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Calcareous seaweed in the diet of growing Japanese quail

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In this way, the present study assessed the replacement of inorganic calcium sources by organic sources in the diet of growing Japanese quails. The study analyzed 300 Japanese quails distributed in a completely randomized design with 3 treatments (control, 0% inclusion; 0.50% inclusion of calcareous seaweed 1; 0.50% inclusion of calcareous seaweed 2, with both inclusions concerning the replacement of calcitic limestone in the diet). Calcium sources did not differ in terms of either the production performance of birds in the growth phase or at the beginning of laying or in terms of yield, weight, organ size or bone quality. The biochemical blood profile did not change, except for the AST index. The replacement treatment affected egg quality, colorimetric fan color, albumen and yolk height, Haugh unit, and the yolk index. Including 0.50% calcareous seaweed as a replacement for calcitic limestone in the diet of quail does not harm production performance, animal health, organ yield, or bone quality in the growth phase. This diet guarantees satisfactory performance and high-quality eggs in the early laying phase.

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Quail farming has aroused the interest of producers and researchers due to its productive functionality, demanding low investments by using a small area and reduced labor. Among the main production aspects of this type of poultry, nutrition stands out. The vast number of options for ingredients with macro- and micronutrients available on the market has drawn attention to the search for alternative means of accessing organic sources for the purpose of supplying minerals [Araújo *et al*. 2012].

In the diet of laying birds, calcium and phosphorus are the most important minerals required for the best function of the reproductive system[Spanivello *et al.* 2022]. These elements are necessary in the growth phase, as they act as protagonists in the formation of the bone mineral matrix and in the production of eggs in the laying phase [Faria *et al*. 2020]. Due to these functions, the nutritional needs for calcium and phosphorus must be met, and these nutrients must be available in greater amounts and proportions in the diet depending on age, race, category, physiological situation, and the production system adopted [Gomes *et al*. 2004, Hakami *et al*. 2022, De Oliveira *et al*. 2023].

The minerals used in animal feed can be inorganic (rocks) or organic (bone meal, shells, or algae). In laying activities, mainly calcium sources from rocks, such as limestone and dicalcium phosphate, are included in the diet, as they have lower costs and are more available for use [Badeca *et al*. 2022].

However, inorganic sources of calcium are nonrenewable mineral resources, and their extraction has a great environmental impact. In this context, organic sources may supply the need for calcium in the diet of birds due to the increased bioavailability of nutrients [Melo *et al*. 2008]. Moreover, animals easily absorb calcium from this type of source, which is not ionic antagonistic [Carvalho *et al*. 2016].

One of the organic sources is seaweed flour, which may be obtained from the skeleton of the seaweed *Lithothamnium calcareum*. It is a rich source of calcium carbonate that is also contained in its magnesium structure and more than 20 trace elements, such as iron, manganese, nickel, copper, and zinc [Rezende *et al*. 2022]. However, studies are needed to determine the bioavailability of calcium and evaluate its interaction with animal feed, considering its high potential for replacing traditionally used calcium sources [Júnior et al., 2018].

In this context, it is necessary to adapt the mineral supply to meet the requirements of quail, as this provides good performance in the growth phase, which directly affects production performance and egg quality. This study assessed the replacement of inorganic calcium (calcitic lime) with organic calcium (limestone) in the diet of growing Japanese quails. We analyzed the influence of this replacement on growing quail performance, biochemical blood profile, organ biometry and bone quality, as well as the impact of the treatments on production performance and egg quality in the early laying phase.

Material and methods

This project was approved by the Research on Animals, which was conducted according to the institutional committee on Animal Use (protocol number 16/2020) of the Universidade Federal da Grande Dourados. The experiment was carried out in the Experimental Quail Aviary of the Universidade Federal Da Grande Dourados, Dourados, Mato Grosso do Sul, Brazil.

The birds were housed in a shed built in masonry that was 6.0 m long, 2.5 m wide, and 3.5 m high, with a concrete floor and roof with fiber cement tiles, 0.60 m short walls, and a 0.50 m eave in length. The autoclave had yellow polyethylene external curtains with manual activation and two air conditioners to control the ambient temperature.

