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SAMPLING TECHNIQUE THE EGGSHELL THICKNESS

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ABSTRACT

The aim of the study was to investigate the shell thickness of table eggs using a destructive method with a focus on the technique of sampling from three locations in the equatorial plane, the sharp and the blunt end of the egg. The subject of the study was the eggshell thickness with membranes of the influence of the sample taken on the egg in equatorial plane 1, equatorial plane 2, equatorial plane 3, at the sharp end and the blunt end of the egg, and a laying hen of breed Dominant aged 61 weeks (3 small-breedings) and 104 weeks (1 small-breeding). The measurement of eggshell thickness was carried out using a DIAL INDICATOR deviation meter, with an accuracy of 0.01 mm and a maximum thickness of 30 mm. The SAS statistical package, version 8.2, was used for statistical evaluation of the results. The influence of the location of the eggshell sample taken in 3 locations of the equatorial plane, at the sharp and blunt end of the egg on its thickness in the range of average values of 0.32 to 0.40 mm was not statistically significant (P>0.05). The influence of laying hen age was statistically significant (P≤0.05) by a higher eggshell thickness in the range of average values of 0.38 to 0.40 mm at 104 weeks compared with eggshell thickness of average values of 0.32 to 0.35 mm at 61 weeks. A strong linear positive correlation in eggshell thickness was found between two variables at all investigated sample locations on eggs laid by laying hens Dominant regardless of age. The overall results of eggshell thickness indicate that its evaluation is a complex process as an indicator of eggshell quality due to multifactorial external and internal influence. Considering the trend of lengthening the laying cycle in laying hens for environmental reasons, it is important to address the influence of age, housing system and breed suitability in relation to optimizing the uniformity of the thickness as part of the shell quality of the eggs.

Keywords: eggshell, thickness, sampling, laying hen, age

INTRODUCTION

The eggshell is important for two main reasons; it protects the edible content of eggs and plays an important role in sale of table eggs for direct consumption. Estimated economic losses of eggs with a cracked shell in the production and distribution cycle represent 8 to 11% of the total egg production **(Hamilton & Bryden, 2021)**. A significant relationship exists between eggshell and percentage of cracked eggs. However, eggshell thickness varies from point to point over the entire surface of the egg. Variations in eggshell thickness from the blunt to the sharp end of the egg are large, while variations in the equatorial plane are small **(Tyler & Geake, 1964)**. Eggshell thickness is one of the most important indirect measurements of eggshell strength. There is a significant curvilinear relationship between eggshell thickness and the percentage of cracked eggs **(Khatkar *et al.*, 1997)**. Eggshell strength is determined by its thickness, which mainly reflects its structural properties **(Bain, 2005)**. Eggshell is a multi-layered bio-ceramic composite containing the mineral component calcium carbonate ($CaCO_3$) in most cases in the polymorphic form of calcite **(Rodriguez-Navarro *et al.*, 2002)**, which is closely connected to the organic matrix **(Ahmed *et al.*, 2005)**. The mineral component consists of several layers, and according to the prevailing theory, eggshell thickness is the main variable that contributes to the mechanical properties of the shell. There is some evidence that eggshell microstructure can also affect mechanical properties **(Rodriguez-Navarro *et al.*, 2002)**. Eggshell thickness ranges from 0.200 mm to 0.400 mm. The main components of eggshells are minerals (96.1%), proteins (3.3%) and water (1.6%) **(Dupoirieux *et al.*, 2001)**. A slightly different composition of the eggshell is found in another study. The eggshell is composed of 96% calcium, 2% organic matter, phosphorus, magnesium, and other trace elements **(Nys *et al.*, 2004).** Several direct and indirect methods exist for evaluating eggshell quality **(De Ketelaere *et al.*, 2000; Dunn *et al.*, 2005; Solomon, 2010)**. Eggshell thickness is usually measured with or without membranes using specific measuring tools **(Peebles & McDaniel, 2004)**. These methods are applicable to broken eggs but cannot be used for hatching eggs during incubation. For this reason, researchers are also discovering new methods for estimating eggshell thickness indirectly **(Arslan & Yamak, 2020)**. The quality of eggshells has long been the subject of genetic selection, and significant improvements in eggshell quality have been noted **(Hocking *et al.*, 2003)**. The eggshell thickness indicator is considered an important feature from many points of view, mainly for selection or monitoring in breeding programs, egg transport, ensuring sufficient calcium content in the feed of laying hens, but also for the disposal of the shell as waste. Eggs with better eggshell thickness uniformity have a stronger shell, which can provide a new way of understanding and a new parameter for breeding and production management programs **(Sun *et al.*, 2012)**. Changes in the physical and chemical properties of eggs are more intense in the case of a thinner layer of eggshells **(Ketta and Tůmová, 2018; Yüceer & Caner, 2021)**. They confirmed these conclusions in their research **Veldsman *et al*. (2020)**, **Ahmed *et al*. (2021)**. The results point to the importance of eggshell thickness and structural integrity as a physical barrier to protect the internal contents of eggs from moisture loss and contamination by pathogenic microorganisms. It is believed that the presence of a denser and thicker eggshell membrane and the eggshell itself could contribute to limiting the movement of water through the shell and prevent dehydration of the egg's internal components **(Kocetkovs *et al*., 2022)**.

