

Research Article



Genetic Differences In Egg Quality Traits Between W-36 and Brown Breeder Strains

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Abstract | In terms of both economic expenses and human health, eggshell quality is critical. In the chicken industry, incubation is one of the most critical variables impacting profitability. As a result, the study's goal is to see how eggshell quality differs between strains. From the two egg type breeder strains, 240 hatched eggs (120 eggs per strain) were picked at random. The layer breeder flocks were tested at four different ages (22, 33, 43, and 64). The purpose of this study is to investigate the differences in egg shell quality between the W-36 and brown hy-lines. Strain and age had a substantial impact on Egg weight, Egg length, Egg width, Shape index, Specific Gravity, Albumen height, Albumen weight, Yolk diameter, Yolk index, Yolk weight, Yolk percent, Yolk albumen ratio, Wet shell weight, and Shell percent, according to the findings. Strain and age had no effect on Albumen weight, Albumen percent, or Yolk height, according to the findings. In terms of hatchery egg shell quality, the w-36 is better than brown.

Keywords | Genetic differences; Egg-shell quality; Hy-line strains; Brown strain; White strain; Egg quality

Received | February 12, 2022; **Accepted** | April 28, 2022; **Published** | August 01, 2022

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Citation | El-Salam AA, Hamouda Y, El-safety S, El-attar A (2022). Genetic differences in egg quality traits between w-36 and brown breeder strains. *Adv. Anim. Vet. Sci.* 10(8):1827-1833.

DOI | <https://dx.doi.org/10.17582/journal.aavs/2022/10.8.1827.1833>

ISSN (Online) | 2307-8316



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INTRODUCTION

In terms of both economic expenses and human health, eggshell quality is critical. Physical features of the egg influenced embryo development and hatching success. Any deviations in these traits can result in the embryo's growth failing (Narushin and Romanov, 2002).

Breeder genotype, age, ambient circumstances, feed additives, and rearing system are all factors that affect hen egg quality, according to (Sarica et al., 2012). Breeds and strains have genetic variances in egg-shell properties. According to Sekeroglu and Duman (2011), there is a positive association between egg-shell colour and certain shell qualities including shell strength and hatchability. Zita et al. (2009) discovered that, in addition to genotype, hen age has a considerable influence on egg quality. Different factors, like as genotype, age, and breeding strategy, might affect the quality of eggs and eggshell.

Layer poultry production has advanced significantly in recent years, and various aspects are now taken into account to assure the quality of eggs produced, including sanity, genetics (differences between strains), management, nutrition, facilities, and some diseases (Bittencourt et al., 2019). Abdullahi et al. (2018) further demonstrated that in all egg metrics, foreign strains (Belgy) outperform indigenous variety (Pearl). Gwaza et al. (2018) found that genotype had a significant impact on laying performance in Nigerian local strains (Fulani and Tiv ecotypes) and exotic commercial layers (Isa brown, Babcock, and Brown Leghorn), respectively. Valentim et al. (2019) compared the performance of two laying hen strains (Hisex Brown[®] and Black Avifran[®]) and found no significant differences in egg weight, albumen weight, yolk weight and shell weight, albumen percentage, yolk percentage and shell percentage, marketable eggs, specific gravity, or egg production. The shell quality is the main concern of the poultry business, according to Eberhart et al. (2021), due to economic losses

caused by variations in this component of the egg. To increase the amount of whole eggs delivered to consumers, the eggshell should be robust. Eggs with low-quality shells are less resistant to industrial processing and do not reach the consumer in the best possible condition, resulting in losses for the producer (Arruda et al., 2019). Almeida et al. (2019) evaluated the egg quality of native chickens and laying hens lineages, finding that the differences in egg size and shape are the most significant differences between these genetic groups.

The goal of study was to look into the different between strains in egg-shell quality, during at ages (22, 33, 43 and 64) of layer breeder flocks. Study the different between strains in egg weight loss during hatch at (10 and 17 days of incubation).

