El estudio de la interacción entre calcio y fósforo y su efecto sobre el rendimiento de los pollitos broiler

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Resumen

El presente trabajo se realizó para determinar el nivel óptimo de calcio y fósforo, las características físico-químicas de diferentes fuentes de Ca (solubilidad) como factores que influyen en el crecimiento, la digestibilidad ileal y la retención de Ca y P, y el contenido de cenizas de la tibia en pollitos de 0 a 14 días. Diseñamos dos experimentos. El primer experimento fue *in vitro*, y se utilizó para comparar la solubilidad de diferentes fuentes de calcio (Ca carbonato, cloruro de Ca y Lipocal, una fuente encapsulada de grasa de fosfato tricálcico) y niveles de Ca a diferentes valores de pH. Los resultados mostraron que el cloruro de Ca tiene la solubilidad de Ca más elevada. El segundo experimento fue un ensayo *in vivo*, 300 pollitos Broiler machos *Ross* se distribuyeron en 60 jaulas, y se sometieron a 12 tratamientos (5 replicas / tratamiento) que diferían en los niveles de NPP (0.3, 0.35, 0.4, 0.45%) y las fuentes de Ca (Ca carbonato, cloruro de Ca y Lipocal) para estudiar sus efectos sobre los rendimientos, la retención de Ca y P, y los parámetros de la tibia. El ADG y BW en el día 14 fue mayor en las aves alimentadas con Lipocal y carbonato de Ca que los alimentadas con cloruro de Ca (P <0,01). El Peso de la tibia fue el más alto en los pollos alimentados con Lipocal y 4 g NPP / kg, y carbonato de Ca con 3,5 g NPP / kg. En cambio, el cloruro de Ca mostró la mayor digestibilidad ileal de Ca, qué a su vez también se aumentó progresivamente con mayores niveles de NPP en la ración. Se puede concluir que un nivel de 3,8 g de NPP / Kg con una sobredosis de fitasa es suficiente para garantizar un buen arranque y formación ósea de pollos broiler del día 0 al día 14. El uso de fuentes de solubilidad alta de Ca puede producir un descenso en el consumo de alimento con respuestas negativas sobre el rendimiento de las aves y la mineralización ósea.

Palabras clave: Calcio; Fosforo; in vitro; Solubilidad; Tibia

Abstract

The present work was conducted to determine the optimal level of calcium and phosphorus, the physicochemical characteristics of different sources of Ca (solubility) as factors influencing growth, ileal digestibility and retention of Ca and P and the ash content of the tibia in chicks from 0-14 days. We designed two experiments. The first experiment was *in vitro*, was used to compare the solubility capacity of different sources of calcium (Ca carbonate, Ca chloride and Lipocal, a source of fat encapsulated tricalcium phosphate) and Ca levels at different pH values. The results showed that the Ca chloride has the highest solubility of Ca. The second experiment was an *in vivo* test, 300 male Ross broiler chicks were distributed in 60 cages and subjected to 12 treatments (5 replicate / treatment) that differed with NPP level (0.3, 0.35, 0.4, 0.45%) and sources of Ca (Ca carbonate, Ca chloride and Lipocal) to study their effects on yields retention of Ca and P, and the parameters of the tibia. The ADG and BW on day 14 was higher in birds fed Lipocal and Ca carbonate that fed Ca chloride (P <0,01). The weight of the tibia was highest in chicks fed Lipocal and 4 g NPP / kg and Ca carbonate with 3.5 g NPP / kg. In contrast, Ca chloride showed the highest Ca ileal digestibility which also increased progressively with higher levels of NPP. It can be concluded that a level of 3.8 g NPP / kg with overdose of phytase is sufficient to guarantee good growth and bone formation for broiler chickens from day 0 to day

14. The use of sources of high-Ca solubility can produce first reductions in feed intake with negative responses on bird performance and bone mineralization.