The aim of our study was to investigate the shell thickness of table eggs using a destructive method with a focus on the technique of sampling from three locations in the equatorial plane, the sharp and the blunt end of the egg.

MATERIAL AND METHODS

The subject of the study was the eggshell thickness of the influence of the sample taken on the egg in equatorial plane 1, equatorial plane 2, equatorial plane 3, at the sharp end and the blunt end of the egg, and a laying hen aged 61 wk (3 small-breedings) and 104 wk (1 small-breeding).

Laying hens and small-breedings. Laying hens of breed Dominant from 4 flocks (n = 10 to 12 hens laying eggs with a brown shell) in the 4 small-breedings in Slovakia were included in the study solution in September 2022 simultaneously, in parallel. Breeding conditions, nutrition and management were ensured in these small farms in accordance with the needs of laying hens, which was sufficiently reflected in the viability and health of laying hens as well as in their egg production. A hen houses with deep litter and a grass area during the growing season was equipped with similar conditions for each flock of laying hens. The concentration of laying hens per unit area was adequate to the recommendations for unrestricted movement and performing natural activities. The hen houses were equipped with a feeder, waterer, nest, and perch. The nest was adapted for manual egg collection. The enclosure was equipped with a feeder and waterer. Laying hens were fed with a common soy-cereal type feed mixture intended for hens laying the table eggs, which was added to the feeder 2 times per day. If the small breeder had small fresh food scraps or eggshells, these crushed ones were used to enrich the feed ration for the laying hens. Clean drinking water was used to feed the hens, which was added to the waterers 2 to 3 times a day as needed. Watering troughs were washed daily.

Data Collection (Table 1). The collection of produced eggs was carried out in the afternoon for 3 days with the aim of obtaining 20 eggs from each flock in 4 small farms.

Table 1 Data collection for measuring eggshell thickness

Breeding	Age	Egg ssamples (n)	Eggshell sample's location (n)				
			EP 1	EP 2	EP 3	SE	BE
1	61wk	20	20	20	20	20	20
2	61 wk	20	20	20	20	20	20
3	61 wk	20	20	20	20	20	20
4	104 wk	20	20	20	20	20	20

Abreviation: EP - equatorial plane, SE - sharp end of egg, BE - blunt end of egg, wk - week

The figure 1 shows the shell sampling locations on the egg to measure its thickness in the equatorial plane (E1), equatorial plane (E2), equatorial plane (E3), at the sharp (SE), and blunt end (BE).

