Ciência Rural, Santa Maria, v.52:10, e20210524, 2022 http://doi.org/10.1590/0103-8478cr20210524

ISSNe 1678-4596
ANIMAL PRODUCTION

Particle size of oyster shell meal and calcium: phosphorus ratios in broiler diets

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ABSTRACT: *The effects of Ca:P total ratio and particle size of oyster shell meal (OSM) were evaluated in broiler diets. In Experiment 1, 800 broilers (22–42 days old) were distributed in a 2×2 factorial design, with two Ca:P ratios (1.7 and 2.0:1) and two OSM particle sizes (coarse = 1,354 µm and fine = 428 µm), totaling four treatments with 10 repetitions with 20 broilers. Feed intake, weight gain, and feed conversion ratio were calculated. In Experiment 2, 1,280 broilers were distributed in a 2×2×2 factorial design (1.7 and 2.0:1 Ca:P ratios; coarse and fine OSM; male and female broilers), with eight treatments and 16 repetitions with 10 broilers. Apparent metabolizability of dry matter, Ca, P, and apparent metabolizable energy (AME), as well as bone resistance, bone weight, ash, Ca, and P content in the tibia were assessed. Growth performance was not affected (P > 0.05). Coarse OSM increased tibia Ca content in male broilers (P < 0.001), and higher Ca:P ratio improved bone ash and bone resistance in both sexes (P < 0.001), but reduced P content in male broilers (P < 0.05); male broilers displayed heavier bones with higher ash content than females (P < 0.05). Metabolizability of Ca was improved with coarse OSM (P < 0.05); whereas metabolizability of DM, P, and AME was not affected (P > 0.05). In conclusion, diets with a Ca:P total ratio of 2.0:1 containing coarser OSM improved bone mineral composition, particularly in male broilers, and coarse OSM improved the metabolizability of Ca in broilers regardless of the Ca:P total ratio or broiler sex.*
Key words: *bone mineralization, calcium, oyster shell, particle size, phosphorus.*

Efeito do tamanho de partícula da farinha de ostras e relações cálcio: fósforo em dietas para frangos de corte

RESUMO: *Dois experimentos foram conduzidos para avaliar os efeitos do tamanho de partícula da farinha de ostras (FO) e relação Ca:P total em dietas para frangos de corte. No primeiro experimento, 800 frangos (22 a 42 dias) foram distribuídos em um delineamento fatorial 2x2: 2 relações Ca:P (1,7 e 2,0:1) e dois tamanhos de partícula da FO (grossa = 1354 µm e fina = 428 µm), totalizando quatro tratamentos com 10 repetições de 20 aves. O consumo de ração, o ganho de peso e a conversão alimentar foram calculados. No segundo experimento, 1.280 frangos foram distribuídos em um fatorial 2x2x2 (relações Ca:P 1,7 e 2,0:1; FO grossa e fina; aves machos e fêmeas) com oito tratamentos e 16 repetições de 10 aves. Foram avaliados: metabolizabilidade aparente da matéria seca, Ca e P, energia metabolizável aparente (EMA), peso e resistência óssea, conteúdo de cinzas, Ca e P na tíbia. As variáveis de desempenho não foram afetadas (P > 0,05). O uso de FO grossa aumentou o conteúdo de Ca na tíbia de frangos machos (P < 0,001), e a relação Ca:P de 2,0:1 aumentou o conteúdo de cinzas e aprimorou resistência óssea em ambos os sexos (P < 0,001), porém reduziu P na tíbia dos machos (P < 0,05); frangos machos também tiveram ossos mais pesados e maior conteúdo de cinzas do que fêmeas (P < 0,05). A metabolizabilidade de Ca foi melhorada com FO grossa, enquanto a metabolizabilidade da matéria seca, P, e EMA não foram afetadas (P > 0,05). Conclui-se que as dietas com relação Ca:P de 2,0:1 e com FO grossa resultaram em melhor composição mineral óssea – particularmente em frangos machos – e a FO grossa melhorou a metabolizabilidade de Ca independentemente da relação Ca:P ou do gênero das aves.*
Palavras-chave: *cálcio, farinha de ostras, fósforo, granulometria, mineralização óssea.*

