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Influence of dietary calcium levels on the performance and egg quality of Canela-Preta free range laying hens

Efeito dos níveis de cálcio na dieta sobre o desempenho e qualidade de ovos de galinhas caipiras da raça Canela-Preta

Claudiane Morais dos Santos[1]*; Marcos Lenyn Rodrigues dos Santos[2]; Nayron Mikael Costa Silva[3]; Jefferson Breno Lopes de Moura[3]; Raimundo Borges Leal Neto[4]; João Roniele de Sousa Silveira[4]; Roseane Madeira Bezerra[5]; Daniel Louçana da Costa Araújo[6]; Leilane Rocha Barros Dourado[6]; João Batista Lopes[6]

Highlights

Calcium requirement in birds depends on factors such as breed and production phase.
Calcium can affect productivity, bone formation, and egg quality.
A decline in dietary calcium leads to its mobilization from bones.

Abstract

This study aimed to evaluate the zootechnical performance and egg quality of Canela-Preta free-range laying hens under diets with different calcium levels, while grazing on Bahia grass (Paspalum notatum). A total of 120 laying hens were housed in an experimental laying facility with access to pasture. The experimental design used was completely randomized in a 4x3 factorial arrangement (Ca X bird age), with dietary calcium levels of 2.3, 3.0, 3.7, and 4.4%, and bird ages of 28, 32, and 36 weeks. Each treatment had five replicates, with six laying hens per experimental unit. Parameters assessed were zootechnical

[1] Dr[a]. in Animal Science, Universidade Federal do Piauí, UFPI, Campus Petrônio Portela, Teresina, PI, Brazil. E-mail: claudianemor2@gmail.com
[2] Animal Science student, Agrarian Sciences Center, UFPI, Petrônio Portela Campus, Teresina, PI, Brazil. E-mail: marcoslenyn@ufpi.edu.br
[3] Agronomy Students, Agrarian Sciences Center, UFPI, Petrônio Portela Campus, Teresina, PI, Brazil. E-mail: nayronmikael@gmail.com; breno.moura@ufpi.edu.br
[4] Veterinary Students, Agrarian Sciences Center, UFPI, Petrônio Portela Campus, Teresina, PI, Brazil. E-mail:rdoneto55@gmail.com; joaoroniele@ufpi.edu.br
[5] Pos Doctor of Animal Science Program, Cinobelina Elvas Campus, UFPI, Bom Jesus, PI, Brazil. E-mail: roseanemadeira@hotmail.com
[6] Profs., of Department of Animal Science, Agrarian Sciences Center, UFPI, Petrônio Portela Campus, Teresina, PI, Brazil. E-mail: danielloucana@ufpi.edu.br; leilane@ufpi.edu.br; lopesjb@ufpi.edu.br
* Author for correspondence

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performance, egg quality, and bone characteristics. The 2.3% calcium level met the laying performance requirements of Canela-Preta laying hens, between 28 to 36 weeks of age, representing the age of optimal feed conversion both per dozen and egg mass. Dietary calcium levels did not affect eggshell length, specific gravity, thickness, eggshell breaking strength, egg shape index, or Haugh unit. However, from 3.03% calcium inclusion onwards, yolk percentage decreased to 4.17% and eggshell percentage declined. Bone parameters were not affected up to 4.4% of calcium inclusion in the diets of Canela-Preta laying hens during the 28 to 32-week period of life.
Key words: Native bird. Feed efficiency. Semi-intensive system. Haugh unit.

Resumo

Este estudo teve como objetivo avaliar o desempenho zootécnico e a qualidade dos ovos de galinhas caipiras Canela-Preta submetidas a dietas com diferentes níveis de cálcio, em pastejo em capim-baiana (Paspalum notatum). Um total de 120 aves foram alojadas em instalação experimental de postura com acesso a pasto. O delineamento experimental utilizado foi inteiramente casualizado em esquema fatorial 4x3 (Ca X idade das aves), com níveis de cálcio na dieta de 2,3, 3,0, 3,7 e 4,4% e idades das aves de 28, 32 e 36 semanas. Cada tratamento contou com cinco repetições, com seis aves por unidade experimental. Os parâmetros avaliados foram desempenho zootécnico, qualidade dos ovos e características ósseas. O nível de cálcio de 2,3% atendeu às exigências de desempenho de postura de galinha Canela-Preta, entre 28 e 36 semanas de idade, representando a idade de conversão alimentar ideal tanto por dúzia quanto por massa de ovos. Os níveis de cálcio na dieta não afetaram o comprimento da casca, a gravidade específica, a espessura, a resistência à ruptura da casca, o índice de forma do ovo ou a unidade Haugh. Entretanto, a partir de 3,03% de inclusão de cálcio, o percentual de gema diminuiu para 4,17% e o percentual de casca do ovo diminuiu. Os parâmetros ósseos não foram afetados até 4,4% da inclusão de cálcio nas dietas das galinhas caipira Canela-Preta durante o período de 28 a 32 semanas de vida.
Palavras-chave: Ave nativa. Eficiência alimentar. Sistema semi-intensivo. Unidade Haugh.

