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The Impact of Different Plumage Color Selected Quail Lines on Egg Quality and Eggshell Properties

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Abstract

This study aimed to explore how different genotypes influence eggshell traits, egg quality, ultrastructure and nutritional value in egg quails during the egg production phase. Two breeder lines selected for six generations, brown quail and white quail, were subject to analysis, with 90 eggs examined for each genotype. At 42 days of age, 180 eggs were used, 90 from each strain for egg quality, eggshell characteristics and shell microstructure. The results indicated that the breed strain had a significant effect on various parameters, including egg weight, length, width, shape index, albumen height, weight and percentage, yolk height, diameter, index, weight and percentage, yolk-albumen ratio, shell weight and shell percentage. In particular, the white strain eggshell had different ultrastructure flaws than the brown strain. These included late fusion, poor confluence and different striations. Additionally, the egg quail showed a high content of polyunsaturated fatty acids. Different genotypes influence eggshell traits, egg quality and ultrastructure. Understanding these genetic variances can lead to improvements in egg production systems' overall performance and egg quality.

Keywords: Albumen yolk; eggshell characteristics; egg quality; quail genotype; shape index and ultrastructure.

1. Introduction

Quail is a small and economically significant poultry species due to its key attributes, such as a short growth cycle, strong reproductive capability, excellent egg production performance, noteworthy nutritional value, minimal investment requirements and substantial economic benefits [1, 2]. Notably, the eggs of wild Japanese quail (*Coturnix japonica*) are lighter than those of domestic varieties [3, 4] and their size is proportionate to weight. Domestic quail lay eggs weighing between 9 and 14 g on average, representing a 20 to 100% increase in weight over their wild counterparts. Egg-laying breeds typically produce smaller eggs weighing around 9- 12 g [5, 6], whereas larger meat-type strains produce yolks weighing between 12 and 14 g [7- 9]. Globally, people raise Japanese quail for both egg and meat production, renowned for their rapid growth [10, 11]. In Europe, the emphasis is on improving quail meat production, but in Asia, egg production comes first [12]. Japanese quail eggs, despite their smaller size, boast three times the nutritive value of chicken eggs [13]. Quails possess significant economic value as they offer a viable alternative to commonly raised chickens. In Egypt, the quail industry is experiencing rapid expansion due to the birds' ability to thrive in compact environments, their manageable nature and their ability to reach sexual maturity within a short span of six weeks [14, 15]. People primarily cultivate the Japanese quail for its meat and eggs, which have gained immense popularity among consumers [16]. Egg quality assessment encompasses both external and internal measurements [17]. External characteristics include egg weight, eggshell thickness, shape index, impact hatchability and chick development [18]. The egg shape index, which indicates the width-to-length ratio, is crucial for determining egg quality. Abnormal egg shapes, whether round or unusually long, pose challenges during shipment and may result in economic losses. Consumers are more likely to reject abnormally shaped eggs and flaws in the ultrastructure of eggshell can cause breakage or poor embryonic development during incubation [19]. The internal egg quality characteristics, including albumen index, yolk index (YI), percentage of albumen and yolk and Haugh unit (HU), serve as essential indicators of egg freshness [20]. HU and YI are particularly significant for assessing internal egg quality, with higher scores indicating superior egg quality [21]. The emergence of functional food products aimed at improving public health has spurred research efforts to engineer feeds, including the production of functional meat or eggs low in cholesterol and rich in n-3 fatty acids or a balanced blend of n-6 and n-3. For example, Indonesia produces palm oil high in n-6 and lemur fish oil high in n-3. Some fatty acids, due to their numerous physiological activities, can influence consumer health. Conjugated linoleic acid is believed to possess anticancer and antioxidant properties, along with immune system benefits [22, 23]. Eggshell formation primarily occurs in the

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hen's uterus and is the longest stage of the egg-forming process, lasting between 10 and 22 hours. Eggshell deposition occurs in three stages: initial mineralization of the mammillary layer, fast mineralization of the palisade layer and final calcification of the vertical crystal layer. During eggshell formation, mammillary knob density, size and thickness can all have an impact on palisade thickness [24]. The crucial time in the mineralization process is when calcium carbonate forms in the mammillary and palisade layers. The uterus' mineralization state has a direct impact on the eggshell's quality. Genetic factors and various environmental factors primarily cause defective eggshells [25–27]. During the egg production phase, the current study aimed to investigate the effects of different genotypes on eggshell features, egg quality, ultrastructure and nutritional composition in quail eggs.

2. Experimental Setup Chemical and Reagents

Data collection

In this investigation, two distinct breeder strains selected for six generations for increased body weight, at the same age, take the same feed and the same condition of management, namely white quail and brown quail (meat line), were employed to assess the quality of eggs. A total of 180 eggs, evenly distributed with 90 eggs from each strain at 42 days of age, were selected for comprehensive assessment. The eggs were subjected to precise weighing using a sensitive balance to ascertain the form index. Furthermore, each egg's length and width were measured to estimate its respective sizes, providing essential information for assessing egg quality and breeder strain performance.

Evaluating the quality of eggs

The following equations were utilized for accurate measurements of egg quality:

Egg shape-index = yolk height / yolk diameter × 100

Eggshell percentage = eggshell weight/ egg weight×100

Internal characteristics were measured by breaking all eggs and the following equations were employed:

Yolk shape index= yolk diameter/ yolk height× 100

Albumen%= albumen weight/ egg weight×100

Yolk albumen ratio (YA) = weight of yolk/ weight of albumen ×100

Chick yield percentage= chick weight (g)/ initial egg weight (g)×100

Yolk percentage= yolk weight / Egg weight×100

Chemical analysis of the Japanese quail egg

At six weeks, 60 samples of albumen, yolk and eggshell were randomly selected from each strain (30 eggs / strain). Chemical analysis of these samples were performed in the Food Science Department's Accredited Research Laboratory at the Food Science National Research Centre. The dry matter, crude protein, crude fat and Ash percentage contents were determined using traditional procedures comprising an oven, Kjeldahl apparatus, Soxhlet extractor and muffle furnace.

Determination of fatty acids

The total fatty acid content of quail dry egg powder was measured in triplicate using the procedures described in AOAC 2010 [28].

Shell microstructure

For microscopic analysis, a total of six eggshell samples were gathered, which included five distinct strains of quail (brown and white). A slice measuring 1 square centimeter from the equatorial area of each egg's shell was made. The shell membranes were removed using a chemical solution. The samples were further analyzed using a JEOL JSM-T330A Scanning Electron Microscope. Various structural measurements, including palisade and mammillary layer lengths, total thickness and mammillary layer thickness, were obtained.

The ultrastructural integrity of each egg was assessed using a comprehensive ultrastructural score, considering features such as confluence, type B, type A, cubic, aragonite and altered membrane. Fusion and cuffing were also evaluated, with corresponding ranks assigned based on the degree of occurrence.

EDAX contains

Eggshell samples, each representing two different strains (brown and white quails) were collected for EDAX examination. A 1 cm² fragment of shell from the equatorial area of each egg was obtained and the shell membranes were eliminated using a chemical solution. The samples were then examined under EDAX. Various structural measurements, including mineral content were obtained.