Keywords: Calcium; Phosphorus; in vitro; solubility; Tibia

Introducción

Calcium requirements for starting chicken range from 0.9 to 1.1 total Ca% (FEDNA, 2008; NRC, 1994). As Ca is mainly stored in bones, Ca requirements for bone mineralization are even higher than those established to optimize body weight gain (Driver et al, 2005). The values are described on a total Ca basis, but ignores that a large proportion of calcium is not soluble in the digestive tract or precipitates to be excreted in the faeces. In fact, an excess of calcium is considered negative for performance as it establishes many negative interactions in the digestive tract. Soluble calcium may form soap precipitates with free saturated fatty acids, decreasing the energy digestibility of the diet (Pepper et al., 1955; Edwards et al., 1960). It may also precipitate mineral phosphates and phytic acid; reducing the activity of endogenous and exogenous phytase (Tamim et al., 2004). However, little efforts have been done to optimize the use and availability of different Ca sources, even considering the opportunity of using sources with different solubility. In the present study we hypothesized that the use of a highly soluble source of calcium will allow the use of low calcium diets for starting chickens. To evaluate this hypothesis we evaluated *in vitro* and *in vivo* three sources of calcium: a highly soluble source ($CaCl_2$), a medium soluble source (Ca carbonate) and a very low soluble source (encapsulated tricalcium phosphate).

Material y Métodos

Experiment 1 (*In vitro*)

The first trial was designed to measure the Ca and P release from Ca carbonate, Ca chloride, and tricalcium phosphate (Lipocal (Lipofoods, Barcelona, Spain)) at different pHs (pH 2.96, 3.53, 4.18, 4.77, 5.27, 6.01, 6.52) and in the presence or absence of phytic P as organic source of P. The seven phosphate-citrate buffer solutions were prepared and pH adjusted before the addition of the phytic acid.

The level of Ca to be added in each solution was calculated to simulate a similar level to that derived from the consumption of diets containing 0.9% Ca (90 mg Ca/ml). The tubes were vortexed and incubated for 60 min at 37ºC to observe the amount of mineral precipitate, and to obtain supernatant samples that were analyzed for soluble Ca and P content. Calcium and phosphorus were analyzed using Optical emission spectroscopy inductively coupled plasma Perkin-Elmer, model Optima 4300DV.

Experiment 2 (*In vivo*)

The experiment was conducted at the experimental farm in the Veterinary Faculty of the Universitat Autònoma de Barcelona.

1. Birds and Diets

Three hundred Day-old Ross broiler male chicks were obtained from a local hatchery where they had received vaccinations for Newcastle Disease and Infectious Bronchitis post hatch. The birds were weighed individually and distributed in 60 battery brooders cages (5 chicks per cage) in order to get a similar initial average body weight for each cage. Chicks were individually labeled in order to register individual body weight as well the group body weight along the experimental period. Each part contained one feeder that allows feeding 5 animals at the same time and free access to fresh water.

All diets were formulated to meet NRC (1994) recommendations, with the exception of Ca and aP. Diets were fed in mash form and contained 0.3% titanium dioxide as an indigestible marker. The phytase used allowed to reach an analyzed activity of 1150 FTU/kg (Quantum Blue, AB Vista Feed Ingredients).

2. Experimental Design

The experimental design was completely randomized with a 3 × 4 factorial arrangement of 3 sources of calcium (0.55% Ca from Calcium Carbonate, Calcium chloride and Lipocal) and 4 levels of NPP (0.3, 0.35, 0.4, and 0.45%). Each treatment was replicated 5 times.

3. Traits measured

a. Animal performance

Individual and group Body weight (BW) and feed intake were measured on Day 1, 7 and 14. From these values average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) from D 1 to 7 and from D 7 to 14 were calculated.

b. Tibia parameters

On day 14, the 3 birds with the closest body weight to the average cage BW were killed by cervical dislocation. Later, the right tibiotarsus was removed, boiled, and cleaned from adherent tissue. As described by Brenes et al. (2003), the bones were dried at 110°C for 12 h, defatted with ether for 48 h, dried again at 110°C for 12 h, and finally ashed at 550°C for 12 h in a muffle.

c. Ileal digesta

The ileal digesta were collected from three animals in each cage from the Meckel's diverticulum to about 2 cm to the ileo-cecal junction, and stored at -20ºC. Samples were digested in nitric perchloric and fluoridric acids and subsequently analyzed for P, Ca, Ti and Zn by flame atomic absorption spectroscopy. Ileal digestibility of calcium and Phosphorus (%) was calculated as follows:

Ileal Ca Digestibility= 1- ($[Ti]_D$/ $[Ca]_D$ / $[Ti]_M$/ $[Ca]_M$)

$[Ti]_{D:}$ the concentration of Ti in the diet; $[Ca]_D$ the Ca or P content in the diet; $[Ti]_{M:}$ the concentration of Ti in the ileal digesta; $[Ca]_M$ the Ca or P content in the ileum digesta.

4. Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the General Linear Model(GLM) of SAS software (SAS, 2008), version 9.2. The statistical model used for the analysis of dependent variables was: $Y_{ijk}= \mu+Ca_i + P_j+ Ca_i*P_j + e_{ijk}$, where Y_{ijk} is the individual observation, μ the experimental mean, Ca_i the Calcium Source effect, P_j the phosphorus effect, Ca_i*P_j the Ca source and P interaction, and e_{ijk} the random error.

Table 1 . Calculated and analyzed composition of experimental diet

[1]Limestone supplied 38% Ca.[2]Calcium chloride supplied 36% Ca.[3]Lipocal supplied 38% Ca. [4]Monocalcium phosphate supplied 16% Ca.

Results

1. Calcium and Phosphorus Solubility

Figure 1 shows the calcium solubility from the different sources. The values are described as depending on the pH, source of calcium (Ca carbonate, Ca chloride or Lipocal) and the addition of phytic acid. Clear differences among Ca sources were observed on calcium solubility. Ca carbonate increased solubility of Ca with values below pH=4, reaching practically twice soluble Ca content with a pH of 2.69. On the other hand, Ca chloride showed a higher Ca solubility in the range between 2.69 and 6.

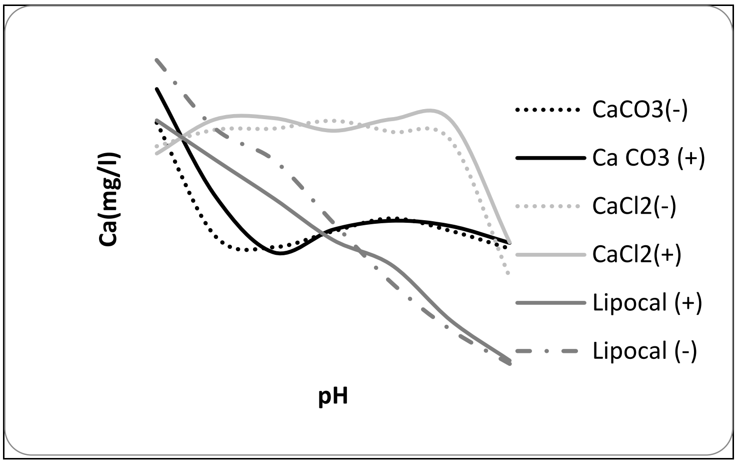

Figure1. Concentration of Ca (mg/L) in the supernatant of Ca chloride Ca carbonate and Lipocal solutions at different pH (from left to right, 2.69, 3.53, 4.18, 4.77, 5.27, 6.01, 6.52) and with the addition or not of phytic acid (+vs-).

Treatment Diet												
Ca source	$CaCO_3$				$CaCl_2$				Lipocal			
NPP (g/kg)	**3**	**3.5**	**4**	**4.5**	**3**	**3.5**	**4**	**4.5**	**3**	**3.5**	**4**	**4.5**
Ingredients (%)												
Corn	23.87											
Wheat	25											
Soybean meal 44%	27.15											
Extruded soybean	13.27											
Na phosphate	0	0	0	0	0.48	0.48	0.48	0.48	0	0	0	0
L-Lis	0.29											
DL-Met	0.33											
L-Thr	0.04											
$CaCl_2$[2]	0	0	0	0	0.7	0.7	0.7	0.7	0	0		0
Limestone[1]	0.66	0.56	0.46	0.36	0.34	0.23	0.13	0.03	0.17	0.17	0.17	0.17
Lipocal [3]	0	0	0	0	0	0	0	0	0.93	0.74	0.54	0.35
Soy oil	6											
Monocalcium phosphate[4]	0.72	0.94	1.16	1.38	0.32	0.55	0.77	0.99	0	0.37	0.74	1.11
Salt	0.57	0.57	0.57	0.57	0.01	0	0	0	0.57	0.57	0.57	0.57
Vit-mineral premix	0.3											
SUCROSE	1.5	1.38	1.26	1.14	1.6	1.49	1.37	1.25	1.78	1.6	1.43	1.25
Titanium dioxide	0.3											
Calculated Composition (%)												
DM %	88.75											
M.E(Kcal/Kg)	2960											
CP	22											
Lys	1.38											
Met	0.64											
Met+Cys	1.01											
Thr	0.86											
Try	0.27											
Ca	0.55											
Total P	0.54	0.59	0.64	0.69	0.54	0.59	0.64	0.69	0.54	0.59	0.64	0.69
Available P	0.29	0.34	0.39	0.44	0.29	0.34	0.39	0.44	0.29	0.34	0.39	0.44
PP	0.25											
NPP	0.3	0.35	0.4	0.45	0.3	0.35	0.4	0.45	0.3	0.35	0.4	0.45
Analyzed Composition (%)												
DM	89.85											
CP	22.33											
Ca	6	5.7	6.4	6.3	7.6	8.1	8.5	8.1	7.4	7.8	6.8	6.8
Total P	6.7	6.7	7.8	8.3	7.1	7.6	8.9	9.5	7.2	7.5	8.1	8.6