INTRODUCTION

Ca and P have remarkably high levels of inclusion in broiler diets because of their great importance and major roles on many biological functions in the organism. In the gastrointestinal tract, Ca and P interact with each other and with other substances, such as vitamin D and phytase, which together influence the intestinal absorption of nutrients (LIU et al., 2013). As stated by RAMA RAO et al. (2006), the excess of dietary Ca can lead to the formation of insoluble Ca-phytate complexes in the upper gut, reducing diet solubility and P absorption; whereas an insufficient supply of Ca may

Received 07.08.21 Approved 11.03.21 Returned by the author 02.04.22
CR-2021-0524.R1
Editors: Rudi Weiblen Wagner Araújo

impair bone mineralization and the animal's growth. Therefore, diet formulation with adequate levels of both minerals, considering not only the separate levels of Ca and P, but rather the dietary Ca:P ratio, has become a standard in poultry nutrition. Studies demonstrate that the Ca:P ratio for broilers can be lowered without harming growth performance (MELLO et al., 2012) or Ca and P digestibility (LIU et al., 2013; ANWAR et al., 2016), which can be both economically and environmentally beneficial.

Common sources of inorganic Ca in broiler diets included rocks, such as calcitic limestone, or marine sources such as oyster shell meal (OSM), and each source has different solubility rates and Ca contents that can affect Ca utilization (ANWAR et al., 2017). Oyster shells are considered a common inorganic Ca source for laying hens (MANANGI & COON, 2007; ANWAR et al., 2017); however, few studies have assessed the use of OSM and its effects on broiler chicken diets.

Another determining factor for Ca solubility and digestibility (*in vitro* and *in vivo*) is the particle size of the source. Studies have reported that the particle size of the supplied Ca source has a direct influence on growth performance variables and bone mineralization characteristics of broilers, mainly because of differences observed in *in vivo* Ca and P digestibility when using finer or coarser sources (MANANGI & COON, 2007; LI et al., 2012; ANWAR et al., 2016; ANWAR et al., 2017).

Based on these considerations, this study evaluated the effect of different dietary Ca and P ratios and OSM particle sizes on growth performance, bone mineralization, and nutrient metabolizability of broiler chickens.

MATERIALS AND METHODS

Birds, facilities, and diets

A total of 2,080 Cobb 500 broilers (1 day-old) were housed in 2.06 m^2 experimental pens (11.6 broilers/m^2) with wood shavings as litter, equipped with nipple drinkers and tube feeders. Incandescent lighting was continuously provided during the first 24 h and gradually reduced after the first day according to the breeder's management guidelines (COBB-VANTRESS, 2018). The temperature was set to 32 °C on day 1 and was gradually reduced to 25 °C until day 21. All pens were checked daily for the removal of dead birds.

Until day 21, all birds received the same basal diet (2,950 kcal/kg AME, 21.05% crude protein; 0.82% Ca, 0.39% available P, 0.72% total P) based on corn-soybean meal offered in mashed form. Food and water were provided *ad libitum*. On day 22, the birds were divided and prepared for the performance and metabolizability assays. Experimental diets (Table 1) were based on corn and soybean meal supplemented with 500 units/kg of exogenous phytase, varying only according to the total Ca:P ratio of each treatment. The Ca source used was OSM at two different particle sizes, which were coarse and fine: 1,354 and 428 µm. The standard particle size of OSM was 1,354 µm, which was ground through a 2-mm screen hammer mill to achieve the particle size of 428 µm. The average *in vitro* solubility of the coarse and fine OSM was 26.86 and 29.02%; respectively, according to the methodology of CHENG & COON (1990).

Growth performance assay

In the first experiment, 800 broilers were reared from 22 to 42 days old in 2.06 m^2 experimental pens (12 broilers/m^2) with wood shavings as litter. The average individual weight of broilers at the beginning of the experiment was 800 g. The birds were distributed in a 2 × 2 completely randomized factorial design, and the dietary treatments consisted of diets formulated with two OSM particle sizes (coarse = 1,354 µm and fine = 428 µm) and two dietary Ca:P total ratios (1.7 and 2.0:1), totaling four treatments with 10 repetitions of 20 birds each. Each pen contained 10 male and 10 female broilers, and a pen was considered an experimental unit. The experimental diets and water were provided *ad libitum* throughout the experimental period with tubular feeders and nipple drinkers.