The research included 300 (21 days old) Japanese quails, with an average weight of 87±3.5 g. The animals were distributed in a completely randomized experimental design with 3 treatments (control, 0 % inclusion; 0.50 % inclusion of calcareous seaweed 1; 0.50 % inclusion of calcareous seaweed 2), with both inclusions concerning the replacement of calcitic limestone in the diet. Each treatment included ten replicates of ten quail. Table 1 shows the difference in the composition of each calcium source. The two commercial products composed of calcareous algae (*Lithothamnium Calcareum*) were purchased from commercial companies and provided in the quantities indicated by the supplier.

Table 1. Chemical composition of calcareous seaweeds (*Lithothamnium Calcareum*) and calcitic limestone

Chemical composition	Calcareous seaweed 1	Calcareous seaweed 2	Calcitic limestone
Ash (%)	96.00	95.00	97.7
Calcium (%)	34.50	32.39	39.9
Magnesium (%)	3.60	5.00	0.32
Sodium (%)	0.340	0.347	–
Potassium (%)	0.040	0.038	–
Phosphorus (%)	0.030	0.034	–
Iron (ppm)	77.6	85.00	90.00
Copper (ppm)	7.20	7.25	–
Zinc (ppm)	4.58	5.50	–
Manganese (ppm)	1.47	1.53	–
Molybdenium (ppm)	0.196	0.250	–
Selenium (ppm)	0.50	0.50	–

Adapted from Dias *et al*. 2000.

The quail were fed diets formulated for the growth phase from 21 to 42 days. Following the National Research Council (NRC) (1994) recommendation, the diets used were isonutritive (Tab. 2). The lighting program included 24 hours of artificial light until the 15th day of life. From this point on until 42 days of age, we used natural lighting so that the birds would not reach sexual maturity prematurely.

The quails were housed in cages arranged in parallel series on three floors. Each cage had the following dimensions: 25 cm wide, 35 cm long, and 20 cm high,

Table 2. Percentage and calculated composition of the experimental diets with different calcium sources in the growth phase (21 to 42 days)

Ingredient	Limestone	Seaweed 1	Seaweed 2
Ground corn 8.51%	49.229	49.229	49.229
Soybean meal 45%	43.351	43.351	43.351
Oil	2.500	2.500	2.500
Inert	2.000	2.000	2.000
Limestone	1.226	0.726	0.726
Calcareous seaweed	0.000	0.500	0.500
Dicalcium phosphate	0.893	0.893	0.893
Salt	0.320	0.320	0.320
DL-methionine	0.165	0.165	0.165
L-lysine	0.116	0.116	0.116
Mineral birds[1]	0.100	0.100	0.100
Vitamin birds[2]	0.100	0.100	0.100
Calculated nutritional composition			
ME (kcal/kg)	2900	2900	2900
Crude Protein (%)	24.36	24.36	24.36
Digestible Lysine (%)	1.095	1.095	1.095
Methionine + Cist. (%)	0.744	0.744	0.744
Digest. Tryptophan (%)	0.186	0.186	0.186
Digest. Threonine (%)	0.733	0.733	0.733
Calcium (%)	1.092	1.092	1.092
Available Phosphorus (%)	0.513	0.513	0.513
Sodium (%)	0.205	0.205	0.205

[1] Vitamin supplement/kg of diet: Folic acid (Min.) 145.4 mg; pantothenic acid (Min.) 5931.6 mg; choline (Min.) 121.8 g; niacin (Min.) 12.9 g; selenium (Min.) 480.0 mg; vitamin A (Min.) 5,000,000.0 IU; vitamin B12 (Min.) 6,500.0 mcg; vitamin B2 (Min.) 2000.0 mg; vitamin B6 (Min.) 250.0 mg; vitamin D3 (Min.) 1,8500.0 IU; vitamin E (Min.) 4,500.0 IU; and vitamin K3 (Min.) 918.0 mg.

[2] Mineral supplement/kg: Copper (Min.) 7,000.0 mg; Iron (Min.) 50.0 g; Iodine (Min.) 1,500.0 mg; Manganese (Min.) 67.5 g; Zinc (Min.) 45.6 g.

corresponding to an area of 175 cm²/housed bird, and was equipped with trough-type feeders and nipple drinkers.

