International Journal of Animal Science and Technology
2025, Vol. 9, No. 2, pp. 74-86
https://doi.org/10.11648/j.ijast.20250902.16

Research Article

Effect of Limestone or Extruded Eggshell on Egg and Eggshell Quality of Laying Hens

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Abstract

The study aimed to determine the effect of limestone or extruded eggshells on the egg and eggshell quality of laying hens. A total of 240 and 60 eggs were collected from diets D_1 (limestone) and D_2 (extruded eggshells), respectively, with two levels of calcium source (L): L_1 (4% limestone or eggshells) and L_2 (8% limestone or eggshells) at 44, 52, 60, 68, and 72 weeks of age. These eggs were obtained from an ongoing project to measure egg quality and dry matter content. Diet D_1 showed higher albumen width and height, Haugh unit, and a lower egg yolk height than D_2 ($p<0.05$). However, the percentage of albumen and yolk weight, as well as the yolk index, tended to be higher in D_2 than in D_1. Other egg quality traits were similar between diets ($p>0.05$). The L_2 was superior to L_1 in both diets for egg quality traits ($p<0.01$), and the L_2 in the extruded eggshell diet performed better than L_2 in limestone diet. The egg, albumen, yolk, and eggshell weight, albumen width, and yolk height were increased but the eggshell strength, yolk color, albumen height, eggshell thickness, and membrane thickness were decreased with advancing the birds' age. Egg, albumen, yolk, and eggshell weight, as well as albumen width and yolk height, increased with the advancing age of the birds, whereas eggshell strength, yolk color, albumen height, eggshell thickness, and membrane thickness decreased. Egg, albumen, and eggshell with membrane weight were influenced by the diet × L interaction, while diet × age interaction affected other traits. Egg quality traits were influenced by L x A interaction, but were not affected by D x L x A interaction. The dry matter content of an egg was similar between diets, but moisture content was higher in D_2 than in D_1. The dry matter content of albumen was influenced by diet x age interaction. Therefore, the 8% extruded eggshell appears to be the most suitable diet for improving egg and eggshell quality.

Keywords

Age, Ca Source Level, Dry Matter, Extruded Eggshell, Egg Quality, Laying Hen

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Received: 29 March 2025; **Accepted:** 8 April 2025; **Published:** 29 April 2025

1. Introduction

The poultry industry has become an unparalleled platform for providing valuable animal protein to humans and for a quick return compared to other agricultural sectors. In recent years, the poultry sector in the globe has gained remarkable development in response to the current market demand.

Eggs produced by the poultry industry are completely natural food items that play a vital role in providing animal protein for everybody. Eggs are highly a nutritious food preferred by people of all ages and religions [1]. In poultry farming, feed costs account for approximately 65–70% of the total production expenses. For layer birds, calcium is the most crucial nutrient due to its role in eggshell formation and the maintenance of the birds' skeletal system. The eggshell is a critical component of the egg, shaping it and protecting its internal contents, which are essential for the successful hatching of day-old chicks. Eggshells contain approximately 96.6% calcium carbonate [2]. To ensure optimal eggshell formation, laying hens must receive an adequate dietary supply of calcium. If there is any deficit of Ca in the layer diet, the laying bird will take Ca from her bone to form an eggshell. Besides, lay a thin-shelled egg or shellless egg. In breeder birds, a thick and strong eggshell is essential for maximizing hatchability of the egg.

Therefore, calcium-rich feed ingredients should be incorporated into the diets of laying hens to promote high quality eggshell formation. Calcium sources feed ingredients like limestone, oyster shell, and di-calcium phosphates are used in the layer diet. However, oyster shells are both costly and less readily available, in addition to containing harmful substances such as aluminum (Al), mercury (Hg), and cadmium (Cd) [3]. Limestone, calcium carbonate, and di-calcium phosphate are available in the market but can significantly impact feed and production costs. Egg production and egg quality of laying hens were improved by replacing fine limestone with 75-100% coarse limestone in their diets [4]. Extruded eggshell is an unconventional calcium-rich feed ingredient preferred by birds [5-7].

In addition to calcium, extruded eggshells contain a certain amount of protein due to the presence of the eggshell membrane, which contributes 6–13% crude protein [6]. These eggshells are widely available as byproducts from hatcheries, hotels, restaurants, kitchens, and factories where eggs are used for food and non-food production. Unfortunately, vast quantities of eggshells are discarded as waste, contributing to environmental pollution. Considering these factors, the present study was designed to evaluate the effect of limestone or extruded eggshell on the quality of eggs and eggshells in laying hens to identify the most suitable dietary group for producing high quality and cost-effective eggs essential for the poultry industry.

2. Materials and Methods

All animal care and data collection procedures in this study were approved by the Animal Research Ethics Committee (AREC) of Gazipur Agricultural University (No. FVMS/AREC/2023/48), and carried out following the Guidelines for Experimental Animals (Livestock and Poultry) of the Ministry of Livestock and Fisheries (Dhaka, Bangladesh).

Experimental site and the time of investigation

The experiments were conducted at the laboratory of the Department of Dairy and Poultry Science at Gazipur Agricultural University (GAU), Gazipur-1706, Bangladesh.

2.1. Egg and Eggshell Quality of Laying Hens

Two hundred forty eggs from 2 dietary groups with 2 levels of Ca sources in each at different ages of the birds were collected from the project “Use of extruded eggshell as a calcium source substituting limestone or oyster shell in the diet of laying hen” funded by the Ministry of Education, Bangladesh to measure egg and eggshell quality (Table 1).

Table 1. *Layout for the investigation of egg and eggshell quality of laying hens fed diet included limestone or extruded eggshell at different levels and ages of the birds.*

Diet (D)	Level (L)	Number of eggs Age (A)					Total
		A_1	A_2	A_3	A_4	A_5	
D_1	L_1	12	12	12	12	12	60
	L_2	12	12	12	12	12	60
D_2	L_1	12	12	12	12	12	60
	L_2	12	12	12	12	12	60

Diet (D)	Level (L)	Number of eggs Age (A)					Total
		A_1	A_2	A_3	A_4	A_5	
	Total	48	48	48	48	48	240

D_1= Diet with limestone; D_2=Diet with extruded eggshell; L_1= 4% limestone or extruded eggshell; L_2= 8% limestone or extruded eggshell; A_1= 44 weeks; A_2= 52 weeks; A_3= 60 weeks; A_4= 68 weeks; A_5= 72 weeks of age of the bird

Data recording

The following egg quality traits were recorded during the investigation using the various digital scientific devices listed in Table 2.

