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RESEARCH ARTICLE

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Comparison of how different feed phosphates affect performance, bone mineralization and phosphorus retention in broilers

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Abstract

The objective of this work was to evaluate the comparative P bio-avalability of different sources of phosphate based on their effects on animal performance, bones mineralization and mineral retention in broilers. To achieve this goal, two experiments were conducted. In Experiment 1, twenty diets were prepared including five different phosphorus sources, either mono-calcium phosphate (MCP) or 4 different batches of di-calcium phosphate, to supplement non phytic P (NPP) levels at 3.0, 3.5, 4.0 and 4.5 g/kg in the diets. In Experiment 2, three treatments were used: the low MCP diet was deficient in NPP (3.1 g/kg for the starter phase and 2.8 g/kg for the grower phase); the high MCP diet and the high TCP (tri-calcium phosphate) diet included adequate levels of NPP (4.4-4.7 g/kg for the starter phase and 4.2-4.3 g/kg for the grower phase). Phytase was not added to experimental diets. Results of Exp. 1 indicated that an increase of NPP in the diet from 3.0 to 4.0 g/kg increased weight gain and feed intake between d 1 and d 21 (Trial 1). Alternatively, tibia weight and ash percentage at d 21 responded up to the level of 4.5 g/kg and showed significant difference with birds of the 4.0 g/kg NPP group. In Trial 2, chickens fed with the high MCP and TCP had improved growth performances and bone mineralization. No differences were observed on the P availability among different mineral P sources. A level of 4.5 g/kg, NPP is recommended when phytase is not included to maximize both performance and bone mineralization in broiler chickens up to d 21.

Additional keywords: mineralization; digestibility; calcium; phosphorus sources.

Abbreviation used: BW (body weight); CP (crude protein); DCP (di-calcium phosphate); DM (dry matter); FI (feed intake); G: F (gain feed ratio); MCP (mono-calcium phosphate); ME (metabolic energy); NPP (non phytic phosphorus); TCP (tri-calcium phosphate); WG (weight gain).

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Introduction

Phosphorus (P) is one of the most expensive nutrients in poultry diets as well a source of environmental concerns regarding excessive P in ground water. In order to reduce the total dietary P, the use of exogenous phytase provides a more efficient use of dietary P and a reduced inclusion of feed phosphates. However, mineral sources of P in diets for broiler chickens are still a need to meet the requirement of poultry and prevent P deficiencies. The phosphate levels in the diets will depend on their P content; and P bio-availability is pivotal to formulate diets at a higher precision and avoid excessive P excretion.

Different P sources are mainly used in the poultry diets, such as di-calcium phosphate (DCP) and monocalcium phosphate (MCP). The MCP has a higher concentration of P (22.6%) than DCP (FEDNA, 2010), which content depends on the degree of hydration. The DCP dihydrate contains typically 17.7% P and 24% Ca and DCP anhydrous contains 20.1% P and 27% Ca (FEDNA, 2010). Commercial products labeled DCP are industrial products resulting from the acidulation of rock phosphate, frequently with sulfuric acid, yielding phosphoric acid, which is neutralized with Ca carbonate after purification (Lima *et al.*, 1997). Alternatively, tri-calcium phosphate (TCP) provides a

Ca:P ratio close to the recommended dietary ratios for growing chickens (17.4% P, and 34% Ca). However, a limited amount of information exists regarding their P availability or retention.

Phosphorus is also a nutrient that linearly reduces growth performance and bone characteristics when levels below P requirements are applied (Hamdi *et al.*, 2015a). Based on this property, different methodologies exist to measure *in vivo* bio-availability of P or to compare P sources including bio-assays based on growth, bone weight and bone ash weight (Ravindran *et al.*, 1995; Lima *et al.*, 1997). These procedures are useful relative measurements for the comparison of different sources of Ca and P (Leske & Coon, 2002). Different sources have been used as a P reference in bio-assay studies, monosodium phosphates (Shastak *et al.*, 2012), MCP (De Groote & Huyghebaert, 1997) or DCP (Ravindran *et al.*, 1995; Fernandes *et al.*, 1999; Coon *et al.*, 2007). It is believed that P in MCP is more digestible than P in DCP (Grimbergen *et al.*, 1985; Eekhout & De Paepe, 1997). Therefore, FEDNA feed evaluation tables describes higher availability for MCP than for DCP. However, Rostagno (2017) described similar P availability and digestibility in broilers for MCP, DCP and TCP. Because most feed phosphates designated as MCP or DCP are mixtures of MCP and DCP, differences within a source may also exist (Petersen *et al.*, 2011).