Introduction

Free-range chicken breeding has evolved in recent years, becoming a significant segment in the production of highly nutritious food, not only for families involved in family farming, but also for a select group of consumers seeking products perceived as being healthier. In this respect, it is essential to adapt techniques that allow for optimizing the productive efficiency of these birds (Sousa et al., 2020).

Feeding represents the largest fraction of production costs, and small improvements in nutrient use in diets can significantly lower costs (Albuquerque et al., 2020). Mineral supplementation is an alternative to enhance the zootechnical performance of these laying hens, contributing to weight, the egg laying rate, feed conversion ratio (FCR), and eggshell quality (Vellasco et al., 2016). It is important to underscore that the nutritional needs of birds vary according to age, sex, breed, nutritional and health status, production phase, and economic purpose (Rostagno et al., 2017).

The first record of Canela-Preta free-range chicken research was in 2008 in Piauí state, Brazil. It is considered an important qualitative source of animal protein (meat and eggs), particularly for the rural population of the state and later in the northeastern region of Brazil (Carvalho et al., 2017).

With the registration of new free-range chicken breeds, there is a need to understand the zootechnical potential of these animals and their nutritional requirements for protein, energy, minerals, and vitamins, among other nutrients. These factors are vital for successful production, given that feed represents the highest production cost. Additionally, providing adequate dietary nutrients is a prerequisite for these animals to achieve their maximum productive potential.

Despite the dissemination of research and the consolidation of manuals on the nutritional requirements of modern laying hens, these animals are highly sensitive to changes in dietary nutrient levels. However, little is known about the requirements of free-range chickens, especially native breeds such as the Canela-Preta that have not undergone genetic improvement.

Calcium is one of the most important minerals in the diet of laying hens, sinces it contributes to performance and correct eggshell formation. It is also used in numerous metabolic processes, including muscle, skeletal and cardiac contraction, membrane permeability, blood clotting, bone formation in developing birds, in addition to acting as an enzyme activator and stabilizer (Pinto et al., 2012; J. P. Silva et al., 2022; Vellasco et al., 2016).

Based on these assumptions, the aim of this study was to assess the calcium requirement of Canela-Preta free-range laying hens aged 28 to 36 weeks, focusing on zootechnical performance and egg quality.

Materials and Methods

The experiment was conducted at the Department of Animal Science (DZO) of the Center for Agricultural Sciences (CCA) at the Federal University of Piauí (UFPI), from February to July 2022, after approval by the Animal Ethics Committee of the Federal University of Piauí (CEUA/UFPI), under protocol number 669/2021.

A total of 120 native Canela-Preta free-range laying hens, initially aged 20 weeks, were acquired from a local producer in the Teresina region, Piauí. The laying hens were housed in 20 boxes in a shed with concrete flooring, each containing six birds, with a density of 0.41 birds/m². They had access to a pasture consisting of 20 paddocks with a density of 1.5 birds/m², where Bahia grass (*Paspalum notatum*) was grown and irrigated. The laying hens had free access to the pasture during the day, characterizing a semi-intensive poultry breeding system according to the Associação Brasileira de Normas Técnicas [ABNT] (NBR 16437, 2016). Each box was equipped with water dispensers, feeders, and wooden nests lined with rice straw to prevent egg cracking.

The birds were individually identified with leg bands, grouped, and distributed into the boxes based on their body weight and pelvic bone opening, measured by a single evaluator using fingers as a measuring unit, where 1, 2, 3, and 4 fingers corresponded to widths of 15, 30, 51, and 63 mm, respectively. For weighing, a digital electronic scale with a capacity of up to 5 kg was used.

A continuous light program was adopted, providing a photoperiod of 17 hours of natural and artificial light (4 to 10 pm).

Sanitary management included daily washing of water dispensers, visual assessment of the birds with the removal of dead animals or those with health issues, cleaning and sanitization procedures for facilities and nests, and quality control of inputs and materials. Additionally, ectoparasite and endoparasite control measures were implemented (NBR 16389, 2015; Domingues & Langoni, 2001). The vaccination program followed the recommendations of Avila et al. (2017).

For feeding management, diets consisted primarily of corn and soybean meal, supplemented with minerals and vitamins to meet the nutritional needs of the birds, except for calcium. Nutritional levels followed the recommendations of Avila et al. (2017) and complied with Associação Brasileira de Normas Técnicas [ABNT] (NBR 16437, 2016). Limestone was added to the diets to replace inert material, resulting in isonutritive diets containing 2.3, 3.0, 3.7, and 4.4% calcium (Table 1).

The birds had ad libitum access to feed and water. Feed was weighed at the beginning and leftovers were weighed at the end of each experimental period, that is, every 28 days, in order to quantify feed consumption per experimental unit.

Throughout the experimental period, environmental variables (Table 2) such as temperature and relative humidity were monitored twice daily at 9 am and 3 pm using maximum-minimum thermometers, dry and wet bulb thermometers, a black globe thermometer, and a digital thermohygrometer placed at bird height in the facility. These data were then converted into the Black Globe Temperature Humidity Index (BGTHI) according to Buffington et al. (1981) methodology.