At days 22 and 42, birds were weighed by pen to determine the mean body weight for body weight gain (BWG) calculations. Feed intake (FI) was calculated by weighing the feed addition and leftovers on days 22 and 42. The feed conversion ratio (FCR) was calculated as the ratio of FI to BWG. Culled and dead birds were weighed, and the performance variables were adjusted by their weight.

Metabolizability and bone characteristics assay

In the second experiment, a total of 1,280 Cobb 500 broilers (640 males and 640 females) were allocated to metabolic battery cages and reared from 22 to 28 days of age. Each battery consisted of four floors with two cages per floor. Each cage was 0.98 m × 0.90 m × 0.50 m (L × W × H) and was equipped with gutter feeders and nipple drinkers. Metal trays lined with plastic canvas were placed under the cages for excreta collection.

The birds were distributed in a 2 × 2 × 2 completely randomized factorial design with two

Table 1 - Ingredients and calculated composition of experimental diets.

Ingredient (%)	T1	T2	T3	T4
Corn	60.90	60.90	60.90	60.90
Soybean meal	31.35	31.35	31.35	31.35
Soybean oil	3.196	3.196	3.196	3.196
Dicalcium phosphate	0.458	0.458	0.458	0.458
Coarse oyster shell meal	1.034	0.000	1.458	0.000
Fine oyster shell meal	0.000	1.034	0.000	1.458
Sodium chloride	0.450	0.450	0.450	0.450
Celite[1]	1.000	1.000	1.000	1.000
DL-Methionine	0.234	0.234	0.234	0.234
Caulin	0.835	0.835	0.411	0.411
L-Lysine	0.224	0.224	0.224	0.224
Vitamin premix[2]	0.100	0.100	0.100	0.100
L-Threonine	0.079	0.079	0.079	0.079
Choline chloride	0.066	0.066	0.066	0.066
Mineral premix[3]	0.050	0.050	0.050	0.050
BHT	0.010	0.010	0.010	0.010
Phytase[4]	0.01	0.01	0.01	0.01
--Calculated composition---				
AME (kcal/kg)	3,050	3,050	3,050	3,050
Crude protein (%)	18.76	18.76	18.76	18.76
Calcium (%)	0.75	0.75	0.91	0.91
Available phosphorus (%)	0.32	0.32	0.32	0.32
Total phosphorus (%)	0.44	0.44	0.44	0.44
Ca:total P ratio	1.70	1.70	2.06	2.06
Ca:available P ratio	2.34	2.34	2.84	2.84
Sodium (%)	0.18	0.18	0.18	0.18
Digestible lysine (%)	1.04	1.04	1.04	1.04
Digestible methionine+cysteine (%)	0.76	0.76	0.76	0.76
Digestible methionine (%)	0.47	0.47	0.47	0.47
Digestible threonine (%)	0.67	0.67	0.67	0.67
Digestible tryptophan (%)	0.19	0.19	0.19	0.19
Digestible valine (%)	0.79	0.79	0.79	0.79
Mongin (meq/kg)	183	183	183	183

[1]Insoluble marker (Celite 400; Celite Corp., Lompoc, CA, US).
[2]Provided per kg of product: Vitamin A: 9,000,000 IU, Vitamin D3: 2,500,000 IU, Vitamin E: 20,000 IU, Vitamin K3: 2,500 mg, Vitamin B1: 1,500 mg, Vitamin, B2: 6,000 mg, Vitamin, B6: 3,000 mg, Vitamin B12: 12,000 mcg, Pantothenic acid: 12 g, Niacin: 25 g, Folic acid: 800 mg, Biotin: 60 mg, Selenium: 250 mg.
[3]Provided per kg of product: Copper: 20 g, Iron: 100 g, Manganese: 160 g, Cobalt: 2,000 mg, Iodine: 2,000 mg, Zinc: 100 g.
[4]Quantum Blue (AB Vista, São Paulo, Brasil).

OSM particle sizes (coarse = 1,354 µm and fine = 428 µm); two total Ca:P ratios (1.7 and 2.0:1); and two sexes (male and female), totaling eight treatments with 16 repetitions (8 with only male broilers and 8 with only female) of 10 birds each. The experimental diets and water were provided *ad libitum*. The experimental diets were the same as those used in the first experiment.