Table 2. *Egg and eggshell quality traits and measuring devices.*

Egg and eggshell quality traits	Devices used
Egg weight	Digital balance
Yolk color	DSM Yolk Color Fan
Albumen width	Digital Slide calipers
Albumen height	Digital spherometer/digital height meter
Albumen weight	Digital balance
Yolk width	Digital Slide calipers
Yolk height	Digital spherometer/ digital height meter
Yolk weight	Digital balance
Eggshell weight	Digital balance
Eggshell with membrane thickness	Digital eggshell thickness gauge/ meter
Eggshell thickness	Digital eggshell thickness gauge/ meter
Eggshell strength	Digital egg force reader/Digital eggshell strength measuring machine
Yolk-albumen ratio	Calculator

2.2. Dry Matter Content of Eggs

A total of 60 eggs from two dietary groups at different levels and ages of the bird were collected from the project "Use of extruded eggshell as a calcium source substituting limestone or oyster shell in the diet of laying hen" funded by the Ministry of Education, Bangladesh to assess the dry matter content of eggs (Table 3). The dry matter of eggs was determined using a hot-air oven at a temperature of 105 ℃ for 24 hours.

Table 3. *Layout for the investigation of dry matter traits of eggs from laying hens fed diet included limestone or extruded eggshell at different levels and ages of the birds.*

Diet (D)	Level (L)	Number of eggs Age (A)					Total
		A_1	A_2	A_3	A_4	A_5	
D_1	L_1	3	3	3	3	3	15
	L_2	3	3	3	3	3	15
D_2	L_1	3	3	3	3	3	15
	L_2	3	3	3	3	3	15
	Total	12	12	12	12	12	60

D_1= Diet with limestone; D_2=Diet with extruded eggshell; L_1= 4% limestone or extruded eggshell; L_2= 8% limestone or extruded eggshell; A_1= 44 weeks; A_2= 52 weeks; A_3= 60 weeks; A_4= 68 weeks; A_5= 72 weeks of age of the bird

Data recording

The following dry matter traits of laying hen eggs were recorded:

1. Whole egg weight 2. Dried eggshell weight 3. Dried albumen weight 4. Dried yolk weight 5. Fresh eggshell weight 6. Fresh albumen weight 7. Fresh yolk weight.

2.3. Statistical Analysis

The data on egg and eggshell quality, and dry matter traits of egg were analyzed in 2 diets x 2 levels of Ca sources x 5 age groups factorial design employing Statistix 10 computer package program.

Statistical model

The following statistical model was used for data analysis:

$$Y_{ijkl} = \mu + D_i + L_j + A_k + (D \times L)_{ij} + (D \times A)_{ik} + (L \times A)_{jk} + (D \times L \times A)_{ijk} + e_{ijkl}$$

Where Y_{ijkl} is the observation in the l^{th} replication of the i^{th} dietary group, the j^{th} level and the k^{th} age group.

μ is the overall mean.

D_i is the fixed effect of the i^{th} dietary group (i =1, 2).

L_i is the fixed effect of the j^{th} level of calcium source (j= 1, 2).

A_k is the effect of the k^{th} age group (k = 1-------5).

$(D \times L)_{ij}$ is the interaction effect of the i^{th} dietary group and the j^{th} level of calcium source.

$(D \times A)_{ik}$ is the interaction effect of the i^{th} dietary goup and k^{th} age group.

$(L \times A)_{jk}$ is the interaction effect of the j^{th} level of calcium source and the k^{th} age group.

$(D \times L \times A)_{ijk}$ is the interaction effect of the i^{th} dietary group, the j^{th} level of calcium source, and the k^{th} age group.

e_{ijkl} is a random error.

3. Results

3.1. Impact of Dietary Limestone or Extruded Eggshell on Egg and Eggshell Quality in Laying Hens

The diets varied significantly for albumen width, albumen height, Haugh unit ($p<0.001$), and yolk height ($p>0.05$) but did not vary for other traits ($p>0.05$) (Table 4). Diet D_1 showed higher albumen width and height and Haugh unit, but a lower yolk height than diet D_2. The percentage of albumen and yolk weight, and yolk index, was non-significantly higher in diet D_2 compared to diet D_1. The other traits such as egg weight, eggshell strength, yolk color and width, eggshell with membrane weight and thickness, eggshell and membrane thickness, and yolk albumen ratio were almost similar between diets.

The level of Ca source (L) was significantly different for eggshell strength ($p<0.05$), yolk width ($p<0.001$), yolk weight ($p<0.05$), eggshell with membrane weight (g/egg or%), eggshell with membrane thickness, and eggshell thickness ($p<0.001$). These traits were found to be better in L_2 (8% Ca sources) than in L_1 (4% Ca sources) for both diets. However, L_2 in D_2 was better than L_2 in D_1 for these traits. The remaining egg quality traits, as shown in Table 4, were almost similar between Ca source levels in both diets ($p>0.05$).

There was a significant difference among age groups for the egg and eggshell quality mentioned in Table 4, except for yolk width, albumen weight, and yolk albumen ratio ($p>0.05$). The egg and eggshell quality traits such as egg weight, albumen weight and width, yolk weight and height, and eggshell with membrane weight increased with the age of the birds. However, eggshell strength, egg yolk color, albumen height, eggshell with membrane thickness, eggshell thickness, and membrane thickness decreased with increasing age of the birds. The yolk width and yolk albumen ratio remained almost the same.

Table 4. Egg quality traits of laying hens fed diets with limestone or extruded eggshell at different levels and ages.

