR of broilers from 8 to 21 d of age while working with fixed Ca concentration. In contrast, Kill *et al.* (2008), Puppo *et al.* (2008) and Cardoso *et al.* (2010), under the same circumstances, did not identify any significant FCR variation. Also, in the case of diets with fixed Ca:nPP ratio, a similar result was also obtained by Mello *et al.* (2012).

The inconsistency of the results showed can be attributed, among other things, to the variation in initial body weight of chicks used in the different studies. As reported by Angel (2011), there are several factors that can justify the different results in determining the nutritional nPP requirements, especially the growth rate of birds during the experimental period.

It was also evidenced in this study that in the two lowest nPP levels (0.25 and 0.35%) tested, the absolute FCR values were, respectively, 6.94 and 5.07% higher in birds fed CaV diets. This may indicate Ca deficiency when diets with CaV were used.

Also, in the lowest levels of nPP (0.25%) tested, the absolute values of TibP were 13.24% higher in birds fed diets with CaV what indicates the importance of a proper Ca:nPP ratio for an ideal bone mineralization. The obtained data on TibCa and TibP in the lowest nPP level tested (0.25%) showed that birds fed diets with CaV prioritized the deposition of both minerals in the tibia at the expense of growth.

As for TibCa, when observing the lowest (0.25%) and highest (0.55%) nPP levels tested, it was found that Ca deposition was compromised when using diets with CaF. Thus, it can be deduced that the deposition of calcium in the bone with deficiently nPP levels or above the requirement for optimal growth (0.45%) is dependent on a proper Ca:nPP ratio, which in this study was 1.6:1.

As for TibAsh content, a significant effect of the increasing nPP levels tested was also observed by Gomes *et al.* (1993), Lima (1995) and Runho *et al.* (2001). The results obtained for TibAsh when the lowest nPP concentration was tested (0.25%), suggests that once the Ca:nPP ratio is maintained at 1.6:1, birds tend to improve bone mineralization even if those minerals are deficient.

Although bone mineralization occurs most efficiently when Ca:nPP ratio is maintained, Cardoso *et al.* (2010) found that broiler chickens fed diets deficient in Ca and aP had a lower TibAsh percentage due to insufficient quantity of those minerals studied for a proper mineralization.

The average values of TibCa:TibP (2.00:1 × 2.02:1) did not differ ($p > 0.05$) when using diets with CaF or CaV. Since Ca:nPP ratio in the diet which has been used CaF ranged from 1.63 to 3.60, it can be inferred that tibia P deposition occurs simultaneously with Ca in a proportion close to 2:1, characterizing interdependence between these minerals. Studying the nPP levels in broiler chicks from 8 to 21 d old on thermoneutral environment conditions, Maia *et al.* (2009) also found proportionality in TibCa:TibP ratio (2:1), regardless of their ratio in the experimental diets.

Based on the results, it is clear that the levels of dietary Ca and nPP are not responsible for this regulation in the TibCa:TibP ratio deposited. In this sense, Crenshaw *et al.* (2011) identified the bone as the main site of synthesis of Fibroblast Growth Factor 23 (FGF 23), responsible for both renal P transport and by regulating vitamin D_3 activation, characterizing the bone tissue as an endogenous gland.

The best performance and bone mineralization of male broilers reared at thermoneutral conditions from 8 to 21 d of age were at nPP concentration of, respectively, 0.480 and 0.459% with the diet in which the level of Ca was kept fixed, corresponding to the estimated nPP intake of 4.46 and 4.26 g, and 0.550% independently of the Ca supply regimen adopted with the diet in which Ca varied proportionally to the nPP level, keeping a fixed Ca:nPP ratio of 1.6:1, corresponding to the estimated nPP intake of 5.14 g. In conclusion, the results from the present study indicate that, under thermoneutral conditions, CaV diets negatively affected growth performance of broilers, while positively affecting bone mineralization especially when low nPP levels are applied.

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