Statistical analysis

The data were analyzed using the XLSTAT software, specifically employing a one-way analysis of variance (ANOVA) based on the general linear model (GLM) in XLSTAT, version 2019 [29]. The primary impacts were linear. The examined traits included egg weight (g), egg length (mm), egg width (mm), shape index, shell thickness (mm), shell weight (g), shell percentage, yolk height (mm), albumen height (mm), yolk weight (g), yolk percentage, albumen weight (g), albumen percentage, Laroch, chemical analysis, fatty acid composition, membrane layer (µm), cuticle layer (µm), mammillary layer (µm) and palisade layer (µm).

The following model was used: $Yij = \mu + Li + eijk$

Where: Yij: The ith observation of the jth variable.µ: the overall mean; Li: the effect of the ith line and eijk: random error.

The data presented are reported as least square means (LSM), with standard errors (SE) indicated. The mean values were separated using Duncan's multiple range test (Duncan, 1955) [30] when a statistically significant difference was discovered, with a significance level of 5%.

3. Results and Discussion

Table (1) presents data highlighting the influence of the white and brown feathers on various egg traits, with a notable focus on egg weight. The overall mean indicates that the white quail feathered strain exhibited higher egg weights (14.28 g) compared to the brown quail feathered strain (12.59 g). This finding contrasts with the reported egg weights of 10.27-12.76 g for quail by Sari et al. [31] and Narinc et al. [32], who observed lower weights. In contrast, Alkan et al. [33] observed a greater mean egg weight of 14.14 g in a Japanese quail line with a high body weight.

The impact of strain on egg length is also significant, with the overall mean showing that the white quail feathered strain produced eggs with greater length (35.21 mm) than the brown quail feathered strain (33.21 mm). This observation aligns with findings published by Abdulfatah et al. [34], Alasahan et al. [35] and Akram et al. [36], indicating a consistent trend. The statistical analysis revealed a significant relationship between the color of eggshells and the presence of spots in both egg weight and egg breadth (P = 0.05).

Similarly, the effect of strain on egg width is evident in the overall mean, with the white quail feathered strain 4 exhibiting greater egg width (26.37 mm) than the brown quail feathered strain (25.51 mm). This outcome is in agreement with the results reported by [34-36]. Regarding the shape index, indicative of overall egg shape, no significant differences were observed between strains. This finding aligns with results reported in the literature by [32-37]. However, Kul and Seker [38] reported shape index value of 74.90%, differing from the current observations

Table 1. Least square means and standard error of the difference between strains in egg quality

Traits	White quail	Brown quail	SE	Probability
Egg weight (g)	14.28^a	12.59^b	0.36	0.001
Egg length (mm)	35.21^a	33.60^b	0.36	0.006
Egg width (mm)	26.37^a	25.51^b	0.27	0.034
Shape index	74.98^a	76.06^a	1.01	0.489

[a and b] Means, within trait and source of variation, followed by different superscripts, differ significantly, Duncan [30].

Table 2. Least square means and standard error of the difference between strains in internal egg quality

Traits	White quail	Brown quail	SE	probability
Yolk height (mm)	11.78^a	10.73^b	0.31	0.046
Albumen height (mm)	5.36^a	5.57^a	0.24	0.589
Yolk weight (g)	4.80^a	4.25^b	4.52	0.015
Yolk percentage	4.80^b	5.60^a	0.07	< 0.0001
Albumen weight (g)	7.30^a	6.45 ^{b}	0.26	0.015
Albumen percentage	51.09^a	51.29^a	1.16	0.91
Laroch	3.93^a	3.93^a	0.23	1

[a and b] Means, within trait and source of variation, followed by different superscripts, differ significantly, Duncan [30].

The data from Table (2) shows that the strain of the hens had a significant impact on the interior egg quality. The mean values for yolk height, yolk weight, yolk percentage and albumen weight were higher for the white quail strain compared to the brown quail strain. Nevertheless, there were no notable disparities observed among the strains regarding albumen percentage, albumen height and Laroch. Özçelik [39] states that albumen index, albumen height, albumen weight and albumen ratio are metrics that indicate the density of albumen quality. These metrics are utilized to calculate the Haugh unit, which is a crucial determinant of egg quality. Moreover, the weights of the yolk and albumen were affected by the different egg weight categories. As the weight of the egg increased, there was a corresponding increase in both the weight of the yolk and the weight of the albumen.

Table (3) displays the data on the impact of different feathered strains on various eggshell traits. While the statistical analysis did not reveal a significant effect of strain on eggshell weight and shell percentage, the overall mean values indicate that the white feathered strain exhibited higher values than the brown feathered strain in both eggshell weight and shell percentage. Interestingly, although the effect of strain on eggshell thickness did not reach statistical significance, the overall mean values suggest that the white quail strain had the greatest eggshell thickness, aligning with the results published by Hassan et al. [40]. Conversely, Al-Kafajy et al. [41] observed that desert quail lines had notably thicker shells, while white quail lines had heavier shells. These findings contradict the results made by Inci et al. [42] and Chimezie et al. [6], who found no noticeable variations in the thickness of eggshells among different quail lines. Furthermore, Drabik et al. [43] presented evidence of differences in the quality of quail eggs depending on the color of their eggshells.

The data in Table (4) reveals significant differences in the chemical analysis of eggs between feathered strains. In the overall egg composition, the statistical analysis indicates the impact of strain on ash, protein, fat and total carbohydrates percentages.

Specifically, the white feathered quail exhibited higher levels of ash% and fat% compared to the brown feathered quail, while the brown feathered quail displayed higher levels of protein% and total carbohydrates. This pattern persists in the yolk, where the brown quail surpassed the white quail in ash%, protein% and total carbohydrates, while the white quail showed a higher fat%. Similarly, the statistical analysis of the shell composition indicated significant strain-related differences in ash, protein, fat and total carbohydrates percentages. Notably, the white quail had a higher ash percentage in the shell compared to the brown quail. The avian egg serves as a crucial nutrient source, encompassing proteins, lipids, vitamins, minerals and growth factors essential for embryonic development. Additionally, eggs contain bioactive substances such as immune proteins and enzymes with diverse functionalities, including antiadhesive, antioxidant, antimicrobial, immunomodulatory, anticancer and antihypertensive properties. These components contribute to human health, emphasizing the role of eggs in disease prevention and treatment [44, 45].

The chemical make-up of quail egg yolk is similar to that of ostrich egg yolk, with a lower fat content and a higher ash content compared to chicken egg yolk [46]. Genchev [47] noted higher crude protein content in quail egg albumen and lower ash content in both albumen and yolk compared to Dudusola's [46] findings. Sinanoglou et al. [48] agreed with Dudusola [46] and Genchev [47] on the yolk dry matter content of quail eggs; however, they found that the fat content was lower and the ash content was higher in the yolk.