Traits	Diet (D)	Level (L)	Age (A)					Mean	LSD value & level of significance+						
			A_1	A_2	A_3	A_4	A_5		D	L	A	D x L	D x A	L x A	D x Lx A
Egg weight (g/egg)	D_1	L_1	58.20	58.18	57.27	58.54	61.67	58.77	1.131^{NS}	1.131^{NS}	1.789^{***}	1.600^{***}	2.530^{NS}	2.530^{NS}	3.577^{NS}
		L_2	56.48	54.97	55.26	58.31	60.17	57.04							
		Mean	57.34	56.57	56.27	58.43	60.92	57.90							
	D_2	L_1	51.50	53.10	56.19	58.53	57.38	55.34							
		L_2	58.65	56.64	56.48	60.88	59.55	58.44							
		Mean	55.07	54.87	56.33	59.70	58.47	56.89							
Eggshell strength (kg/egg)	D_1	L_1	3.10	3.08	2.93	3.64	3.47	3.34	0.205^{NS}	0.205^{*}	0.323^{*}	0.289^{NS}	0.457^{NS}	0.457^{NS}	0.646^{NS}
		L_2	3.39	3.79	3.28	3.54	3.64	3.53							
		Mean	3.24	3.44	3.10	3.59	3.55	3.38							
	D_2	L_1	3.07	3.69	3.36	3.74	3.08	3.39							
		L_2	3.11	3.33	3.77	3.78	3.664	3.531							

Traits	Diet (D)	Level (L)	Age (A)						LSD value & level of significance+						
			A_1	A_2	A_3	A_4	A_5	Mean	D	L	A	D x L	D x A	L x A	D x Lx A
		Mean	3.09	3.51	3.57	3.76	3.372	3.26							
Egg yolk color (DSM)	D_1	L_1	7.75	8.00	8.08	5.75	7.42	7.40	0.153^{NS}	0.153^{NS}	0.243^{***}	0.217^{NS}	0.343^{*}	0.343^{***}	0.485^{NS}
		L_2	7.75	7.75	7.75	6.50	7.17	7.38							
		Mean	7.75	7.87	7.92	6.13	7.29	7.39							
	D_2	L_1	7.75	7.83	7.83	6.42	7.30	7.43							
		L_2	7.92	8.00	8.08	7.00	6.83	7.57							
		Mean	7.83	7.92	7.96	6.71	7.08	7.50							
Albumen width (mm)	D_1	L_1	77.25	76.50	76.61	76.96	83.72	78.21	1.281^{**}	1.281^{NS}	2.025^{***}	1.812^{NS}	2.864^{***}	2.864^{***}	4.051^{*}
		L_2	76.27	76.71	78.18	85.17	79.51	79.17							
		Mean	76.76	76.60	77.40	81.07	81.62	78.69							
	D_2	L_1	72.02	73.13	81.66	79.60	74.11	76.10							
		L_2	77.58	76.58	81.31	81.33	71.15	77.59							
		Mean	74.80	74.86	81.48	80.46	72.63	76.85							
Albumen height (mm)	D_1	L_1	10.53	10.76	10.09	9.83	8.92	10.03	0.259^{***}	0.259^{NS}	0.409^{***}	0.366^{NS}	0.578^{***}	0.578^{**}	0.818^{NS}
		L_2	10.37	9.72	9.63	9.31	9.67	9.74							
		Mean	10.45	10.24	9.86	9.57	9.30	9.88							
	D_2	L_1	9.70	9.95	8.07	11.15	8.16	9.42							
		L_2	9.71	9.90	8.85	10.06	8.59	9.40							
		Mean	9.71	9.93	8.46	10.60	8.37	9.41							
Yolk width (mm)	D_1	L_1	38.75	38.09	38.03	39.34	38.85	38.61	0.484^{NS}	0.484^{***}	0.765^{NS}	0.684^{NS}	1.082^{*}	1.082^{NS}	1.53^{NS}
		L_2	38.40	38.24	38.13	39.33	39.06	38.63							
		Mean	38.58	38.16	38.08	39.34	38.96	38.62							
	D_2	L_1	37.16	37.21	39.99	40.02	39.06	38.69							
		L_2	39.07	37.55	39.05	39.85	39.09	38.92							
		Mean	38.12	37.38	39.52	39.94	39.08	38.81							
Yolk height (mm)	D_1	L_1	18.89	18.43	18.54	18.59	18.72	18.63	0.190^{*}	0.190^{NS}	0.300^{*}	0.268^{NS}	0.424^{*}	0.424^{NS}	0.600^{NS}
		L_2	18.71	18.38	18.74	18.55	19.12	18.70							
		Mean	18.80	18.41	18.64	18.57	18.92	18.67							
	D_2	L_1	18.68	18.50	18.30	19.26	18.95	18.74							
		L_2	18.64	19.00	18.92	19.34	19.26	19.04							
		Mean	18.66	18.75	18.61	19.30	19.11	18.89							
Albumen weight (g/egg)	D_1	L_1	37.14	37.48	33.96	35.78	38.89	36.65	0.943^{NS}	0.943^{NS}	1.491^{**}	1.334^{**}	2.109^{NS}	2.109^{NS}	2.983^{NS}