MATERIALS AND METHODS

DATA COLLECTION

This research was conducted in collaboration with Misr Poultry Company Farms and the Department of Poultry Production, Faculty of Agriculture, Ain Shams University, and the Department of Animal Production, National Research Center. The current investigation used two layer breeder strains (Hy-Line White (W36) and Hy-Line Brown parents). Brown and White breeders were grown in semi-closed houses on deep litter with densities of five and six birds per square metre, respectively. During the laying time, the breeders were subjected to conventional management circumstances. According to the practical user's handbook for each genetic group, each hen was fed the necessary amount of feed to maintain a high hatching egg production rate. Throughout the day, there was plenty of water available. A 16-hour photoperiod was offered at all times. Prior to incubation, hatching eggs were fumigated using formaldehyde gas. After sanitation, eggs were stored in a vertical posture (large end up) for 3-7 days at 15°C and 80% relative humidity (RH) until incubation. Eggs were transported to the pre-heating room for eight hours before incubation to progressively raise the temperature of hatching eggs. After that, all of the eggs were moved to setter units. The incubator was set to 37.7°C and 60% relative humidity, while the hatcher was set to 37.3°C and 70% relative humidity.

The layer breeder flocks were tested at four different ages (22, 33, 43, and 64 weeks). Each age yielded two hundred and forty viable eggs (120 eggs each strain). Sixty fertile eggs (30 from each strain) were incubated at each age to evaluate hatching traits, while the remaining sixty fertile eggs were utilised to study egg quality. All eggs are numbered and weighted separately before incubation. To calculate the egg weight decrease, all eggs were individually weighed at 10 days and 17 days of incubation. To analyse

shell characteristics and architectures, egg samples (25 eggs per age of incubated eggs) were gathered. Fifty eggs were taken to the hatcher to finish hatching.

EGG WEIGHT

A total of 240 eggs (60 eggs per age) were tested for egg quality. To determine the form index, the egg weight was measured using a sensitive balance, as well as the egg length and width.

$$\begin{aligned} \text{Egg shape-index} &= \text{Egg length} / \text{Egg width} \times 100 \\ \text{Egg-shell \%} &= \text{Egg-shell weight} / \text{Egg weight} \times 100 \end{aligned}$$

All eggs were broken to measure the internal characters using the following equation:

$$\text{Yolk shape-index} = \text{Yolk height} / \text{Yolk height} \times 100$$

Albumen height and the height of yolk were measured at the top by spherometer. The yolk diameter was measured using digital calipers.

Specific gravity (SG) was calculated using the following formulas:

$$\text{SG} = [\text{Egg weight} / (0.968 \text{ Egg weight} - 0.4759 \text{ The shell weight})]$$

Albumen percentage was calculated by this formula (albumen weight/egg weight) x100

Albumen weight was calculated using the following formula:

$$\text{Egg weight} - (\text{yolk weight} + \text{Shell weight})$$

Yolk albumen ratio was calculated using the following formula:

$$\text{YA\%} = \text{weight of yolk} / \text{weight of albumen.}$$

Chick yield (%) was calculated using the following formula:

$$\text{Chick weight (g)} / \text{Intimal egg weight (g)} \times 100$$

Yolk percentage was estimated using the following formula:

$$(\text{Yolk weight} / \text{Egg weight}) \times 100.$$

The individual body weight was weighed using a digital balance to the nearest of 0.01 g accuracy after chick hatch.

STATISTICAL ANALYSIS

All parameters which have been studied were subjected to two way analysis of variance using General Liner Model

(GLM) procedure of SAS User's Guide (2009) according to the following model;

$$Y_{ijkf} = \mu + St_i + Ak + (St \cdot A)_{ik} + E_{ikf}$$

Where; μ = overall means; St_i = breeder strains effect ($i=1, 2$); A_k = layer breeder flock age ($k=1, \dots, 4$); $(St \cdot A)_{ik}$ = interaction between strain and age; E_{ikf} = experimental error.

RESULTS AND DISCUSSION

EFFECT OF STRAIN AND BREEDER'S AGE ON EGG QUALITY

The influence of strain and breeder age on egg quality was shown in Table 1. Strain has a considerable effect on egg weight, according to the study. Brown-egg had a higher average value (57.53) than White-egg (55.61). These findings corroborate what others have observed (Vits et al., 2005; Singh et al., 2009; Rayan et al., 2013) Brown egg layers were heavier than white egg layers, they discovered. Age had a substantial impact on the total mean ($P \leq 0.001$). The highest value was found in the 43rd week of life, followed by the 64th, 33rd, and 22nd weeks of life, in that order. These findings support those of (Rizzi and Chiericato, 2005; Johnston and Gous, 2007; Rayan et al., 2013). They discovered a link between egg weight and the age of the hens. In this feature, the interaction between breeder age and strain was not significant. This is owing to the fact that the brown hen's body weight is more than the white hen's.