For the metabolizability analysis, a period of 22 to 24 days was considered an adaptation period to the diets and facilities. From 25 to 28 days, excreta samples were collected using the total collection method. Excreta were collected twice per day with the aid of plastic spatulas, immediately placed in duly identified plastic bags, and frozen at -18 °C. The samples were subsequently thawed to room temperature and dried in a force-ventilation oven at 55 °C for 72 h. Feed and excreta samples were then ground to a 0.5 mm particle size. Dry matter (DM) was determined by oven drying at 105 °C for 12 h, and Ca (method 927.02) and P (method 965.17) contents were analyzed according to guidelines of the Association of Official Analytical Chemists (AOAC, 1999). The gross energy (GE) of the samples was determined using a calorimetric bomb (Ika 134 Werke C2000 Control Oxygen Bomb Calorimeter, Ika-Werke GmbH &Co, Staufen, Germany).

Based on the results, the coefficients of apparent metabolizability (CAM) of DM, Ca, and P were calculated according to Sakomura and Rostagno (2007): CAM (%) = [(Nutrient in the diet (g) – Nutrient in the excreta (g))/Nutrient in the diet (g)] × 100. The apparent metabolizable energy (AME) was calculated as the difference between GE in the diet and GE in the excreta.

On day 28, one male and one female from each experimental unit were sacrificed by cervical dislocation, and both tibia bones were manually removed. Bones were cleaned with ether to remove any remnants of fat and muscle, and oven-dried at 105 °C for 12 h. Bones from the right leg were used for bone resistance (BR) and bone weight measurements. The BR test was conducted using a TA XT Plus Texture Analyzer (Texture Technologies Corp., Hamilton, USA) and the Exponent Stable Micro Systems software (Stable Micro Systems Ltd., Godalming, UK). The left tibia bones were ashed in a muffle furnace at 600 ºC for 3 h, and ash (method 942.05), Ca (method 927.02), and P (method 965.17) contents were analyzed according to AOAC (1999).

Statistical analysis

Residue normality of all collected data was determined using the Shapiro-Wilk test. After a normal distribution was detected, data from the first and second experiments were subjected to two-way and three-way analyses of variance. When significant interactions between factors were detected, their breakdown means were compared by Tukey’s test at a 5% probability level.

RESULTS

Growth performance

There was no significant interaction between the factors for the performance variables analyzed in the first experiment (P > 0.05; Table 2). The performance variables were also not affected by the total Ca: P ratio or OSM particle size (P > 0.05).

Bone characteristics

No three-way interactions were observed for the bone characteristic variables analyzed in the second experiment (P > 0.05; Table 3). Two-way interactions were detected between Ca:P total ratio and sex for P content in the bone (P < 0.05) and between OSM particle size and sex for Ca content (P < 0.001). The statistical breakdown of these interactions (Table 4) showed that male broilers fed diets with a 2.0:1 Ca:P total ratio had lower P content in the tibia compared to other treatments, whereas male broilers fed diets with coarse OSM had greater tibial Ca content.

The ash content of the tibia bones was affected by Ca:P total ratio (P < 0.001) and sex (P < 0.001). The ash content of broilers fed diets with 2.0:1 total Ca:P was greater than those fed with 1.7 total Ca:P, and male broilers had greater ash content in the tibia than females (P < 0.001). Body weight was also affected by sex, as male broilers had heavier bones (P < 0.001). A higher Ca:P ratio of 2.0:1 also resulted in a higher BR (P < 0.05).

Metabolizability

No three-way or two-way interactions were detected for the CAM variables (P > 0.05; Table 5). When evaluating the main effects separately, only the CAM of Ca was affected by OSM particle size (P < 0.001), as broilers fed the diets containing coarser OSM (1,354 µm) had greater CAM of Ca. The CAM of DM, P, and AME were not affected by the proposed treatments (P > 0.05).

DISCUSSION

Performance

There was no significant interaction between the factors for the performance variables

Table 2 - Feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) of broilers fed with diets containing oyster shell meal of two particle sizes and formulated with two total Ca:P ratios at 22 to 42 days of age.