Consequently, in this study we tested the hypothesis that different feed phosphates, varying in the amount of P and Ca, will affect the dietary P retention and overall productivity of broiler chickens. Two experiments were conducted to test this hypothesis and the objectives were to evaluate the effect of the P source provided at different levels in broiler diets on the availability of P and their effects on performance and bone mineralization for broilers. In the first experiment, P availability was compared between MCP and four different batches of DCP in broilers chickens up to d 21. In the second experiment, the inclusion of a source of TCP as a single source of Ca and P was compared to MCP in broiler diets with respect to their effects on performance, bone mineralization and P retention in broilers up to d 35.

Material and methods

All the animal experimentation procedures used in the two experiments were approved by the animal Ethics Committee of the Universitat Autònoma de Barcelona and were in compliance with the European Union guidelines for the care and use of animals in research (European Parliament, 2010).

Experiment 1

Five hundred broiler male chickens (Ross 308) were included in the study. Animals were randomly distributed into twenty experimental groups according to the experimental treatment and continuously controlled for 21 days. Birds were individually weighed and distributed in 100 battery brooder cages (5 chicks per cage) to get a similar initial average body weight for each cage. Chicks were wing-tagged to record individual body weight as well the group body weight during the experimental period. The brooder temperature was maintained at 35ºC during the 4 first days post-hatch, and then was progressively reduced to 25ºC from d 14 to d 21. The light cycle was 24 h/d from d 1 to d 2, 23 h/d from d 3 to d 10 and 18 h/d from d 11 to d 21.

An identical basic mixture of ingredients (wheat, 250 g/kg; corn, 287.2 g/kg; soybean meal, 248.8 g/kg; and soybean oil, 42.5 g/kg) was formulated and prepared to contain adequate levels of nutrients (ME (metabolizable energy), 2,960 kcal/kg; DM (dry matter), 882 g/kg; and CP (crude protein), 220 g/kg) to meet and exceed nutritional requirements in broilers (FEDNA, 2008), except for P. All the diets were presented in mash form. A total of 20 experimental treatments were prepared by including four levels of non-phytic phosphorus (3.0, 3.5, 4.0 and 4.5 g NPP (non phytic phosphorus)/kg) from five different feed phosphate sources in diets that all contained 9.0 g Ca/kg. Phytase was not added to experimental diets. The five main sources of phosphate were MCP-monohydrate (22.3% P, 16.8% Ca), DCP1-dihydrate (18.7% P, 23.9% Ca), DCP2-dihydrate (18.7% P, 26.1% Ca), DCP3-dihydrate (17.9% P, 25.6% Ca) and DCP4-dihydrate (17.9% P, 26.9% Ca). Table 1 shows the P concentration and the levels of incorporation of each source of P in experimental diets. The study will allow comparing P bio-availability among four different batches of DCP as well as their comparative differences to MCP. All P sources were obtained from independent distributors rather than manufacturers of the products. The broiler chickens had free access to drinking water served via a nipple system and to feeders containing the feed in a mash form. The feed intake (FI), weight gain (WG) and gain feed ratio (G:F) were calculated. On d 21, three birds were euthanized and the left tibia was collected for bone-ash determination.