Table 1
Ingredients and calculated composition of the experimental diets on a natural matter basis

	Calcium Levels			
Ingredients (kg)	2.3%	3.0%	3.7%	4.4%
Maize	61.492	61.492	61.492	61.492
Soybean meal	24.124	24.124	24.124	24.124
Soybean oil	1.004	1.004	1.004	1.004
Buriti oil	0.400	0.400	0.400	0.400
Bicalcium phosphate	1.715	1.715	1.715	1.715
Premix – APP1	0.500	0.500	0.500	0.500
Limestone	4.636	6.459	8.282	10.105
Lys-HCl	0.039	0.039	0.039	0.039
Common salt	0.401	0.401	0.401	0.401
DL-Methionine	0.211	0.211	0.211	0.211
L-Valine	0.009	0.009	0.009	0.009
Inert (Sand)	5.469	3.646	1.823	0.000
Total	100.000	100.000	100.000	100.000
Calculated composition (%)				
Linoleic acid	2.075	2.075	2.075	2.075
Calcium	2.300	3.000	3.700	4.400
Met. Energ. Birds= (Mcal/kg)	2.750	2.750	2.750	2.750
Available phosphorus	0.400	0.400	0.400	0.400
Dig. Lysine Birds	0.760	0.760	0.760	0.760
Dig. Met.+cyst. Birds	0.660	0.660	0.660	0.660
Dig. Arginine Birds	0.959	0.959	0.959	0.959
Dig. Treonine Birds	0.547	0.547	0.547	0.547
Dig. Triptophan Birds	0.174	0.174	0.174	0.174
Dig. Valine Birds Aves	0.670	0.670	0.670	0.670
Raw Protein	15.950	15.950	15.950	15.950
Potassium	0.638	0.638	0.638	0.638
Sodium	0.170	0.170	0.170	0.170

[1] Supplies/kg of product. Fe – 10.00g; Cu – 2500mg; Co -60.00g; Choline – 100.00mg; Zn – 20.00mg; I – 208.00mg; Mn- 20.00g; Niacin – 5025mg; Methionine – 152g; Folic acid – 74mg; Biotin – 5mg; Se – 75.00mg; Vit. B12 – 2400mg; Vit. B6 – 250mg; Vit. A – 2000.000IU; Vit. D3 – 625,000IU; Vit. E – 3000IU; Vit. K – 395mg; Vit. B1 – 250mg; Vit. B2 – 1000mg; Calcium pantothenate – 1085mg.

Table 2
Mean temperature, relative humidity (RH) and black globe temperature and humidity index (BGTHI), recorded inside the shed during the experimental period

	Temperature, ºC			RH, %			BGTHI
Period	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Mean
Feb/Mar	30.47	27.00	27.73	99.00	80.84	90.40	80.09
Mar/Apr	31.02	25.92	28.25	98.32	69.23	87.48	81.11
Apr/May	31.18	24.30	28.23	98.12	61.29	85.06	81.32
Overall mean geral	30.89	25.74	28.07	98.48	70.45	87.64	80.84

The experiment lasted for five months and was divided into four data collection periods: a 30-day adaptation and quarantine period, followed by three 28-day periods corresponding to the birds' ages of 28, 32, and 36 weeks, when the following parameters were collected and assessed:

Feed Consumption (FC) - calculated from the amount of feed supplied during each 28-day period minus any leftovers, adjusted for mortality (Sakomura & Rostagno, 2016). The result was divided by the number of birds in each experimental unit to express feed consumption in grams per bird per day.

Egg Production (EP) - eggs were collected and recorded daily at 11 am and 4 pm, stored in trays. The average egg production was calculated based on the number of birds housed per experimental unit, adjusted for broody hens, and expressed as egg production per bird per day.

Egg Weight (EW) - all eggs from the three days preceding and completing each 28-day assessment period were individually weighed using a digital scale with a precision of 0.01g. The average egg weight (g) was calculated by dividing the total weight of collected eggs by the number of eggs collected per experimental unit.

Egg Mass (EM) - the total egg mass was calculated by dividing egg weight by egg production per bird per day, expressed in grams per bird per day.

Feed Conversion Ratio per dozen eggs (FCR/DzE) and per egg mass (FCR/EM) - initially, feed consumption was adjusted for mortality, and the corrected feed conversion ratio (FCRc) per dozen eggs was calculated based on the ratio between feed consumption and dozen egg production per experimental unit over each 28-day period. The feed conversion ratio per egg mass was determned by dividing feed consumption by egg mass, resulting in FCRc expressed in kg per DzE and kg per kg of egg mass.

Egg Shape Index - initially, egg size (mm) was determined using a digital caliper accuate to 0.01mm, resulting in the egg shape index, calculated as follows: Egg Shape Index (%) = (smallest diameter/largest diameter) x 100.

Specific Gravity - to determine specific gravity, all intact eggs collected over three days during each 28-day period were immersed in nine saline solutions (water + NaCl) with densities ranging from 1.060 to 1.100 g/cm³, at intervals of 0.05 kg/l. The density of each solution was measured using a densimeter (Araujo & Albino, 2011).