Table 3. Least square means and standard error of the difference between strains in shell quality

Source of variation	White quail	Brown quail	SE	Probability
Shell weight(g)	0.048^a	0.027^b	0.004	0.001
Shell %	2.180^a	1.887^b	0.098	0.063
Shell thickness	15.259^a	14.945^a	0.628	0.749

$^{a\text{ and }b}$ Means, within trait and source of variation, followed by different superscripts, differ significantly, Duncan [30]

Table 4. Least square means and standard error of the difference between egg strains in chemical analysis

Egg ingredients	Source of variation	White quail	Brown quail	SE	Probability
Egg albumen	Ash %	5.21^a	4.97^b	0.006	< 0.0001
	Protein %	80.22^b	82.53^a	0.008	< 0.0001
	Fat %	3.29^a	0.23^b	0.01	< 0.0001
	Total carbohydrates %	11.29^b	12.27^a	0.006	< 0.0001
Egg yolk	Ash %	3.71^a	3.52^b	0.01	< 0.0001
	Protein %	33.43^a	30.52^b	0.003	< 0.0001
	Fat %	59.30^b	64.81^a	0.007	< 0.0001
	Total carbohydrates %	3.56^a	1.12^b	0.01	< 0.0001
Eggshell	Ash %	86.64^a	85.60^b	0.006	< 0.0001
	Protein %	0	0		
	Fat %	0	0		
	Total carbohydrates %	0	0		

$^{a\text{ and }b}$ Means, within trait and source of variation, followed by different superscripts, differ significantly, Duncan [30]

Table (5) reveals significant differences in fatty acid composition between quail strains. The statistical analysis shows that there are strain-related effects on the total egg's lauric acid, myristic acid, palmitic acid, palmitoleic acid, heptadecanoic acid, cis-10-heptadecanoic acid, stearic acid, oleic acid, linoleic acid, α-linoleic acid and Arachidic acid. Oleic acid, on the other hand, did not show a statistically significant difference. There were more palmitoleic acid, heptadecanoic acid, stearic acid, linoleic acid, α-linolenic acid and arachidic acid in the white quail strain than in the brown quail strain. There were more lauric acid, myristic acid, palmitic acid, Cis-10-heptadecanoic acid, oleic acid and linoleic acid in the brown quail strain. People recognize eggs for their nutritive value and functional properties, which make them a significant source of animal protein. However, some have raised concerns about the high cholesterol content in eggs and its potential association with coronary heart disease [49]. Polat et al. [50] conducted a study to examine the fatty acid composition of yolks from several chicken species, including quail eggs, in their natural habitat. Researchers discovered that quail eggs had a higher proportion of monounsaturated fatty acids (45%) in the yolk compared to chicken eggs (39.1%) and a lower amount of polyunsaturated fatty acids (25.1% vs. 31.3%, respectively). The total content of saturated fatty acids in the yolk was comparable across the two species. Furthermore, it is important to mention that linoleic acid and alpha-linolenic acid, as well as their long-chain derivatives (EPA; eicosapentaenoic acid; 20:5 n-3; and DHA; docosahexaenoic acid; 22:6 n-3) are essential constituents of animal and plant cell membranes [51].

Table (6) presents significant correlations among egg quality traits in both the white and brown quail strains. Positive relationships were found in the white strain between egg length and egg weight, as well as between egg width and egg weight. In addition, there were favorable associations observed between the shape index and both the length and width of the eggs. A direct relationship was established between the thickness of the shell and the shape index. Additionally, positive relationships were observed between the weight of the shell and the weight, length and width of the egg. Furthermore, positive correlations were noted between shell percentage and shell thickness and weight. The height of the yolk showed a positive link with the length of the egg, but a negative correlation with the form index. Albumen height displayed positive correlations with egg weight, egg length, shell weight, shell and yolk height.

Positive associations were seen in the brown feathered strain between egg weight and many characteristics, including egg length, egg width, shell thickness, shell weight, yolk weight and albumen weight. However, a negative correlation was observed with the shape index. Egg length correlated positively with shell thickness, shell weight, yolk weight and albumen weight, but negatively with shape index. Egg width exhibited positive correlations with shape index, yolk weight and albumen weight, but a negative correlation with yolk height. Negative correlations were identified between egg length, shell thickness, shell weight and yolk height. Shell thickness correlated positively with yolk height, yolk weight, albumen height and albumen weight, but negatively with shape index. Shell weight showed positive correlations with shell percentage and yolk weight, but negative correlations with shell thickness and albumen percentage. Shell percentage correlated positively with yolk weight and Laroch, but negatively with albumen weight, albumen height and albumen percentage. Yolk height had positive correlations with albumen height and albumen percentage, but a negative correlation with yolk weight. Albumen height showed positive correlations with albumen weight and albumen percentage, but negative correlations with yolk weight. Yolk weight displayed negative correlations with albumen percentage. The yolk percentage had positive correlations with Laroch, while albumen weight correlated positively with albumen percentage but negatively with Laroch.

The results of this study are consistent with previous research conducted by Alkan et al. [33] and Khurshid et al. [52]. These studies provide more evidence that some internal characteristics of eggs, including the height and width of the albumen, can reliably predict the weight of the yolk. The positive correlations underscore the shared genetic control of these internal traits, suggesting their potential as predictors for egg weight. This information is valuable for breeding and improvement plans, as emphasized by Ige [53]. The positive relationships between egg weight and egg length/width can be explained by the spatial occupancy of the egg yolk, which affects the overall weight of the egg. Monira et al. [54] and Abanikannda et al. [55] have found similar relationships, which confirm the ability of egg length and width to predict egg weight. Tebesi et al. [56] and Apuno et al. [57] also discovered substantial connections between the weight of eggs and their length/width, which aligns with the findings of the present investigation.

Table 5. Least square means and standard error of the difference between egg strains in fatty acid composition

Traits	White quail	Brown quail	SE	Probability
Lauric acid (C12:0)	0.00^b	0.55^a	0.041	0.001
Myristic acid (C14:0)	0.76^b	2.03^a	0.005	< 0.0001
Palmitic acid (C16:0)	21.20^b	26.78^a	0.041	< 0.0001
Palmitoleic acid (C16:1) n_9	4.22^a	1.88^b	0.006	< 0.0001
Heptadecanoic acid (C17:0)	0.32^a	0.26^b	0.006	0.002
Cis-10-heptadecanoic acid (C17:1)	0.15^b	0.24^a	0.006	0.0001
Stearic acid (C18:0)	13.03^a	10.44^b	0.006	< 0.0001
Oleic acid (C18;1n9c)	37.20^b	42.83^a	0.041	< 0.0001
Oleic acid (C18:1n9t)	0.00	1.18	0.408	0.110
Linoleic acid (C18:2n6c)	18.84^a	10.65^b	0.006	< 0.0001
Linoleic acid (C18:2n6t)	0.34^b	0.49^a	0.006	< 0.0001
α - linoleic acid (C18:3n3)	1.23^a	1.02^b	0.006	< 0.0001
Arachidic acid (C20:0)	2.71^a	1.65^b	0.008	< 0.0001

[a and b] Means, within trait and source of variation (S.O.V), followed by different superscripts, differ significantly, Duncan [30].