Experiment 2

Ninety-six male broiler chickens (Ross 308) were used in a growth performance study during 35 days. Animals were randomly distributed into three experimental groups according to the experimental treatment. Chicks were wing-tagged to monitor individual BW and

Table 1. Composition of the experimental diet (Experiment 1)

	NPP (g/kg)			
	3.0	**3.5**	**4.0**	**4.5**
Ingredients[1]				
− P source inclusion (%)				
MCP[2]	0.70	0.92	1.14	1.36
DCP1[3]	0.86	1.13	1.39	1.66
DCP2	0.86	1.13	1.39	1.66
DCP3	0.90	1.18	1.45	1.73
DCP4	0.90	1.18	1.46	1.74
Total P (g/kg diet)	5.5	6.0	6.5	7.0
PP	2.5	2.5	2.5	2.5
Available P	2.8	3.3	3.7	4.2
− Limestone inclusion (%)				
MCP	1.57	1.47	1.36	1.26
DCP1	1.36	1.19	1.02	0.86
DCP2	1.31	1.13	0.94	0.76
DCP3	1.29	1.11	0.92	0.73
DCP4	1.26	1.07	0.87	0.67
Analyzed composition				
− Total P (g/kg diet)				
MCP	6.5	6.9	7.3	7.4
DCP1	6.4	6.7	7.2	7.9
DCP2	6.5	6.6	7.3	8.0
DCP3	5.7	6.7	7.4	7.9
DCP4	6.1	6.9	6.9	7.7
− Total Ca (g/kg diet)				
MCP	13.1	13.0	13.4	12.2
DCP1	15.1	13.1	12.5	14.5
DCP2	13.2	13.0	12.9	13.4
DCP3	11.7	11.9	13.3	12.1
DCP4	12.3	13.1	12.2	12.8

[1]Basal diet: Ingredients (wheat, 250 g/kg; corn, 287.2 g/kg; soybean meal, 248.8 g/kg; and soybean oil, 42.5 g/kg), nutrients (ME, 2,960 kcal/kg; DM, 882 g/kg; and CP, 220 g/kg). [2]MCP, mono-calcium phosphate. [3]DCP; di-calcium phosphate.

distributed in 24 battery brooder cages (4 chicks per cage) to get a similar initial average body weight for each cage. The brooder temperature was maintained at 35ºC during the 4 first days post-hatch, and was progressively reduced to 25-22ºC on d 14 to d 35 day. The light cycle was 24h/d from d 1 to d 2, 23h/d from d 3 to d 10 and 18h/d from d 11 to d 35.

All diets met or exceeded the nutrient requirements for broilers (FEDNA, 2008), with the exception of P (Table 2). All birds received two experimental diets, the first one in the starter phase (from d 1 to d 21) and the second diet during the grower phase (from d 21 to d 35). The low MCP treatment included limestone (38% Ca) and MCP (22.6% P and 17.8% Ca) as sources of Ca and P and was formulated to be adequate in Ca (9.0 g/kg and 8.5 g/kg for the starter and grower phase, respectively), but deficient in NPP (3.1 g/kg and 2.8 g/kg for the starter and grower phase, respectively). The high MCP treatment was formulated to be adequate in NPP (4.4 g/kg and 4.2 g/kg for the starter phase and grower phase). The high TCP treatment was formulated with the use of Lipocal® (Lipofoods; Barcelona, Spain) (a fat-encapsulated tricalcium phosphate, supplying 17.4% P and 34% Ca) as a single mineral source of Ca and P. The Ca content was 8.0 g/kg for the starter phase and 7.3 g/kg for grower phase. The NPP was 4.7 g/kg for the starter

Table 2. Day 1 to day 35 broiler starter and grower experimental diets (Experiment 2).

Ingredients[1] (g/kg)	Starter phase			Grower phase		
	Low MCP[2]	High MCP	High TCP[3]	Low MCP	High MCP	High TCP
Corn	191.1	184.7	200.5	254.6	248.4	263.8
Wheat	350.0	350.0	350.0	350.0	350.0	350.0
Soybean meal	212.6	213.8	210.7	151.0	152.0	14.96
Extruded soybean	150.0	150.0	150.0	180.0	180.0	180.0
Limestone	15.8	13.1	0	15.3	12.7	0
Lipocal	0	0	1.89	0	0	1.71
MCP[2]	7.7	13.4	0	6.4	12.1	0
Salt	4.3	4.2	4.3	4.0	4.0	4.0
Vit-mineral premix	3.0	3.0	3.0	3.0	3.0	3.0
Calculated composition (g/kg)						
DM	889.0	889.0	889.0	885.0	885.0	885.0
ME (kcal/kg)	3000	3000	3000	3140	3140	3140
CP	210.0	210.0	210.0	198.0	198.0	198.0
Ca	9.0	9.0	8.0	8.5	8.5	7.3
Total P	5.6	6.9	7.2	5.3	6.6	6.8
Available P	3.0	4.2	4.2	2.7	3.9	3.9
PP	2.5	2.5	2.5	2.5	2.5	2.5
NPP	3.1	4.4	4.7	2.8	4.2	4.3
Analyzed composition (g/kg)						
Ca	9.4	10.0	9.5	11.4	11.2	10.5
Total P	5.7	7.2	7.0	5.4	6.5	7.0

