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Substitution of limestone for eggshell powder in the diet of Japanese laying quails

Substituição do calcário calcítico pela farinha de casca de ovos de codorna na dieta de codornas japonesas em postura

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ABSTRACT

Were evaluated the effects of substituting limestone with quail eggshell powder on the zootechnical performance, egg quality, and economic efficiency of Japanese quails during peak egg production (11–20 weeks old). The experimental period was 63 days, divided into three 20-day periods. A completely randomized experimental design was used with five treatments (0, 25, 50, 75, and 100% substitution of limestone by eggshell powder), six repetitions, and seven birds per experimental unit, for a total of 210 quails. Feed intake, production of egg/bird/day, egg production per bird housed, marketable egg production, egg viability, feed conversion per egg mass, Seedor index, tibia calcium content, and percentage of calcium excreted were determined. The following variables for egg quality were analyzed: egg weight, specific weight, percentage of egg yolk, albumen and shell, and shell thickness. Economic efficiency was evaluated in fresh and industrialized eggs. Statistical analyses were performed using the SAS software, version 9.2 (2010). Substitution of limestone for eggshell powder had no significant effect on zootechnical performance, tibia calcium content, or egg quality. However, there were significant effects on the percentages of albumen and calcium excreted. The substitution of limestone by eggshell powder in quail feed resulted in higher economic efficiency indexes. These results indicate that the use of 100% eggshell powder in the feed of Japanese laying quails is viable.

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Keywords: zootechnical performance, Seedor index, calcium content.

RESUMO

Com o objetivo de avaliar os efeitos da substituição do calcário pela farinha de casca de ovo de codorna sobre o desempenho zootécnico, a qualidade dos ovos e a eficiência econômica de codornas japonesas no pico de postura (11 a 20 semanas de idade), foram utilizadas 210 codornas em um período experimental com duração de 63 dias, divididos em três períodos de 21 dias cada. Foi utilizado o delineamento experimental inteiramente casualizado, composto de cinco tratamentos (0, 25, 50, 75 e 100% de substituição de calcário calcítico por casca de ovo), seis repetições e sete aves por unidade experimental. Avaliou-se o consumo de ração, produção de ovo/ave/dia, produção de ovo ave/alojada, produção de ovos comercializáveis, viabilidade, conversão alimentar massa de ovos, índice de *Seedor*, teor de cálcio na tíbia e porcentagem de cálcio excretado. Para avaliação da qualidade dos ovos, foram analisadas as seguintes variáveis: peso do ovo, peso específico, porcentagem da gema, de albúmen e de casca e espessura da casca. A eficiência econômica foi avaliada no ovo *in natura* e no ovo industrializado. As análises estatísticas foram realizadas utilizando o software SAS, 2010 (*Statistical Analysis System*, versão 9.2). Não houve efeito significativo da substituição do calcário calcítico por farinha de casca de ovo sobre o desempenho zootécnico, porcentagem de cálcio na tíbia e qualidade de ovos. Entretanto, a porcentagem de albúmen e a porcentagem de cálcio excretado foram alterados significativamente. A substituição do calcário pela farinha de casca de ovo nas rações resultou na melhora dos índices de eficiência econômica. É possível substituir em 100% a farinha de casca de ovo em rações para codornas japonesas em postura.

Palavras-chave: desempenho zootécnico, índice de *Seedor*, teor de cálcio.

INTRODUCTION

Quail production has assumed worldwide importance because of the potential for egg production (Silva et. al 2019). However, there is a lack of studies on quail nutrition to decrease economic production costs (Sarcinelli et al. 2020). In Brazil, the total production of quail eggs amounted to 297.3 million dozens in 2018 (STATISTA, 2020). Most of these eggs are processed and sold as preserved eggs. The commercialization of preserved quail eggs increases the profitability of the producer and is the main driver for consumption since it facilitates product distribution and access.