Strain had a considerable effect on egg length and width, according to the data in Table 1. Brown eggs had a longer average length (55.24) than their white counterparts (54.11). Brown eggs had a higher average egg width (43.06) than white eggs (42.67). According to Abdullahi et al. (2018) and Abanikannda et al. (2011), the breed effect was substantial on egg length and egg breadth, with the Ross breed having the longest egg length and the Anak breed having the shortest. Age had a substantial impact on the total mean ($P \leq 0.001$). The highest value was found in the 43rd week of life, followed by the 33rd, 64th, and 22nd weeks of life, in that order. The significant difference observed in egg width as a result of varied genotype is well documented (Abdullahi et al., 2018)

Strain had a substantial effect on shape index, according to the analysis of variance in Table 1. Brown eggs had a higher average value (78.88) than white eggs (78). This finding is consistent with Abanikannda et al. (2011), who discovered that the ross breed had the greatest shap index value, while the Anak breed had the lowest. By age, the overall mean differed considerably ($P \leq 0.05$). The highest value was found in the 43rd week of life, followed by the 22nd, 33rd, and 64th weeks of life, in that order. Choprakarn et al. (1998), Gunlu et al. (2003), and Brand et al. (2004)

all all reached the same conclusion. Rayan et al. (2013) discovered that as the age of the layers increased, the egg shape index decreased. Because shape index is directly proportional to egg width and inversely proportional to egg length, the form index of the eggs decreased with age, implying that the rate at which eggs develop longer is quicker than the rate at which they become wider.

Table 1: Effect of strain and age of breeders on egg quality before hatch.

Age(A)	Strain (S)		Overall mean	Prob.		
	White	Brown		S	A	S*A
Egg weight (g)						
22	52.96± 0.6	55.57± 0.68	54.26 ^c ±0.64	**	***	NS
33	55.65± 1.28	57.25± 0.81	56.51 ^b ±1.045			
43	58.97± 0.76	58.2± 0.66	58.58 ^a ±0.71			
64	54.96± 1.65	59.15± 0.91	57.28 ^{ab} ±1.28			
Overall	55.61 ^b ± 1.07	57.53 ^a ± 0.76				
Egg length (mm)						
22	52.78± 0.32	54.25± 0.35	53.52 ^b ±0.33	***	***	NS
33	53.97± 0.43	55.53± 0.41	54.81 ^a ±0.42			
43	55.29± 0.26	55.21± 0.26	55.25 ^a ±0.26			
64	54.55± 0.53	55.95± 0.43	55.32 ^a ±0.48			
Overall	54.11 ^b ± 0.38	55.24 ^a ± 0.36				
Egg width (mm)						
22	41.84± 0.19	42.46± 0.22	42.15 ^c ±0.20	*	***	NS
33	42.76± 0.42	42.99± 0.22	42.88 ^b ±0.32			
43	43.8± 0.22	43.56± 0.23	43.68 ^a ±0.22			
64	42.27± 0.33	43.28± 0.21	42.83 ^b ±0.27			
Overall	42.67 ^b ± 0.29	43.06 ^a ± 0.22				
Shap index						
22	78.31± 0.64	79.3± 0.54	78.81 ^a ±0.59	*	*	NS
33	77.47± 0.73	79.26± 0.83	78.3 ^{ab} ±0.59			
43	78.9± 0.31	79.24± 0.39	79.07 ^a ±0.59			
64	77.39± 0.47	77.53± 0.64	77.45 ^b ±0.59			
Overall	78 ^b ± 0.53	78.88 ^a ± 0.6				
Specific gravity						
22	1.087± 0.0005	1.093± 0.001	1.09 ^a ± 0.0007	***	***	NS
33	1.082± 0.0008	1.087± 0.0009	1.084 ^{bc} ± 0.0008			
43	1.082± 0.0009	1.088± 0.0007	1.085 ^b ± 0.0008			
64	1.078± 0.0007	1.088± 0.001	1.082 ^c ± 0.0008			
Overall	1.082 ^b ± 0.0007	1.089 ^a ± 0.0009				

a,b and c overall Means within the same row or column with different letters are significantly differed. S= strain, A= age, NS: not significant, *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$; g: grams; mm: Micrometre.