[1]Provided per kg of feed: vitamin A (retinil acetate), 10000 UI; vitamin D (vitamin D3) (colecalciferol), 2000 UI; vitamin D (25- hidroxicolecalciferol, 25 µg equivalent at 1000 UI), 1500 UI; vitamin E/acetate de tot-rac-3- tocopheril), 75 mg; vitamin K3 (MNB, menadione nicotinamide bisulfite), 5 mg; vitamin B1 (thiamine mononitrate), 2 mg; vitamin B2 (riboflavin), 5 mg; vitamin B6 (piridoxin chlorhidrate), 4 mg; vitamin B12 (cyanocobalamine), 0.015 mg; nicotinic acid (niacin), 25 mg; pantotenic acid (calcium D-pantotenate), 10 mg; biotin (D-(+)-biotin), 0.15 mg; folic acid, 1 mg; iron ($FeSO_4$), 46 mg; zinc (ZnO), 125 mg; manganese (MnO), 150 mg; iodine ($Ca(IO_3)_2$), 2 mg; selenium (Na_2SeO_3), 0.3 mg; cobalt ($CoCO_3$), 0.5 mg; copper ($CuSO_4$), 20 mg; DL-methionin, 500 mg; etoxiquin, 0.1332 mg; endo-1,3(4)-betaglucanase EC 3.2.1.6, 10 FBG; endo-1,4-beta-xylanase EC 3.2.1.8, 150 FXU; malic acid, 60 mg; fumaric acid, 75 mg; sepiolite, 400 mg; calcium carbonate, 4 g. [2]MCP, mono-calcium phosphate. [3] TCP, tri-calcium phosphate.

phase and 4.3 g/kg in growth phase. Phytase was not incorporated in the experimental diets.

Individual BW (body weight) as well as the group BW was monitored at the start (d 1) and d 7, d 14, d 21, d 28 and d 35 post-hatch. From these values the FI, WG and G:F ratio were determined. Feed intake was registered and excreta were collected from d 19 to d 21 and from d 33 to d 35 in order to determine the retention of Ca and P. At d 35, three chickens per cage were euthanized and the left tibia was collected for bone-ash determination.

Laboratory analyses

Diets and excreta samples were analyzed for DM, Ca and P. Dry matter was determined by placing samples in a drying oven at 105ºC for 24h. Diets, excreta samples were digested in a nitric perchloric and fluorhydric acid mixture, and P and Ca concentrations were subsequently determined by performing inductively coupled, plasma-optical emission spectroscopy (ICP-OES) using an Optima 4300DV Perkin-Elmer optical emission spectrometer.

The tibias were defatted for 48 h in ethyl alcohol followed by a 48 h extraction in ethyl ether; they were then dried for 12 h at 110ºC and then ashed overnight at 550ºC (Brenes *et al.*, 2003).

Statistical analyses

Data were analyzed by using the Generalized Linear Model procedure of SAS software (SAS, 2008, vers 9.2). In Exp. 1, the main factors used in the model were P level (4 levels), sources (5 sources) and their interaction. In Exp. 2, the dietary treatments and the source of P were taking into

account. The pen of the chicks served as the experimental unit. The results are presented as least square means. Probability was considered significant when $p \leq 0.05$.