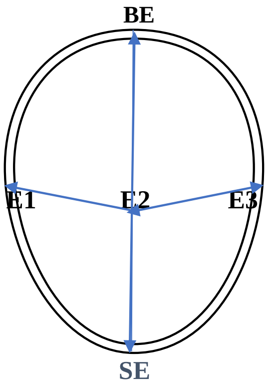

Figure 1 Distribution of points of measured eggshell samples on the egg

Measuring eggshell thickness

The collected egg samples were marked with numbers from 1 to 20 and according to small-breeding 1 to 4. Eggshell thickness measurement was performed after breaking the egg in the equatorial plane and separating it from the internal egg content. The eggshell together with the membranes was washed under running water and dried in an HS 62 A dryer at a temperature of 55 °C for 8 hours.
The measurement of the eggshell sample of the blunt end of the egg and the equatorial plane using the DIAL INDICATOR deviation meter shows figure 2 (an accuracy of 0.01 mm and maximum thickness of 30 mm). Eggshell samples with an area approximately of 1.8 to 2.4 cm^2 were taken from the equatorial plane of the egg. The samples from the equatorial planes were irregular in shape. The condition for eggshell measurement was the presence of membranes on the inner side of the shell for all samples.

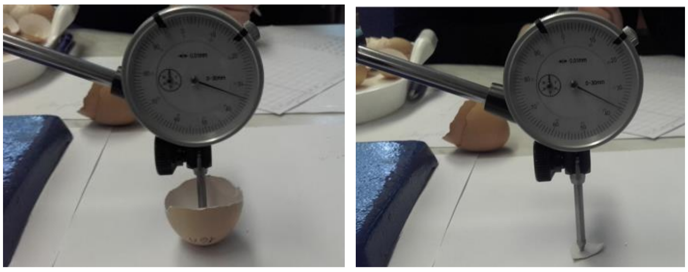

Figure 2 Measurement of eggshell thickness using the deviation meter DIAL INDICATOR

Statistical Analysis

The results in the study are interpreted as mean, standard deviation (SD), minimum and maximum value. The hypothesis of equality of means was tested using ANOVA. Scheffe's test was used at the significance level of P≤0.05 to compare the difference between eggshell thickness sampling sites and laying hen age. Pearson's correlation coefficient (r) was used to express the degree of relationship between the two variables of the locations of the shell samples collected on the egg at the age of 61 and 104 wk, i.e., according to small-breeding, and without considering age. The interpretation of the results of the correlation coefficient (r) was done according to **Cohen (1988)** as a relationship trivial up to 0.1, weak 0.1 to 0.3, moderate 0.3 to 0.5 and strong above 0.5. The results of the correlation coefficient were evaluated by statistical significance at P≤0.05, P≤0.01 and P≤0.001. The SAS statistical package, version 8.2, was used for statistical evaluation of the results.

RESULTS AND DISCUSSION

The influence of the location of eggshell sampling on eggshell thickness

Basic statistical evaluation of the eggshell thickness is shown Table 2. The variances in the eggshell thickness data groups differed statistically significantly P<0.001, which was confirmed the F-test, which was verified the assumption of equality of variances. The null hypothesis H0 about the absence of difference between groups was rejected (Table 2). The average value of eggshell thickness was 0.350±0.045 mm.

Table 2 Basic statistical evaluation of the eggshell thickness

Indicator	n	Egshell (mm)	Statistical significance
		Mean ± SD	*F* value
Thickness	400	0.350±0.045	<0.001