Strain had a substantial effect on specific gravity, according to the analysis of variance Table 1. Brown-egg had a higher average value (1.089) than white-egg (1.082). Age had a substantial impact on the total mean ($P \leq 0.001$). The highest value was found in the 22nd week of life, followed by the 43rd, 33rd, and 64th weeks of life, in that order. There was no significant relationship between breeder age and strain.

Strain had no significant effect on yolk height, according to the data in Table 2. Brown-eggs had a lower average value (15.17) than white-eggs (15.7). Age had no discernible effect on the overall mean. The highest value was found in the 33rd week of life, followed by the 22nd, 43rd, and 64th weeks.

Table 2 shows that strain had a significant effect on yolk diameter based on the analysis of variance. The effect of strain on the average yolk diameter was statistically significant ($P 0.001$). Brown eggs had a lower average value (37.02) than white eggs (39.69). Age had a substantial impact on the total mean ($P \leq 0.001$). The highest value was found in the 64th week of life, followed by the 43rd, 33rd, and 22nd weeks of life, in that order. There was no significant relationship between breeder age and strain.

The analysis of variance in Table 2 indicated that strain had a significant effect on yolk index. The average value of brown-eggs was higher (41.23) than the white ones (39.69). The overall mean varied significantly ($P \leq 0.001$) by age. The highest value was recorded at the 22th week of age followed by 33th, 43th, and 64th week of age, respectively. These results were in agreement with Padh et al. (2013). Who said the yolk index showed significant ($P \leq 0.05$) difference between different age of measurement. The interaction between breeder's age and strain was significant at ($P \leq 0.001$).

Table 2 shows that strain had a substantial impact on yolk weight based on the analysis of variance. Brown eggs had a lower average value (15.12) than white eggs (16.45). Age had a substantial impact on the total mean ($P \leq 0.001$). The highest value was found in the 64th week of life, followed by the 43rd, 33rd, and 22nd weeks of life, in that order. These findings corroborated those of Suk and Park (2001), Silversides et al. (2006), Rajkumar et al. (2009), and Padh et al. (2013), who found that the yolk weight rose with chicken age. At ($P 0.01$), the interaction between breeder age and strain was significant.

Table 2 shows that strain had a substantial impact on yolk percentage based on the analysis of variance. Brown eggs had a lower average value (27.22) than white eggs (28.58). Age had a substantial impact on the total mean ($P \leq 0.001$). The highest value was found in the 64th week

of life, followed by the 43rd, 33rd, and 22nd weeks of life, in that order. The yolk percent grew as the age of measures increased, as reported by (Silversides and Scott, 2001; Tumova and Gous, 2012; Padh et al., 2013). There was no significant relationship between breeder age and strain.

Table 2: Effect of strain and age of breeders on yolk quality before hatch.

Age(A)	Strain (S)		Overall mean	Prob.		
	White	Brown		S	A	S*A
Yolk height (mm)						
22	15.77±0.22	15.1±0.24	15.43±0.23	NS	NS	***
33	16.29±0.18	15.64±0.17	15.99±0.175			
43	15.06±0.25	15.59±0.27	15.32±0.26			
64	15.64± 0.25	14.25± 0.47	15.02±0.36			
Overall	15.7± 0.22	15.17± 0.28				
Yolk diameter (mm)						
22	37.05± 0.26	33.59±0.42	35.32 ^b ±0.34	***	***	NS
33	40.08± 0.32	38.51±0.37	39.35 ^a ±0.34			
43	41.13± 0.33	38.26±0.76	39.55 ^a ±0.54			
64	40.61± 0.53	38.23±0.48	39.69 ^a ±0.50			
Overall	39.69 ^a ±0.36	37.02 ^b ±0.50				
Yolk index						
22	42.58± 0.66	45.05± 0.97	43.82 ^a ±0.81	*	***	***
33	40.67± 0.46	40.67± 0.69	40.67 ^b ±0.57			
43	36.65± 0.69	40.96±1.095	38.81 ^c ±0.89			
64	38.64± 0.89	37.36± 1.36	38.07 ^c ±1.12			
Overall	39.69 ^b ±0.67	41.23 ^a ±1.02				
Yolk weight (g)						
22	15.19± 0.44	12.67± 0.37	13.93 ^c ±0.40	***	***	**
33	15.74± 0.3	14.67± 0.29	15.24 ^b ±0.29			
43	16.88± 0.37	17.22± 0.28	17.05 ^a ±0.32			
64	18.02± 0.44	16.21± 0.31	17.22 ^a ±0.37			
Overall	16.45 ^a ±0.38	15.12 ^b ±0.31				
Yolk %						
22	27.33±0.72	23.93±0.66	25.63 ^b ±0.69	*	***	Ns
33	27.48±0.31	26.41±0.37	26.99 ^b ±0.34			
43	28.99±0.49	29.22±0.43	29.11 ^a ±0.46			
64	30.54±0.85	29.87±1.31	30.24 ^a ±1.08			
Overall	28.58 ^a ±0.59	27.22 ^b ±0.69				
Yolk albumen ratio						
22	0.43±0.01	0.27±0.007	0.35 ^c ±0.008	***	***	NS
33	0.43±0.007	0.29±0.004	0.37 ^c ±0.005			
43	0.47±0.012	0.32±0.004	0.39 ^b ±0.008			
64	0.5±0.02	0.33±0.014	0.43 ^a ±0.017			
Overall	0.46 ^a ±0.012	0.3 ^b ±0.007				

