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Digestibility of different sources and particle sizes of calcium in diets for laying hens during the laying phase

Abstract – The objective of this work was to evaluate the digestibility of different calcium sources and particle sizes in diets for commercial laying hens during the laying phase. Ninety-five Hisex Brown hens, with 26 weeks of age, were used, distributed in a completely randomized experimental design, in a 3x2 factorial arrangement with three Ca sources (oyster shell meal and the Furquim and Supercal commercial calcitic limestones) and two particle sizes (fine and coarse). The following eight experimental diets were prepared: six in which corn starch was replaced with the evaluated Ca sources and particle sizes; one without Ca; and a basal diet. The Ca digestibility coefficients were similar between the diets containing Furquim limestone and oyster shell meal, which presented greater values than the diets containing Supercal limestone. The results of the in vitro solubility of the Ca particle sizes indicated a relationship between particle size and solubility, where in vitro solubility decreases with the increase in particle diameter. The coarse particle size significantly reduced the digestibility coefficients of the feed and Ca sources analyzed. Oyster shell meal and Furquim calcitic limestone are equivalent sources in terms of the Ca digestibility coefficient, and the use of fine particles increases the digestibility coefficients of the feed and Ca sources.

Index terms: *Gallus gallus*, animal nutrition, calcitic limestone, oyster shell meal.

Digestibilidade de diferentes fontes e granulometrias de cálcio em dietas para poedeiras na fase de postura

Resumo – O objetivo deste trabalho foi avaliar a digestibilidade de diferentes fontes e granulometrias de cálcio em dietas para poedeiras comerciais em fase de postura. Foram utilizadas 95 poedeiras da linhagem Hisex Brown com 26 semanas de idade, distribuídas em delineamento experimental inteiramente casualizado, em arranjo fatorial 3x2, com três fontes de Ca (farinha de concha de ostra e os calcários calcíticos comerciais Furquim e Supercal) e duas granulometrias (fina e grossa). Foram elaboradas as seguintes oito dietas experimentais: seis em que o amido de milho foi substituído pelas fontes e pelas granulometrias de Ca avaliadas; uma isenta de Ca; e uma dieta basal. Os coeficientes de digestibilidade do Ca foram semelhantes entre as dietas contendo calcário Furquim e farinha de ostras, cujos valores foram maiores que os das dietas contendo calcário Supercal. Os resultados de solubilidade in vitro das granulometrias das fontes de Ca indicaram uma relação entre granulometria e solubilidade, em que a solubilidade in vitro diminui com o aumento do diâmetro das partículas. A granulometria grossa reduziu substancialmente os coeficientes de digestibilidade da ração e das fontes de Ca analisadas. A farinha de concha de ostra e o calcário calcítico Furquim são fontes equivalentes em relação ao coeficiente de digestibilidade de Ca, e o uso de partículas finas aumenta os coeficientes de digestibilidade da ração e das fontes de Ca.

Termos de indexação: *Gallus gallus*, nutrição animal, calcário calcítico, farinha de concha de ostra.

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Introduction

Constant genetic improvement has created highly productive birds, which require adjustments in calcium supplementation in their diets for an optimal laying performance (Kiarie & Mills, 2019; David et al., 2021). However, the determination of Ca digestibility has not received much attention in the past because calcitic limestone, the main inorganic Ca source used in poultry feed, presents a low cost and abundant availability (Anwar & Ravindran, 2020). Furthermore, it is generally assumed that the ingredients present in the feed, including Ca, are fully available for absorption by the birds' bodies, which still needs to be confirmed since the data published on Ca digestibility in layer diets remain limited (Anwar & Ravindran, 2020).

During the laying phase, it is essential that hens receive Ca from the diet to ensure a higher availability of the circulating nutrient, which can be influenced by the Ca source and particle size used in the feed (Matuszewski et al., 2020). Besides calcitic limestone, other sources can be used, which could reduce the impact on nonrenewable natural Ca reserves (Morris et al., 2016; Matuszewski et al., 2020; Vieira et al., 2020). Among these alternatives, organic minerals, such as oyster shell meal (OSM), stand out because, in addition to being a source of calcium carbonate, they also contain proteins, which are absorbed by intestinal carriers of amino acids and peptides, not solely through the classic mineral transport system (Vieira, 2008). This avoids competition between minerals for absorption channels, consequently increasing their availability (Vieira et al., 2020).