Results

Experiment 1 (Comparison between MCP and DCP) - Growth performance and bone mineralization

Animal performance along the experimental period is presented in Table 3 as least square means. Productive performance was not significantly affected by the source of phosphorus or their interaction with NPP levels. However, higher BW at d 21, WG, FI and G:F were observed ($p<0.001$) from broilers in the 4.0 and 4.5 g NPP/kg group as compared to broilers in the 3.0 and 3.5 g NPP/kg group.

The effect of different sources and levels of P on bone mineralization (tibia weight and ash) and tibia weight/BW is reported in Table 4. Results are presented as least square means. No significant interaction effect was observed between P sources and NPP levels. On d 21, tibia weight and bone ash percentage were increased ($p<0.05$) by the dietary P supplementation, regardless of the source of P utilized. Broilers in the 4.5 g NPP/kg level had a greater tibia weight ($p<0.001$) than broilers in the 4.0 g NPP/kg group. Broiler chickens fed diets supplemented with 4.0 and 4.5 g NPP/kg showed ($p<0.001$) a higher tibia ash (%) and tibia weight/BW than those fed diets supplemented with 3.0 and 3.5 g NPP/kg.

Experiment 2 (Comparison between TCP and MCP) - Growth performance and bone mineralization

The feed intake and growth performance responses of broiler chickens to different levels and sources of Ca and P are presented in Table 5. Higher BW was observed ($p<0.05$) at d 21 and d 35 for broilers fed the high MCP and high TCP compared to birds fed the low MCP group. Higher WG and FI ($p<0.05$) from d 1 to 21 and from d 21 to d 35 was also observed for birds of the high MCP and high TCP. As a consequence, G:F was also greater for broilers chicken of the high MCP and high TCP compared to those birds of the low MCP.

The effect of different treatments on bone mineralization (ash content, %), tibia weight (g, and % as total BW) are presented in Table 6. Tibia weight (g and %/BW) had significantly higher values ($p<0.001$) for broilers on the high MCP and TCP diets than in birds fed the low MCP diet. Tibia ash (%, g/tibia) was significantly lower ($p<0.001$) in

Table 3. Effect of different P sources and levels on feed intake and growth performance of broilers between d 1 and d 21 of age (Experiment 1).

	BW[5] d 21 (g)	WG[6] d 1-21 (g/d)	FI[7] d 1-21 (g/d)	G:F[8] d 1-21
Source				
MCP[1]	728	32.6	46.4	0.70
DCP[2]1	733	32.9	46.8	0.70
DCP2	737	33.1	46.7	0.71
DCP3	744	33.5	47.5	0.70
DCP4	737	33.0	46.4	0.71
NPP (g/kg)				
3.0	670[b]	30.0[c]	43.8[c]	0.69[b]
3.5	714[b]	32.1[b]	46.1[b]	0.70[b]
4.0	771[a]	34.7[a]	48.1[a]	0.72[a]
4.5	788[a]	35.4[a]	49.1[a]	0.72[a]
SEM[3]	21.4	0.04	1.13	0.018
p value[4]				
Source	0.879	0.818	0.638	0.930
NPP	<0.0001	<0.0001	<0.0001	0.002
Source*NPP	0.461	0.617	0.803	0.630

[1]MCP, mono-calcium phosphate. [2]DCP, di-calcium phosphate. [3]SEM, standard error of the mean. [4] [a,b,c] Values in the same column with different letters are significantly different ($p<0.05$). [5]BW, body weight. [6]WG, average daily weight gain. [7]FI, average daily feed intake. [8]G: F, Gain: Feed ratio.

Table 4. Effect of different P sources and level on tibial weight and ash content in birds from d 1 to d 21 of age (Experiment 1).

	Tibial weight (g)	Tibial ash (%)	Tibial weight (%/BW)	Tibial ash (mg/tibia)
Source				
MCP	1.57	50.83	0.215	0.798
DCP1[1]	1.62	51.07	0.216	0.831
DCP2[2]	1.60	51.05	0.215	0.816
DCP3	1.59	51.53	0.214	0.821
DCP4	1.58	50.63	0.213	0.800
NPP (g/kg)				
3.0	1.41^{c}	48.29^{c}	0.207^{c}	0.682^{c}
3.5	1.52^{d}	50.80^{b}	0.211^{b}	0.770^{d}
4.0	1.66^{b}	52.11^{a}	0.217^{a}	0.868^{b}
4.5	1.76^{a}	52.88^{a}	0.223^{a}	0.932^{a}
SEM[3]	0.053	0.530	0.0038	0.028
p value[4]				
Source	0.619	0.179	0.875	0.409
NPP	<0.0001	<0.0001	<0.0001	<0.0001
Source*NPP	0.809	0.234	0.570	0.6113