a,b and c overall Means within the same row or column with different letters are significantly differed. S: strain; A: age; NS: not significant; *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$, g: grams.

Table 2 shows that strain had a substantial impact on yolk percentage based on the analysis of variance. Brown eggs had a lower average value (27.22) than white eggs (28.58). Age had a substantial impact on the total mean ($P \leq 0.001$). The highest value was found in the 64th week of life, followed by the 43rd, 33rd, and 22nd weeks of life, in that order. The yolk percent grew as the age of measures increased, as reported by (Silversides and Scott, 2001; Tumova and Gous, 2012; Padh et al., 2013). There was no significant relationship between breeder age and strain.

Strain had a substantial effect on albumen height and weight, according to the analysis of variance in Table 3. The strain effect on albumen height average was considerable ($P \leq 0.001$). Brown eggs had a lower average value (6.66) than white eggs (7.87). In terms of albumen weight, brown eggs had a higher average value (49.39) than white eggs (35.72). Age had a substantial impact on the total mean ($P \leq 0.001$). The highest value was found in the 33rd week of life, followed by the 22nd, 43rd, and 63rd weeks of life, in that order. This is comparable to what Silversides et al. (2006), Rajkumar et al. (2009), discovered (2013). Albumen height and weight were significantly affected by the breeder's age ($P \leq 0.05$). In albumen height, there was no significant interaction between breeder age and strain. In albumen weight, the interaction between breeder age and strain was significant ($P \leq 0.05$).

Table 3: Effect of strain and age of breeders on albumen quality before hatch.

Age(A)	Strain (S)		Overall mean	Prob.		
	White	Brown		S	A	S*A
Albumen height (mm)						
22	8.02± 0.36	6.54± 0.35	7.28 ^b ± 0.35	***	***	NS
33	9.15± 0.22	7.04± 0.39	8.17 ^a ± 0.30			
43	7.35± 0.29	7.11± 0.46	7.23 ^b ± 0.37			
64	6.91± 0.31	5.89± 0.34	6.46 ^c ± 0.32			
Overall	7.87 ^a ± 0.29	6.66 ^b ± 0.38				
Albumen weight (g)						
22	34.77±0.6	46.61±0.56	40.69 ^c ±0.58	***	***	*
33	36.22±0.57	49.65±1.2	42.45 ^b ±0.88			
43	35.9± 0.47	52.52± 0.69	44.21 ^a ±0.58			
64	36.02±0.84	48.93± 1.55	41.76 ^{bc} ±1.195			
Overall	35.72 ^b ±0.62	49.39 ^a ±1				
Albumen %						
22	62.56±0.71	64.86±0.21	63.71±0.46	NS	NS	NS
33	63.24± 0.33	63.67± 0.17	61.22±0.25			
43	61.7±0.55	62.41±0.13	61.17±0.34			
64	60.82±0.82	60.23±0.37	60.83±0.59			
Overall	62.09±0.60	62.79±0.22				

a,b and c overall means within the same row or column with different letters are significantly differed. S: strain; A: age; NS: not significant; *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$; g: grams; mm: Micrometre.

