













Calcified seaweed in the diet of semi-heavy hens during the rearing and pre-laying phases

Alga marinha calcária na dieta de poedeiras semipesadas nas fases de recria e pré-postura

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Abstract: Calcareous seaweed (*Lithothamnium calcareum*) is a source of organic minerals and has been used as an alternative food in poultry nutrition. The objective is to evaluate the levels of calcareous algae in the diet of chickens during the rearing and pre-laying phases, as also on the performance and thermoregulation variables. One hundred and forty-four semi-heavy Hisex Brown hens, aged 12 to 18 weeks, were used in the rearing and pre-laying stages. They were fed with four levels of calcareous algae (0%, 10%, 20%, 30%), which were used in place of the calcitic limestone. Six replications and six birds were used per experimental unit, distributed in a completely randomized design. Feed intake, weight gain, feed conversion, body weight, viability, and thermoregulation through cloacal temperature (°C), mean body temperature (°C), and mean skin temperature (°C) were evaluated. There was no significant difference ($P > 0.05$) in the thermoregulation of the birds. There was a difference ($P < 0.05$) in the feed intake during the pre-laying phase, however, the other parameters showed no difference ($P > 0.05$). The inclusion of up to 30% calcareous algae in the diet of semi-heavy laying hens in the rearing and pre-laying phases did not affect the performance or thermoregulation of the birds. Therefore, it is possible to substitute calcitic limestone with up to 30% of the calcareous algae in the diet of semi-heavy laying hens.

Keywords: Poultry, *Lithothamnium calcareum*, Organic Mineral.

Resumo: A alga calcária (*Lithothamnium calcareum*) é fonte de minerais orgânicos e tem sido utilizada como alimento alternativo na nutrição de aves. O objetivo foi avaliar níveis de alga calcária na dieta de poedeiras semipesadas nas fases de recria e pré-postura sobre variáveis de desempenho e termorregulação. Foram utilizadas 144 galinhas semipesadas da linhagem Hisex Brown, com idade entre 12 a 18 semanas, nas fases de recria e pré-postura, alimentadas com 4 níveis de alga calcária (0%, 10%, 20%, 30%) em substituição ao calcário calcítico, seis repetições e seis aves por unidade experimental, distribuídas em delineamento inteiramente casualizado. Foram avaliados consumo de ração, ganho de peso, conversão alimentar, peso corporal e viabilidade, termorregulação através da

Received: November 08, 2023. Accepted: March 26, 2024. Published: July 05, 2024.

temperatura cloacal (°C), temperatura corporal média (°C) e temperatura média da pele (°C). Não houve diferença significativa ($P>0,05$) na termorregulação das aves. Houve diferença ($P<0,05$) para o consumo de ração na fase de pré-postura, os demais parâmetros não apresentaram diferença ($P>0,05$). A inclusão de até 30% de alga calcária na dieta de poedeiras semipesadas nas fases de recria e pré-postura não afetou o desempenho e a termorregulação das aves. Portanto, é possível substituir até 30% de alga calcária por calcário calcítico na dieta de poedeiras semipesadas.

Palavras-chave: Aves, *Lithothamnium Calcareum*, Mineral Orgânico.

1. Introduction

Poultry egg production is a highly profitable activity in Brazil, primarily aimed at egg production. The national scenario is extremely favorable due to the ideal climatic conditions for poultry farming, as well as the affordable cost of eggs, catering to all social classes. According to data from the Brazilian Animal Protein Association⁽¹⁾, the sector currently has approximately 113,979,595 laying hen accommodations, resulting in an impressive annual production of 52,068,585,438 eggs.

To enhance eggshell quality and the performance of laying hens, it is crucial to pay attention to minerals, as they are related to physiological processes that maintain performance and account for approximately 90% of eggshell formation⁽²⁾.

In this context, the search for alternative sources of calcium, such as organic minerals, has intensified in poultry farming, aiming to replace conventional sources. These organic sources can meet the calcium needs of poultry due to their more effective bioavailability⁽³⁾. Calcium from these sources is readily absorbed without presenting ionic antagonism.