Table 6. Correlation (pearson) among egg quality traits in quail strains

Variables	Egg weight (g)	Egg length (mm)	Egg width (mm)	Shape index	Shell thickness (mm)	Shell weight (g)	Shell percentage	Yolk height (mm)	Albumen height (mm)	Yolk weight (g)	Yolk percentage	Albumen weight (g)	Albumen percentage	Laroch
Egg weight (g)	**1**	**0.57**	**0.62**	0.04	0.03	**0.62**	0.04	0.18	**0.43**	**0.62**	**0.62**	**0.81**	0.12	**0.37**
Egg length (mm)	**0.78**	**1**	0.13	**-0.65**	0.15	**0.35**	0.01	**0.60**	**0.45**	**0.67**	**0.67**	**0.27**	**-0.24**	0.22
Egg width (mm)	**0.53**	0.12	**1**	**0.67**	-0.21	**0.35**	-0.02	0.09	0.16	**0.41**	**0.41**	**0.49**	0.07	**0.24**
Shape index	**-0.28**	**-0.74**	**0.58**	**1**	**-0.28**	0.01	-0.02	**-0.38**	-0.22	-0.18	-0.18	0.18	**0.23**	0.01
Shell thickness (mm)	**0.48**	**0.46**	-0.08	**-0.43**	**1**	**-0.30**	**-0.39**	0.14	-0.13	0.19	0.19	0.05	0.06	0.19
Shell weight (g)	**0.52**	**0.45**	0.16	**-0.27**	0.05	**1**	**0.81**	0.02	**0.49**	**0.37**	**0.37**	**0.24**	**-0.37**	0.16
Shell percentage %	0.15	0.18	-0.05	-0.18	-0.15	**0.92**	**1**	-0.12	**0.31**	0.02	0.02	**-0.30**	**-0.56**	-0.09
Yolk height (mm)	-0.076	0.16	**-0.43**	**-0.42**	**0.26**	0.00	0.08	**1**	**0.43**	**0.36**	**0.36**	0.03	-0.15	**0.35**
Albumen height (mm)	0.21	0.08	0.03	-0.05	**0.56**	-0.14	**-0.25**	**0.46**	**1**	0.22	0.22	**0.27**	-0.07	**0.23**
Yolk weight (g)	**0.64**	**0.52**	**0.55**	-0.05	**0.26**	**0.48**	**0.27**	**-0.42**	**-0.24**	**1**	**1.00**	0.12	**-0.56**	0.14
Yolk percentage	-0.081	-0.08	0.05	0.11	-0.18	-0.07	-0.03	-0.14	-0.14	-0.04	**1**	0.12	**-0.56**	0.14
Albumen weight (g)	**0.68**	**0.50**	**0.28**	-0.21	**0.45**	-0.09	**-0.43**	0.18	**0.52**	-0.04	-0.05	**1**	**0.68**	**0.39**
Albumen percentage %	-0.04	-0.09	-0.15	-0.03	0.15	**-0.62**	**-0.71**	**0.30**	**0.50**	**-0.68**	-0.01	**0.70**	**1**	0.20
Laroch	0.02	0.19	-0.02	-0.16	-0.17	0.21	**0.27**	0.12	-0.11	0.12	**0.26**	-0.16	**-0.28**	**1**

Values in bold are different from 0 with a significance level alpha=0.05, Phenotypic correlations for brown quail are presented below the diagonal and Phenotypic correlations for white quail above the diagonal. N = 90 /lines

Shell microstructure

This study delves into the microstructure of eggshells from two distinct quail strains, aiming to scrutinize potential variations and discern any consequential impacts on egg quality. The figures presented herein elucidate the influence of different quail strains on the ultrastructure of eggshells.

Initially, eggs were procured from two quail strains: strain brown and strain white. Subsequently, a meticulous examination was conducted using a Scanning Electron Microscope (SEM) to delve into the microstructure of their eggshells. The investigation focused on three pivotal aspects: shell thickness, crystal structure and general microstructural features. Figures (1 to 4) showcase the resultant images, providing insights into how these strains exert influence on the overall structure of eggshells. Upon scrutinizing the microstructure of eggs from the commercial brown strain, as depicted in Figures (1 and 2), a noticeable degradation in eggshell structure becomes apparent. This deterioration is characterized by the presence of type B crystals, late fusion, poor confluence and alignment issues. Similarly, the white strain, illustrated in Figures (3 and 4), exhibits a decline in eggshell structure, with discernible instances of late fusion, poor confluence and alignment challenges.

The findings underscore the importance of microstructural analysis in comprehending the subtle yet significant differences between quail strains, shedding light on potential implications for overall egg quality. These insights contribute to our understanding of how specific strains may impact the structural integrity of eggshells, thus informing considerations for breeding and production practices. After investigating the influence of genotype on eggshell ultrastructure, samples from each group underwent scanning electron microscopy and subsequent analysis was conducted using ImageJ software. Table (7) and Figures (5-10) illustrate that the cuticle layer was more prominent in the white strain compared to the brown strain. In addition, the eggshell's cross-sectional structure displayed cone-shaped mammillary knobs that were in direct contact with the inner eggshell, as well as a columnar basal layer. The size of mammillary knobs varied between strains, with the white strain exhibiting a smaller average diameter, indicating a higher mammillary layer density in the brown strains. The palisade layer's average diameter exhibited no significant variation between the white and brown strains, while the membrane layer was more substantial in the brown strain than in the white strain.

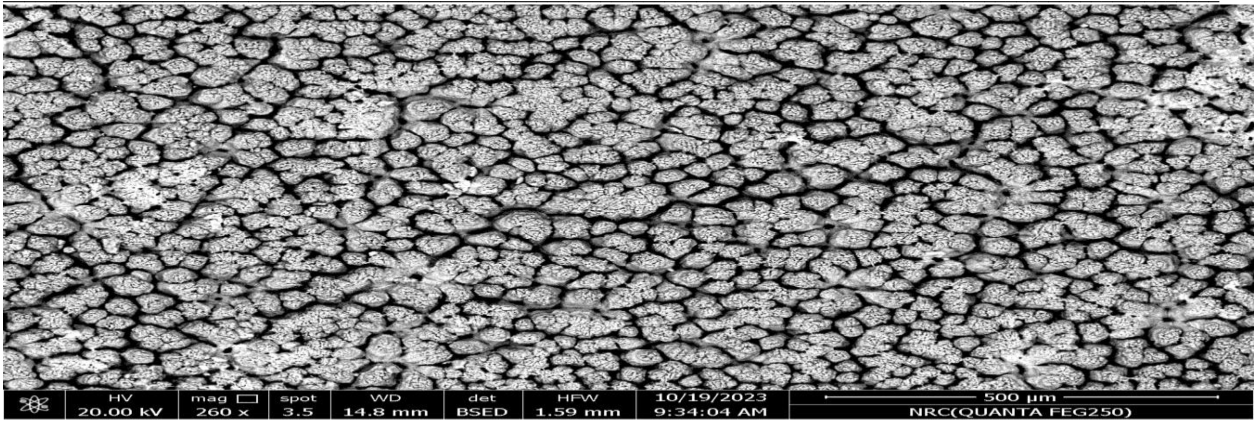

Figure 1. Eggshell ultrastructure of brown strain showed type B's and late fusion (arrowed) in eggshell.