Egg Yolk Color - assessment of egg yolk color was performed using a subjective analysis method with the DSM® yolk color fan, as described in the DSM® yolk pigmentation guide. The assessment was conducted on a flat, non-reflective glass surface by a single evaluator.

Egg Component Weight (ECW) - initially, each intact egg was weighed, then broken to separate the yolk, albumen, and shell. Yolks and albumens were weighed individually, while shells were washed under running water, dried in an oven at 65°C for 24 hours, and then individually weighed. The weight of each egg component was obtained by subtracting the weight of the yolk and albumen from the weight of the egg.

Eggshell Thickness - after being dried in an oven, eggshell thickness was measured using a digital caliper accurate to 0.01mm at both the vegetal and animal poles, and the equatorial region of the egg. Eggshell thickness was determined by calculating the arithmetic mean of these three measurements and expressed in millimeters (mm).

Haugh Unit – in order to determine the Haugh unit, eggs were weighed, cracked open, and their contents (albumen + yolk) placed on a flat, non-reflective glass surface to measure albumen height (mm). A digital caliper accurate to 0.01mm was used. The weight and albumen height values were used to calculate the Haugh unit (HU) applying the formula adapted by Barbosa (2004):

Equation: $HU = 100 \log (h + 7.57 - 1.7W^{0.37})$

where:

h - Albumen height (mm)

W - Egg weight (g)

Percentage of Mineral and Organic Matter in Eggshells - after drying and weighing, eggshells were grouped by experimental unit and period (every 28 days), and ground using a ball mill. The method described by D. J. Silva and Queiroz (2002) was used to determine dry matter and mineral matter content.

Bone Strength - at the end of each experimental period (84 days), one bird from each experimental unit was euthanized by cervical dislocation, the left tibias removed, and the flesh stripped from the bone without damaging it or cartilage. Tibia weight, length and width were measured using a digital balance and caliper. The tibias were then frozen at -20°C. Bone strength was assessed using a texture analyzer (TexturePro CT®) following the test model: compression, test target = distance, reference value = 10, trigger load = 10g, and speed = 3 mm/s, using the TA-TPB device. A computer program recorded the force (kg) required to completely break the bone.

Percentage of Dry Matter, Mineral Matter, and Bone Density - after bone strength analysis, left tibias were dried in a forced air oven at 105°C for 72 hours, then reweighed to obtain dry bone and dry matter weight, following the methodology described by Kim et al. (2004).

For mineral matter analysis, the bones used to determine dry matter were ground in a ball mill and then calcined in a muffle furnace at 600°C for four hours. The ashes were then weighed to determine the percentage of ash based on dry matter, in accordance with Detmann et al. (2021). The Seedor index (SI) was determined to indicate bone density by dividing bone ash weight (mg) by its length (mm) (Seedor et al., 1991).

Percentage of Calcium and Phosphorus in Eggshells and Bones - calcium and phosphorus analysis of eggshells and bones used the powder from dried and ground eggshells and fresh bone meal. All samples were analyzed in duplicate using an X-ray fluorescence (XRF) spectrometer equipped with a silver anode X-ray tube, silicon drift detector (SDD), with a maximum voltage of 50 kV, 200 µA current, and power of 2 W. The equipment has four filters: main, low, high, and light, each sensitive to a set of chemical elements. Samples were placed in a 32 mm collimator with a 4 µm-thick polypropylene film and analyzed under an atmosphere for 105 seconds, using the spotless mode (irradiation area from 7 to 50 mm²).

Data were submitted to analysis of variance and the mean comparison test using PROC NLIN of the SAS® OnDemand for Academics, Free online version (Statistical Analysis System Institute [SAS Institute], 2024), followed by regression analysis. Means of the sum of the score of the variables in each treatment were compared using the Friedman chi-square test. A significance level of $\alpha = 0.05$ was established.

Results and Discussion

Average minimum and maximum temperatures, relative humidity, and black globe temperature and humidity index (BGTHI) during the experimental period were 24.3 to 31.18°C, 61.29 to 99%, and 80.09 to 81.32, respectively (Table 2). These data indicate that the birds were exposed to temperatures and humidity levels outside the thermoneutral range. According to Medeiros et al. (2005) and Abreu and Abreu (2011), ambient temperatures around 20°C, relative humidity between 60 and 70%, and BGTHI ranging from 69 to 77 are ideal for healthy adult birds to express their maximum productive potential.

The environmental data obtained in the present study demonstrate high temperatures, relative humidity, and BGTHI values. In the free-range system, climatic conditions are not 100% controllable, meaning it cannot be affirmed that when Canela-Preta free-range laying hens are exposed to thermal comfort conditions, they would exhibit better performance and egg quality.

For performance variables (Table 3), there was no interaction between dietary calcium levels and bird age ($P > 0.05$). Thus, it was observed that dietary calcium levels did not influence ($P > 0.05$) feed consumption, percentage egg production, egg weight, egg mass, feed conversion per dozen eggs, and per egg mass during the experimental period, indicating that even at the 2.3%

calcium level, the nutritional requirements for Canela-Preta egg production during the laying period between 28 and 36 weeks of age were met.