2. Influence of Ca source and P levels on early bird performance and bone mineralization Phosphorus and Calcium Ileal digestibility

The nutrients of the diets are presented in Table 1. It is worth noting that Ca was higher than formulated likely as a consequence of Ca carbonate added as filler of vitamin and mineral premixes.

a. Bird performance

Dietary P did not influence (P>0.05) feed intake or growth performance. Dietary source of Ca influenced ADFI (P<0.05), and growth performance (P<0.01) from Day 7 to 14 and from Day 1 to 14. The feed intake was higher on birds fed the Lipocal diet than birds fed the Ca chloride diet. Birds fed on Calcium Carbonate showed intermediate values. The increase in ADG and BW on day 14 was higher in birds fed Lipocal and Calcium Carbonate than birds fed Ca chloride.

Table 2. Influence of Ca source and P levels on feed intake and growth performance of broilers from Day 1 to 14

b. Bone mineralization

Tibia weight and tibia ash content (%, mg/tibia) are presented in Table 3. Tibia weight was influenced by a Ca source x P level interaction. Tibia weight was the highest in birds fed Lipocal at 4 g NPP/kg, and Ca carbonate at 3.5 g NPP/kg; and was the lowest for treatments including Ca chloride and 3.5 and 4 g NPP/kg in the diet. Bone weight and mineralization as affected by main factors are also presented. Changing the source of Ca in the diet promoted significant effects on the tibia weight and tibia ash content (% and mg/tibia). Tibia weight was significantly higher for birds fed Lipocal and Ca carbonate than Ca chloride. However, tibia ash percent was significantly higher for Lipocal than for Ca carbonate and Ca chloride. As a consequence, the ash content per tibia was the greatest for birds fed Lipocal, and the lowest for birds fed the Ca chloride diet. Ca carbonate showed intermediate results.

c. Phosphorus and Calcium Ileal digestibility

The calcium and phosphorus ileal digestibility were not influenced by the Ca source x P level interaction (P>0.05). Then, average values for main factors (source of Calcium and NPP levels) are shown in Table 4. Calcium ileal digestibility was influenced by both, source of Ca and NPP levels. Birds fed Ca chloride have the highest Ca ileal digestibility as compared to birds fed Ca carbonate and Lipocal. Calcium ileal digestibility was also progressively increased with higher levels of NPP, being significantly higher in birds fed 4.5 g NPP/Kg than 3g NPP/Kg. P ileal digestibility was influenced by the level of NPP, being the highest with the level 4.5g NPP/Kg and the lowest with 3 g NPP/ Kg .