Treatment	FI (g)	BWG (g)	FCR (g/g)
---Effect of Ca:P total ratio---			
1.7	3,275	1,999	1.639
2.0	3,253	1,999	1.628
---Effect of oyster shell particle size---			
1,354 µm	3,254	1,987	1.639
428 µm	3,244	1,978	1.640
---Effect of interaction---			
1.7 × 1,354 µm	3,277	1,993	1.645
1.7 × 428 µm	3,273	2,005	1.633
2.0 × 1,354 µm	3,277	2,015	1.627
2.0 × 428 µm	3,229	1,982	1.630
SEM[1]			
---P-values---			
Ca:P total ratio	0.435	0.993	0.432
Particle size	0.359	0.645	0.740
Interaction	0.452	0.326	0.553

[1]Standard error of the mean.

analyzed in the first experiment (P > 0.05; Table 2). The performance variables were also unaffected by Ca:P total ratio and the OSM particle size (P > 0.05). Although, performance was not affected by Ca:P total ratio in this study, LI et al. (2012) reported lower FI and BWG in broilers fed a high Ca:P total ratio (4.0:1), using limestone as the Ca source, compared to diets with a 2.0:1 ratio. The authors attributed this decrease in performance to a negative effect on P metabolism caused by high levels of dietary Ca. DELEZIE et al. (2015) suggested that poultry birds can regulate their FI based on dietary Ca levels to maintain appropriate Ca:P intake; thus, FI is reduced when diets have increased Ca.

According to SCHOULTEN et al. (2003), raising the levels of Ca and, consequently, the Ca:P ratio in broiler starter diets based on corn and soybean meal will negatively affect BWG. The superior BWG was obtained by those authors with diets containing 0.46% Ca. AMERAH et al. (2014) evaluated four different Ca:P ratios (1.4:1, 2.1:1, 2.8:1, and 3.5:1) and observed that increasing the Ca:P ratio resulted in a quadratic reduction in BWG and FI, as well as a quadratic increase in FCR. High concentrations of Ca in the gastrointestinal tract will promote an interaction with phytate, leading to the formation of an insoluble phytate-Ca complex that reduces the digestibility and absorption of Ca, P, and other nutrients, and consequently performance. This can be mitigated by phytase supplementation and reduction of dietary Ca (AKTER et al., 2016; BEESON et al., 2017). However, in the current study, the utilized Ca levels were likely insufficient to induce adverse effects on growth performance variables.

The OSM particle size had no influence on performance variables, in agreement with BRADBURY et al. (2016), who showed that the particle size of the Ca source (limestone or highly soluble Ca with particles > 0.5 mm or < 0.5 mm) had no effect on broiler performance, but it was influenced by the Ca concentration and solubility of the material.. In contrast, MANANGI & COON (2007) tested the use of limestone with eight different particle sizes for broilers and observed greater BWG in broilers fed small-to-medium particle sizes (between 137 and 388 µm) compared to the smallest (28 µm) or largest particle (1,306 µm) sizes. Those authors stated that limestone with smaller particles

Table 3 - Ash, Ca, P, bone weight (BW), and bone resistance (BR) of tibia of 28 day old male and female broilers fed diets containing oyster shell meal of two particle sizes and formulated with two Ca P total ratios.

Treatment	Ash (%)	Ca (%)	P (%)	BW (g)	BR (kgf[1])
-----Effect of Ca:P total ratio-----					
1.7	50.12 b	17.65	8.86	11.02	47.55 b
2.0	51.88 a	18.21	8.70	11.14	50.88 a
-----Effect of oyster shell particle size-----					
1,354 µm	51.33	18.24 a	8.89	10.97	49.25
428 µm	50.70	17.60 b	8.64	11.20	49.18
-----Effect of sex-----					
Male	51.75 a	18.80 a	8.84	12.14 a	50.48
Female	50.27 b	17.06 b	8.68	10.03 b	48.96
-----P-values-----					
Ca:P total ratio	0.001	0.082	0.165	0.653	0.027
Particle size	0.139	0.040	0.069	0.397	0.966
Sex	0.001	0.001	0.224	0.001	0.093
Ratio×Particle size	0.918	0.679	0.696	0.387	0.584
Ratio×Sex	0.269	0.974	0.013	0.396	0.407
Particle size×Sex	0.067	0.001	0.349	0.325	0.177
Ratio×Particle size×Sex	0.275	0.745	0.967	0.727	0.151

[1]kilogram-force.
Means followed by equal letters (vertical) do not differ statistically by Tukey's test at 5%.

led to increased FI and therefore to increased intake and retention of Ca and P. However, it should be reinforced that the variability between studies is caused by using different Ca sources with different chemical and physical characteristics.