Traits	Diet (D)	Level (L)	Age (A)					Mean	LSD value & level of significance+						
			A_1	A_2	A_3	A_4	A_5		D	L	A	D x L	D x A	L x A	D x Lx A
		L_2	35.61	33.19	34.53	35.41	37.14	35.18							
		Mean	36.38	35.33	34.24	35.59	38.02	35.91							
	D_2	L_1	32.32	33.17	35.92	35.69	36.29	34.68							
		L_2	36.53	35.37	35.10	37.62	36.55	36.23							
		Mean	34.43	34.27	35.51	36.66	36.42	35.46							
Albumen weight (%)	D_1	L_1	63.76	64.44	59.45	61.10	63.21	62.39	1.148^{NS}	1.148^{NS}	1.815^{NS}	1.623^{NS}	2.567^{NS}	2.567^{NS}	3.630^{NS}
		L_2	63.05	60.39	62.51	60.63	61.72	61.66							
		Mean	63.40	62.42	60.98	60.86	62.46	62.03							
	D_2	L_1	62.58	62.41	63.88	61.02	63.42	62.66							
		L_2	62.47	62.43	62.16	61.68	61.49	62.04							
		Mean	62.53	62.42	63.02	61.35	62.46	62.35							
Yolk weight (g/egg)	D_1	L_1	14.22	14.09	13.68	15.09	15.74	14.56	0.410^{NS}	0.410^{*}	0.648^{***}	0.579^{NS}	0.916^{NS}	0.916^{NS}	1.295^{NS}
		L_2	13.91	14.36	13.75	15.50	15.45	14.59							
		Mean	14.06	14.22	13.71	15.29	15.59	14.58							
	D_2	L_1	12.88	13.23	13.82	15.27	15.14	14.07							
		L_2	14.07	14.42	14.49	15.73	15.51	14.84							
		Mean	13.48	13.83	14.15	15.50	15.33	14.46							
Yolk weight (%)	D_1	L_1	24.48	24.15	23.89	25.77	25.38	24.73	0.480^{NS}	0.480^{NS}	0.760^{***}	0.679^{NS}	1.074^{NS}	1.074^{NS}	1.519^{NS}
		L_2	24.63	26.07	24.85	26.66	25.68	25.58							
		Mean	24.56	25.11	24.37	266.22	25.53	25.16							
	D_2	L_1	25.00	24.97	24.67	26.06	26.34	25.41							
		L_2	23.96	25.51	25.64	25.92	25.91	25.39							
		Mean	24.48	25.24	25.16	25.99	26.12	25.40							
Eggshell with membrane weight (g/egg)	D_1	L_1	6.52	6.26	6.47	7.51	6.85	6.72	0.172^{NS}	0.172^{***}	0.271^{***}	0.243^{*}	0.383^{NS}	0.383^{**}	0.542^{NS}
		L_2	6.66	6.28	6.70	7.19	7.44	6.85							
		Mean	6.59	6.27	6.59	7.35	7.14	6.79							
	D_2	L_1	5.62	6.08	6.32	7.46	6.35	6.36							
		L_2	6.86	6.30	6.68	7.36	7.34	6.91							
		Mean	6.24	9.19	6.50	7.41	6.84	6.63							
Eggshell with membrane weight (%)	D_1	L_1	11.22	10.80	11.32	12.86	11.10	11.46	0.231^{NS}	0.231^{***}	0.365^{***}	0.326^{NS}	0.516^{NS}	0.516^{***}	0.729^{NS}
		L_2	11.80	11.44	12.14	12.33	12.39	12.02							
		Mean	11.51	11.12	11.73	12.60	11.74	11.74							

Traits	Diet (D)	Level (L)	Age (A)					Mean	LSD value & level of significance+						
			A_1	A_2	A_3	A_4	A_5		D	L	A	D x L	D x A	L x A	D x Lx A
	D_2	L_1	10.94	11.46	11.21	12.74	11.03	11.47							
		L_2	11.73	11.12	11.81	12.14	12.34	11.83							
		Mean	11.34	11.29	11.51	12.44	11.68	11.65							
Eggshell with membrane thickness (mm)	D_1	L_1	0.41	0.42	0.38	0.42	0.38	0.40	0.007^{NS}	0.007^{***}	0.011^{***}	0.010^{NS}	0.016^{***}	0.016^{***}	0.022^{NS}
		L_2	0.42	0.42	0.43	0.44	0.42	0.43							
		Mean	0.42	0.42	0.41	0.43	0.40	0.41							
	D_2	L_1	0.42	0.41	0.40	0.42	0.37	0.40							
		L_2	0.44	0.38	0.43	0.45	0.42	0.42							
		Mean	0.43	0.39	0.41	0.43	0.39	0.41							
Eggshell thickness (mm)	D_1	L_1	0.38	0.38	0.35	0.39	0.36	0.37	0.007^{NS}	0.007^{***}	0.011^{***}	0.010^{NS}	0.016^{**}	0.016^{***}	0.023^{NS}
		L_2	0.39	0.38	0.39	0.41	0.38	0.39							
		Mean	0.38	0.38	0.37	0.40	0.37	0.38							
	D_2	L_1	0.39	0.37	0.36	0.38	0.34	0.37							
		L_2	0.41	0.34	0.39	0.42	0.39	0.39							
		Mean	0.40	0.36	0.37	0.40	0.36	0.38							
Membrane thickness (mm)	D_1	L_1	0.033	0.038	0.035	0.033	0.021	0.032	0.002^{NS}	0.002^{NS}	0.004^{***}	0.003^{*}	0.005^{NS}	0.005^{NS}	0.008^{NS}
		L_2	0.032	0.036	0.040	0.036	0.035	0.036							
		Mean	0.033	0.037	0.038	0.034	0.028	0.034							
	D_2	L_1	0.035	0.033	0.038	0.039	0.031	0.035							
		L_2	0.029	0.038	0.036	0.035	0.029	0.034							
		Mean	0.032	0.036	0.037	0.037	0.030	0.034							
Haugh unit	D_1	L_1	101.88	102.83	100.23	98.87	92.95	99.35	1.29^{**}	1.29^{NS}	2.04^{***}	1.83^{NS}	2.89^{***}	2.89^{**}	4.08^{NS}
		L_2	101.66	98.99	98.51	96.40	97.20	98.55							
		Mean	101.77	101.91	99.37	97.64	95.07	98.95							
	D_2	L_1	99.97	100.54	90.87	104.32	90.76	97.29							
		L_2	98.28	99.66	94.54	99.38	92.57	96.89							
		Mean	99.12	100.10	92.70	101.85	91.66	97.09							
Yolk index	D_1	L_1	0.49	0.48	0.49	0.47	0.48	0.48	0.007^{NS}	0.007^{NS}	0.011^{*}	0.009^{NS}	0.015^{**}	0.015^{NS}	0.021^{NS}
		L_2	0.49	0.48	0.49	0.47	0.49	0.49							
		Mean	0.49	0.48	0.49	0.47	0.49	0.48							
	D_2	L_1	0.50	0.50	0.46	0.48	0.49	0.49							
		L_2	0.48	0.51	0.49	0.49	0.49	0.49							