[1]MCP, mono-calcium phosphate. [2]DCP, di-calcium phosphate. [3]SEM, standard error of the mean. [4 a,b] Values in the same column with different letters are significantly different (p<0.05).

Table 5. Effect of MCP[1] and TCP[2] diets on feed intake and growth performance in broilers between d 1 and d 35 (Experiment 2).

	BW[5], g		WG[6], g/d		FI[7], g/d		G:F[8]	
	d 21	d 35	d 1-21	d 21-35	d 1-21	d 21-35	d 1-21	d 21-35
Low MCP	714^{b}	1680^{b}	32.1^{b}	70.9^{b}	48.2^{b}	118.7^{b}	0.665^{b}	0.554^{b}
High MCP	824^{a}	2108^{a}	37.3^{a}	91.1^{a}	51.4^{ab}	146.9^{a}	0.725^{a}	0.620^{a}
High TCP	832^{a}	2087^{a}	37.7^{a}	89.6^{a}	52.8^{a}	145.8^{a}	0.713^{a}	0.615^{a}
SEM[3]	20.9	41.5	0.99	2.67	1.18	3.48	0.0105	0.0106
p value[4]	0.001	<0.0001	0.001	<0.0001	0.034	<0.0001	0.001	0.0003

[1]MCP, mono-calcium phosphate. [2]TCP, tri-calcium phosphate. [3] SEM, standard error of the mean. [4 a,b] Values in the same column with different letters are significantly different (p<0.05). [5]BW, body weight. [6]WG, average daily weight gain. [7]FI, average daily feed intake. [8]G:F, Gain: Feed ratio.

the low MCP group than in the high MCP and TCP groups. The amount of tibia ash (%, and g/tibia) for birds fed the high MCP was not different to those fed high TCP.

Apparent retention of calcium and phosphorus

The retention of P and Ca expressed on a percent basis and g/day is shown in Table 7. On days 19 to 21, P retention (%) was significantly (p<0.05) affected by the dietary treatment, with greater values observed for birds in the low MCP (59.46%) diet compared to the high TCP (44.57%) diet. Intermediate values were observed for chicks of the high MCP treatment (50.66%). Ca retention was not affected by the dietary treatment. Furthermore, during the finishing phase (d 33 to d 35), no significant differences were observed on the P retention (%) among treatments. However, broilers in the high MCP (1.28 g/day) and TCP (1.51 g/day) groups had (p<0.001) a higher daily retention of P than the low MCP (0.88 g/day) group.

Discussion

Present results showed that feed consumption, bird performance and bone mineralization were decreased when low NPP levels (3.0 to 3.5 g/kg) were included in

Table 6. Effect of MCP[1] and TCP[2] on tibial weight and ash content in 35-day-old broilers (Experiment 2).

	Tibial weight (g)	Tibial ash (%)	Tibial weight (%/BW)	Tibial ash (g/tibia)
Low MCP	3.41[b]	48.1[b]	0.203[b]	1.64[b]
High MCP	4.82[a]	52.9[a]	0.228[a]	2.55[a]
High TCP	4.57[a]	52.3[a]	0.219[a]	2.39[a]
SEM[3]	0.151	0.47	0.0057	0.079
p value[4]	<0.0001	<0.0001	0.013	<0.0001

[1]MCP, mono-calcium phosphate. [2]TCP, tri-calcium phosphate. [3]SEM, standard error of the mean. [4 a,b] Values in the same column with different letters are significantly different ($p<0.05$).

Table 7. Effect of MCP[1] and TCP[2] on P retention, Ca and P digestibility (%) and Ca and P retention (g/day) in 35-day-old broilers[1] (Experiment 2).