On the other hand, the collection period influenced ($P \leq 0.05$) all the parameters assessed. Thus, higher feed consumption was obtained in both the 32nd and 36th weeks, with similar values and higher than those recorded in the 28th week ($P \leq 0.05$).

The results are consistent with those reported by Vargas et al. (2003), highlighting that birds primarily regulate consumption according to the energy demands of the body in general. Birds have the ability to regulate calcium consumption to meet their needs, and calcium-deficient diets tend to increase consumption. This may explain why there was no change in feed consumption based on calcium levels, since the experimental diets were isoenergetic, and the 2.3% calcium level in the diets met the birds' requirements.

Improved feed conversion ($P < 0.05$) was observed in the 28th and 32nd weeks of age for a dozen eggs and egg mass. Another variable with a higher value was egg weight (51.54g), which stood out in the 32nd week compared to other periods.

Lower egg production percentage (Table 3) was observed at 36 weeks ($P \leq 0.05$) when compared to the 28th and 32nd weeks, which were similar ($P > 0.05$). The decline in egg production percentage is consistent with the findings of Lemos et al. (2014), who observed that as laying hens age, the interval between ovulations increases, leading to a decrease in the laying rate accompanied by an increase in egg size, given that the same amount of yolk from hepatic synthesis is deposited in fewer follicles. This lower egg production percentage may indicate that Canela-Preta free-range laying hens reached their peak production at 32 weeks of age, similar to improved breeds such as EMBRAPA 051 (Alves et al., 2021).

Egg weight was not affected ($P > 0.05$) by the dietary calcium level but peaked at 51.54 g at 32 weeks. It should be noted that factors such as genetics, nutrition, bird age, and laying period can influence egg weight (Galeano-Vasco et al., 2018).

According to the weight criteria guideline no. 634/2022 of the Agriculture Defense Department, eggs are classified into six types: 1) jumbo – weighing 66 g and higher; 2) extra – 60 to 65.99 g; 3) large – 55 to 59.99 g; 4) medium – 50 to 54.99 g; 5) small – 45 to 49.99 g; and 6) very small – less than 45 g (MAPA, 2022). Based on the the aforementioned egg classification and considering the values obtained (Table 3), Canela-Preta free-range laying hens eggs at 28 weeks of age fall into the small category, while at 32 and 36 weeks, they are considered medium-sized. It is important to underscore that these birds did not undergo any artificial selection process for this characteristic; nevertheless, they produce eggs with standard commercial weights.

Table 3
Productive performance of Canela-Preta free-range laying hens fed different dietary calcium levels and grazing on Bahia grass

Feed consumption, g/kg							
Laying hens age	Calcium levels, %				Means	P value Linear	P value Quadratic
	2.3	3.0	3.7	4.4			
28th week	98.92	96.20	93.49	92.33	95.24^{b}		
32th week	99.39	96.81	101.74	98.60	99.14^{a}		
36th week	94.41	98.90	102.68	97.90	98.47^{a}		
Means	97.58	97.31	99.30	96.28		0.7851	0.2982
		CV, %			6.11		
Egg production, %							
	2.3	3.0	3.7	4.4			
28th week	48.12	57.86	47.36	62.83	54.04^{a}		
32th week	54.27	46.89	46.62	48.67	49.11^{a}		
36th week	48.16	44.61	36.17	40.14	42.27^{b}		
Means	50.18	49.79	43.38	50.55		0.5993	0.0593
		CV, %			17.88		
Egg weight, g							
	2.3	3.0	3.7	4.4			
28th week	49.70	50.30	49.54	47.50	49.26^{b}		
32th week	52.42	50.76	51.69	51.30	51.54^{a}		
36th week	50.56	49.06	50.27	50.22	50.03^{b}		
Means	50.89	50.04	50.50	49.67		0.1722	0.2668
		CV, %			3.96		
Egg mass, g							
	2.3	3.0	3.7	4.4			
28th week	23.96	29.11	23.34	29.82	26.56^{a}	0.3974	0.1247
32th week	28.31	23.87	23.97	24.99	25.28^{a}		
36th week	24.30	21.75	18.30	20.13	21.12^{b}		
Means	25.52	24.91	21.87	24.98			
		CV, %			19.34		
Feed conversion/Dozen eggs, kg/Dz							
	2.3	3.0	3.7	4.4			
28th week	2.59	2.36	2.56	1.97	2.37^{b}	0.8692	0.2425
32th week	2.45	2.86	2.73	2.55	2.65^{a}		
36th week	2.64	2.85	3.51	2.80	2.95^{a}		
Means	2.56	2.69	2.93	2.44			
		CV, %			CV (%)	22.87	

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Feed conversion/Egg mass, kg/kg							
	2.3	3.0	3.7	4.4			
28th week	4.34	3.45	4.23	3.11	3.78[b]	0.5032	0.1810
32th week	3.60	4.60	4.23	4.03	4.12[b]		
36th week	3.96	4.72	6.21	5.02	4.98[a]		
Means	3.96	4.26	4.89	4.05			
		CV, %			26.73		

CV=coefficient of variation; means followed by the same letter on the same line do not differ according to the LSM test.