Traits	Diet (D)	Level (L)	Age (A) A_1	A_2	A_3	A_4	A_5	Mean	LSD value & level of significance+ D	L	A	D x L	D x A	L x A	D x Lx A
		Mean	0.49	0.50	0.47	0.48	0.49	0.49							
Yolk-Albumen ratio	D_1	L_1	0.39	0.38	0.63	0.42	0.40	0.44	0.053^{NS}	0.053^{NS}	0.083^{NS}	0.075^{NS}	0.118^{NS}	0.118^{NS}	0.167^{NS}
		L_2	0.39	0.43	0.40	0.44	0.42	0.42							
		Mean	0.39	0.40	0.52	0.43	0.41	0.43							
	D_2	L_1	0.40	0.40	0.39	0.43	0.42	0.41							
		L_2	0.39	0.41	0.41	0.42	0.42	0.41							
		Mean	0.39	0.41	0.40	0.43	0.42	0.41							

+NS, P>0.05; *, P<0.05; **, P<0.01; ***, P<0.001; D_1 = Diet with limestone; D_2 = Diet with extruded eggshell; L_1= Diet with 4% limestone or extruded eggshell; L_2= Diet with 8% limestone or extruded eggshell; A_1= 44 weeks; A_2= 52 weeks; A_3= 60 weeks; A_4= 68 weeks; A_5= 72 weeks of age of the bird

The interaction between diet and level of Ca source influenced egg weight, albumen weight, eggshell with membrane weight, and membrane thickness, but not other egg quality traits. The diet significantly interacted with the age of the birds for the egg quality traits, except for egg weight, eggshell strength, albumen weight, yolk weight, and eggshell with membrane weight (p>0.05). The Ca source level (L) interacted with age group (A) for the egg quality traits, except for egg weight, eggshell strength, yolk width and height, albumen and yolk weight, membrane thickness, yolk index, and yolk albumen ratio. No interaction of diet x calcium source level x age group was found for the egg quality traits (p>0.05), except for albumen width (p<0.05).

3.2. Dry Matters of Eggs of Laying Hen-fed Diet Included Limestone or Extruded Eggshell at Different Levels and Ages of Birds

The percentage of fresh egg weight, moisture content, dry yolk (p<0.05), and dry albumen weight (p<0.01) differed significantly between diets (D_1 and D2) (p<0.05). However, other traits, such as fresh egg, dry egg, albumen, yolk, and eggshell weight (g/egg), and the percentage of fresh albumen, yolk, eggshell, and dry eggshell weight were identical between two diets (p>0.05) (Table 5). The percentage of fresh egg, dry albumen, and dry yolk weight was higher in diet D_1 compared to diet D_2. Consequently, the egg in diet D_2 contained higher moisture (%) than in diet D_1. The other dry matter traits of eggs were nearly identical between the two diets. As for the effect of the level of Ca sources, no significant disparity was observed between levels in both diets (p>0.05). Therefore, dry matter traits of eggs were nearly the same between the two levels of Ca sources in both diets. Age significantly affected fresh yolk weight, eggshell weight (g/egg), eggshell weight (%) (p<0.01), and dry albumen weight (g/egg) (p<0.05), dry albumen weight (%) (p<0.01), and dry eggshell weight (%) (p<0.05), while the other traits were not affected by age (p>0.05). The fresh albumen and eggshell weight percentages increased, whereas dry albumen and eggshell weight percentages decreased with increasing bird age. The other dry matter measurements of eggs were statistically similar among the dietary groups.

Table 5. *Dry matter content of eggs of laying hens fed diets with limestone or extruded eggshell at different levels and ages.*

Traits	Diet (D)	Level (L)	Age (A) A1	A2	A3	A4	A5	Mean	LSD value & level of significance+ D	L	A	D x L	D x A	L x A	D x L x A
Fresh egg weight (g/egg)	D_1	L_1	56.44	56.05	57.93	54.24	57.86	56.50	2.294^{NS}	2.294^{NS}	3.627^{NS}	3.244^{NS}	5.129^{NS}	5.129^{NS}	7.254^{NS}
		L_2	51.79	54.31	57.77	58.74	62.23	56.97							

Traits	Diet (D)	Level (L)	Age (A)					Mean	LSD value & level of significance+						
			A1	A2	A3	A4	A5		D	L	A	D x L	D x A	L x A	D x L x A
		Mean	54.11	55.18	57.85	56.49	60.05	56.73							
	D$_2$	L$_1$	55.56	54.06	60.70	58.98	55.16	56.89							
		L$_2$	58.05	57.96	60.02	53.80	54.56	56.88							
		Mean	56.80	56.01	60.36	56.39	54.86	56.89							
Fresh albumen weight (g/egg)	D$_1$	L$_1$	35.95	36.83	35.79	32.82	36.22	35.43	1.675^{NS}	1.675^{NS}	2.649^{NS}	2.369^{NS}	3.746^{NS}	3.746^{NS}	5.298^{NS}
		L$_2$	32.31	34.33	36.28	34.99	38.75	35.33							
		Mean	34.13	35.36	36.04	33.91	37.49	35.38							
	D$_2$	L1	35.63	33.64	38.37	36.83	34.21	35.74							
		L2	36.00	37.28	36.64	32.89	33.00	35.16							
		Mean	35.82	35.46	37.51	34.86	33.61	35.45							
Fresh yolk weight (g/egg)	D$_1$	L$_1$	14.06	13.06	14.61	14.63	15.07	14.29	0.675^{NS}	0.675^{NS}	1.067^{**}	0.955^{NS}	1.509^{NS}	1.509^{NS}	2.135^{NS}
		L$_2$	13.11	13.18	15.16	16.25	16.21	14.78							
		Mean	13.58	13.12	14.89	15.44	15.64	14.53							
	D$_2$	L1	13.89	13.99	15.60	15.12	14.60	14.64							
		L2	15.16	14.13	16.26	14.12	14.51	14.84							
		Mean	14.53	14.06	15.93	14.62	14.55	14.74							
Fresh eggshell weight (g/egg)	D$_1$	L$_1$	6.22	6.47	6.95	6.57	6.22	6.49	0.252^{NS}	0.252^{NS}	0.399^{**}	0.357^{NS}	0.564^{NS}	0.564^{NS}	0.798^{*}
		L$_2$	6.25	6.66	6.21	7.30	6.25	6.54							
		Mean	6.24	6.57	6.58	6.94	6.24	6.51							
	D$_2$	L$_1$	5.83	6.17	6.65	7.17	5.83	6.33							
		L$_2$	6.63	6.37	6.75	6.61	6.63	6.60							
		Mean	6.23	6.27	6.70	6.89	6.23	6.47							
Fresh egg weight (%)	D$_1$	L$_1$	30.50	33.09	32.06	30.20	29.84	31.14	0.828^{*}	0.828^{NS}	1.310^{NS}	1.171^{NS}	1.852^{NS}	1.852^{NS}	2.619^{NS}
		L$_2$	31.36	33.30	31.46	31.27	30.51	31.58							
		Mean	30.93	33.19	31.76	30.74	30.17	31.36							
	D$_2$	L1	29.82	30.07	30.76	30.39	30.73	30.35							
		L2	30.31	30.31	30.39	30.35	30.05	30.28							
		Mean	30.07	30.19	30.58	30.37	30.39	30.32							
Fresh albumen weight (%)	D$_1$	L$_1$	63.60	64.80	61.99	60.43	62.63	62.69	1.438^{NS}	1.438 NS	2.273 NS	2.033 NS	3.215 NS	3.215 NS	4.546^{NS}
		L$_2$	62.41	63.64	62.71	59.58	62.07	62.08							
		Mean	63.01	64.22	62.35	60.01	62.35	62.39							
	D2	L1	64.07	62.21	63.01	62.45	61.97	62.74							
		L2	62.00	64.25	61.08	61.08	60.49	61.78							