Temperature and relative humidity (RH) were monitored daily at 08:00 h by a digital thermohygrometer positioned in the center of the shed at the height of the back of the quails. The equipment obtained average maximum and minimum temperatures of 32.6°C and 19.01°C, respectively, and maximum and minimum relative humidities of 84.62 % and 33.75 %, respectively.

Assessment of performance in the growth phase

We assessed weight gain (WG; g/bird/day), feed intake (FI; g/bird/day), and feed conversion (FC) according to the following methodology by Valentim *et al*. [2023].

WG: Average weight gain per bird/day was determined by weighing at 21, 28, 35, and 42 days of age, always in the morning. FI: Average feed intake was determined by dividing the difference between the feed provided during the phase and the leftover feed consumed at the end of the study by the number of birds in the plot. Leftovers were also weighed on a scale; the averages were summed to determine the average feed consumption per bird in the plot. FC: Feed conversion was calculated by dividing the average feed intake by the average weight gain of the birds in the plots under study.

Organ and visceral weight characteristics

At 42 days of age, the birds were fasted for 8 hours and had access to water. Then, one bird from each plot – with an average weight within the range of ±10% of the average weight of the experimental unit – was selected, weighed, and identified, for a total of 30 slaughtered birds. These birds were stunned by cervical dislocation and manually bled through a jugular cut. After mild scaling at a temperature of 56°C for 2 minutes, the birds were manually plucked.

To perform percent calculations viscera included: liver, gizzard, and heart; proventriculus, small intestine (duodenum, jejunum, and ileum), cecum, ovary, oviduct, and abdominal fat were weighed on a semi analytical scale accurate to 3200 g. To measure gizzard weight, the feed that was inside the organ was removed, keeping the coilin membrane. The proventriculus, duodenum, jejunum, ileum, and cecum were slightly compressed to eliminate the interior content. Then, the clean tissue was weighed on a scale accurate to 0.5 grams following Valentim *et al*. [2017].

The percentage of visceral and abdominal fat was calculated by the ratio of the average weight of the representative cut of each replicate to the carcass weight according to the following formula:

$$\text{Yield x} = (\text{variable weight}/\text{carcass weight}) \times 100$$

Digestive and reproductive tract biometry

After identifying each segment of the digestive and reproductive tract, duodenum, jejunum, jejunum + ileum, cecum, ovary, and oviduct, the segments were sectioned, and the intestinal contents were subsequently emptied. A 90 cm measuring tape accurate to 0.1 mm was used to measure all segments. To obtain the relative length values, the measurements of each segment were divided by the total length of the organ, and the result was multiplied by 100.

Biochemical blood profile

Blood samples were collected at 42 days of age from the quail when the growth phase was completed. Blood samples were collected by heart puncture just before the slaughtering process. After collection, the samples were immediately centrifuged to separate the serum and then frozen at -20°C until biochemical analysis. The latter comprised the assessment of aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin (g/dL), and total protein (g/dL). For biochemical tests, we used Cobas 111® commercial kits following the principles of spectrophotometry.

Bone quality

After the birds were slaughtered, the left thighs were identified, packaged, and stored at -15°C for 120 days to assess bone quality. The following parameters were evaluated: the seedor index, tibia bone weight (g), length (mm), moisture (%), strength (kg), and ash content.

For determination of moisture content, the tibias were carefully deboned, dried at room temperature for 24 hours, and weighed before and after being placed in an oven at 105°C for 24 hours. Subsequently, without lipid extraction, as described by Yan *et al.* [2005], these parts were incinerated in a muffle furnace at 600°C for 8 hours to determine the ash content. Before the plants were sent to the muffle furnace, the tibia bone length was measured to determine the seedor index. For that purpose, we divided the weight of the bone by its length, as proposed by Seedor [1995].

The bones were also subjected to bone strength assessment through shear force analysis using the texture analyzer device TA. XT Plus and the Blade Set probe HDP/BS were used at a speed of 4 mm/s and a distance of 10 mm in the pretest. This equipment was adapted to allow free span of the bone diaphysis to reach 6.0 cm, preventing length from influencing the results.