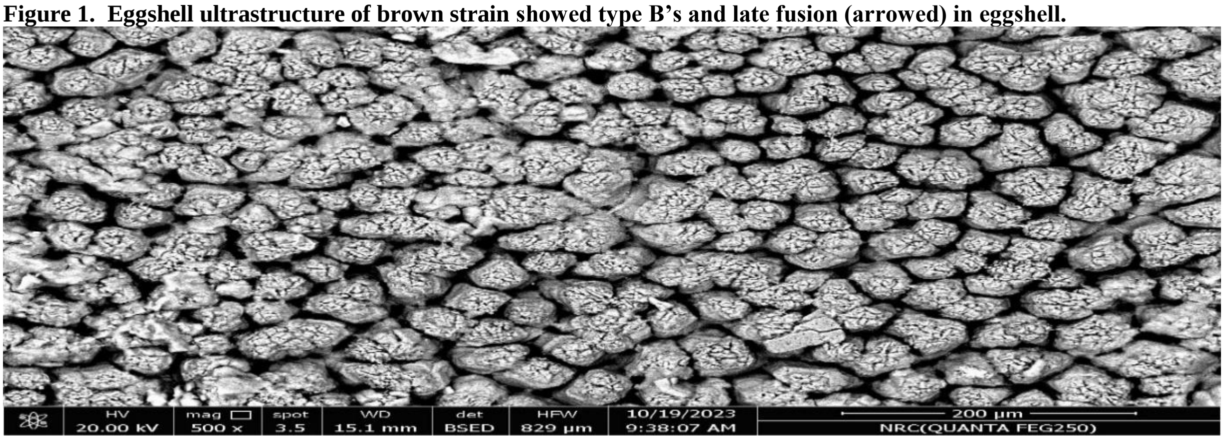

Figure 2. Eggshell ultrastructure of brown strain showed the poor confluence and alignment (arrowed) in eggshell.

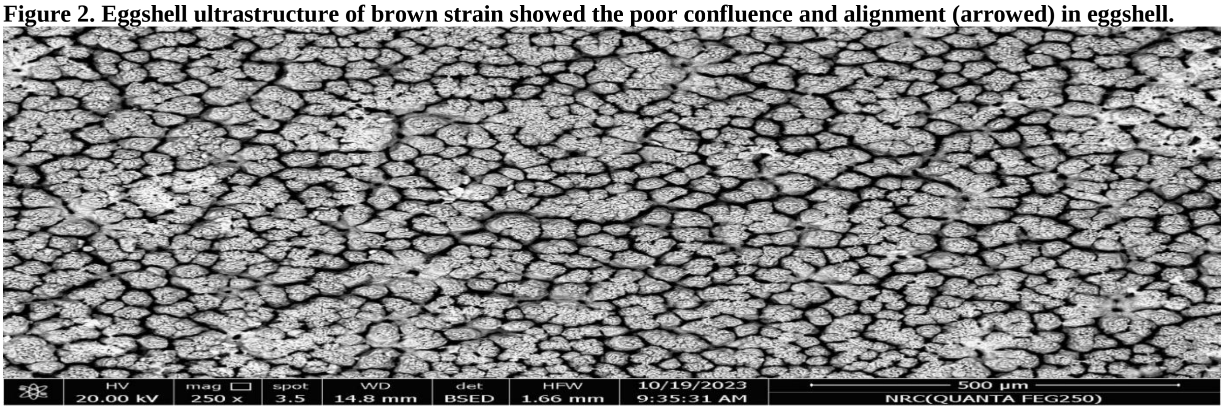

Figure 3. Eggshell ultrastructure of white strain showed late fusion, poor confluence,

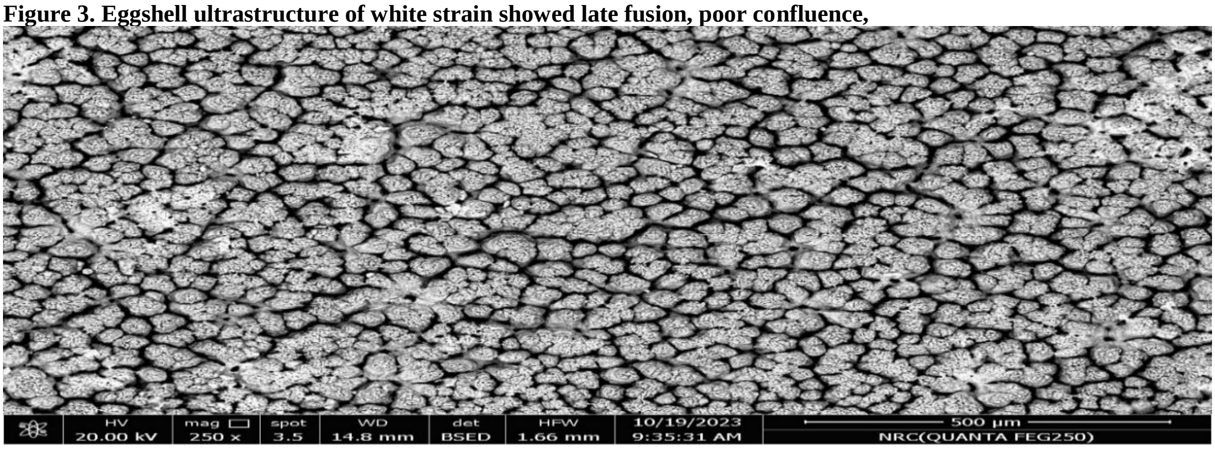

Figure 4. Eggshell ultrastructure of white strain showed alignment challenges.

Table 7. The least square means and standard error of the difference between egg strains in the ultrastructure of the eggshell

Source of variation	White quail	Brown quail	SE	Probability
Membrane layer (µm)	49.405 [b]	65.864 [a]	3.095	0.007
Cuticle layer (µm)	14.008 [a]	7.152 [b]	3.91	0.003
Mammillary layer (µm)	35.418 [b]	51.344 [a]	1.09	0.024
Palisade layer (µm)	118.700 [a]	116.074 [a]	2.055	0.398

[a and b] Means, within trait and source of variation, followed by different superscripts, differ significantly, Duncan [30].

Multiple intricate elements influence the strength of eggshells, with the ultrastructure playing a crucial role in defining their quality [58]. Research has established the influence of probiotics on eggshell durability, but it has not thoroughly investigated their effects on the ultrastructure. The eggshell possesses a well-structured composition consisting of inner and outer membranes, a mammillary layer, a palisade layer, a vertical crystal layer and a cuticle. The effective thickness is calculated by subtracting the effective mammillary thickness from the overall thickness. Typically, an increased density of the mammillary layer, a smaller mastoid knot and a thicker palisade layer all contribute to a higher level of strength in the eggshell [59]. A mammillary layer with an irregular structure might decrease the ability of the eggshell to resist fractures and degrade its resistance to external forces [59, 60]. The palisade layer, comprising calcite crystals with an embedded organic matrix, represents approximately 70% of the total eggshell thickness [61]. These columnar-shaped structures provide high strength against breakage [62]. It was thought that blood ionic constituents directly affect the quality of eggshells, but our results show that as hens get older, the palisade layer ratio and mammillary knob density decrease in the eggshell ultrastructure. The mammillary layer, the innermost calcified portion of the eggshell, plays a crucial role in eggshell strength. The overall durability of the eggshell intricately links to the cohesive interaction between the mammillary knob and the eggshell membrane. The mammillary knob's reduced density lowers the binding force, resulting in a decrease in eggshell strength. Ensuring a consistent size and form of mammillary knobs, as well as a robust connection with the shell membrane, are critical factors in achieving high eggshell strength [59, 60]. Fathi et al. [63] found that type A mammillary bodies, which have a rounded shape and are evenly distributed in size, optimize their connection to the outer membrane fibers. Type A bodies exhibit limited interaction with membrane fibers, resulting in a conical shape at the level of the mammillary layer [64]. On the other hand, mammillary knobs contain debris in the form of rounded type B bodies. They do not contribute to the formation of the palisade layer, but instead weaken its base. This leads to a shell that is more susceptible to breaking [65].