Calcified seaweeds have high levels of minerals retained from the marine environment, with 32.5% bioavailable calcium, justifying their use in poultry farming. Calcium is the primary mineral involved in the production processes of these birds, aiding in eggshell formation, bone growth, and the physiological processes in production animals⁽⁴⁾. The limitation in their use is due to the varied composition and extraction cost, with closer proximity to the sea resulting in lower acquisition costs⁽⁵⁾.

Therefore, the objective of this study is to evaluate the levels of calcified seaweed as a replacement for calcitic limestone in the diet of semi-heavy laying hens during the rearing and pre-laying phases, focusing on the performance and physiological parameters of body temperature.

2. Materials and Methods

The experiment was conducted at the Poultry Farming Sector of the Experimental Farm of Zootechny and Agronomy at the Federal University of Mato Grosso - Campus Cuiabá, located in Santo Antônio do Leverger, MT, with a total duration of 49 days, divided into two production phases: rearing hens (12 to 15 weeks of age) and pre-laying hens (16 to 18 weeks of age). The project was submitted, analyzed, and approved by the Ethics Committee for Animal Use (CEUA) under number (23108.073261/2022-60).

The experimental poultry house was constructed in an East–West direction and was equipped with an external curtain system on the sides with mechanical operation through winches, fans, and a misting system for temperature and humidity control inside. The roof was made of clay tiles.

The poultry house system was cage-free, divided into four lines arranged laterally, with 15 screened boxes in each line. The birds were housed in boxes (experimental units), with dimensions of 1.76 m x 1.53 m (length x width), providing an area of 0.448 m² per bird. The boxes were equipped with tubular feeders and hanging drinkers and the floor was covered with rice straw. Weighing and distribution of the birds into their respective plots was carried out on the day of housing. Each bird was individually weighed and the average weight was calculated. The birds were then selected based on approximately 10% of the overall average weight.

A total of 144 semi-heavy hens of the Hisex Brown lineage, aged 12 to 18 weeks, were used, with six replicates, totaling 24 experimental units, with six birds in each. A completely randomized design was used with four treatments as follows: T1: control diet with 0% calcified seaweed replacing the calcitic limestone; T2: diet with 10% the calcified seaweed replacing calcitic limestone; T3: diet with 20% calcified seaweed replacing the calcitic limestone; T4: diet with 30% calcified seaweed replacing the calcitic limestone. The difference in composition of each source was expressed in (Table 1), adapted from Dias ⁽⁶⁾. The experimental diets (Tables 2 and 3) were formulated based on corn and soybean meal, and they were isoenergetic and isoproteic ⁽⁷⁾.

Table 1 Chemical composition of calcareous algae and calcitic limestone.

Chemical composition	Calcareous algae	Calcitic limestone
Ash (%)	93.00	97.7
Calcium (%)	32.39	39.9
Magnesium (%)	5.00	0.32
Sodium (%)	0.347	----
Potassium (%)	0.038	----
Phosphorus (%)	0.034	----
Iron (ppm)	85.00	90.00
Copper (ppm)	7.25	----
Zinc (ppm)	5.50	----
Manganese (ppm)	1.53	----
Molybdenum (ppm)	0.250	----
Selenium (ppm)	0.50	----

Table 2 Percentage and calculated composition of the experimental diets T1 (0% calcareous algae), T2 (10% calcareous algae), T3 (20% calcareous algae), and T4 (30% calcareous algae) replacing the calcitic limestone in the recreation phase 12 to 15 weeks of life.