Laying performance

At the end of the growth phase (42 days), the birds received a single ration from the 42nd to the 63rd day of age, still following the distribution used in the previous phase. The diets were prepared without the inclusion of different calcareous seaweeds, only with calcitic limestone, with the aim of assessing the repercussions of the growth phase on the early laying phase. The single diet was calculated following the recommendations of Rostagno *et al*. [2017] – Table 3.

Daily management consisted of collecting and counting the eggs (the number of broken, cracked, soft-shelled, and shellless eggs was computed daily), providing the feed, cleaning the egg trimers, and reading the temperature and humidity. In the early laying phase (42 to 63 days), we provided 16 hours of light daily. This light supply was controlled by an automatic timer, which allows the lights to be turned on and off during the night and at dawn, according to the procedure adopted on commercial farms.

Production performance was also evaluated in the postlaying phase. The feed leftovers from each plot were weighed and deducted from the amount of offered feed to obtain the feed consumption. In the case of dead birds during the period, their average consumption was deducted and corrected, obtaining the actual average consumption for the experimental unit.

The daily weight gain and total weight gain of the poultry were evaluated by weighing individual experimental plots at the beginning and at the end of the production cycle. The average egg production was obtained by computing the number of eggs produced, including broken, cracked, and abnormal eggs (soft-shelled and shell-less eggs). The values are expressed as a percentage of the average number of

Table 3. Percentage and calculated composition of the experimental feed in the early laying phase

Ingredient	%
Corn	54.30
Soybean meal	33.00
Oil	2.50
Limestone	7.40
Dicalcium phosphate	1.05
Mineral premix[1]	0.10
Vitamin premix[2]	0.10
DL-methionine	0.30
L-lysine	0.20
Choline	0.10
Salt	0.45
Inert	0.50
Calculated composition	
Metabolizable energy (Kcal/kg)	2800
Crude protein (%)	19.46
Digestible Lysine (%)	1.08
Digestible Methionine + Cystine (%)	0.94
Digestible tryptophan (%)	0.23
Digestible threonine (%)	0.68
Calcium (%)	3.07
Available phosphorus (%)	0.3
Sodium (%)	0.16
Crude fiber (%)	2.74

[1] Vitamin supplement/kg of diet: Folic acid (Min.) 145.4 mg; pantothenic acid (Min.) 5,931.6 mg; choline (Min.) 121.8 g; niacin (Min.) 12.9 g; selenium (Min.) 480.0 mg; vitamin A (Min.) 5,000,000.0 IU; vitamin B12 (Min.) 6,500.0 mcg; vitamin B2 (Min.) 2,000.0 mg; vitamin B6 (Min.) 250.0 mg; vitamin D3 (Min.) 1,8500.0 IU; vitamin E (Min.) 4,500.0 IU; and vitamin K3 (Min.) 918.0 mg.

[2] Mineral supplement/kg: Copper (Min.) 7,000.0 mg; Iron (Min.) 50.0 g; Iodine (Min.) 1,500.0 mg; Manganese (Min.) 67.5 g; Zinc (Min.) 45.6 g.

birds in the period (egg/bird/day) and of the number of birds housed at the beginning of the experiment (egg/bird housed).

Egg quality

To check the external and internal quality of the eggs, in the last 3 days of the experimental period (63rd day), 3 intact eggs were collected from each plot in the morning, totaling 270 eggs. First, the eggs were individually weighed on a semianalytical scale; then, specific gravity analysis was performed.

Specific gravity was determined by immersing the eggs in saline solutions of different densities, ranging from 1.065 to 1.125 with a variation of 0.005 for each solution, according to the methodology proposed by Castelló *et al*. [1989]. Densities were adjusted with a densimeter, and the eggs were submerged from the lowest to the highest saline concentration.

Afterwards, the eggs were broken, and the albumen, yolk, and shell were manually separated. The yolks were individually weighed on a precision scale. Subsequently, the shells were washed in running water and allowed to dry naturally for 72 hours at room temperature. Then, soon after the procedure, the plants were individually weighed. The albumen weight was obtained by subtracting the whole egg weight, yolk weight, and shell weight.

Yolk and albumen height and yolk diameter were measured with the aid of a digital caliper and a tripod. Yolk height was measured in the central region, and albumen height was measured approximately 1 cm from the yolk. The yolk index was calculated by the ratio of the height to the diameter of the structure.