Egg quality is directly related to shell quality and consequently, to productivity, influencing the rate of marketable eggs, egg weight, and feed conversion. Calcium is an essential mineral for laying quails, which is linked to bone and eggshell formation.

The manufacturing of preserved quail eggs generates shell as a by-product. Quail eggshell represents 10% of the total egg weight, has a high calcium carbonate ($CaCO_3$) content, and a considerable content of protein; however, it has limited perceived value as a co-product of the egg processing

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industry. Therefore, eggshell disposal remains a problem for the industry, and the main destination is agriculture.
Although eggshell has economic potential for producers, the inappropriate disposal of shells can have negative environmental impacts. The substitution of limestone is an alternative to avoid negative environmental impacts due to the extraction of limestone rocks, since they are non-renewable reserves. An alternative use for eggshell is in animal feed as a source of calcium, substituting limestone. Quail eggshell powder is a raw material with great potential as a calcium source in animal feed, since it does not contain high levels of protein or sodium, which increase renal calcium excretion (Weinsier; Krumdieck, 2000). Moreover, the solubility of eggshell powder is higher than that of limestone, indicating higher bioavailability. Thus, eggshell powder may be an alternative for the use of solid waste in the egg processing industry, and an organic and renewable source of calcium in animal feed. The objectives of this study were to evaluate the effects of substituting limestone with quail eggshell powder on the zootechnical performance, egg quality, and economic efficiency of Japanese quails during peak egg production (11-20 weeks old).

MATERIAL AND METHODS

The experiment was conducted in the quail farming area of the Academic Department of Animal Science of the Federal Institute of Education, Science and Technology of Southeast Minas Gerais, Rio Pomba *Campus*, Minas Gerais, with the approval of the Animal Ethics Committee (Annex 1). The pre-experimental period (adaptation) lasted five days and the experimental period lasted 63 days, which was divided into three periods of 21 days each, from June to August 2017.
Quails were allocated treatment based on body weight (160.0 ± 7 g). Quails were housed in metallic cages in a brick shed. Each compartment was considered an experimental unit measuring 23.5 × 47.5 × 21 cm (width, depth, and height), with a density of 159.46 cm² per bird.
The eggshells were dried in a forced air circulation oven at 65°C for 24 h. Then, the shells were ground in a cutting mill with a 2 mm sieve. The mean values of total calcium and available phosphorus in the eggshell powder were 36.13 and 0.314%, respectively. A total of 210 Japanese quails within their laying peak (11–20-weeks old) were used in a completely randomized experimental design with five treatments (0, 25, 50, 75, and 100% of eggshell powder as a substitute to limestone), with six repetitions and seven birds per experimental unit.
The experimental feeds were prepared with corn and soybean meal, following the nutritional food characteristics and requirements for quails recommended by Rostagno et al. (2017). The diets were produced using the dilution technique, as previously described (SAKOMURA; ROSTAGNO, 2016).

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Table 1. Percent composition of the experimental feeds used in the treatments.

Ingredients	**Diet**	
	100% limestone	**100% Quail eggshell powder**
Corn	57.15	56.19
Soybean meal	31.03	31.00
Limestone	7.24	---
Eggshell powder[1]	---	8.41
Soybean oil	2.00	2.00
Dicalcium phosphate	1.27	1.09
DL-methionine	0.40	0.40
Salt	0.36	0.36
L-lysine	0.27	0.27
Vitamin-mineral supplement [2]	0.15	0.15
Choline chloride	0.10	0.10
L-tryptophan	0.02	0.02
BHT	0.01	0.01
Total	**100.00**	**100.00**
Calculated nutritional composition		
Metabolizable energy (kcal/kg)	2.832	2.800
Crude protein (%)	19.062	18.988
Digestible lysine (%)	1.105	1.106
Digestible methionine + cystine (%)	0.909	0.907
Digestible methionine (%)	0.478	0.478
Sodium (%)	0.177	0.177
Calcium (%)	3.157 (3.44*)	3.150 (3.74*)
Available phosphorus (%)	0.327 (0.47*)	0.327 (0.46*)
Digestible tryptophan (%)	0.232	0.232
Digestible threonine (%)	0.640	0.637