Laying hens age influenced (P ≤ 0.05) feed conversion per dozen eggs and per egg mass. The findings in this study demonstrate that the 2.3% calcium level was sufficient to meet the mineral demand for these laying hens between 28 and 36 weeks of age. This is significant since reducing calcium levels contributes to lowering feed costs, even though calcium is not one of the costliest nutrients.

There was no interaction between egg quality parameters (Table 4) and collection periods (P > 0.05). Dietary calcium levels did not influence (P > 0.05) egg length (mm), specific gravity (g cm3), albumen percentage, shell thickness (mm), shape index (%), and Haugh unit.

Table 4
Canela-Preta free-range egg quality, in laying hens aged between 28 and 36 weeks, fed different dietary calcium levels

Egg length, mm							
Age of laying hens	Calcium levels, %				Means	P value Linear	P value Quadratic
	2.3	3.0	3.7	4.4			
28th week	53.89	54.32	53.28	53.32	53.70b		
32th week	54.18	54.22	53.13	53.83	53.84ab		
36th week	50.50	53.77	54.48	54.27	54.26a		
Means	54.19	54.10	53.63	53.81		0.0890	0.2713
		CV, %			1.4		
Egg width, mm							
	2.3	3.0	3.7	4.4			
28th week	40.57	40.56	40.16	39.73	40.26	0.0271	0.8123
32th week	40.53	40.03	40.23	40.34	40.28a		
36th week	40.59	40.59	40.44	40.01	40.41a		
Means	40.56	40.40	40.28	40.03			
		CV, %			1.60		
Shape index, %							
	2.3	3.0	3.7	4.4			
28th week	75.30	74.92	75.38	74.59	75.05a	0.5712	0.3130
32th week	74.89	73.90	75.86	75.00	74.92a		
36th week	74.52	75.56	74.28	73.86	74.56a		
Means	74.90	74.79	75.18	74.48			
		CV, %			1.78		
Yolk color							
	2.3	3.0	3.7	4.4			
28th week	7.18	6.30	5.93	6.59	6.50a	0.7184	0.0120
32th week	6.67	5.59	6.49	6.83	6.39a		
36th week	6.81	5.98	6.96	7.11	6.72a		
Means	6.89	5.96	6.46	6.84			
		CV, %			13.25		
Yolk, %							
	2.3	3.0	3.7	4.4			
28th week	32.91	31.96	31.40	32.52	32.20a	0.0076	0.0.0026
32th week	32.81	33.78	30.25	32.64	32.37a		
36th week	32.62	33.18	31.48	30.78	32.02a		
Means	32.78	32.97	31.05	31.98			
		CV, %			4.10		

continue...

continuation...

			Albumen, %				
	2.3	3.0	3.7	4.4			
28th week	60.31	60.53	61.06	60.87	60.69[a]	0.6201	0.1891
32th week	59.42	59.77	62.35	61.31	60.72[a]		
36th week	60.05	58.59	59.06	57.73	58.86[b]		
Means	59.93	59.63	60.82	59.97			
		CV, %			3.81		
			Haugh unit				
	2.3	3.0	3.7	4.4			
28th week	91.96	91.93	93.01	92.82	92.43[a]	0.4944	0.5027
32th week	93.13	92.87	95.25	93.21	93.62[a]		
36th week	90.88	90.78	90.56	91.60	90.95[b]		
Means	91.99	91.86	92.94	92.54			
		CV, %			3.71		
			Eggshell, %				
	2.3	3.0	3.7	4.4			
28th week	9.52	9.32	9.88	9.21	9.48[a]	0.6593	0.0285
32th week	8.74	8.60	9.32	8.82	8.80[b]		
36th week	9.18	9.72	9.65	8.92	9.32[a]		
Means	9.15	9.22	9.62	8.72			
		CV, %			5.85		
			Eggshell thickness, mm				
	2.3	3.0	3.7	4.4			
28th week	0.45	0.46	0.48	0.45	0.46[a]	0.3983	0.1350
32th week	0.46	0.43	0.50	0.48	0.47[a]		
36th week	0.39	0.42	0.39	0.38	0.40[b]		
Means	0.43	0.44	0.46	0.44			
		CV, %			7.76		
			Specific gravity, g/cm^3				
	2.3	3.0	3.7	4.4			
28th week	1.093	1.092	1.095	1.093	1.093[a]	0.4329	0.0880
32th week	1.092	1.091	1.096	1.093	1.093[a]		
36th week	1.084	1.088	1.088	1.084	1.086[b]		
Means	1.090	1.090	1.093	1.090			
		CV, %			0.34		

CV=coefficient of variation; means followed by the same letter on the same line do not differ according to the LSM test.

Egg width (EW) decreased ($P \leq 0.05$) with a rise in dietary calcium (x) according to the equation: $EW = -0.2443x + 41.136$ ($R^2 = 0.9768$). Yolk color (YC), yolk percentage (YP), and shell percentage (SP) exhibited quadratic effects according to their respective equations: $YC = 0.6684x^2 - 4.4281x + 13.461$ ($R^2 = 0.7837$; minimum point = 3.31%); $YP = 0.3776x^2 - 3.1467x + 38.268$ ($R^2 = 0.4652$; minimum point = 4.17%); $SP = -0.3929x^2 + 2.5907x + 5.1981$ ($R^2 = 0.5985$; maximum point = 3.30%).