Bone characteristics

Tibia ash content was improved with a 2.0:1 total Ca:P ratio compared to the lower ratio of 1.7:1; however, the higher ratio reduced P content in the tibia of male broilers, as observed with the interaction between the Ca:P total ratio and sex. Some studies have reported the negative effects of higher Ca:P ratios on bone formation and mineralization. Greater concentrations of Ca will foster the formation of insoluble Ca-phytate complexes, reducing the absorption and digestibility of Ca and P, and eventually the deposition of mineral residues of P and Mn in the bone (SCHOULTEN et al., 2003; LI et al., 2012; AMERAH et al., 2014). This may explain why a higher ratio reduced the P content in the tibia of males. However, according to RAMA RAO et al. (2006), the ash and mineral contents of the tibias of broiler chickens are highest when the Ca:P ratio is maintained at 2:1. Therefore, the use of a 2.0:1 ratio in the current study improved tibial mineralization overall, as observed with the greater tibial ash content and stronger BR, regardless of other factors, whereas lowering the dietary Ca:P total ratio impaired these aspects.

The OSM particle size influenced Ca content in the tibia in relation to sex, as male broilers fed diets with coarse OSM had greater tibial Ca content. These results agreed with ANDERSON et al. (1984), who reported that the use of a Ca source with finer particles (powdered limestone of 74 µm) resulted in lower mineral content in the tibia of broilers when compared to medium particles (ranging from 150 to 1,000 µm). Apparently, young broilers can shunt excess Ca through the digestive system more easily in the form of medium particles rather than finer particles. In contrast, GUINOTTE et al. (1991) reported that Ca sources with ground particles (less than 150 µm) improved bone ossification in

Table 4 - Statistical breakdown of the interaction between the Ca:P total ratio × broiler sex and between particle size of oyster shell meal × broiler sex on ash, Ca, P, bone weight (BW), and bone resistance (BR) of tibia bones.

Treatment	Ash (%)	Ca (%)	P (%)	BW (g)	BR (kgf[1])
---Effect of Ratio×Sex---					
1.7 × female	49.16	16.77	8.77 ab	10.09	46.90
1.7 × male	51.11	18.51	8.95 a	11.96	48.19
2.0 × female	51.38	17.35	8.92 a	9.97	49.01
2.0 × male	52.38	19.07	8.41 b	12.31	52.77
SEM	0.43	0.32	0.13	0.28	1.48
---Effect of Particle size×Sex---					
428 µm × female	50.36	17.31 b	8.78	10.28	46.91
428 µm × male	51.01	17.90 b	8.49	12.11	51.46
1,354 µm × female	50.17	16.82 b	8.91	9.78	48.99
1,354 µm × male	52.48	19.67 a	8.87	12.15	49.50
SEM	0.43	0.32	0.13	0.28	1.48

[1]kilogram-force.
Means followed by equal letters (vertical) do not differ statistically by Tukey's test at 5%.

broilers, and BRADBURY et al. (2016) found no effect of the particle size (< 0.5 or > 0.5 mm) of limestone or highly soluble Ca on foot ash percentage of broilers. This divergence between studies is most likely related to the origin of the different Ca sources.

Differing from males, the Ca and P tibial contents of female broilers were not affected by the Ca:P total ratio or OSM particle size. When comparing sexes separately, male broilers had heavier bones with greater ash and Ca contents. These distinctions between sexes could be linked to differences in the metabolism of Ca and P, as male broilers have larger bones due to their faster growth, and thus have greater requirements for dietary Ca and P (BAR et al., 2003; DRIVER et al., 2005).