	P retention				Ca retention			
	d 19-21		d 33-35		d 19-21		d 33-35	
	(%)	(g/day)	(%)	(g/day)	(%)	(g/day)	(%)	(g/day)
Low MCP	59.46[a]	0.64	55.43	0.88[b]	35.24	0.63	37.05	1.24
High MCP	50.66[ab]	0.73	50.16	1.28[a]	28.19	0.56	38.65	1.73
High TCP	44.57[b]	0.60	53.07	1.51[a]	29.08	0.56	37.96	1.66
SEM[3]	3.05	0.05	1.88	0.09	4.52	0.08	4.06	0.20
p value[4]	0.005	0.252	0.166	0.0003	0.50	0.811	0.962	0.199

[1]MCP, mono-calcium phosphate. [2]TCP, tri-calcium phosphate. [3]SEM, standard error of the mean. [4 a,b] Values in the same column with different letters are significantly different ($p<0.05$).

the diet as compared to 4 and 4.5 g P/kg. Similar results were observed by Akter *et al.* (2016) on the growth performance, and by Viveros *et al.* (2002) on bone mineralization. Through its involvement in metabolic and structural processes, P is essential for animals to attain their optimum genetic potential in growth and feed efficiency as well as skeletal integrity and development (Applegate & Richert, 2008). Thus, based on the effect a deficiency has on the body, P is classified as nutrient type II. Individuals with a Type II deficiency are stunted in growth and have no visual signs or differences from 'normal' individuals.

In our experiment, no differences in animal performance were observed between broilers fed the 4.0 and 4.5 g NPP/kg diets. However, greater NPP levels, from 4.0 to 4.5 g/kg, increased tibia weight and tibia ash (mg/tibia). Hamdi *et al.* (2015a) concluded that a level of 3.8 g NPP /kg improved the growth of chicks and increased bone mineralization on d 14, but no further increases were observed with 4.5 g NPP/kg in diets including the addition of phytase. The lower required levels observed for this experiment as compared to the actual results may be due to the effect of phytase inclusion in the diet and also the duration of the experiment.

Yan *et al.* (2001) reported that NPP requirements for BW gain and feed conversion were considerably less than required for tibia ash for broilers of 3 to 6 weeks of age in the absence of phytase. They suggested NPP levels of 0.330%; 0.186% and 0.163% to optimize tibia ash, BW gain and G:F ratio, when no phytase was included in the diet. However, with 800 FTU (phytase activity is expressed as phytase units or FTUs; one FTU is the activity of phytase required to liberate 1 µmol of inorganic phosphorus per minute at pH 5.5 from an excess of 15 M sodium phytate at 37°C), phytase diets, the suggested NPP were lowered to 0.240%, 0.151% and 0.109% respectively to optimize tibia ash, BW gain and BW G: F ratio. Ravindran *et al.* (1995) also observed that bone-mineralization criterion is a good, sensitive indicator of the P status of the birds. Despite phosphorus is being largely contained in whole-body tissues, bone is the main storage organ for P, containing 85% of the body's total P.

Létourneau-Montminy *et al.* (2008) showed that P deficiency may be aggrieved by high dietary Ca concentrations. In the present study the levels of Ca were formulated to remain constant, which increased the Ca:P ratios as lower were the NPP values. Hamdi *et al.* (2015a) also described lower performance with the use of diets with high levels of Ca (9 g/kg) containing limiting values of NPP (2.5 g/kg) for broiler at 14 d of age as compared to lower levels of Ca (5-7 g/kg). Al

Masri (1995) showed that greater dietary Ca levels and its ratio to P may decrease P absorption. Calcium has the capacity to interact with organic P in the gut lumen limiting mineral and P absorption (Lonnerdal *et al.*, 1989) and can have interference with other micromineral absorption (Simpson & Wise, 1990). Calcium may decrease the amount of P in a suitable form for absorption through the formation of precipitates of orthophosphate in the gut and of insoluble complexes with the phytate molecule (Wise, 1983; Maenz *et al.*, 1999). Furthermore, soluble Ca may increase the intestinal pH and reduce mineral solubility and availability, as reported by Shafey & McDonald (1991). Ca-phytate complexes precipitate at pH values between 4 and 6, which coincide with the pH values of the intestine, where metal ion absorption, occurs (Tamim & Angel, 2003). Létourneau-Montminy *et al.* (2007) concluded in a literature review including 158 treatments from 14 references, that reduction in dietary P and Ca to (6.0 g Ca/kg and 3.1 g NPP/kg) allowed similar performance and bone mineralization to currently recommended levels by NRC (1994) (10.0 g Ca/kg and 4.5 g NPP/kg).