Table 3. Influence of Ca and P levels on tibia weight and tibia ash of 14-d-old broilers

Treatment	Tibia weight(g)	Tibia ash (%)	Tibia ash (mg/tibia)
Calcium Source			
$CaCO_3$	0.80^{a}	50.57^{b}	407^{ab}
$CaCl_2$	0.75^{b}	50.75^{b}	383^{b}
Lipocal	0.81^{a}	51.29^{a}	415^{a}
S.E.M	0.014	0.148	7.58
NPP Level (g/Kg)			

Treatment		Day 7 BW (g)	Day 14 BW (g)	ADFI 1-14 d (g/d)	ADG 1-14 d (g/d)
Calcium Source					
$CaCO_3$		149.4	437.7^{a}	33.3^{ab}	28.1^{a}
$CaCl_2$		141.9	410.7^{b}	31.6^{b}	26.3^{b}
Lipocal		149.9	441.2^{a}	33.9^{a}	28.4^{a}
S.E.M		4.30	7.19	0.66	0.51
NPP Level (g/Kg)					
3		146.3	426.7	32.9	27.4
3.5		147.9	426.2	32.4	27.4
4		146.5	435.0	33.5	28.0
4.5		147.7	431.8	33.2	27.8
S.E.M		5.02	8.38	0.77	0.59
Calcium Source	NPP (g/Kg)				
$CaCO_3$	3	150.5	442.2	33.6	28.4
	3.5	144.3	436.4	31.9	28.2
	4	155	441.2	34.3	28.5
	4.5	147.7	431.1	33.4	27.8
$CaCl_2$	3	138.6	413.3	32.1	26.5
	3.5	149	410.1	32	26.3
	4	135.3	402.9	31	25.8
	4.5	144.3	416.7	31.7	26.8
Lipocal	3	149.6	424.5	33	27.3
	3.5	150.1	432.1	33.4	27.8
	4	149	460.7	35	29.9
	4.5	151	447.5	34.5	28.9
S.E.M		9.34	15.59	1.43	1.11
		Probability			
ANOVA					
Calcium Source		0.321	0.006	0.045	0.007

NPP Level	0.993	0.849	0.778	0.819
Calcium Source × NPP Level	0.874	0.686	0.855	0.692

3		0.78	50.69	50.69
3.5		0.79	50.88	50.88
4		0.79	50.80	50.80
4.5		0.80	51.10	51.10
S.E.M		0.017	0.172	9.11
Calcium Source	NPP(g/Kg)			
$CaCO_3$	3	0.78^{ab}	50.51	398
	3.5	0.83^{a}	50.35	418
	4	0.79^{ab}	50.65	405
	4.5	0.80^{ab}	50.77	407
$CaCl_2$	3	0.77^{ab}	50.61	393
	3.5	0.74^{b}	50.46	377
	4	0.70^{b}	50.68	359
	4.5	0.78^{ab}	51.24	404
Lipocal	3	0.77^{ab}	50.96	394
	3.5	0.78^{ab}	51.81	406
	4	0.89^{a}	51.08	450
	4.5	0.80^{ab}	51.27	409
S.E.M		0.031	0.320	16.95
			Probability	
ANOVA				
Calcium Source		0.011	0.002	0.011
NPP Level		0.883	0.382	0.800
Calcium Source× NPP Level		0.029	0.291	0.058

Table 4 . Influence of Ca and P levels on Ca ileal digestion and P ileal digestion of 14-d-old broilers

	Calcium Source			P-value		NPP (g/Kg)				P-value	
	Calcium Carbonate	Calcium chloride	Lipocal	*S.E.M*	S Ca	3	3.5	4	4.5	*S.E.M*	P
Ca ileal digestibility (%)	67.1^{b}	73.7^{a}	66.8^{b}	1.43	0.001	65.8^{b}	69.7^{ab}	68.8^{ab}	72.5^{a}	1.67	0.047
P ileal digestibility (%)	82.2	83.0	79.7	1.33	0.188	76.8^{b}	81.3^{ab}	82.3^{ab}	86.1^{a}	1.55	0.001

Discussion

1.Calcium and Phosphorus Solubility

Precipitation obtained in the case of Ca carbonate and Lipocal at low pH is consistent with Selle *et al*. (2000), who have suggested that most mineral complexes are soluble at low pH's (less than 3.5) with maximum insolubility occurring between 4 and 7. Champagne (1988) has reported that Ca-PP complexes precipitate at pH's between 4 and 6 which is the approximate pH of the intestine where the Ca ions should be absorbed. Previous *in vitro* research in corn-based diets would suggest limestone is approximately 80% soluble in the acidic medium of the gastrointestinal tract but decreased to 77% solubility in neutral conditions of the intestine, suggesting no further dissolution of Ca in the intestinal phase (Walk et al., 2012c).