Ingredients (%)	0%	10%	20%	30%
Corn bran	85.70	85.70	85.70	85.70
Soybean meal	9.00	9.00	9.00	9.00
Calcitic limestone	1.25	1.125	1.00	0.875
Calcareous algae	0.00	0.125	0.250	0.375
Dicalcium phosphate	1.50	1.50	1.50	1.50
Common salt	0.40	0.40	0.40	0.40
Growth core	1.87	1.87	1.87	1.87
L-lysine HCl	0.20	0.20	0.20	0.20
DL-Methionine	0.07	0.07	0.07	0.07
L-Threonine	0.01	0.01	0.01	0.01
Nutritional Composition				
Energy metabolizável (kcal/kg)	2850	2850	2850	2850
Crude protein (%)	10.85	10.85	10.85	10.85
Digestive lysine (%)	0.343	0.343	0.343	0.343
Methionine+Cystine digestível (%)	0.465	0.465	0.465	0.465
Tryptophan digestível (%)	0.116	0.116	0.116	0.116
Digestive threonine (%)	0.395	0.395	0.395	0.395
Calcium (%)	0.840	0.840	0.840	0.840
Available phosphorus (%)	0.390	0.390	0.390	0.390
Sodium (%)	0.160	0.160	0.160	0.160

Composition core growth: Calcium (min) 165 g/kg, Calcium (max) 200 g/kg Phosphorus (min) 35 g/kg, Sodium (min) 30 g/kg, Methionine (min) 18.30 g/kg, Lysine (min) 18 g/kg, Vitamin A (min) 250000 IU/kg, Vitamin D3 (min) 62500 IU/kg, Vitamin E (min) 435 IU/kg, Vitamin K3 (min) 50 mg/kg, Vitamin B1 (min) 37 mg/kg, Vitamin B2 (min) 185 mg/kg, Vitamin B6 (min) 50 mg/kg, Vitamin B12 (min) 370 mcg/kg, Folic Acid (min) 12.5 mg/kg, Pantothenic Acid (min) 375 mg/kg, Biotin (min) 1.50 mg/kg, Choline (min) 6000 mg/kg, Niacin (min) 625 mg/kg, Copper (min) 2672 mg/kg, Iron (min) 600 mg/kg, Iodine (min) 20 mg/kg, Manganese (min) 1400 mg/kg, Selenium (min) 7 mg/kg, Cobalt (min) 4 mg/kg, Zinc (min) 1000 mg/kg, Fluoride (max) 370 mg/kg.

Table 3 Percentage and calculated composition of the experimental diets T1 (0% calcareous algae), T2 (10% calcareous algae), T3 (20% calcareous algae), and T4 (30% calcareous algae) to replace calcitic limestone in the pre-laying phase 16 to 18 weeks of life.

Ingredients (%)	0%	10%	20%	30%
Corn bran	77.45	77.45	77.45	77.45
Soybean meal	13.68	13.68	13.68	13.68
Calcitic limestone	4.80	4.32	3.84	3.36
Calcareous algae	0.00	0.48	0.96	1.44
Dicalcium phosphate	1.50	1.50	1.50	1.50
Common salt	0.38	0.38	0.38	0.38
Posture core	1.87	1.87	1.87	1.87
L-lysine HCl	0.20	0.20	0.20	0.20
DL-Methionine	0.10	0.10	0.10	0.10
L-Treonine	0.02	0.02	0.02	0.02

Nutritional Composition				
Energy metabolizável (kcal/kg)	2850	2850	2850	2850
Crude protein (%)	12.66	12.66	12.66	12.66
Digestive lysine (%)	0.431	0.431	0.431	0.431
Methionine+Cystine digestível (%)	0.554	0.554	0.554	0.554
Tryptophan digestível (%)	0.149	0.149	0.149	0.149
Digestive threonine (%)	0.466	0.466	0.466	0.466
Calcium (%)	2.2	2.2	2.2	2.2
Available phosphorus (%)	0.440	0.440	0.440	0.440
Sodium (%)	0.160	0.160	0.160	0.160

Core laying composition: Calcium (max) 230g/kg, Calcium (min).210g/kg, Phosphorus (min) 36.6g, Methionine (min) 52.6g, Lysine (min) 5600mg, Vitamin A (min) 187500 I.U., Vitamin D3 (min) 60000 I.U., Vitamin E (min) 562 I.U., Thiamine (B1) (min) 46.88 mg, Riboflavin (B2) (min) 121.88 mg, Pyridoxine (B6) (min) 75 mg, Vitamin B12 (min) 468.75 mcg, Vitamin K3 (mini) 46.88 mg, Folic Acid (min) 18.75 mg, Niacin (mini) 750 mg, Choline (min) 3875 mg, Sodium (min) 40g, Manganese (min) 2000 mg, Zinc (min) 1750 mg, Copper (min) 200 mg, Iron (min) 1250 mg, Iodine (min) 25 mg, Selenium (min) 7.5 mg, Phytase (min) 12500 FTU and Fluoride (max) 366 mg.