The availability of Ca both from inorganic and organic sources varies according to the mineral composition and particle size of these sources (Roland, 1986; Matuszewski et al., 2020). Regarding particle size, in layers, larger particles are retained for a longer time in the gizzard, allowing of a continuous supply of Ca to the bird, even during the night, making this nutrient more available throughout the eggshell formation process, whereas smaller particles show an increased solubility due to the larger specific surface area of the feed particles, allowing of a better contact with the HCl produced by the proventriculus (Anwar et al., 2017; Kim et al., 2018; Zhang & Coon, 1997).

The objective of this work was to evaluate the digestibility of different calcium sources and particle sizes in diets for commercial laying hens during the laying phase.

Materials and Methods

The experiment was conducted in the Poultry Sector of the Department of Animal Production and Food at the Center for Agro-Veterinary Sciences of Universidade do Estado de Santa Catarina (UDESC), located in the municipality of Lages, in the state of Santa Catarina, Brazil. The project was approved by the animal experimentation ethics committee of UDESC, under protocol number 7082260623.

For the study, 95 Hisex Brown laying hens, with 26 weeks of age, were used. The layers were individually weighed (1.751 kg ±4%) and randomly allocated to 40 metabolic cages (0.5x0.5x0.5 m); each cage was considered as an experimental unit. A completely randomized design was used, in a 3x2 factorial arrangement, with three Ca sources (OSM and two commercial limestones) and two particle sizes (fine and coarse). The cages were equipped with a front trough feeder, a nipple drinker with an individual water reservoir, and a plastic-coated excreta collection tray for easy excreta collection. Feed and water were provided ad libitum throughout the experimental period, under a lighting regimen of 16 hours light and 8 hours dark.

The two commercial calcitic limestones (animal feed supplements) used as Ca sources, both in fine and coarse forms, were: CF, commercial Furquim (Calcário Furquim, Minérios Furquim Ltda., Rio Branco do Sul, PR, Brazil), with respective geometric mean diameters of 0.37 and 2.04 mm; and CS, Supercal (Supercal Pains Ltda., Arcos, MG, Brazil), with geometric mean diameters of 0.55 and 3.35 mm. The used OSM source (CYSY: Calcário de Conchas, Jaguaruna, SC, Brazil), also in fine and coarse forms (FA001 and FA003, respectively), had geometric mean diameters of 0.37 and 2.97 mm, respectively. The particle sizes of the Ca sources are within the limits defined by Zanotto & Bellaver (1996) for ingredient particles classified as coarse (> 2.00 mm) and fine (< 0.60 mm). The geometric standard deviation of the Ca sources showed values below 2%, indicating a high uniformity of the particles.

To conduct the experiment, eight diets were prepared: six in which corn starch was replaced with each of the evaluated Ca sources and particle sizes; one without Ca, used to determine endogenous Ca loss; and a basal diet with known and calculated nutritional levels, without the inclusion of a Ca source and with 6.80% corn starch in its composition. Each diet had five replicates of two birds, except for the Ca-free diet, which had five birds per replicate. The nutritional and calculated composition of the diets used in the experiment were formulated according to the recommendations of Rostagno (2017), as shown in Table 1. Phosphorus was added in the form of monosodium phosphate (NaH_2PO_4) to all diets, as low P concentrations in the diet reduce the availability of Ca in the body of the birds (Anwar et al., 2016). The birds also received ultrapure water, with care taken to ensure that no external source of Ca was provided.

Table 1. Nutritional composition and calculated values of the experimental diets.