For egg specific gravity, albumen percentage, shell thickness, and Haugh unit, weeks 28 and 32 obtained similar values ($P > 0.05$) and were higher than those at 36 weeks ($P \leq 0.05$). With respect to eggshell percentage, the 28^{th} and 36^{th} weeks showed similar values ($P > 0.05$), which were higher than those at 32 weeks.

In the present study, egg length and width varied across different dietary calcium levels, ranging between 53.63 and 54.19 mm long and 40.03 and 40.56 mm wide, respectively, which are close to the results reported by Almeida et al. (2019) in native birds, with egg lengths and widths for Caneluda chickens at 58.13 and 44.29 mm, Barbuda chickens at 56.01 and 42.44 mm, and Peloco chickens at 54.08 and 41.19 mm.

The Canela-Preta free-range laying hens in this study showed an average shape index of 74.8% (Table 4). Thus, it can be affirmed that these eggs exhibit a normal shape and do not pose packaging problems, thereby reducing transport losses.

Yolk pigmentation decreased up to the 3.31% dietary calcium level and increased thereafter, while bird age had no influence on yolk color, which ranged from 5.96 to 6.89 on a scale of 1 - 15 on the DSM® yolk color fan scale. These results were unexpected due to the available forage, leading to higher carotenoid intake for laying hens aged 28 to 32 weeks.

Eggs produced by birds in alternative systems can have more pigmented yolks (7 to 10) because they have access to carotenoid-rich green forage. It is important to underscore that yolk color is highly appreciated by consumers, who prefer eggs with a yolk color above eight, that is, yellow-orange, on the DSM colorimetric fan scale (Fassani et al., 2019).

Laying hens age influenced ($P > 0.05$) albumen percentage, with a decline observed at 36 weeks of age. With respect to yolk percentage, dietary calcium decreased to 4.17% dietary calcium, while for the shell percentage, it increased to 3.30%. Literature reports emphasize that as a laying hen ages, in addition to egg weight, yolk percentage rises while albumen and shell percentages decline due to lower intestinal absorption and calcium mobilization capacity (Garcia et al., 2010).

Although dietary calcium levels did not influence the eggshell thickness of Canela-Preta free-range laying hens, bird age did, whereby thickness decreased from 0.47 to 0.40 mm between the ages of 32 to 36 weeks. B. L. Oliveira and Oliveira (2013) underscore that eggshell thickness is related to genetics, hen age, weight, and egg size, and that thicker shells are stronger.

According to Vellasco et al. (2016), increased dietary calcium availability is reflected almost exclusively in the external egg quality, that is, shell quality, a fact observed in this study where eggshell quality improved up to the 3.30% dietary calcium level.

In regard to specific gravity, B. L. Oliveira and Oliveira (2013) consider densities between 1.080 and 1.084 g/cm^3 as normal, whereas values above and below denote excellent and poor shell quality, respectively. The specific gravity values for Canela-Preta free-range laying hens aged 28 to 36 weeks (Table 4), ranging between 1.090 and 1.093, characterize these eggs as having excellent shell quality.

The Haugh unit (HU) was not affected ($P > 0.05$) by dietary calcium levels. In general, this parameter is used worldwide to determine internal egg quality, where higher HU values indicate better egg quality. Eggs are classified as low, medium, high, and excellent quality, represented by values of 0 to 29, 30 to 59, 60 to 71, and 72 to 100, respectively (United States Department of Agriculture [USDA], 2000; B. L. Oliveira & Oliveira, 2013).

The results of shell breakage resistance, mineral matter, organic matter, calcium, and phosphorus of Canela-Preta free-range laying hens fed diets with different calcium levels, at ages between 28 and 36 weeks, are shown in Table 5.

Table 5

Means of eggshell breaking strength (EBS), mineral matter (MM), organic matter (OM), calcium (Ca) and phosphorus (P) of Canela-Preta free-range laying hens, from 28 to 36 weeks and fed different dietary calcium levels

Calcium levels (%)	Laying hens age in weeks			Means	CV (%)
	28 weeks	32 weeks	36 weeks		
Eggshell breaking strength (kg/f)					
2.3	3.63	4.43	3.56	3.78	22.99
3.0	3.68	3.22	4.03	3.64	
3.7	4.08	3.83	4.15	4.02	
4.4	3.28	3.95	3.70	3.64	
Means	3.67a	3.86a	3.66a		
P value – Linear				0.7727	
P value – Quadratic				0.1962	
Mineral Matter (%)					
2.3	53.72	53.92	53.51	53.72	0.55
3.0	53.99	53.36	53.08	53.48	
3.7	53.42	53.79	53.78	53.66	
4.4	53.83	53.44	53.88	53.72	
Means	53.74a	53.63a	53.56a		
P value – Linear				0.6046	
P value – Quadratic				0.1231	
Calcium (%)					
2.3	37.00	36.85	37.46	37.10	2.35
3.0	37.11	37.78	37.65	37.51	
3.7	37.52	37.62	37.76	37.63	
4.4	38.32	37.29	38.06	37.89	
Means	37.48a	37.39a	37.74a		
P value – Linear					
P value – Quadratic					
Phosphorus (%)					
2.3	0.210	0.256	0.234	0.233	19.10
3.0	0.214	0.242	0.186	0.214	
3.7	0.200	0.244	0.202	0.215	
4.4	0.232	0.214	0.210	0.218	
Means	0.214ab	0.239a	0.208b		
P value – Linear				0.3864	
P value – Quadratic				0.7034	

Means followed by the same letter on the same line do not differ according to the LSM test.