The feeding was carried out twice a day, and water was provided *ad libitum*. The maximum and minimum air temperatures and relative humidity inside the poultry house were measured and recorded twice a day using a digital thermometer, with maximum and minimum sensors positioned at the height of the birds' backs, at 8:00 AM and 2:00 PM, throughout the experimental period.

Following the Hisex Brown manual⁽⁸⁾, it was assumed that up to the seventeenth week of life, the birds would receive 10 to 12 hours of light per day. After the seventeenth week, a light program was adopted, by increasing 30 minutes of light each week until it reached 16 hours of daily light, using both natural and artificial light. The light supply was controlled by an automatic timer.

At the beginning and end of each production phase, all birds were weighed, to determine the weight gain, with values expressed in g/bird. Feed consumption (g/bird/day) was calculated by the difference between the amount of feed provided and the leftovers during each phase. Feed conversion was calculated by dividing the average feed consumption by the average weight gain of the birds.

During the experimental period, body weight variation was evaluated by measuring the difference in weight at the beginning and end of the life phase, using a scale with a precision of 0.001 g. The number of dead birds were recorded daily, and the remaining feed in the parcel on the day of occurrence was weighed for corrections in feed consumption.

Data related to the birds' thermoregulatory variables were collected. The evaluation of body temperature was always performed at the same time, during the hottest periods of the day, using a sample of two birds/box from each experimental unit. Data on temperatures of head, leg, back, and wing were collected using an infrared thermometer (Infrared Digital Laser Sight Thermometer), with the laser sight at a distance of 15 cm from the animal's skin. The cloacal temperature was determined using a digital clinical thermometer with a rigid tip (iColor- THGTH150B - White - G-Tech), which was inserted into the birds' cloaca, from which a sound was emitted when the temperature stabilized.

Subsequently, the physiological data were used to calculate the average skin temperature (AST) and average body temperature (ABT) of the birds, according to the equation proposed by Richards⁽⁹⁾. Considering the surface temperatures and the rectal temperature of the birds: $AST = (0.70BT + 0.12WT + 0.09HT + 0.09LT)$, where BT = back temperature (°C); WT = wing temperature (°C); HT = head temperature (°C); LT = leg temperature. $ABT = (0.3AST + 0.7CT)$, where CT = cloacal temperature (°C).

For statistical analysis, the evaluated parameters were subjected to analysis of variance at a 5% probability level using the R statistical software⁽¹⁰⁾, with prior verification of the residual normality by the Shapiro-Wilk test, homogeneity of variances by the Bartlett test, and error independence using the Durbin-Watson test. Subsequently, the effects of the limestone levels in the seaweed were estimated through analysis of the variables, using the linear and quadratic regression models, according to the best fit obtained.

Contrasts were tested using the Dunnett test at a 5% probability level, comparing the treatment without seaweed limestone inclusion (control) with the others (10%, 20%, and 30%). The data from the statistical analyses of variables that did not meet any of the ANOVA assumptions were first transformed using the logarithmic function $Y' = \log(Y + 0.5)$. Then, these non-parametric variables were subjected to the Kruskal-Wallis test with Dunn-Bonferroni post-hoc, at a 5% significance level.

3. Results and Discussion

During the rearing phase, temperatures ranged from a maximum of 31.65°C to a minimum of 23.30°C, even as relative humidity ranged from a maximum of 88.12% to a minimum of 54.62%. In the pre-laying phase, the maximum temperature was 32.85°C and the minimum was 24.67°C, whereas, relative humidity ranged from a maximum of 87.94% to a minimum of 56.63%. Therefore, the birds experienced periods of heat stress, as the experiment was conducted in a region with a hot climate.