Ingredient (g kg^{-1})	Calcium-free diet	Basal diet
Corn	-	53.2
Soybean meal	-	23.5
Wheat bran	-	14.9
Corn starch	64.6	6.80
Sugar	15.0	-
Inert kaolin	16.4	-
KCl	0.86	-
NaH_2PO_4	1.31	0.76
Salt	0.32	0.32
Lysine	0.75	0.12
Methionine	0.46	0.10
Premix(1)	0.30	0.30
Total (kg)	100	100
Calculated composition		
Metabolizable energy (kcal kg^{-1})	2,850	2,852
Crude protein (%)	0.00	15.14
Total calcium (%)	0.00	0.09
Available phosphorus (%)	0.31	0.31
Sodium (%)	0.26	0.17
Potassium (%)	0.44	0.73
Chlorine (%)	0.40	0.42
Linoleic acid (%)	0.00	1.34
Lysine (%)	0.75	0.75
Methionine (%)	0.40	0.40
Tryptophan (%)	0.00	0.26

(1)Vitamin and mineral supplement, containing per kilogram: 9,333.34 mg zinc bacitracin, 100,000.00 U phytase (min.), 123.40 g methionine (min.), 99.90 mg folic acid (min.), 6.66 mg biotin (min.), 2,998.80 mg copper (min.), 55.33 g choline (min.), 16.66 g iron (min.), 333.20 mg iodine (min.), 1,831.50 mg pantothenic acid (min.), 24.34 g manganese (min.), 994.00 niacin (min.) mg, 99.90 mg selenium (min.), 2,797,200.00 IU vitamin A (min.), 283.05 mg vitamin B1 (min.), 2331.00 mcg vitamin B12 (min.), 999.00 mg vitamin B2 (min.), 932,400.00 IU vitamin D3 (min.), 1,998.00 IU vitamin E (min.), 399.60 mg vitamin K3 (min.), and 23.32 g zinc (min.).

The layers fed diets with coarse particle sizes underwent a 20 day adaptation period before the trial, in which basalt pebbles were added on top of the feed in the feeders. This adaptation aimed to promote gizzard hypertrophy and hyperplasia, facilitating the subsequent grinding of the diets with Ca sources with coarser particle sizes (Kiarie & Mills, 2019).

The geometric mean diameter and geometric standard deviation of the Ca sources were determined following the methodology outlined by Zanotto et al. (2016). For this, the quantities retained on sieves were recorded and analyzed using the Granucalc software (Embrapa Suínos e Aves, 2013). In addition, the in vitro solubility of the Ca sources was obtained using the method of percentage of weight loss described by Cheng & Coon (1990).

The experimental period lasted nine days, of which five days were used for layer adaptation to the experimental diets and four days, for total excreta collection. Excreta were collected once daily, weighed, stored in labeled plastic bags by replicate, and frozen at -18°C. The samples were subsequently thawed, homogenized, dried in a forced-ventilation oven at 55°C for 72 hours, and ground using a Wiley-type knife mill (Silva & Queiroz, 2002). All steps involved in laboratory analyses were conducted in triplicate, from material drying to the analysis of the Ca samples.

Nutrient contents in the experimental diets and excreta were analyzed for dry matter and mineral matter following the methods in the study of Silva & Queiroz (2002). The Ca content in the samples was determined by ethylenediaminetetraacetic acid complexometric titration according to the analytical procedure described by Raij (1966). The obtained results were used in the equations provided by Sakomura & Rostagno (2016), in order to calculate the apparent and true digestibility coefficients of the diets and Ca sources.

The data were subjected to the analysis of variance, and, when differences were observed, means were compared by Tukey's test, at 5% probability, using the PROC GLM procedure of the SAS statistical software (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

The coefficient of mineral matter digestibility (DCMM) was influenced by the Ca source used (Table 2). The DCMM did not differ statistically for CS and OSM, whose results were superior to those obtained for CF. The differences observed in digestibility among the used sources can be attributed to mineral bioavailability, which is influenced by various factors such as mineral processing conditions, physical form, and interactions with other minerals and nutrients in the diets (Miles & Henry, 2000). Vieira et al. (2020) added that, when ionized, minerals can form complexes with other components in the source or diet, hindering mineral transport, absorption, or metabolism in layers.

DCMM was also influenced by particle size. Lower values were obtained with coarser particle sizes, which negatively affected mineral digestibility due to a reduced surface area and accessibility to the digestive system (Kim et al., 2019).

The coefficient of dry matter digestibility (DCDM) was influenced by Ca sources and the interaction between these factors, but not by particle size. As previously described, ionized minerals can form complexes with other diet ingredients, which affects their transport and absorption (Miles & Henry, 2000). This factor likely influenced the different dry matter digestibility results observed among the evaluated Ca sources.