The Haugh unit was calculated according to the formula given by Alleoni & Antunes [2001]:

$$HU = 100 \log (H + 7.57 - 1.7 W 0.37)$$

where H – dense albumen height (mm); w – egg weight (g).

Statistical analysis

The normality of the residual daa was checked using the Shapiro–Wilk test, and the homogeneity of variance was checked using the Levene test. The data were subsequently subjected to analysis of variance using the SAS MIXED procedure (SAS 9.3). In the case of a significant effect, means were compared using the Tukey test. The 5% significance level was used for all analyses.

Results and discussion

The growth phase (21 to 42 days) performance of Japanese quail did not significantly differ between the treatments involving calcareous seaweed and those involving calcitic limestone (Tab. 4).

Table 5 shows the lack of significant differences in the biochemical blood profile ALT (UI/L), albumin, and total protein (g/dL) levels, except for the AST (IU/L) level, which reflects the effect of calcium sources, with seaweed 2 having lower levels.

The weights of the organs treated with seaweeds 1 and 2 did not differ between the organic sources and the control group (Tab. 6). Differences in the weight of organs in the initial phase are indicative of bird development.

No significant changes occurred in the bone quality parameters of the birds (Tab. 7). Bone density and strength did not decrease with different calcium sources in the diet for quails in the growth phase. Thus, there is no evidence of physiological changes promoted by sources of calcium over a period of 21 to 42 days of supplementation.

Production performance in the early laying phase did not differ significantly between the previous treatments, and the results were not affected by the consumption of the seaweeds 1 and 2 in the growth phase. The performance variables remained similar for the treatments, reinforcing the possible use of seaweed as a substitute for limestone (Tab. 8).

Table 4. Performance by phase of growing Japanese quails (21 to 42 days) fed different calcium sources in the diet

Variable	Calcium source			SEM	P value
	Limestone	Seaweed 1	Seaweed 2		
21 to 28 days					
daily weight gain 21-28 d (g)	4.102	4.071	3.965	0.121	0.8972
weight gain 21-28 d (g)	29.955	28.500	27.760	0.795	0.5451
feed intake 21-28 d (g)	12.881	12.494	13.270	0.288	0.5642
feed conversion 21-28 d (g/g)	3.037	3.100	3.242	0.091	0.6686
average weight 28 d (g)	110.100	111.200	109.200	0.000	0.1586
21 to 35 days					
daily weight gain 21-35 d (g)	3.5930	3.7810	3.5380	0.0770	0.4168
weight gain 21-35 d (g)	51.6090	51.9390	50.8440	0.997	0.9059
feed intake 21-35 d (g)	15.90	15.77	15.83	0.242	0.9806
feed conversion 21-35 d (g/g)	4.45	4.18	4.57	0.1010	0.2773
average weight 35 d (g)	131.900	134.600	129.800	0.172	0.1698
21 to 42 days					
daily weight gain 21-42 d (g)	3.465	3.510	3.401	0.047	0.6636
weight gain 21-42 d (g)	74.069	72.715	72.728	1.011	0.8314
feed intake 21-42 d (g)	17.276	17.218	17.444	0.046	0.1180
feed conversion 21-42 d (g/g)	4.998	4.851	5.183	0.084	0.2813
average weight 42 d (g)	154.300	155.300	151.800	0.001	0.3509

SEM – standard error of the mean.

Table 5. Biochemical analyses of the blood serum of growing Japanese quails fed different calcium sources

Variable	Calcium source			SEM	P value
	Limestone	Seaweed 1	Seaweed 2		
ALT (IU/L)	2.110	1.625	2.013	0.125	0.2869
AST (IU/L)	231.65^{A}	180.96A^{B}	170.51^{B}	9.929	0.0160
Albumin (g/dL)	0.990	0.970	0.813	0.040	0.1461
Total protein (g/dL)	2.450	2.350	1.980	0.117	0.2314

^{AB}Means followed by different letters in the line differ statistically at the 5% probability level.
SEM – Standard error of the mean.

The different types of calcium sources in the diet of growing quail influenced the color of the color of the color fan, the albumen height, the yolk height, the Haugh unit, and the yolk index. The different sources did not influence the other variables significantly (Tab. 9).