[1] Ca = 36.13%; P = 0.314%
[2] Vitamin-mineral supplement per kg of the product: vit. A 8,000.00 IU; vit. B1 1,000.00 mg; vit. B2 3,000.00 mg; vit. D3 2,100,000 IU; vit. E 7,000 IU; vit. B6 700.00 mg; vit. B12 6,000.00 mcg; vit. K3 2,000.00 mg; biotin 10.00 mg; niacin 20.00 g; folic acid 100.00 mg; pantothenic acid 10,000.00 mg; cobalt 100.00 mg; copper 6,000.00 mg; iron 50.00 g; selenium 200.00 mg; iodine 1,000.00 mg; manganese 55.00 g; zinc 22.50 g.
*Nutritional composition analyzed.

Table 2. Levels of quail eggshell powder (QESP) and limestone (CL) added to the experimental diets.

Diet, %	Levels of quail eggshell powder added				
	0%	25%	50%	75%	100%
100% CL	100	75	50	25	0
100% QESP	0	25	50	75	100
Total	100	100	100	100	100

The cages contained had polyvinyl chloride (PVC) feeders with the same length as the experimental unit, and nipple drinkers (one nipple: seven birds), with the feeder and drinker placed at the front and back of the unit, respectively.

Water was offered *ad libitum* during the entire experiment, and feed was given three times a day (07:00 AM, 1:00 PM, and 5:00 PM).

Temperature and humidity were controlled throughout the experiment with curtains and monitored using a six thermometers, and a dry and wet bulb thermometers. Thermometers reading were taken daily, twice a day (8:00 AM and 4:00 PM) during the experimental period.

The light cycle was controlled by an automatic timer throughout the experimental period, allowing 16 h of light daily, as used in commercial farms.

At the end of each experimental period, the remaining feed in each parcel was weighed and deduced from the amount of feed provided to calculate feed intake. If any quails died during the study period, the mean intake was deduced and corrected, in order to obtain accurate feed intake for the experimental unit.

Feed conversion per dozen eggs was calculated by the relation of total feed intake in kilograms per 12 eggs produced (kg/dz), and feed conversion per egg mass was calculated by feed intake in kilograms by the number of eggs produced multiplied by the mean weight (kg/kg).

Bird mortality was determined daily; therefore, the bird viability rate was obtained at the end of the experimental period, calculated as the difference in number of live and dead birds, and converted into a percentage.

Egg production was calculated by the number of eggs produced, including broken, cracked, and abnormal (i.e., soft or absent shell) eggs, divided by the mean number of birds in the period (egg/bird/day), and then divided by the number of housed birds at the beginning of the period (egg/housed bird), both expressed as a percentage.

Marketable egg production was determined by the number of broken, cracked, and abnormal eggs deduced from the total number of eggs produced. Then, the production of intact eggs was divided by the mean number of birds during the period (marketable eggs/bird/day), and expressed as percentage.

All intact eggs produced in each repetition were weighed in the last 3 days of each 21-day period to obtain the mean weight (g). The mean weight of the eggs was multiplied by the production of eggs/bird/day to obtain the total mass of eggs (g/bird/day).

To analyze the physical quality of eggs, four eggs from each repetition were weighed individually to the nearest 0.0001 g on days 19, 20, and 21 of each 21-day period.

Specific egg weight was determined on days 19, 20, and 21 of each 21-day period by immersion in saline solution with densities ranging from 1.055 to 1.095 g/cm³, at intervals of 0.005 g/cm³, calibrated with a densimeter as described by Oliveira (2013).