There was an interaction between factors for DCMM and DCDM. The OSM with a fine particle size resulted in a higher mineral and dry matter digestibility, differing significantly from both limestone sources. The OSM with a coarse particle size also showed a higher digestibility, which did not differ from that of CS; both of these sources were superior to CF.

Differences in dry matter and mineral digestibility can be attributed to the processes of nutrient digestion and absorption, strongly related to the accessible surface area for HCl from the proventriculus, particle pore dimensions, chemical composition, and polarity of sources (Kiarie & Mills, 2019). These factors can interact to enhance dry matter and mineral absorption or adversely complex with other diet ingredients, reducing mineral and dry matter availability (Kim et al., 2019).

Table 2. Coefficients of mineral matter digestibility (DCMM), dry matter digestibility (DCDM), apparent digestibility of feed (CDAF), true digestibility of the calcium present in the feed (CDVF), and apparent (CDAS) and true (CDVS) digestibility of Ca in its respective sources, as well as the interaction between particle size and Ca sources for the DCMM and the DCDM[(1)].

Treatments		DCMM	DCDM	CDAF	CDVF	CDAS	CDVS
		(%)					
Calcium source	Oyster shell flour	64.3a	67.2a	82.3a	82.5a	83.2a	83.6a
	Furquim limestone	57.7b	63.8b	81.7a	81.8a	82.5a	83.0a
	Supercal limestone	63.0a	66.7a	79.7b	79.9b	80.5b	81.0b
Particle size	Fine	62.9a	66.0	81.9a	82.1a	82.8a	83.2a
	Coarse	60.4b	65.8	80.5b	80.7b	81.3b	81.8b
Calcium source		0.0001	0.0044	0.0006	0.0006	0.0010	0.0012
Particle size		0.0164	0.8797	0.0077	0.0087	0.0101	0.0125
Sources x particle size		0.0354	0.0112	0.3500	0.3692	0.3579	0.3814
CV[(2)] (%)		4.25	3.31	1.64	1.64	1.74	1.73
SEM[(3)]		2.62	2.18	1.33	1.33	1.43	1.43
		DCMM (%)			DCDM (%)		
		Oyster shell flour	Furquim limestone	Supercal limestone	Oyster shell flour	Furquim limestone	Supercal limestone
Particle size	Fine	65.5Aa	60.5Ac	62.6Bb	67.0Aa	65.6Ab	65.3Bb
	Coarse	63.1Ba	54.8Bb	63.4Aa	67.4Ba	62.1Bb	68.1Aa

[(1)]Means followed by different letters, in the columns, differ significantly by Tukey's test, at 5% probability. For the interaction, means with different lowercase letters, in the columns, and uppercase letters, in the rows, differ statiscally. [(2)]CV, coefficient of variation. [(3)]SEM, standard error of the mean.

Significant differences were observed among the evaluated Ca sources for the coefficients of apparent and true digestibility of Ca. The digestibility coefficients were higher for CF and OSM, but lower for CS.

The different digestibility of the tested Ca sources could be explained by the fact that, in ionized form, minerals may bind with other diet constituents, which reduces their solubilization and, consequently, their absorption by the animal's organism (Vieira et al., 2020). According to Anwar et al. (2016), impurities and other minerals are common in the limestone used in animal feed. In this line, Vieira (2008) found that Ca has the potential to interact with other minerals present in limestone, including trace elements such as Mg, Fe, S, Zn, and Cu, causing mutual antagonisms that can potentially reduce nutrient absorption and metabolism rates.

The apparent and true digestibility coefficients of Ca in the tested diets and sources were higher for fine particle sizes, which allowed of a prompt Ca release for absorption. This result can be attributed to the greater contact area of finely ground OSM and calcitic limestone particles with HCl, facilitating food digestion (Kim et al., 2018), which contrasts with the less-soluble coarser Ca sources. This result is in alignment with that of Kim et al. (2018), who also found that particle size directly influences mineral solubility, with fine particles exhibiting a higher solubility than the coarse ones.