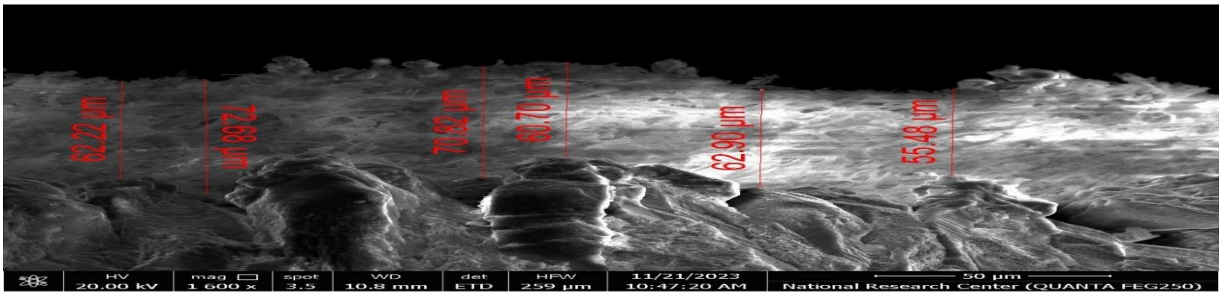

Figure 5. Membranes layer in eggshell (brown strain).

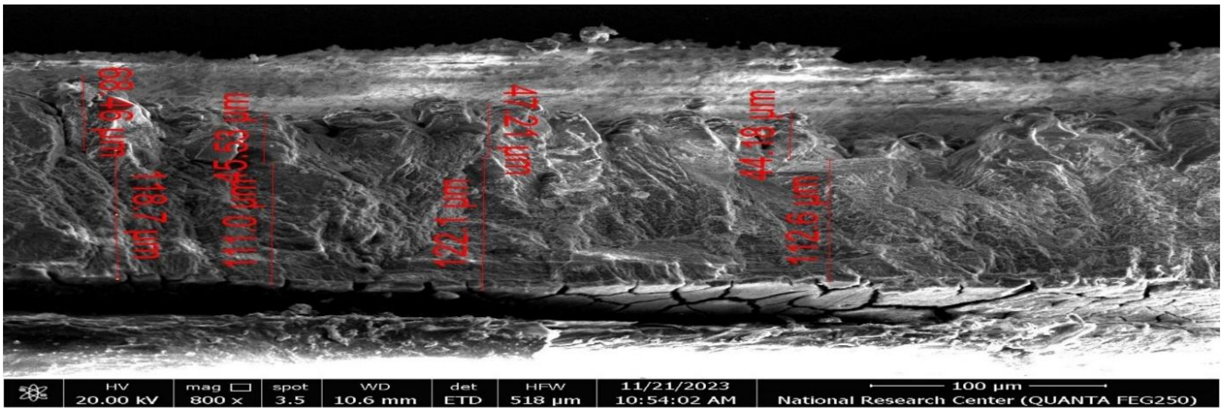

Figure 6. Mammillary and palisade layers in eggshell (brown strain).

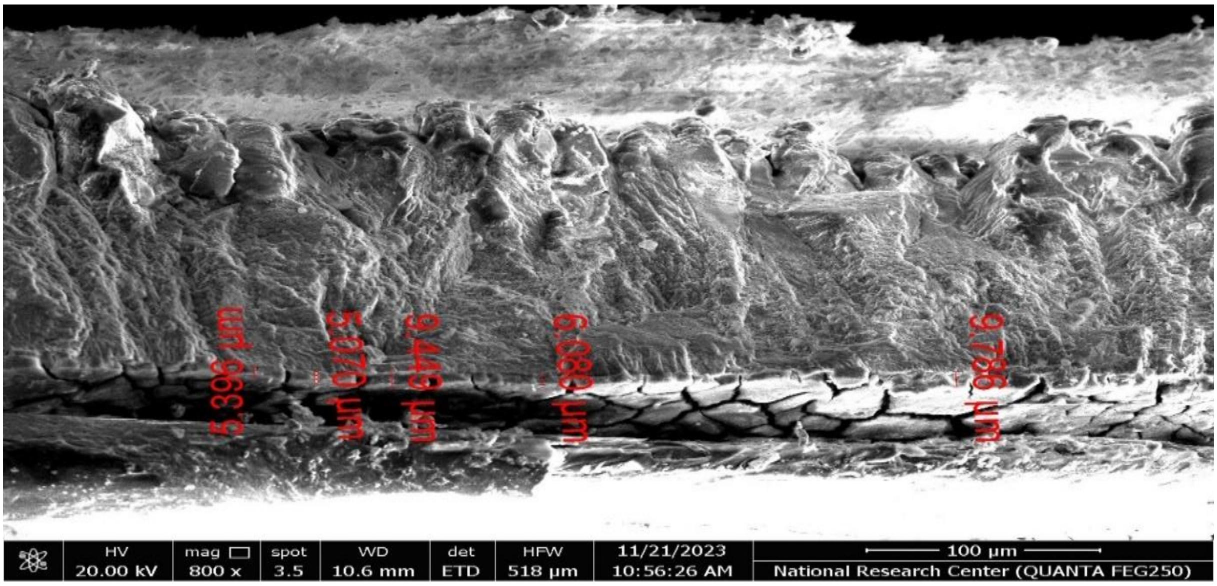

Figure 7. Cuticle layer in eggshell (brown strain).

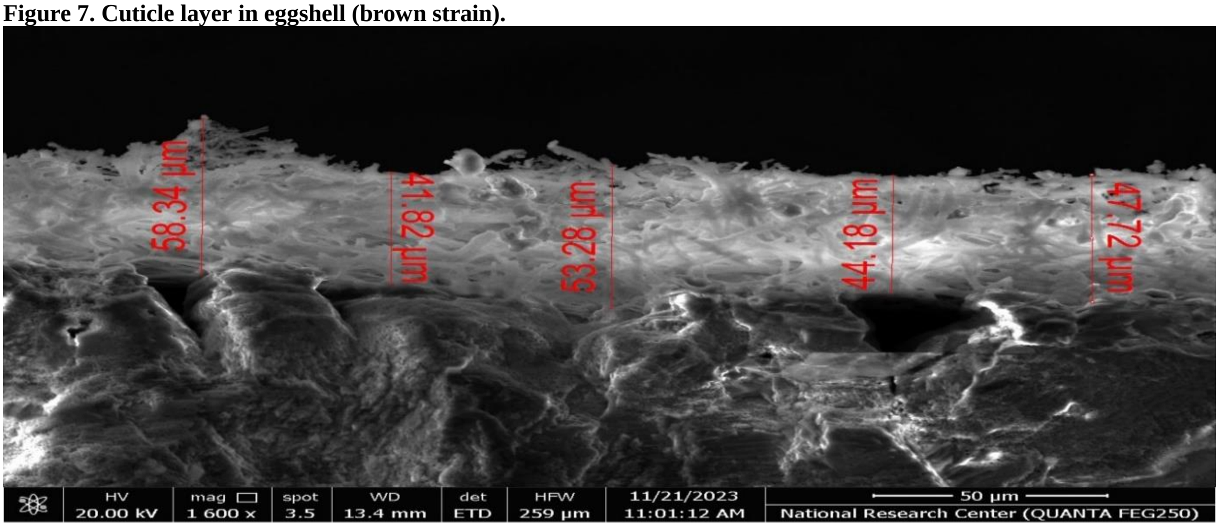

Figure 8. Membranes layer in eggshell (white strain).