Traits	Diet (D)	Level (L)	Age (A)					Mean	LSD value & level of significance+						
			A1	A2	A3	A4	A5		D	L	A	D x L	D x A	L x A	D x L x A
		Mean	63.04	63.23	62.04	61.76	61.23	62.26							
Fresh yolk weight (%)	D_1	L_1	25.01	23.40	25.10	27.02	26.01	25.31	1.154^{NS}	1.154^{NS}	1.825^{NS}	1.632^{NS}	2.581^{NS}	2.581^{NS}	3.650^{NS}
		L_2	25.28	24.56	26.35	27.67	26.27	26.03							
		Mean	25.14	23.98	25.73	27.35	26.14	25.67							
	D_2	L1	25.05	25.89	25.87	25.64	26.52	25.80							
		L2	26.13	24.43	27.10	26.19	26.60	26.09							
		Mean	25.59	25.16	26.48	25.91	26.56	25.94							
Fresh eggshell weight (%)	D_1	L_1	11.01	11.57	11.99	12.14	10.80	11.50	0.405^{NS}	0.405^{NS}	0.641^{**}	0.573^{NS}	0.906^{NS}	0.906^{NS}	1.282^{NS}
		L_2	12.08	12.29	10.75	12.43	10.15	11.54							
		Mean	11.55	11.93	11.37	12.29	10.47	11.52							
	D_2	L_1	10.52	11.43	10.99	12.15	10.60	11.14							
		L_2	11.43	11.01	11.24	12.39	12.16	11.65							
		Mean	10.97	11.22	11.12	12.27	11.38	11.39							
Dry egg weight (g/egg)	D_1	L_1	17.20	18.55	18.72	16.38	17.28	17.63	0.807^{NS}	0.807^{NS}	1.276^{NS}	1.141^{NS}	1.804^{NS}	1.804^{NS}	2.551^{NS}
		L_2	16.25	17.94	18.16	18.36	18.95	17.93							
		Mean	16.73	18.24	18.44	17.37	18.11	17.78							
	D_2	L_1	16.56	16.26	18.64	17.93	16.96	17.27							
		L_2	17.59	17.56	18.26	16.33	16.40	17.23							
		Mean	17.08	16.91	18.45	17.13	16.68	17.25							
Moisture%	D_1	L_1	69.50	66.91	67.95	69.80	70.16	68.86	0.828^{*}	0.828^{NS}	1.310^{NS}	1.171^{NS}	1.852^{NS}	1.852^{NS}	2.619^{NS}
		L_2	68.65	66.70	68.55	68.73	69.49	68.42							
		Mean	69.07	66.81	68.25	69.27	69.83	68.64							
	D_2	L1	70.18	69.93	69.24	69.62	69.27	69.65							
		L2	69.69	69.69	60.61	69.65	69.95	69.72							
		Mean	69.93	69.81	69.43	69.63	69.61	69.68							
Dry albumen weight (g/egg)	D_1	L_1	4.73	5.62	5.06	3.73	4.25	4.68	0.348^{NS}	0.348^{NS}	0.550^{*}	0.492^{NS}	0.778^{NS}	0.778^{NS}	1.100^{NS}
		L_2	3.90	4.94	4.92	4.08	4.51	4.47							
		Mean	4.32	5.28	4.99	3.91	4.38	4.57							
	D_2	L_1	4.68	3.81	4.86	4.39	4.21	4.39							
		L_2	4.32	4.85	4.38	3.91	3.99	4.29							
		Mean	4.50	4.33	4.62	4.15	4.10	4.33							
Dry yolk weight (g/egg)	D_1	L_1	7.34	7.24	7.80	7.31	7.79	7.50	0.493^{NS}	0.493^{NS}	0.779^{NS}	0.679^{NS}	1.102^{NS}	1.102^{NS}	1.558^{NS}