Roland & Bryant (1999) concluded that the substitution of fine-particle size Ca sources with coarse-particle size sources should not exceed 50%, to avoid affecting layer consumption and diet-component digestibility. This factor may have contributed to the reduced Ca digestibility in the treatments with a coarse particle size, which is considered more effective than a fine particle size when layers receive inadequate Ca levels or are exposed to factors that reduce the use of this nutrient (Roland, 1986). Since, in the present study, Ca was provided according to the recommended requirements, there was likely no influence on the need for a greater use of minerals with a coarse particle size by the layer organisms.

Ca percentage was influenced by Ca sources and particle sizes, but with no interaction between these factors (Table 3). The percentage of Ca was lower in larger particles, probably because this nutrient is not uniformly distributed throughout the particle. The differences observed in the Ca concentrations in the limestone and OSM sources may be explained by the differences in the composition of these sources, which may vary depending on their extraction site, geological origin, physical properties, and chemical composition (Saunders-Blades et al., 2009; Kim et al., 2019).

In vitro solubility was also influenced by Ca sources and particle sizes. The decrease in particle size increased solubility values, which is consistent with the findings of Anwar et al. (2017), who observed that in vitro solubility was higher for fine Ca particles. The results of the present study also indicated the relationship between particle size and solubility, in alignment with the findings of Cheng & Coon (1990).

Solubility percentage was influenced by Ca sources and particle sizes, but with an interaction between these factors. A greater solubility was observed for OSM with a coarse particle size, being significantly superior to that of the calcitic limestone sources with a coarse particle size. The flat and elongated surface of coarse OSM particles (>2.00 mm) provides a greater surface area, facilitating acid reaction and resulting in a higher solubility compared with that of limestone with a similar particle size (Saunders-Blades et al., 2009).

Table 3. In vitro solubility and calcium content of the used sources and interaction between particle size and Ca sources for solubility percentage[1].

Treatments		Solubility (%)	Ca (%)
Calcium source (CS)	Oyster shell meal	24.2a	35.8b
	Furquim limestone	15.8c	36.9a
	Supercal limestone	20.5b	36.1b
Particle size	Fine	26.5a	36.8a
	Coarse	13.8b	35.7b
CS		0.0001	0.0001
Particle size		0.0001	0.0001
CS x particle size		0.0001	0.7828
CV[2] (%)		1.49	1.64
SEM[3]		0.30	133
	Solubility (%)		
Particle size	Oyster shell meal	Furquim limestone	Supercal limestone
Fine	27.7Aa	25.3Ac	26.6Ab
Coarse	20.8Ba	6.30Bc	14.4Bb

[1]Means followed by different letters, in the columns, differ significantly by Tukey's test, at 5% probability. For the interaction, means with different lowercase letters, in the columns, and uppercase letters, in the rows, differ statiscally. [2]CV, coefficient of variation. [3]SEM, standard error of the mean.

The obtained results show that particle size is an important factor influencing the values of in vitro solubility, but that it is not the only one, as solubility differed between sources with the same particle size. Although CF and OSM had a similar geometric mean of 0.37 mm, the latter source with a fine particle size showed a higher in vitro solubility percentage, which is an indicative that mineral composition and physical characteristics are other factors that influence limestone solubility (Kim et al., 2019).

Ca digestibility is not only influenced by particle size, but also by ingredient solubility. Zhang & Coon (1997) highlighted that a lower in vitro solubility increases retention time in the gizzards of layers, which enhances mineral digestion, representing an inversely proportional relationship.

CF had the lowest in vitro solubility and highest Ca digestibility in laying layers, which is consistent with the findings of Zhang & Coon (1997). However, this inverse proportional relationship does not hold for OSM, which showed a higher in vitro solubility than the limestone sources, but also a higher Ca digestibility, similar to that of CF. This divergence may be explained by the fact that, in addition to its higher in vitro solubility, OSM is of organic origin, presenting a high in vivo solubility and intestinal mineral absorption. When bound to proteins, the minerals present in the oyster shell are absorbed by intestinal amino acid and peptide carriers rather than solely through classical mineral transport channels (Vieira, 2008). This factor avoids competition among minerals for absorption channels, increasing their availability and favoring Ca digestibility in layers fed OSM (Vieira et al., 2020).

Conclusions

1. Oyster shell flour and Furquim commercial calcitic limestone are equivalent sources regarding calcium digestibility in laying Hisex Brown hens.
2. Ca with a fine particle size enhances the digestibility coefficients of the used feed and Ca sources.

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