According to the Hisex Brown lineage manual⁽⁸⁾, a pleasant environment for birds is characterized by temperatures ranging from 18°C to 28°C, along with relative humidity between 60% and 70%. These temperatures and humidities allow the birds to be in thermal comfort and express their full genetic potential.

There was no significant difference ($P > 0.05$) in the temperatures measured in the bodies of birds fed with diets that had partial substitution of calcitic limestone by seaweed limestone, during the rearing and pre-laying phases of Hisex Brown hens (Table 4).

Table 4. Cloacal temperature (TC°C), mean skin temperature (TMP°C), and mean body temperature (TMC°C) of Hisex Brown chickens fed calcareous algae levels instead of calcitic limestone.

Recreation Phase (12 to 15 weeks of life)							
Parameters	Calcareous algae levels (%)				CV (%)	EPM	P- Value
	0	10	20	30			
TC°C	41.25	41.3	41.31	41.13	0.61	0.050	0.602
TMP°C	30.12	31.02	31.54	30.89	5.95	0.365	0.617

TMC°C	37.91	38.22	38.38	38.06	1.68	0.126	0.628
Pre-posture phase (16 to 18 weeks of life)							
TC°C	40.69	40.27	40.59	40.58	0.63	0.058	0.461
TMP°C	31.37	31.52	31.65	31.54	1.63	0.100	0.817
TMC°C	37.89	37.64	37.91	37.86	0.71	0.055	0.287

CV = coefficient of variation; SEM = Standard Error of the Mean; Means followed by an asterisk on the same line differ from the control group by Dunnett's test ($P < 0.05$).

According to Marchini *et al.*⁽¹¹⁾, in a thermoneutral environment, the cloacal temperature of birds is normally between 41°C and 41.5°C. In the rearing phase, the cloacal temperature values were as mentioned in the literature. In the pre-laying phase, despite no statistical difference in the absolute values, these birds were better able to dissipate heat, as they exhibited a lower cloacal temperature compared to the data found in the literature.

Birds are homeothermic animals and use processes of radiation, conduction, convection (non-evaporative), and evaporation (which is a latent mechanism of specific heat transfer) to dissipate body heat into the environment^(12, 13). These mechanisms allow birds to regulate their body temperature and carry out their activities efficiently, avoiding performance losses.

Performance is one of the parameters most affected when birds face heat stress. One sign of this stress is a reduction in feed intake, which occurs as an attempt to reduce the rate of metabolic heat production. However, this can result in worsened weight gain and feed conversion⁽¹⁴⁾.

Table 5 presents the results for the variables: Weight gain, feed consumption, feed conversion, body weight, and viability for the rearing and pre-laying phases. There was no significant difference ($P > 0.05$) in any of the parameters evaluated during the rearing phase. However, in the pre-laying phase, there was a significance, in that, $P < 0.05$ for feed consumption.

Table 5 Weight gain, feed intake, feed conversion, body weight and viability of Hisex Brown chickens fed calcareous algae levels instead of calcitic limestone.

Recreation Phase (12 to 15 weeks of life)							
Parameters	Calcareous algae levels (%)				CV (%)	EPM	P- Value
	0	10	20	30			
Weight gain (g/bird)	0.303	0.330	0.320	0.337	14.89	0.009	0.646
Feed consumption (g/bird/day)	71.59	72.53	75.18	71.30	4.36	0.682	0.169
Feed conversion (kg/kg)	6.22	5.77	6.26	5.53	12.9	0.159	0.306
Body Weight (kg/bird) ¹	1.238	1.261	1.251	1.262	3.71	0.009	0.785
Viability (%)	100	100	100	100	-	-	-
Pre-posture phase (16 to 18 weeks of life)							
Weight gain (g/bird)	0.270	0.302	0.280	0.284	10.96	0.006	0.357

Feed consumption (g/bird/day) ²	86.57	85.25	86.31	80.74*	3.86	0.792	0.012
Feed conversion (kg/kg)	6.74	5.96	6.55	6.10	11.71	0.156	0.247
Body Weight (kg/bird) ³	1.508	1.564	1.531	1.547	3.45	0.010	0.337
Viability (%)	100	100	100	100	-	-	-

CV = coefficient of variation; SEM = Standard Error of the Mean; Means followed by an asterisk on the same line differ from the control group by Dunnett's test ($P < 0.05$); ¹ Body weight at 15 weeks; ² linear effect; ³ Body weight at 18 weeks.