Our results show that Ca chloride was more soluble than Ca carbonate and Lipocal. Taylor (1965, Manangi and Coon (2008)) has suggested that the primary factor affecting PP utilization is the Ca ion concentration in the small intestine where insoluble Ca-PP-complexes form. Thus, a precipitated PP-mineral complex would not be accessible for hydrolysis or absorption in the intestine. Recent studies suggest that a more digestible calcium source may be effective on performance and reduced risks of leg problems if a higher amount of phytase is included in the diets.

2.Influence of calcium source and its interaction with NPP levels on performance, bone mineralization and ileal digestibility

The calcium source promoted significant differences on feed intake. The introduction of tricalcium phosphate (Lipocal) in the diet promoted higher ADFI in comparison with the more soluble Ca source

(Ca chloride). This difference directly affected the growth of the animals, being birds fed on Ca carbonate and Lipocal the ones showing the highest body weight gain. Then, it could be suggested that sources of calcium with a lower solubility may allow better performances than the high soluble Ca chloride. This result agrees well with the result obtained by Walk et al., (2012c) who found that broiler chickens fed 0.90% Ca from limestone ate more and were heavier than birds fed 0.90% Ca from a high soluble calcium source (HSC). Due to the soluble nature of the HSC source, the authors suggested that feeding 0.90% Ca from HSC may have reduced broiler performance as a result of a wide Ca:P ratio and an increase in calcium phosphate or calcium phytate precipitation. The authors also described that N digestibility was reduced as Ca from the high soluble source increased, most likely due to the buffering capacity of high inclusion levels of the HSC, which may have reduced pepsin efficacy in the proventriculus and gizzard (pH optimum at 2.8, Bohak, 1969).

A similar response was observed by Coon and Manangi (2008) when they evaluated the effect of different Ca particles sizes (from 28 to 1306 µm) in broilers, The higher weight gains were obtained in chicks fed intermediate $CaCO_3$ particles sizes, between 137 and 388 µ, compared to the gains obtained with the smallest (28 µm) or largest particles sizes. Our study included limestone with an average particle size of 200µm. Both *in vivo* and *in vitro* studies (Coon and Manangi, 2008) indicated that limestone with a high solubility (>70.0%, ie. 28 microns) limited phytate hydrolysis to provide available P for growth and bone ash formation.

It has been suggested that the effects of soluble Ca limiting phytate hydrolysis could be counteracted by an overdose of phytase. Walk et al (2012) described that an overdose of phytase in feed to reach 2,000-2,500 U/kg was able to increase the performance of broilers fed on 0.9% Ca of a high soluble Ca source to values close to those presented by a limestone source without phytase. Similar performance were observed with lower levels of Ca, which suggest that reductions in dietary Ca may be obtained with high soluble sources of Ca while maintaining broiler performance and bone ash.

In the present study, however, all the diets were overdosed with phytase to reach an analyzed phytase activity of 1,150 U/kg. The results also showed that Ca digestibility was higher in Ca chloride than limestone and Lipocal, but no differences were observed in P ileal digestibility or not responses were observed to the NPP supplementation, which make questionable the explanation of a limited P digestibility with the high soluble source of Ca.

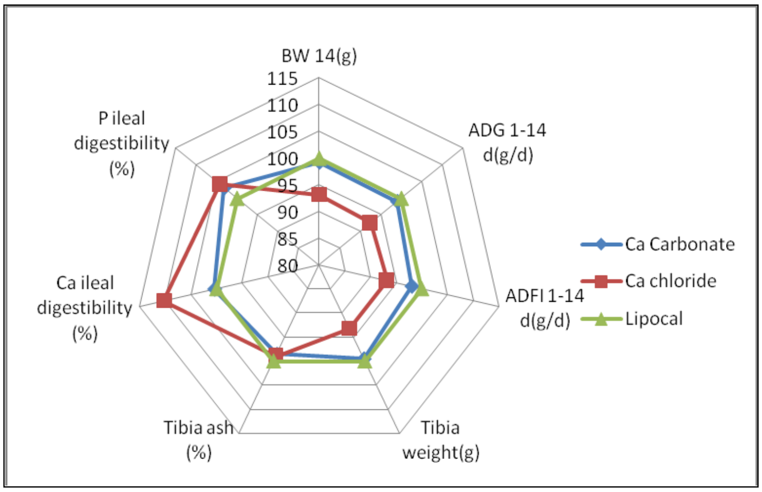

Figure 2 . Effect of calcium sources on the various parameters (the results obtained for the Lipocal represent 100%, the other results for calcium Carbonate and calcium chloride are compared relative to Lipocal).