Considering the differences on P solubility observed in the literature among different phosphate sources, and the likely interactions among minerals on P precipitation in the poultry digesta, we hypothesized that phosphate sources would differ on P availability. Therefore, our study focused on the comparison of the broiler performance and bone mineralization from MCP, DCP or TCP sources, as well as the comparison among different batches of DCP. Diets were prepared on a total P basis in order to allow that likely differences on their P availability (between MCP and DCP in Exp.1, and between MCP and TCP in Exp. 2) could promote differences on performance or bone mineralization.

Results indicated that no differences were observed among P sources (MCP, DCP and TCP) with respect to their effects on the productive performance and bone mineralization of broiler chicken at ages of 21 and 35 days. Lima *et al.* (1997) also confirmed the lack of differences on broiler performance when they evaluated seven DCP sources from different origins. Similarly, Shastak *et al.* (2012) did not observe significant effects of the P source on feed intake and BW gain for broilers at 3 and 5 weeks of age, when comparing mono-sodium phosphate and DCP anhydrous in the diets. However, Gillis *et al.* (1962) reported higher P availability in purified grade MCP diets than in DCP diets. The authors suggested the differences on the hydration degree of P sources as explanation to differences on P availability. Specifically, P in the anhydrous DCP form is less available for poultry than the hydrated salt. It is noteworthy to describe that during the DCP manufacturing, conditions including temperature are responsible for the formation of the dihydrate or anhydrate product. Grimbergen *et al.* (1985) showed that the growth response was lower when anhydrous-DCP was included in the diet as compared to MCP or hydrated-DCP. In addition, Grimbergen *et al.* (1985), did not detect any difference in the growth response between MCP and hydrated DCP. According to Rucker *et al.* (1968), the dihydrate form of DCP dissolved more rapidly in an acid environment than the anhydrous form.

Moreover, Lima *et al.* (1997) also suggested that the particle size of phosphate may have a role also on P availability. Specifically, the larger particle size phosphate sources are retained longer in the gizzard than smaller particles, likely allowing a higher solubility and availability (Burnell *et al.*, 1990; Lima *et al.*, 1997). This criterion of particle size was not registered or evaluated in this study.

No significant difference was either observed between high MCP and TCP in animal performance and bone mineralization in Exp. 2. However, Wilcox *et al.* (1954) reported that the P in TCP was poorly utilized by the turkey poults. The use of TCP for young broilers decreased the P retention. However, no difference was observed between high MCP and TCP in the grower phase. Hamdi *et al.* (2015b) showed that the supplementation of broilers diet with TCP, improved FI and WG at d 14 but ileal digestibility of Ca and P was lower for diets including TCP and limestone as compared to diets including Ca chloride, MCP and sodium phosphate. This difference could be associated with solubility of the P sources in birds of different ages.

The results of this experiment also showed that the dietary P retention (% values) is increased when the levels of NPP are lower in the diet. However, the results were not affected by the P source (MCP, TCP). Result is consistent with Leske & Coon (2002) who demonstrated that the retention of P from different P sources depends on the amount of the inorganic P included. They found that P retention from MCP declined from 98% to 59% when NPP was increased from 1.6 to 4.5 g/kg. Wasserman & Taylor (1973) suggested the existence of a saturable component in P absorption, which can be responsible for the decrease in P absorption in the intestine.

Our results allow us to conclude that despite the highly different physical structure and chemical properties among P sources (MCP, DCP and TCP), no evidences were observed of differences in their vivo P availability in growing broilers. Increasing the dietary P have a direct impact on performance and bone mineralization, but higher NPP levels are required in the broiler diets to optimize bone mineralization than to optimize growth performance.

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