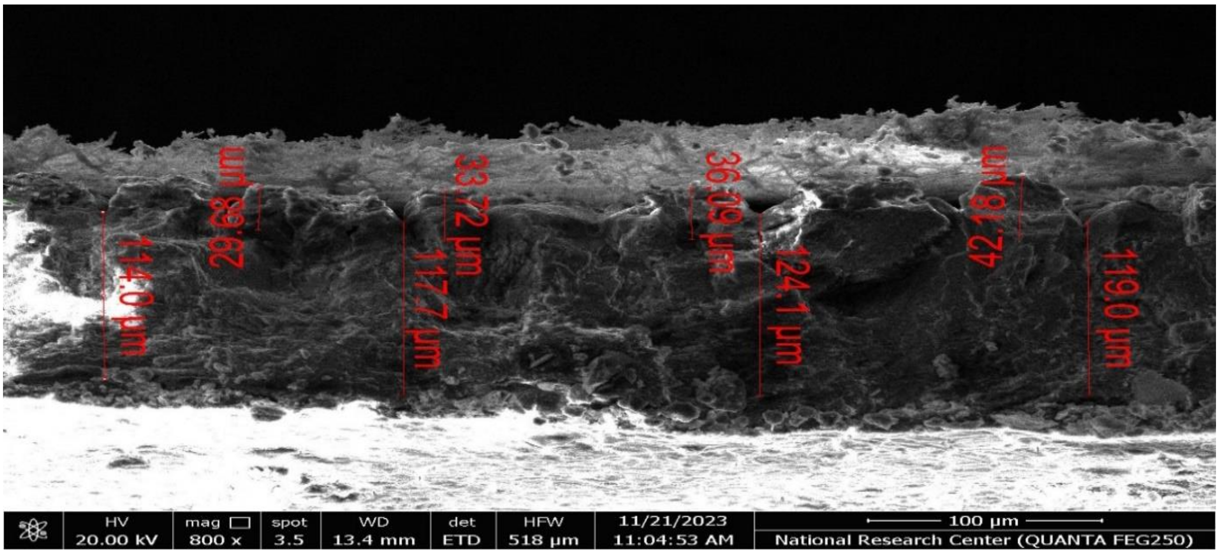

Figure 9. Mammillary and palisade layers in eggshell (white strain).

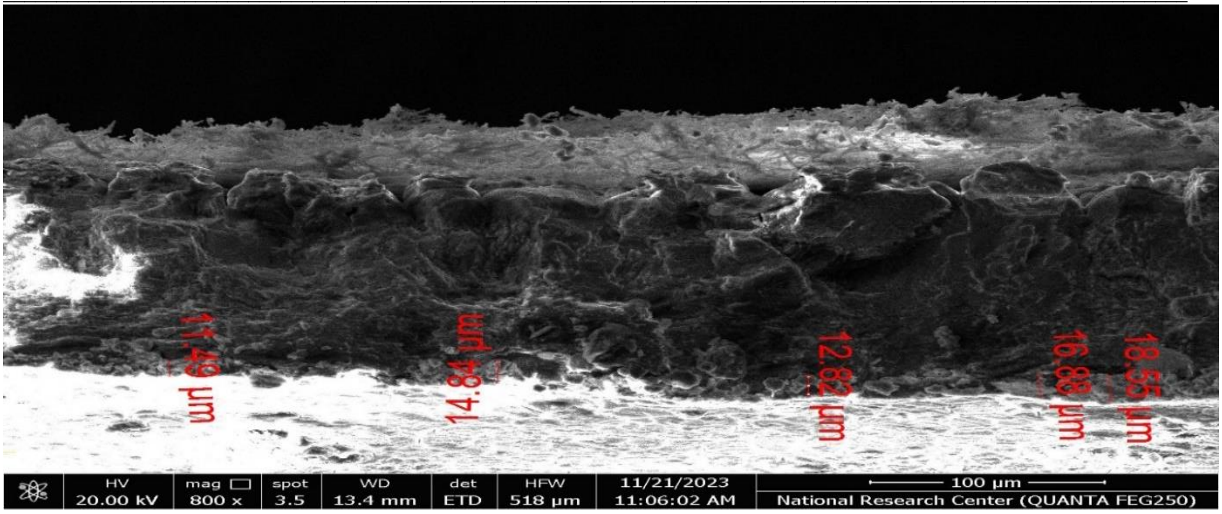

Figure 10. Cuticle layer layers in eggshell (white strain).

EDAX (Energy Dispersive X-ray Spectroscopy) analysis

The data provides details of an EDAX (Energy Dispersive X-ray Spectroscopy) analysis conducted by the EDAX team on a sample. The data provides information on the sample, the added spectra and the analysis results. The results of the EDAX analysis on the brown quail sample provide detailed information about the elemental composition and analytical parameters used. The analysis reveals the presence and quantities of various elements, including carbon, oxygen, sodium, magnesium, phosphorus, chlorine and calcium. The data includes weight percentage, atomic percentage, net intensity and error percentage for each element. The sample has oxygen as the most prevalent element, followed by calcium, carbon and sodium. The thoroughness of the data allows for a comprehensive understanding of the sample's chemical make-up and facilitates further interpretation and analysis. The eggshell of a chicken can serve as a viable supply of calcium, which can be utilized to fulfill the daily requirement for calcium intake. This can serve as a dietary addition to support bone formation, since the recommended daily intake ranges from 700 to 1300 mg [66]. Experts advise individuals with osteoporosis to consume 400–500 mg of calcium daily as a dietary supplement [67], sourced from eggshells. Furthermore, it can serve as a supplementary component in animal diet formulation [68]. Magnesium is a vital element for living beings. People with a deficiency can transform chicken eggshells into a viable source of magnesium supplements by processing them. In humans, a lack of this substance can lead to the development of pre-eclampsia, arrhythmias, arteriosclerosis, diabetes mellitus and metabolic syndrome. Thus, administering magnesium supplements to these patients can be advantageous in the majority of instances [69]. Potassium is a vital element for the existence of all types of plant and animal life. In humans, it plays a crucial role in nerve function and is highly significant in maintaining the balance of water, electrolytes and acid-base levels in the bloodstream and tissues [70]. The primary portion of the data provides the elemental composition of the sample, specifically indicating the presence of carbon, oxygen, sodium, magnesium, phosphorus, chlorine and calcium. The document presents the weight percentage, atomic percentage, net intensity and error percentage for each element for instance, the weight proportion of oxygen is 50.04%, while the atomic proportion is 61.39%. The net intensity and error percentage for each element are additionally supplied. The data essentially summarizes the results of an EDAX analysis on a sample, providing detailed information about the sample's elemental composition and the analytical parameters used.