Metabolizability

A higher Ca:P ratio can reduce both Ca and P digestibility, mainly because of Ca binding with phytate at high concentrations in the gastrointestinal tract, hampering the activity of phytase and endogenous phosphatases to break down phytate and release phytate-P (AMERAH et al., 2014; ANWAR et al., 2016). Similarly, overly low dietary Ca levels will imbalance the Ca:P ratio and be equally harmful for dietary nutrient utilization and promote negative impacts on performance and bone quality (SCHOULTEN et al., 2003; ZHANG et al., 2020). In the current study, the lack of effect by the Ca:P ratio to the CAM variables and AME suggests there was no imbalance in the supply of these minerals that would harm their utilization by broilers. Therefore, the supplementation of diets with exogenous phytase could have led to an appropriate availability of Ca and P regardless of the ratio.

The inclusion of coarse OSM (1,354 µm) improved the CAM of Ca, in agreement with other studies that have shown that coarser Ca particle sizes in broiler diets increased the utilization of Ca by improving aspects such as ileal digestibility and total tract retention (ANWAR et al., 2016; ANWAR et al., 2017; LI et al., 2021). MANANGI & COON (2007) also reported on coarser Ca carbonate (1,306 µm) improving phytate hydrolysis. Similar to the results of this research, ANWAR et al. (2017) studied different particle sizes of OSM, in comparison with limestone, and observed greater true ileal digestibility of Ca when using both coarser OSM and limestone (< 0.5 mm × 1–2 mm). As suggested by the aforementioned studies, these improvements are caused by the longer retention time of coarser particles, which slows down the release of Ca and increases its *in vivo* solubility and availability, whereas finer particles tend to have greater *in vitro* solubility because of the larger surface area for the acid reaction (ANWAR et al., 2017). The increase in

Table 5 - Coefficient of apparent metabolizability (CAM) of dry matter (DM), Ca, P, and apparent metabolizable energy (AME) of 28 day old male and female broilers fed diets containing oyster shell meal of two particle sizes and formulated with two Ca:P total ratios.

Treatment	CAM DM (%)	CAM Ca (%)	CAM P (%)	AME (kcal)
---Effect of Ca:P total ratio---				
1.7	72.32	71.81	70.19	3,496
2.0	72.66	70.47	69.12	3,480
---Effect of oyster shell particle size---				
1,354 µm	72.69	72.67 a	69.97	3,479
428 µm	72.29	69.61 b	68.35	3,495
---Effect of sex---				
Male	72.56	71.49	68.81	3,484
Female	72.42	70.75	69.50	3,490
---P-values---				
Ca:P ratio	0.122	0.236	0.127	0.107
Particle size	0.072	0.008	0.228	0.110
Sex	0.513	0.485	0.603	0.504
Ratio×Particle size	0.922	0.279	0.323	0.330
Ratio×Sex	0.926	0.584	0.888	0.231
Particle size×Sex	0.125	0.119	0.976	0.087
Ratio×Particle size×Sex	0.351	0.481	0.506	0.247

Means followed by equal letters (vertical) do not differ statistically by Tukey's test at 5% probability.

bone Ca content in male broilers fed coarser OSM is apparently a result of the greater CAM of Ca.

The sex of broilers did not influence the CAM variables; although, both sexes had distinct capacities to metabolize Ca and P (BAR et al., 2003; DRIVER et al., 2005). This difference was evidenced in the current study, since even though males and females had similar nutrient metabolizability, male broilers were more effective in utilizing dietary minerals for bone mineralization and growth, as they produced heavier bones with greater ash and Ca contents.

CONCLUSION

The different Ca:P total ratios and particle sizes of oyster shell meal did not influence broiler growth performance from 22 to 42 days of age. Increasing Ca:P total ratio from 1.7:1 to 2.0:1 improved bone mineralization characteristics by increasing the ash content in the tibia and bone resistance. Male broilers fed diets with a 2.0:1 Ca:P total ratio also had higher Ca contents in the tibia. The inclusion of a coarser oyster shell meal (1,354 µm) in broiler diets is recommended to increase the metabolizability of Ca and the deposition of Ca in the bone.

ACKNOWLEDGEMENTS

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) – Brasil– Finance Code 001.

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

All animal procedures were approved by the Ethics Committee on the Use of Animals of Embrapa Swine and Poultry, Concórdia, SC, Brasil.

DECLARATION OF CONFLICT OF INTEREST

We have no conflict of interest to declare.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the conception and writing of this manuscript. All authors critically revised the manuscript and approved the final version.

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