Eggshell quality, including thickness, depends on many factors. Various studies have shown this that eggshell quality is predisposed by factors such as age, strain, housing system and so on feeding a balanced diet along with supplements and essential minerals **(Coutts and Wilson, 2007; Butcher & Miles, 2009; Venglovska *et al*., 2014).** These factors are divided into internal and external. The main internal factors include e.g. time of egg mass formation in the oviduct, age, and genotype of laying hens. External factors include the housing system, nutrition, microclimate, etc. **(Campo *et al.*, 2007)**. Among the many factors that determine eggshell quality with relevant implications for hen welfare is nutrition, which is an important modulator **(Roberts, 2004; Islam *et al*. 2021)**. The importance of a balanced diet for laying hens was emphasized by **Stefanello *et al.* (2014), Qiu *et al.* (2020)** who point out the importance of mineral resources used in the feed of laying hens derived from inorganic compounds such as carbonates, phosphates, oxides, and sulfates in the formation of eggshells.
The intention of our experiment was designed for the same conditions of breeding laying hens – resources and management. Animals differed in age in the small-breedings. Laying hens consumed a soy-cereal type feed mixture. For laying hens in a small farm, the feed mixture is sometimes enriched in a small amount with fresh kitchen waste and grass in the paddock, which was also the case in our experiment. No deviations from normal behavior were observed in the behavior of the laying hens. Laying hens performed their natural activities, unrestricted access to feed and water, free movement, pecking and scratching, and laying eggs in the nest.
The shell is responsible for protecting the egg from mechanical shock and allows controlled exchange of fluids and gases through the pores, in addition to providing protection against microbial contamination **(Pires *et al.*, 2019)**. There are conflicting results in studies about the qualitative characteristics of eggs. Some quality characteristics are better for eggs from the cage system compared to alternative systems **(Englmaierová *et al*., 2014)**. Less shell thickness was observed in eggs produced in alternative systems compared to eggs produced in cages (**Pavlovski *et al*., 2000)**.
Sokolowicz *et al.* (2018) found the effect of housing system on eggshell thickness. Different parameters of eggshell quality are related to the type of housing. Eggshell quality can be improved by optimizing the housing system. In the current period, when promoting alternative housing systems for laying hens to European conditions, it is appropriate to focus on the solution of the nest floor material and the selection of a genotype suitable for a specific housing system, as well as the mineral balance of the feed, regarding housing and genotype **(Ketta & Tůmová, 2016)**. Also, **Leyendecker *et al*. (2001)** found that a lower eggshell thickness was obtained in cage-raised eggs, with the highest value in free-range eggs. In addition, several studies reported that shell thickness was lower in eggs also from cages than in the litter system **(Hidalgo *et al*., 2008)**. Different genotypes of laying hens show different parameters of eggshell quality **(Ketta & Tůmová, 2016)**.
Considering the mentioned parameter, we chose laying hens of the Dominant hybrid in all four small-breedings. Scientists have been researching an effective technique or tool for several decades to optimize the evaluation of eggshell thickness and strength in order of reduce economic losses due to its cracking. **Sun *et al*. (2012)** introduced a new parameter called eggshell thickness uniformity. The authors justified this parameter as the reciprocal coefficient of variation of the thickness of the eggshell from several positions. Eggshell thickness uniformity has a significant positive correlation with ultimate strength. The eggshell thickness uniformity indicator is accepted as a tool for evaluating eggshell quality. **Yan *et al*. (2014)** investigated the relationship between eggshell thickness uniformity and Lohmann Brown eggshell quality. These authors state that eggshell thickness uniformity was positively correlated with eggshell thickness (0.297), ultimate

strength (0.430), static stiffness (0.409), and fracture toughness (0.171) and recommend that the eggshell uniformity index be used to evaluate eggshell quality. Basic statistical evaluation of the shell thickness of the influence of the eggshell sample's location on the egg is shown in Table 3 and statistical significance in thickness of the influence of the eggshell sample's location on the egg in Table 4. The evaluation of eggshell thickness was carried out in three locations of the equatorial plane 1, 2, 3, at sharp and blunt end for eggs laid in parallel by laying hens at the age of 61 wk in three small-breedings and at the age of 104 wk in one small-breeding. The average value of shell thickness of eggs laid by hens at the age of 61 wk was found in the range of 0.33±0.04 mm to 0.35±0.04 mm in locations 1, 2 and 3 of the equatorial plane.