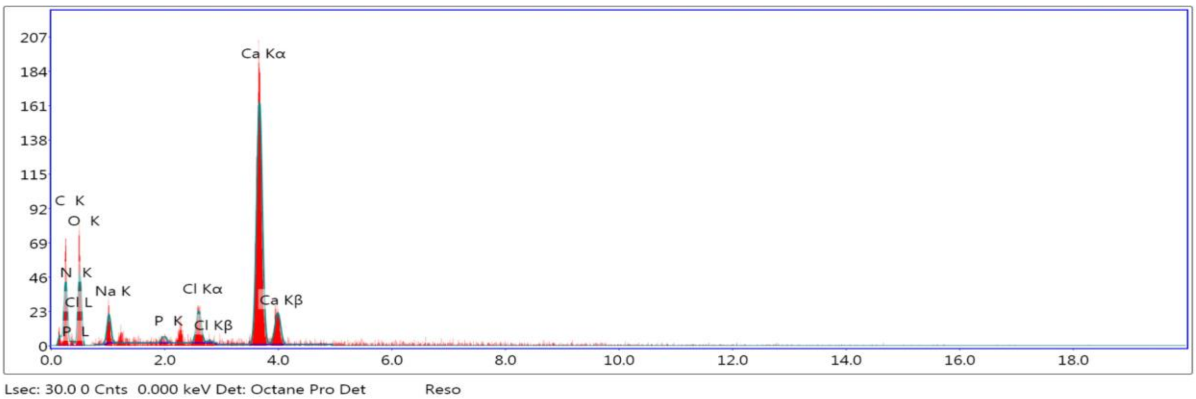

Figure 11. EDAX (Energy Dispersive X-ray Spectroscopy) analyses for egg shill of white quail line.

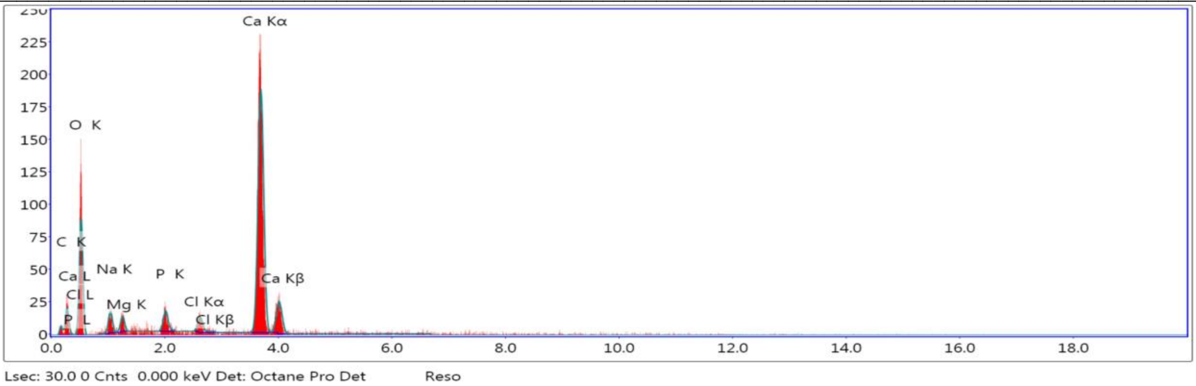

Figure 12. EDAX (Energy Dispersive X-ray Spectroscopy) analysis for egg shill of brown quail line

The analysis offers insights into the relative abundance of different elements within the sample, shedding light on its chemical characteristics. The thoroughness of the elemental composition data enables a comprehensive understanding of the sample's composition and supports further interpretation and analysis of its properties. The data provides information about a sample analysis conducted by the EDAX team. The document includes details about the sample, the date of creation and the analysis results. The tabular presentation of the analysis results reveals the presence of various elements in the sample. The elements analyzed include carbon, nitrogen, oxygen, sodium, phosphorus, chlorine and calcium. The analysis provides the weight percent and atomic percent of each element present in the sample, along with the net intensity and error percent. The results indicate that the sample consists of carbon, with a weight percentage ranging from 21.34 to 31.94% and an atomic percentage ranging from 11.88 to 31.94%. Nitrogen is also present in the sample, with a weight percentage ranging from 9.17 to 11.77% and an atomic percentage ranging from 2.24% to 30.01%. Oxygen is another significant element, with a weight percentage ranging from 34.76 to 39.05% and an atomic percentage ranging from 14.21 to 39.05%. Additionally, sodium is present in the sample, with a weight percentage ranging from 3.48 to 4.45% and an atomic percentage ranging from 11.4 to 16.74%. The sample contains trace amounts of phosphorus, with a weight percentage of 0.41% and an atomic percentage of 2.74%. Chlorine is present with a weight percentage ranging from 1.13 to 2.22% and an atomic percentage ranging from 14.22 to 15.49%. Calcium is the most prominent element in the sample, with a weight percentage ranging from 12.4 to 27.65% and an atomic percentage ranging from 3.35 to 132.77%. The data in Figures (11 and 12) showed that the brown (31.24) has a higher calcium content in eggshell than the white (27.65); also, the brown (1.6) has a higher phosphorus content in eggshell than the white (0.41). Magnesium was found in the shell brown strain (1.62), but not in the white strains. The white shell is higher in carbon content (21.34) than the brown (10.38). This finding aligns with the similar patterns seen by Saeb et al. [71] and Aygun [72]. Fernandes et al. [73] and Yew et al. [74] discovered that eggshells consist of 94% calcium carbonate, 1% magnesium carbonate, 1% calcium phosphate and 4% organic content, typically protein, collagen and sulfated polysaccharides. Eggshells primarily contain calcium carbonate, which plays a crucial role in eggshell production [75]. Previous investigations have identified calcium carbonate as the primary component of the shell, accounting for 96% of its structure. The other components include magnesium, phosphorus, copper, zinc and iron [76], as well as various trace elements such as lithium, strontium and barium [77]. They stated that all eggshells consist primarily of pure calcite, with little amounts of additional elements like S, Mg, P, Al, K and Sr, which are present in parallel chemical entities. The laboratory study focused on analyzing the ultrastructure and crystal architecture of quail eggshells [78]. An X-ray diffractometer identified the eggshell crystal structure. The results showed that calcite dominated the crystal structure of the eggshells of quail eggs. The results of research by Cahya and Marfuah [79] and Murakami et al. [80] aligned with these findings. The EDAX team's analysis provides detailed insights into the sample's elemental composition. Carbon and nitrogen are present.

4. Conclusion

The eggshell features, egg quality and ultrastructure are influenced by various genotypes of layer breeder quail strains. Gaining insight into these genetic variations can result in enhancements in overall efficiency and egg quality within egg production systems.

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Ethics approval

The National Research Centre's Ethics Committee set the rules for this study's conduct. Approval number: “130504010323”

* **Consent to participate**

The authors consent to the journal’s policy

* **Consent for publication**

The authors agree to the journal’s policy

* **Availability of data and materials**

All data produced or examined throughout the present investigation are encompassed in this published publication.

Competing interests

There are no competing interests.

Authors' contributions

All the authors worked together to conduct this investigation. E. M. El-Komy and G.S. Ramadan originated the concept, formulated the experimental design and oversaw the research. A. A. Abd El-Halim, A. M. Abdelsalam and E. M. El-Komy conducted the experiments and collaborated on the paper writing. G.S. Ramadan and F.S. Nassar examined the data. H. H. Abd ElRahman, F. S. Nassar and E. M. El-Komy thoroughly reviewed and made significant improvements to the manuscript. All authors read and approved the final manuscript.

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