The lack of significant results may be related to the ability of birds to absorb calcium similarly between the sources offered. This may be explained by calcium capacity for nutritional assimilation, as younger the bird is, the better its capacity of calcium nutritional assimilation [Moura *et al.* 2020]. In the present research, growing quails showed ease of absorption of this nutrient, and dietary Ca may have been high enough that differences in Ca digestibility could not emerge.

New sources of calcium that are not derived from rocks are extremely necessary to sustainably meet the nutritional demand of animals, maximize animal performance and reduce production costs [Melo and Moura 2009].

Table 6. Relative and absolute weight and organ length of growing quails fed different calcium sources

Variable	Calcium source			SEM	P value
	Limestone	Seaweed 1	Seaweed 2		
Liver (g)	3.160	3.453	3.007	0.103	0.2115
Heart (g)	1.461	1.490	1.390	0.036	0.5365
Gizzard (g)	3.953	4.173	4.076	0.101	0.6828
Proventriculus (g)	0.776	0.663	0.684	0.025	0.0800
Liver (%)	3.156	3.313	3.052	0.120	0.6927
Heart (%)	1.450	1.428	1.377	0.030	0.6245
Gizzard (%)	3.917	4.006	4.089	0.107	0.8200
Proventriculus (%)	0.779	0.639	0.654	0.032	0.1460
Oviduct (g)	2.863	2.095	2.641	0.402	0.7403
Ovary (g)	1.653	1.278	1.984	0.408	0.7887
Reproductive tract (cm)	18.600	13.944	15.428	1.328	0.3226
Reproductive tract (g)	4.516	3.373	4.625	0.416	0.7568
Oviduct (%)	2.481	1.597	2.273	0.377	0.6274
Ovary (%)	1.745	1.283	2.243	0.457	0.7051
Duodenum (g)	1.440	1.397	1.453	0.029	0.7534
Jejunum + ileum (g)	2.062	2.157	2.001	0.055	0.5316
Cecum (g)	1.327	1.224	1.287	0.073	0.8519
Duodenum (%)	1.378	1.351	1.387	0.028	0.8745
Jejunum + ileum (%)	2.057	2.069	2.013	0.064	0.9394
Cecum (%)	1.173	1.180	1.286	0.060	0.7158
Gastrointestinal tract (cm)	52.700	51.510	51.666	1.048	0.8847
Duodenum (cm)	10.500	10.000	10.666	0.220	0.4524
Jejunum + ileum (cm)	37.500	37.100	35.722	0.917	0.7313
Cecum (cm)	7.150	7.500	7.944	0.183	0.2201
Duodenum (% cm)	20.237	19.471	20.824	0.533	0.6040
Jejunum + ileum (% cm)	70.913	72.081	69.049	0.707	0.2257
Cecum (% cm)	13.622	14.583	15.454	0.324	0.0683

SEM – standard error of the mean.

Table 7. Bone parameters evaluated in the tibia of growing quails fed different calcium sources

Variable	Calcium source			SEM	P value
	Limestone	Seaweed 1	Seaweed 2		
Seedor index	10.603	11.051	10.991	0.152	0.4355
Tibia bone weight (g)	0.495	0.520	0.521	0.009	0.4478
Length (mm)	46.703	47.067	47.367	0.364	0.7732
Moisture (%)	10.386	10.358	10.213	0.234	0.9544
Strength (kg)	1.932	1.900	1.958	0.112	0.9798
Ash	51.979	51.991	52.088	1.088	0.9991

SEM – standard error of the mean.

According to Araujo *et al.* [2008], not only is bioavailability superior but also organic minerals are readily transported to tissues, where they remain stored for longer periods than inorganic minerals. The present results corroborate those of Carlos *et al.* [2011], as they found no significant difference in the feed intake of chickens fed *Lithothamnium* seaweed. However, these authors reported a decrease in feed conversion, correlating with a decrease in weight gain and the absence of significant differences in feed consumption associated with seaweed treatment.

Table 8. Performance in the early laying phase of growing quails fed different calcium sources

Variable	Calcium source			SEM	P value
	Limestone	Seaweed 1	Seaweed 2		
42 to 63 days					
final body weight (g)	157.20	158.00	154.20	0.001	0.1556
gain/bird/day (g)	1.548	1.747	1.635	0.084	0.6436
accumulated body gain (g)	32.520	36.703	32.220	1.705	0.1989
feed intake (g)	29.635	29.300	29.789	0.195	0.5960
egg production (%)	42.226	40.543	45.135	1.745	0.5677

SEM – Standard error of the mean.