Subsequently, the egg yolk and albumen were separated. The yolks were weighed to the nearest 0.0001 g. The albumen weight was calculated based on the difference between egg weight minus the yolk and shell weight, and was the weight obtained after the shells were washed and dried in a forced air circulation oven at 65°C for 24 hours.

The percentage of each component, yolk, albumen, and shell, was calculated using the equation: percentage of component (%) = (component weight/egg weight) × 100.

Shell thickness was measured with a digital micrometer after the shell was weighed. Shell fragments were measured at both poles and in the middle of the egg. Shell thickness in each repetition was determined by the arithmetic mean of the three measurements.

Excreta were collected for further analysis by installing trays covered with plastic sheets under the cages and used to analyze the percentage of calcium. The material was collected in the last 3 days of each experimental period, with the addition of 1% synthetic iron oxide (Fe_2O_3) in the feed on the first and last day of collection to trace the beginning and the end of the collection period; these were determined by the reddish color of the excreta.

After feathers, feed residue, and other sources of contamination were removed, the excreta collected in each experimental unit were transferred to labelled plastic bags, weighed, and stored in freezer (-16°C) until the end of the collection. Then, the excreta were thawed, weighed, and homogenized, and 150 g aliquots were taken from each repetition for pre-drying in a forced air circulation oven at 55°C for 72 h.

The samples were then ground and stored until analysis. For calcium analysis, the samples were dried in oven at 105°C for 1 h. Then, 0.2 g of the sample was digested with 6 mL of nitric acid + perchloric acid (4:1) in a digestion block at 210°C for 4 h. The extract was transferred to a 500 mL volumetric flask, and the volume was increased using distilled water. An aliquot of 0.5 mL was taken and diluted to 10 mL with a solution of lanthanum oxide and measured on AAS. All analyses were performed in triplicate.

At the end of the experimental period, three birds from each treatment group were euthanized by cervical dislocation and the right and left tibia were collected. The left tibias, identified by treatment and repetition, with adjacent cartilages and without muscular tissue, were placed in a forced air circulation oven (65°C) for 72 h, and then pre-defatted in a Soxhlet extractor for 4 h. Next, the samples were ground in a ball mill, weighed to the nearest 0.0001 g, and stored in a freezer. Subsequently, the tibias were dried in an oven at 105°C for 16 h for quantification of dry matter. Samples were then calcined at 600°C for 4 h to determine calcium levels, which were expressed as a percentage of dry matter of the pre-defatted tibia.

Prepared right tibias were evaluated to determine weight (mg) in an electronic scale to the nearest 0.0001 g; and the longest length (mm), using a digital pachymeter (100-179J Digimess®). Using these data, the Seedor index (mg/mm) was obtained by dividing bone weight (mg) by length (mm) (SEEDOR, QUARTUCCIO; THOMPSON, 1991).

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Analysis of the economic viability of QESP inclusion considered the cost of feed when all other costs were the same among experimental treatments. The cost of feed/diet to produce 1 kg of eggs was determined by considering the quantity of feed necessary to produce 1 kg of eggs, and the price per kg of diet/feed. Economic feasibility was analyzed by adapting the "Economic Feed Efficiency" (EFE) index proposed by Houndonougbo et al. (2009) for both the marketing of fresh and industrialized eggs. The EFE equation is composed of feed intake (kg), feed price, egg production (kg), and egg price (per kg). The higher the EFE index value, the better the relative cost-benefit ratio of production. The index was calculated as: EFE = (kg egg produced × egg price/kg) ÷ (feed intake × price/kg feed). The costs of the ingredients used in the experimental diet formulations were those quoted during the experimental period in the region of Rio Pomba, MG.

The data were analyzed by analysis of variance using a General Linear Model (PROC GLM) in the SAS software (version 9.2) at the 5% significance level.

When significant differences were found, the levels of eggshell powder were analyzed by linear and/or quadratic regression, depending on the best fit of the model to the data.