A decreasing linear effect was observed (Figure 1) on feed consumption in the different treatments, with treatment four standing out, in which 30% of seaweed limestone was included as substitution for calcitic limestone. In this treatment, the lowest result was observed, but this decrease did not affect the weight gain and body weight of the birds.

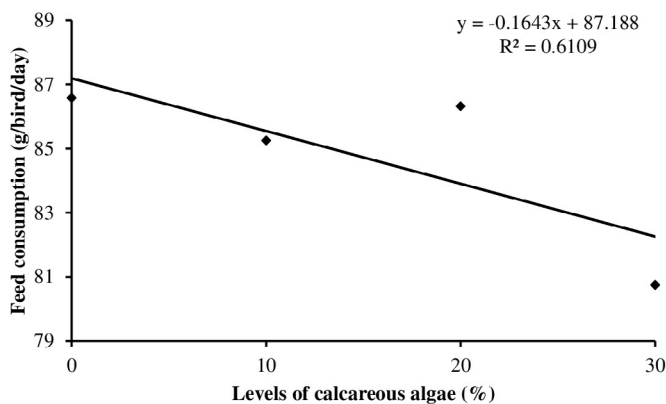


Figure 1 Feed consumption of Hisex Brown hens in the pre-laying phase.

These results corroborate the studies of Silva⁽¹²⁾, who, in his study, evaluated the inclusion of a calcareous seaweed meal in the diet of commercial layers. He observed a reduction in feed consumption in birds receiving a diet containing 1% inclusion of calcareous seaweed meal, without negatively affecting the other performance variables.

Feed consumption is the primary precursor to bird growth, essential for physiological processes, such as, bone and muscle formation, feathering, and development of the reproductive system⁽¹⁵⁾. Despite this decrease, the feed consumption obtained was by the lineage's manual recommendations (approximately 85 g).

Weight gain, feed conversion, body weight, and viability showed no significant differences ($P > 0.05$) in both production phases. However, the values found in the research align with the Hisex Brown lineage manual⁽⁸⁾. These results were consistent with those found by Rezende⁽¹⁶⁾, who, when evaluating calcareous seaweed in the diet of Japanese quails (*Coturnix coturnix japonica*) during the rearing phase, observed that the inclusion of calcareous seaweed did not influence feed consumption, average weight, daily weight gain, total weight gain, or feed conversion.

The author concluded that calcareous seaweed could replace calcitic limestone in the diet of Japanese quails during the rearing phase, without negatively impacting their performance. According to Moura *et al.*⁽¹⁷⁾, the younger birds have a greater ability to assimilate nutrients efficiently. This explains the lack of significant results, as it is associated with the bird's ability to absorb calcium from among the different sources provided in this research.

The results indicate that the partial substitution of calcitic limestone with seaweed limestone had no significant effects on the temperature and performance variables in the rearing phase. However, in the pre-laying phase, there was a reduction in feed consumption, but it did not compromise weight gain and body weight. The inclusion of seaweed limestone in the diet might be a viable and promising alternative, as it did not negatively affect bird performance and could contribute to better nutrient absorption due to the presence of bioavailable calcium in its composition.

4. Conclusion

The inclusion of up to 30% seaweed limestone in the diet of semi-heavy laying hens during the rearing and pre-laying phases did not impair the performance and thermoregulation. Therefore, it is possible to replace calcitic limestone with up to 30% seaweed limestone in the diet of semi-heavy laying hens.

Conflict of interests

The authors declare no conflict of interest.

Author contributions

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Acknowledgment

This work was carried out with the support of the Coordination for the Improvement of Higher Education Personnel – Brazil (CAPES) – Financing code 001.

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