Table 9. The quality of the eggs of quails fed different calcium sources in the growth phase

Variable	Calcium source			SEM	P value
	Limestone	Seaweed 1	Seaweed 2		
Fan	4.8^A	4.5^A	3.5^B	0.101	0.0001
L	56.05	55.86	55.87	0.196	0.9113
a*	-2.40	-2.70	-2.77	0.073	0.0927
b*	41.321	40.46	41.60	0.325	0.3345
Gravity	1.07	1.07	1.07	0.001	0.4189
Egg weight (g)	10.551	10.440	10.314	0.072	0.4113
Yolk weight (g)	3.511	3.526	3.340	0.038	0.0845
Yok diameter (mm)	21.872	22.271	21.842	0.092	0.1037
Albumen height (mm)	4.477^A	3.759^B	3.835^B	0.066	<0.0001
Yolk height	9.939^A	9.385^B	9.375^B	0.068	0.0004
Haugh unit	90.121^A	86.149^B	87.064^B	0.353	<0.0001
Yolk index	0.453^A	0.421^B	0.429^B	0.003	0.0003
Shell weight (g)	0.851	0.834	0.829	0.008	0.5787
Albumen weight	6.19	6.08	6.14	0.056	0.7330
% Yolk	33.26	33.79	32.16	0.301	0.0784
% Albumen	58.68	58.21	59.50	0.324	0.2587
% Shell	8.06	8.00	8.05	0.064	0.9202
Shell thickness (mm)	0.197	0.193	0.201	0.001	0.2687

Means followed by different letters on the line differ from each other at the 5% level of significance. SEM – standard error of the mean. L* – brightness, a* – red/green content. b* – yellow/blue content.

Different concentrations of minerals in the diet may affect tissues and body fluids, leading to biochemical changes that interfere with physiological functions. This results in the emergence of metabolic disorders related to the protein, energy, and mineral metabolism of animals [Freitas *et al.* 2013].

However, despite the significant statistical value of the AST in the present study, AST concentrations above 350 IU/L represented moderate increases in the enzyme, while values above 800 UI/L represented marked increases. When accompanied by biliverdinuria or biliverdinemia, the latter indicates severe hepatocellular damage [Thrall *et al.* 2004, Capitelli and Crosta 2013]. Therefore, in the present study, all AST values remained within the expected normality value, and the alteration in AST may be due to possible liver activity against the source and concentration of calcium.

Quails are animals that have a high tolerance to dietary calcium and phosphorus fluctuations and are efficient at excreting excess minerals [Zhao *et al.* 2020]. This factor may explain the lack of a significant difference between treatments in the present study. Calcium is the most metabolically active macromineral and functions in the regulation of muscle contraction, transmission of nerve impulses, blood coagulation, activation of enzyme systems and cell adhesion, reproduction, and egg formation [Vieira *et al*. 2012].

Research using the calcareous seaweed *Lithothamnium* has shown that this seaweed has alkalinizing characteristics, maintains the acid–base balance, and provides minerals with high bioavailability. Moreover, nutrient adsorption on the cell wall and nutrient assimilation are facilitated by the porous structure of seaweed [Melo and Moura 2009, Carlos *et al*. 2011]. This may explain the good development of bird organs without harming animal health.

According to Lobaugh *et al*. [1981], calcium is absorbed either passively by paracellular diffusion or actively via enterocytes, with its main absorption occurring in the small intestine. The absorptive efficiency of this mineral varies depending on the calcium source, the ratio of Ca:P to vitamin D, the intestinal pH, and the physiological profile. This characteristic characterizes an ingestion in which the greater the need for this mineral is, the greater the efficiency of absorption [Mcdowell 1992]. All the sources had similar absorption capacities, given the absence of differences between the data.

Over the years, the process of selecting laying birds has focused on egg production and quality traits. Thus, the literature has not sufficiently addressed the skeletal structure of birds, especially with regard to long bones, which are necessary for carcass support and as a reserve matrix to meet the demand for egg formation [Moura *et al.* 2020].