RESULTS AND DISCUSSION

The thermal comfort temperature for quails is 22–24°C, with relative humidity around 60%. (Castro et al., 2017). The mean minimum and maximum temperatures and relative humidity measured daily during the experiment were, respectively, 13.9°C ± 1.9; 24.5 ± 2.0°C; and 73.5 ± 9.4%. Although quails were subjected during the experimental period to a little cold stress, zootechnical performance and egg quality was not influenced ($p>0.05$).

No significant difference ($p>0.05$) on zootechnical performance was observed following the substitution of limestone for eggshell powder (Table 3). These results confirm the nutritional compatibility between limestone and eggshell powder, and demonstrate the potential use of eggshell powder in the diet of laying quails.

Table 3. Zootechnical parameters of Japanese quails fed with feed substituting limestone with eggshell powder.

Parameter	Levels of limestone substituted for quail eggshell powder	p value	CV (%)

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	0%	25%	50%	75%	100%		
Feed intake (g/bird/day)	28.53	29.54	29.18	28.57	27.49	0.0906	4.43
Production of egg/bird/day (%)	96.91	97.32	93.79	95.50	94.23	0.1459	2.74
Production of egg/ housed bird (%)	95.08	97.32	91.35	95.50	93.26	0.0882	3.90
Production of marketable egg (%/bird/day)	94.00	97.28	90.44	95.31	92.81	0.0758	4.33
Viability (%)	99.74	100.00	99.47	100.00	99.47	0.3393	0.60
Feed conversion (kg/dozen)	0.359	0.365	0.386	0.359	0.362	0.3223	6.70
Feed conversion (kg/kg)	2.800	2.882	3.055	2.813	2.818	0.1993	7.10
Egg mass (g/bird/day)	10.23	10.27	9.71	10.17	10.07	0.2912	4.82
Seedor index	12.64	11.18	11.58	11.84	10.20	0.1597	9.37

CV = Coefficient of variation.

The total substitution of limestone with eggshell powder in laying quail feed was recommended by Ribeiro et al. (2016). Those authors noted that it was possible to substitute up to 100% limestone with eggshell powder in the diet of Japanese quails aged 11–25 weeks, with no reduction in the productive performance. This supports the results of Reis et al. (2012), who reported that substitution of limestone with eggshell powder in the diet of Japanese quails from 40 to 52 weeks of age had a significant effect on egg and marketable egg production. Those authors concluded that the inclusion of up to 50% eggshell powder in the feed of Japanese laying quails had no effect on productive performance. Olgun et al. (2015) concluded that eggshell powder can be used as a calcium source without damaging zootechnical performance and shell quality in Hy-Line W36 hens at 25 weeks of age.

Seedor index is a bone density indicator, and the higher the value, the greater the tibial bone density. In this study, the treatments had no influence on the Seedor index, indicating complete bone fill. Sousa (2016) studied Japanese quail during the post-peak egg laying period, and reported Seedor index values of 15.88 in the right tibia, consistent with the values found in the present study, at 12.63 in the limestone treatment group (Table 3).

The substitution of limestone for eggshell powder had no effect ($p>0.05$) on egg quality characteristics, such as egg weight, specific weight, yolk percentage, and shell percentage and thickness (Table 4). However, a positive linear effect was found on albumen percentage ($60.912 + 0.0219X$). These results support those reported by Gongruttananun (2011), who studied the substitution of limestone for eggshell powder in the diet of laying hens, and found no significant difference on egg weight, shell percentage and thickness, and specific weight, indicating that limestone can be completely substituted by eggshell powder. Additionally, Reis et al. (2012) did not reported any significant difference in the external quality of eggs, in terms of egg weight, specific weight, yolk percentage, and shell weight and percentage. These results did not support previous findings relating to albumen percentage, for which Gongruttananun (2011) and Reis et al. (2012) reported no significant difference.