Despite the fact that strain had no significant effect on albumen percentage, the analysis of variance Table 3 revealed that it did. Brown eggs had a higher average value (62.79) than white eggs (62.09). The age of the participants had no effect on the aggregate mean. The highest value was found in the 22nd week of life, followed by the 33rd, 43rd, and 64th weeks of life, in that order. The interaction between the breeder's age and the strain had no effect.

Strain had a substantial effect on wet shell weight, according to the analysis of variance in Table 4. Brown eggs had a higher average value (7.02) than white eggs (6.4). This finding is consistent with (Scott and Silversides, 2000; Renema et al., 2001). Brown eggs had a greater eggshell weight than white eggs, according to the researchers. The weight of the shell rose with age.

Table 4: Effect of strain and age of breeders on shell quality before hatch.

Age(A)	Strain (S)		Overall mean	Prob.		
	White	Brown		S	A	S*A
Wet shell weight (g)						
22	6.86± 0.11	7.59± 0.12	7.22 ^a ± 0.11	***	***	NS
33	6.16± 0.08	6.75± 0.14	6.43 ^c ± 0.11			
43	6.44± 0.15	7.04± 0.13	6.74 ^b ± 0.14			
64	6.16± 0.13	6.6± 0.24	6.36 ^c ± 0.18			
Overall	6.4 ^b ± 0.11	7.02 ^a ± 0.15				
Dry shell weight (g)						
22	5.6± 0.06	5.92± 0.11	5.76 ^a ± 0.08	NS	**	NS
33	5.29± 0.07	5.62± 0.11	5.45 ^b ± 0.09			
43	5.41± 0.12	6.05± 0.1	5.73 ^{ab} ± 0.11			
64	5.1± 0.12	5.64± 0.22	5.34 ^b ± 0.17			
Overall	5.35± 0.09	5.82± 0.13				
Shell %						
22	10.1± 0.1	11.2± 0.2	10.65 ^a ±0.15	***	***	NS
33	9.26± 0.14	10.13± 0.16	9.67 ^b ±0.15			
43	9.29± 0.17	10.27± 0.12	9.78 ^b ±0.14			
64	8.62± 0.13	10.3± 0.35	9.37 ^c ±0.24			
Overall	9.32 ^b ±0.13	10.5 ^a ± 0.20				

a,b and c overall Means within the same row or column with different letters are significantly differed. S: strain; A: age; NS: not significant; *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$; g: grams.

Age had a substantial impact on the total mean ($P \leq 0.001$). The highest value was found in the 22nd week of life, followed by the 43rd, 33rd, and 64th weeks of life, in that order. Scott and Silversides (2000), Renema et al. (2001), Suk and Park (2001), Rajkumar et al. (2009), and Padh et al. (2013) also saw a similar trend in shell weight as they became older. Suk and Park (2001) and Rajkumar et al. (2009) discovered that shell weight differed between strains and that the relationship between breeder age and strain was not significant.

Despite the fact that the analysis of variance in Table 4 revealed that strain had no influence on dry shell weight, Brown eggs had a higher average value (5.82) than white eggs (5.35). These findings contradict those of Curtis et al. (1986), Silversides and Scott (2001), and Rayan et al. (2013), who found that brown eggs had a much greater shell percentage than white eggs, possibly due to strain differences. When it comes to the age effect, the results show that the percentage of shells reduces as the hens get older. Age had a substantial impact on the total mean ($P \leq 0.001$). The highest value was found at the 22nd week of age, followed by the 43rd, 33rd, and 64th weeks of age, respectively, however there was no significant interaction between breeder age and strain.

Strain had a substantial effect on shell %, according to the analysis of variance Table 4. Brown eggs had a higher average value (10.5) than white eggs (9.32). Age had a substantial impact on the total mean ($P \leq 0.001$). The highest value was found in the 22nd week of life, followed by the 43rd, 33rd, and 64th weeks of life, in that order. There was no significant relationship between breeder age and strain.

CONCLUSIONS AND RECOMMENDATIONS

In summary, this study found that strain and age had a substantial impact on egg-shell quality in layer breeds flocks of various ages. The difference in shell quality between strains has an impact on the quantity of eggs to incubate and hatch efficiency.

AUTHOR'S CONTRIBUTION

All author's contributed equally.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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