Traits	Diet (D)	Level (L)	Age (A)					Mean	LSD value & level of significance+						
			A1	A2	A3	A4	A5		D	L	A	D x L	D x A	L x A	D x L x A
		L_2	7.01	7.07	8.10	8.29	8.60	7.82							
		Mean	7.18	7.16	7.95	7.80	8.20	7.66							
	D_2	L_1	7.08	7.09	8.19	7.63	7.68	7.53							
		L_2	7.69	7.27	8.39	7.15	7.15	7.53							
		Mean	7.39	7.18	8.29	7.39	7.41	7.53							
Dry eggshell weight (g/egg)	D_1	L_1	5.13	5.68	5.86	5.34	5.24	5.45	0.219 NS	0.219 NS	0.346 NS	0.310 NS	0.489 NS	0.489 NS	0.692*
		L_2	5.33	5.92	5.14	5.99	5.83	5.64							
		Mean	5.23	5.80	5.50	5.66	5.54	5.55							
	D_2	L_1	4.80	5.36	5.59	5.91	5.07	5.35							
		L_2	5.58	5.44	5.49	5.27	5.27	5.41							
		Mean	5.19	5.40	5.54	5.59	5.17	5.38							
Dry albumen weight (%)	D_1	L_1	13.08	15.37	14.00	11.30	11.71	13.10	0.497**	0.497 NS	0.786** *	0.703 NS	1.112** *	1.112 NS	1.572 NS
		L_2	12.08	14.34	13.44	11.67	11.60	12.63							
		Mean	12.89	14.86	13.72	11.49	11.66	12.86							
	D_2	L_1	13.11	11.29	12.56	11.91	12.29	12.23							
		L_2	12.00	12.98	11.91	11.89	12.06	12.17							
		Mean	12.55	12.14	12.23	11.90	12.17	12.20							
Dry yolk weight (%)	D_1	L_1	52.18	55.47	52.73	49.90	51.55	52.37	1.259*	1.259 NS	1.991 NS	1.781 NS	2.815 NS	2.815 NS	3.981 NS
		L_2	53.23	53.56	53.40	51.04	53.02	52.85							
		Mean	52.71	54.52	53.07	50.47	52.29	52.61							
	D_2	L_1	50.97	50.69	52.50	50.42	52.42	51.40							
		L_2	50.76	51.44	51.55	50.40	49.27	50.69							
		Mean	50.87	51.07	52.03	50.41	50.84	51.04							
Dry eggshell weight (%)	D_1	L_1	82.45	87.80	84.26	81.41	85.79	84.34	2.548 NS	2.548 NS	4.029*	3.604 NS	5.698 NS	5.698 NS	8.058 NS
		L_2	85.25	88.96	82.73	81.95	93.22	86.42							
		Mean	83.85	88.38	83.49	81.68	89.50	85.38							
	D_2	L_1	82.34	86.79	84.07	82.59	86.91	84.54							
		L_2	84.03	85.39	81.31	79.57	79.41	81.94							
		Mean	83.19	86.09	82.69	81.08	83.16	83.24							

+NS, P>0.05; *, P<0.05; **, P<0.01; ***, P<0.001; D_1 = Diet with limestone; D_2 = Diet with extruded eggshell; L_1= Diet with 4% limestone or extruded eggshell; L_2= Diet with 8% limestone or extruded eggshell; A_1= 44 weeks; A_2= 52 weeks; A_3= 60 weeks; A_4= 68 weeks; A_5= 72 weeks of age of the bird

No interaction between diet and Ca source levels for dry matter in eggs was found. The interaction between diet and age was observed only for dry albumen weight percentage ($p < 0.001$), where dry albumen weight percentage decreased with increasing bird age. The rest of the dry matter parameters of the eggs were not influenced by the interaction between diet and age (D x A). Furthermore, diet and Ca source levels (D x L) did not interact in terms of the dry parameters of the egg. Accordingly, no interaction among diet, Ca source levels, and age (D x L x A) was observed for the dry parameters, except for fresh eggshell weight (g/egg) ($p < 0.05$).

4. Discussion

4.1. Impact of Dietary Limestone or Extruded Eggshell on Egg and Eggshell Quality in Laying Hens

The egg quality traits were almost similar between the two diets (D_1 and D_2), except for albumen width and height, Haugh unit. Diet D_1 showed a higher Haugh unit due to a higher albumen height and similar egg weight between the two diets (D_1 and D_2). On the other hand, diet D_2 evidently, showed a higher percentage of albumen and yolk weight, yolk index compared to diet D_1. Since the yolk height was higher in D_2, the higher yolk index in diet D_2, also indicated better quality eggs. This finding was consistent with the findings of [8], who reported a non-significant difference among dietary groups (0, 5, 10, and 15% eggshell meal) for eggshell strength in laying hens. This finding is also supported by [5, 6, 9], but partially contradicted by [10, 11]. They found non-significant differences among dietary groups when extruded eggshells, limestone, and oyster shells alone or their combination were used in the laying hen diet. Scheideler, 1998 [12] found improved eggshell quality when combining extruded eggshell and large particle-size limestone or oyster shell in laying hen diets.

L_2 (8% Ca sources) performed better than L1 (4% Ca sources) in both diets for eggshell strength, yolk width, yolk weight, eggshell with membrane weight and thickness, and eggshell thickness. Hence, L_2 was better in the diet D_2 than L_2 in the diet D_1 for these traits. Although the other egg quality traits were almost similar between the Ca source levels in both diets, L_2 in D_2 was found to be better than L_2 in D_1. Therefore, the 8% extruded eggshell was better than the 8% limestone for egg and eggshell quality. This finding contradicted by [8], as they found no significant difference among diets (0, 5, 10, and 15% eggshell meal of laying hens) for eggshell strength. Scientists [13] observed non-significant differences among dietary groups (0, 1.75%, 3.5%, and 7% eggshell diets) for egg quality; however, they found a tendency to improve egg quality in the 7% eggshell diet over the others.

This study investigated increasing egg and albumen weight, albumen width, yolk weight and height, and eggshell with membrane weight with the increasing age of the birds. It also observed that eggshell strength, egg yolk color, albumen height, eggshell with membrane thickness, eggshell thickness, and membrane thickness decreased with the increasing age of the birds, which corroborated the findings of [14, 15, 5, 6]. They reported improved egg and eggshell weight and a decrease in eggshell thickness and strength with increasing age of the birds.