Table 4 – Parameters of egg quality in Japanese quails fed with feeds substituting limestone for eggshell powder.

Parameter	**Levels of limestone substituted by quail eggshell powder**					**p value**	**CV (%)**
	0%	**25%**	**50%**	**75%**	**100%**		
Egg weight (g)	10.81	10.68	10.53	10.65	10.71	0.4667	2.23
Specific weight (g/cm^3)	1.075	1.074	1.074	1.072	1.073	0.7736	0.14
Yolk (%)	30.59	30.04	30.19	30.32	29.98	0.5021	2.13
Albumen (%)*	60.68	61.88	61.69	62.49	63.21	<0.0001	0.83
Shell (%)	8.13	8.04	8.05	7.90	7.98	0.3983	2.56
Shell thickness (mm)	0.290	0.296	0.293	0.288	0.290	0.1790	2.00

* $y = 60.912 + 0.0219X$. $p<0.05$
CV = Coefficient of variation.

The results found here on egg quality characteristics and zootechnical performance are consistent with those reported by Yasothai and Kavithaa (2014), who evaluated eggshell powder as a source of calcium in laying hen diets. Those authors concluded that body weight gain, egg production, egg weight, shell percentage, and thickness did not significantly differ between the different treatment groups supplemented with oyster shell flour, limestone, and eggshell.

The substitution of limestone for eggshell powder had no significant effect on the percentage of calcium in the tibia; however, there was a significant difference ($p<0.05$) in the percentage of calcium excreted (Table 5).

Table 5 – Percentages of calcium in the tibia and excreted in Japanese quails fed with feeds substituting limestone with eggshell powder feed.

Parameter	**Levels of limestone substituted for quail eggshell powder**					**p value**	**CV (%)**
	0%	**25%**	**50%**	**75%**	**100%**		
Calcium in tibia (%)	18.76	20.61	20.62	20.04	20.57	0.0827	4.08
Calcium excreted (% in DM*)	7.24	6.71	7.20	7.71	7.98	0.0004	6.02

*$y = 6.8683 + 0.01X$. $P<0.05$
CV = Coefficient of variation.
DM = Dry matter

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Reis et al. (2012) substituted limestone for eggshell powder and found no significant effects on the percentage of calcium in the bones of Japanese quails at 40–52 weeks of age. They concluded that the level and source of calcium efficiently maintained quail bone integrity.

The percentage of calcium in quail excreta increased linearly (y = 6.8683 + 0.01X) when quails were fed with feed containing eggshell, with 100% eggshell resulting in the highest level of calcium excretion. The maximum inclusion of quail eggshell powder increases the excretion of calcium by a percentage unit, therefore, this increase is not expressible from an environmental point of view when compared with the amount produced from the quail eggshell powder residue.

Table 5 presents the index of economic efficiency for fresh and industrialized eggs. The results show that, in this study, the inclusion of eggshell powder in the feed increased economic efficiency.

Except for albumen percentage and calcium excretion, no significant differences were found for productivity and egg quality; thus, the economic criterium is decisive for the use of eggshell powder in laying quail farming. In both, nutritional and economic aspects, the total substitution of limestone for eggshell powder in the feed of laying Japanese quails is viable.

Table 6 – Economic analysis of Japanese quail feeds with substitution of limestone for eggshell powder.

Parameter	Levels of quail eggshell powder added				
	0%	**25%**	**50%**	**75%**	**100%**
EFE fresh egg	1.75	1.71	1.65	1.78	1.79
EFE industrialized egg	3.93	3.84	3.70	4.00	4.03

EFE = Economic Feed Efficiency index (kg produced egg × price per kg egg)/(feed intake × price kg feed)

CONCLUSIONS

In conclusion, these results indicate that eggshell powder can replace limestone in the feed of laying Japanese quails by up to 100%. This substitution is economically viable and does not compromise zootechnical indexes.

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