Therefore, it is extremely important to offer nutritional supplementation of this macromineral in the early life of birds to meet bone demands. This will ensure a greater reserve of calcium in the bones, favoring the laying phase without compromising the body structure [Almeida *et al.* 2009].

On a large scale, bone constitution reflects the conditions of the skeleton, which are directly correlated with the poultry diet and the nutritional state of the birds. Calcium is the most abundant mineral in the body of birds. Of the total absorbed lipids, 99% are present in bones [Bertechini 2012]. Zhao *et al.* [2020] concluded that diets with low calcium content may facilitate the development of osteoporosis and structural bone loss, decreasing bone quality and strength as well as egg production and quality. This did not occur in the present study, demonstrating that the inclusion of alternative sources of calcium made it possible to meet the requirements.

High demands of calcium for the production of eggs in commercial laying hens induce resorption or breakage of the bone matrix and release of the contained mineral. A deleterious consequence of this process is the weakening of these bones, which can result in possible fractures [Toscano *et al*. 2020].

This process thus leads to physiological disturbances, bone fractures, and damage to animal welfare. In the present study, the tibias of all the treatment groups were resistant to breakage, which demonstrated the efficiency of calcium sources from calcareous seaweed in maintaining the quality of the bone matrix in laying quail. Diets that promote greater mineral deposition in the bones of birds—as in the present study—are of interest because they can reduce the adverse effects of the high demand for minerals [Souza *et al.* 2017].

When 50% of the calcitic limestone was replaced with eggshell flour in the diet of quail, Reis *et al.* [2012] did not observe any difference in the performance of the birds. However, when 100% replacement was used, these authors found lower values for the laying rate. The strategy adopted in this study, partial replacement, is more efficient than the one adopted by the authors and has a positive impact on egg production.

The importance of calcium for laying birds stems from its participation in bone matrix formation and maintenance, egg shell formation, and metabolism. The correct use of calcium levels in the poultry diet, considering the requirement in the growth phase, is essential for ensuring good performance of the bird in the laying phase. The formation of an adequate reserve in the bones during the growth phase positively affects the laying phase [Almeida *et al*. 2009] when the diet meets the mineral requirements of the birds.

Using limestone as the calcium source increased the albumen and yolk height. On the other hand, these variables decreased with the inclusion of seaweed 1 and seaweed 2. The Haugh unit is a mathematical correlation between the weight and height of thick albumen and measures the internal quality of eggs [Spanivello *et al.* 2022]. Despite the difference between the treatments, the results were in the range recommended in the literature, being above the minimum value of 72 recommended by the Egg Grading Manual [2000].

Londero [2019] concluded that supplementation with seaweed calcium considerably improved eggshell quality but did not affect bird performance. In the present research, however, shell thickness did not differ among the treatments, which is satisfactory. The quality also remained similar between treatments, indicating the possibility of replacing calcium sources from calcitic limestone.

Cedro *et al.* [2011] reported that calcium is extremely important for eggshell formation. According to the author, a single egg has approximately three grams of calcium, necessitating adequate supplementation of this mineral in laying birds. As eggshell quality did not differ in the present study, it is possible to indicate the use of calcareous seaweed without harming eggshell quality, weight, or percentage.

The search for new sources of calcium for birds is interesting and necessary. In this context, calcareous seaweed is more sustainable than traditional seaweed and has ecologically correct production and extraction qualities. Its recommendation for use in laying diets depends on its benefits for egg production. As the present study did not find significant differences in production performance, bird health, or bone or egg quality, the authors could indicate the replacement of calcitic limestone by calcareous seaweed in the diet of these animals.

The results support the feasibility of replacing calcitic limestone with calcareous seaweed, offering a promising avenue for addressing environmental concerns associated with the extraction of inorganic calcium sources. The study underscores the importance of considering alternative calcium sources in poultry nutrition to meet the nutritional needs of birds while promoting sustainability in the production system

Conclusion

Including 0.50% calcareous seaweed as a replacement for calcitic limestone in the diet of quail does not harm production performance, animal health, organ yield, or bone quality in the growth phase. This treatment guarantees satisfactory performance and high-quality eggs in the early laying phase.

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The authors report that there are no competing interests to declare.

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