The interaction of diet (D) and Ca source level (L) influenced egg, albumen, and eggshell weight and thickness. The interaction of diet (D) and age (A) affected egg quality traits, except for the egg, albumen, yolk weight, eggshell with membrane weight, and eggshell strength. Egg quality traits improved with the interaction of Ca source level (L) and age (A), but the eggshell strength, yolk width, and height, albumen and yolk weight, membrane thickness, yolk index, and yolk albumen ratio were not influenced by the interaction of Ca source level (L) and age (A). The interaction of diet, Ca source level, and age of the bird did not influence egg quality traits, but it did affect albumen width ($p<0.05$). No literature on these interaction effects on egg quality traits was found.

4.2. Dry Matters of Eggs of Laying Hen-fed Diet Included Limestone or Extruded Eggshell at Different Levels and Ages of Birds

Dry egg, albumen, and yolk weight in gram (g) were almost identical between diets, nevertheless, the percentage of dry albumen and yolk was higher in D_1 compared to D_2. On the other hand, the level of Ca sources had no influence on the dry matter content of eggs, which may be attributed to the effect of other nutrients present in the feed ingredients rather than the Ca content itself. No previous studies were found on these effects. The dry matter content of eggs decreses with the advancing age of the bird, as corroborated by [5, 6]. The dry traits of eggs were not influenced by the interaction of D x L, D x A, L x A, and D x Lx A, except for the percentage of dry albumen, which was affected by the interaction of D x A, where age reduced the dry traits of the egg, as supported by [14].

5. Conclusion and Recommendation

This study reveals that the diet with extruded eggshells proved more effective than the limestone-based diet in enchanging egg quality traits and dry egg weight. The L_2 (8% Ca sources) was found to be superior to the L_1 (4% Ca sources) in both extruded eggshell and limestone based diets in terms of egg quality traits. Notably, the 8% extruded eggshell diet (L2) outperformed the 8% limestone diet (L2) in terms of egg quality traits. As the effect of age, egg, albumen, yolk, and eggshell weight, as well as albumen width and yolk height increased with age. However, eggshell strength, albumen height, Haugh unit, and eggshell thickness decreased as the birds aged. The interaction between diet and Ca source level influenced egg, albumen, and eggshell with membrane weight. Therefore, incorporating extruded eggshells at an 8% level in the diet of laying hens may be beneficial to improve egg quality.

Abbreviations

AREC	Animal Research Ethics Committee
FVMAS	Faculty of Veterinary Medicine and Animal Science
LSD	Least Significant Difference

Acknowledgments

The authors are grateful to the Ministry of Education, Government of the People's Republic of Bangladesh, and the Department of Dairy and Poultry Science, Gazipur Agricultural University, Bangladesh for supporting in carrying out research and preparing the scientific article.

Author Contributions

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Funding

The author received no specific funding for this work.

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] DLS. (2020). Livestock Economy at a Glance. Retrieved from DLS web portal: https://dls.portal.gov.bd

[2] El-Ishaq, A., & Kida, H. D., 2011. Comparative analysis of calcium carbonate content in eggshell of hen, duck and guinea fowl. IRAON, 3, 81-87.

[3] Schaafsma, A., Pakan, I., Hofstede, G. J. H., Muskiet, F. A., Van Der Veer, E., & De Vries, P. J. F. (2000). Mineral, amino acid and hormonal composition of chicken eggshell powder and the evaluation of its use in human nutrition. Poultry Science, 79(12), 1833–1838.

[4] Khosht, A. R., Abdel -Khalek, A. M. & Abed El-Magid, M. A. (2020). Influences of calcium levels with limestone particle size on laying performance, and eggshell quality traits of layers. Egyptian Poultry Science Journal, 40(3), 631-645. http://www.epsj.journals.ekb.eg/

[5] Islam, A. (2020). The final report of the project "Use of extruded eggshell as a calcium source substituting limestone of oyster shell in the diet of laying hen". Final Report, Ministry of Education, BANBES, Dhaka, Bangladesh.

[6] Islam, M. A. & Nishibori, M. (2021). Use of extruded eggshell as a calcium source substituting limestone or oyster shell in the diet of laying hens. Veterinary Medicine and Science, 7, 1948-1958. http://doi.org/10.1002/vms3.544

[7] Islam, M. A., Das, N. C., & Nishibori, M. (2022**)**. Effect of extruded eggshell, limestone and oyster shell on egg production performance of laying hens. Journal of Agricultural and Crop Research, 10(3), 43-51. http://sciencewebpublishing.net/jacr/archive/2022/June/pdf/Islam%20et%20al.pdf

[8] Cufadar, Y. (2014). Effect of Alternative Calcium Sources on Performance and Eggshell Quality in Laying Hens. The Journal of Macro Trends in Applied Science, 21, 587-592.

[9] Lichovnikova, M. (2007). The effect of dietary calcium source, concentration and particle size on calcium retention, eggshell quality and overall calcium requirement in laying hens. British Poultry Science, 48(1), 71–75.

[10] Gongruttananun, N. (2011). Effects of using ground eggshells as a dietary calcium source on egg production traits, hatching performance and eggshell ultrastructure in laying hens. Kasetsart Journal. Natural Sciences, 45, 209-220.

[11] Olgun, O., Yildiz, A. Ö., & Cufadar, Y. (2015). The effects of eggshell and oyster shell supplemental as calcium sources on performance, eggshell quality and mineral excretion in laying hens. Indian Journal of Animal Research, 49(2), 205.

[12] Scheideler, S. E. (1998). Eggshell Calcium Effects on Egg Quality and Ca Digestibility in First- or Third-Cycle Laying Hens. Journal of Applied Poultry Research, 7(1), 69–74.

[13] Sim, J. S., Aw-Yong, L. M., & Bragg, D. B. (1983). Utilization of eggshell waste by the laying hen. Poultry Science, 62, 2227-2229.

[14] Chung, S. H. & Lee, K. W. (2014). Effect of Hen Age, Storage Duration and Temperature on Egg Quality in Laying Hens. International Journal of Poultry Science, 13(11), 634-636.

[15] Padhi, M. K., Chatterjee R. N., Haunshi S., & Rajkumar, U. (2013). Effect of age on egg quality in chicken. Indian Journal of Poultry Science, 48(1), 122-125.