There was no interaction between dietary calcium levels and laying hens age (P > 0.05). Thus, it was observed that calcium levels influenced shell calcium percentage (P ≤ 0.05), according to the equation: CC = 0.3557x + 36.341 (R^2 = 0.9542), while the other parameters were not affected (P > 0.05). With respect to age, there was interference between the parameters assessed, with only shell phosphorus content affected, and the highest values found at 28 and 32 weeks, which did not differ (P > 0.05).

It should be noted that calcium declined significantly during the egg production process, considering that the eggshell represents 9 to 10% of egg weight, with the shell consisting of 90% minerals, 93% of which is calcium carbonate, 1% magnesium carbonate, 1% calcium phosphate, and 5% organic matter, including proteins, pigments, and others. Calcium is present in larger amounts, reaching up to 40% in powdered form (B. L. Oliveira & Oliveira, 2013; D. L. Oliveira et al., 2014).

For the bone parameters such as fresh bone weight (FBW), dry bone weight (DBW), bone length (BL), bone diameter (BD), bone breaking strength (BBS), mineral matter (MM), Seedor index (SI), calcium (Ca), and phosphorus (P) of Canela-Preta free-range laying hens tibia bone, assessed at the end of the experiment at 36 weeks of age and fed with different dietary calcium levels (Table 6), there was no interaction between calcium levels and laying hen age (P > 0.05).

Table 6
Canela-Preta free-range laying hens bone parameters with 36 weeks of age, fed different dietary calcium levels

Variables	Calcium levels (%)				Prob. ANOVA	Regression	Prob. regression	CV (%)
	2.3	3.0	3.7	4.4				
FEW (g)	14.79	16.88	15.22	15.15	0.4947	ns	0.5839	14.87
DEW (g)	9.30	10.42	9.39	10.04	0.4969	ns	0.8450	13.96
BL (mm)	118.90	124.50	122.30	122.09	0.6006	ns	0.5158	5.29
DO (mm)	7.31	7.48	7.07	7.31	0.7617	ns	0.9327	8.26
RQO (kgf)	32.44	30.88	30.64	31.97	0.9654	ns	0.8698	20.61
MM (%)	49.20	47.66	49.72	50.83	0.5386	ns	0.4140	6.89
ISEED (mg/mm)	38.43	38.26	38.14	41.98	0.6221	ns	0.4316	13.60
Ca (%)	16.29	16.27	17.17	17.74	0.5630	ns	0.3680	10.74
P (%)	5.26	5.35	5.61	5.93	0.6629	ns	0.4726	1610

FEW=Fresh egg weight; DEW=dry egg weight; BL=bone length; BD=bone diameter; BBS=bone breaking strength; MM=Mineral material; SI= Seedor index; Ca=Calcium; P=phosphorus; ns= not significant; CV=coefficient of variation.

In studies on the bone characteristics of broiler chickens, Oliveira et al. (2014) and Almeida Paz et al. (2009), reported decreases in bone weight, mineral matter percentage, and Seedor index with reduced dietary calcium levels.

The results obtained for the average percentage of calcium and phosphorus in fresh tibia bones were 32.97 and 13.17%, respectively, totaling 47% for these two minerals combined. According to Olgun and Aygun (2016) in their study with commercial birds, bone tissue stiffness results from calcium and phosphorus deposition, these two minerals being responsible for 70% of bone ash composition.

Overall analysis shows that bone density in the laying hens followed the egg production behavior, which was 50.18, 49.79, 43.38, and 50.55% for calcium levels of 2.3, 3.0, 3.7, and 4.4%, respectively. Study results have demonstrated the relationship between increased mineral, calcium, and phosphorus content with greater bone breaking strength, and that nutritional deficiencies may lead to lower egg production and worse quality, as well as increased bone calcium mobilization (Olgun & Aygun, 2016; Shim et al., 2012). In the present study, the first situation was observed, albeit with some instability, primarily in relation to phosphorus; however, no bone calcium mobilization was observed.

Conclusion

The 2.3% calcium level meets the egg-laying performance requirements of Canela-Preta laying hens, corresponding to the phase from 28 to 36 weeks of age, representing the age of optimal feed conversion per dozen eggs.

Dietary calcium levels do not interfere with the length, specific gravity, shell thickness and breaking strength, shape index, and Haugh unit of the egg. However, yolk percentage decreases to the 4.17% level, and eggshell percentage increases from 3.03% calcium inclusion in the diet

Bone parameters are not affected up to the 4.4% dietary calcium inclusion in Canela-Preta laying hens aged 28 to 32 weeks of age.

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