On the other hand, the results showed a consistent effect of Ca chloride on feed intake and weight gain, which could suggest that broiler, could have reduced feed consumption after detecting a high soluble Ca and P source in the beak and the crop. However, further research should be performed in order to find a likely explanation to these results.

The tibia weight, percentage of tibia ash and mg ash/ tibia were influenced by changes in the calcium source, with Lipocal and Ca carbonate showing higher results than that obtained in the case of Ca chloride. The results are coherent with differences observed on feed intake and weight gain, but not with the results on Ca and P digestibility. Moreover, it is remarkable the significant interaction observed between the calcium source and level of NPP. Ca chloride at average levels of NPP (3.5-4 g NPP/kg) in the diets caused a lower weight of the tibia relative to Lipocal (0.70 vs 0.89 g) and Ca carbonate (0.83g), respectively. It is difficult to find an explanation to this effect on bone mineralization which it appears to be larger than the effects observed in feed intake and performance.

Thus, the results could suggest that bone mineralization is a more complex process which requires providing daily total digestible Ca and P amounts, but also appropriate rates of absorption for each mineral. It appears this rate could be better when derived from less soluble sources. However, this hypothesis deserves further studies.

On the other hand, limestone and Lipocal showed similar responses on performance, bone mineralization and Ca and P digestibility.

In the present experiment, we studied also the calcium and phosphorus ileum digestibility as affected by the Ca source and the NPP level. Increasing the level of NPP increased the apparent ileal P digestibility, which it agrees well with the results of Rodehutscord and Dieckmann, (2005). Al Masri (1995) saw that the values of dietary Ca and its ratio with P may affect phosphorus absorption; with lower values on P absorption as higher were the ratios between Ca and P in the diet.

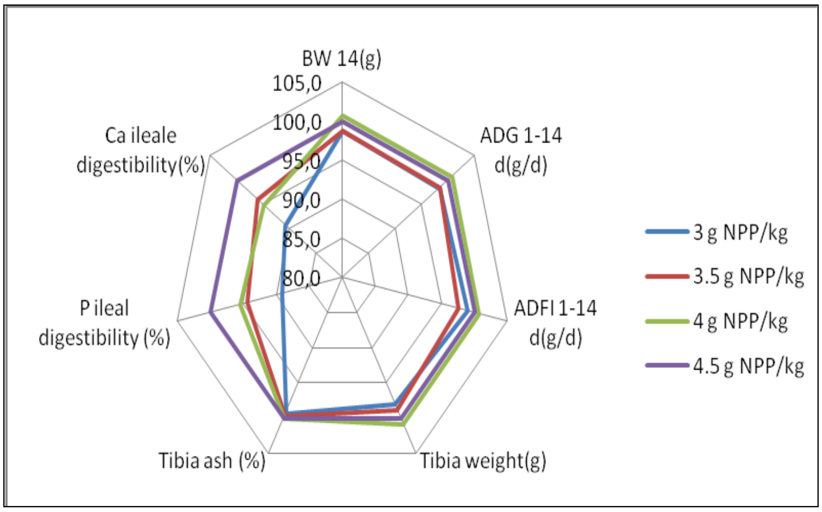

Figure 3 . Effect of NPP on the various parameters (The results obtained for the level 4,5 g NPP/kg represent 100%, the other results are compared relative to this level).

It is also relevant that higher levels of NPP also promoted an increase on calcium digestibility, which it could reflect the changes on the main mineral ingredients used in each diet. Angel (2013) has recently described that true digestibility of Ca for limestone (34.1%) is lower than for monocalcium phosphate (67.9%). The increase on ileum P digestibility with increasing levels of NPP on the Lipocal treatments, also suggest that calcium digestibility is also lower for tricalcium phosphate than monocalcium phosphate. Then a change on the levels of inclusion of mineral ingredients, as those promoted when phytase are included in the diet, are expected to promote also changes on calcium digestibility.

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