The minimum measured value of eggshell was found in the range of 0.23 mm to 0.29 mm and the maximum value in the range of 0.39 to 0.44 mm in sites 1, 2 and 3 of the equatorial plane. The difference in the average value of shell thickness between sites 1, 2 and 3 of the equatorial plane of eggs laid by laying hens at the age of 61 wk was not statistically significant (P>0.05). The average value of shell thickness of eggs laid by hens at the age of 104 wk was found in the range of 0.38±0.03 mm to 0.39±0.03 mm in locations 1, 2 and 3 of the equatorial plane. The minimum measured eggshell value was found to be 0.31 mm and the maximum value was 0.43 mm in locations 1, 2 and 3 of the equatorial plane. The difference in the average value of shell thickness between locations 1, 2 and 3 of the equatorial plane of eggs laid by laying hens at the age of 104 wk was not statistically significant (P>0.05).

Statistical significance in the thickness of the influence of the eggshell sample's location on the egg is shown in Table 5. The differences in eggshell thickness were not statistically significant (P>0.05) between the locations of equatorial plane 1, equatorial plane 2, equatorial plane 3, sharp end and blunt end of eggs. The effect of the location of the shell sample taken on the egg was comparable. The eggshell thickness with the membrane was measured of 7 positions from blunt to sharp end, while the average values ranged from 0.357 to 0.380 mm with a fluctuation of the measured values ±0.022 to 0.031 expressed by the standard deviation **(Sun *et al.*, 2012)**. Similar, slightly different eggshell thickness results were measured in our experiment.

In another study, the thickness of the eggshell and the membrane were measured separately. According to the results of the study, the thickness of the eggshell ranged from 0.356 to 0.366 mm and the membrane from 0.020 to 0.060 mm. No statistically significant difference was observed between the thickness of the eggshells tested **(Kocetkovs *et al.*, 2022)**. The conclusions of the above authors were confirmed by our experiment.

Table 3 Basic statistical evaluation of the shell thickness of the influence of the eggshell sample's location on the egg

Indicator	Eggshell (mm)			
	n	Mean ± SD	Min	Max
Age 61 wk[1]				
Equatorial plane 1	20	0.35ᵃ±0.04	0.29	0.44
Equatorial plane 2	20	0.35ᵃ±0.04	0.28	0.44
Equatorial plane 3	20	0.35ᵃ±0.04	0.28	0.44
Sharp end	20	0.36ᵃ±0.06	0.27	0.47
Blunt end	20	0.34ᵃ±0.06	0.23	0.49
Age 61 wk[2]				
Equatorial plane 1	20	0.33ᵃ±0.04	0.25	0.40
Equatorial plane 2	20	0.33ᵃ±0.04	0.23	0.39
Equatorial plane 3	20	0.33ᵃ±0.04	0.23	0.39
Sharp end	20	0.34ᵃ±0.07	0.18	0.46
Blunt end	20	0.32ᵃ±0.05	0.23	0.40
Age 61 wk[3]				
Equatorial plane 1	20	0.33ᵃ±0.04	0.26	0.39
Equatorial plane 2	20	0.34ᵃ±0.03	0.27	0.40
Equatorial plane 3	20	0.33ᵃ±0.04	0.26	0.40
Sharp end	20	0.33ᵃ±0.06	0.21	0.42
Blunt end	20	0.33ᵃ±0.06	0.24	0.50
Age 104 wk				
Equatorial plane 1	20	0.39ᵃ±0.03	0.31	0.43
Equatorial plane 2	20	0.39ᵃ±0.03	0.32	0.43
Equatorial plane 3	20	0.38ᵃ±0.03	0.31	0.43
Sharp end	20	0.40ᵃ±0.04	0.34	0.49
Blunt end	20	0.38ᵃ±0.03	0.31	0.43

Abbreviations: age 61 wk[1, 2, 3], small-breeding 1, 2, 3 Marking with the same letter at the average value in a column means a statistically no significant difference (P>0.05)

The influence of laying hen age on shell thickness in the shell sampling locations on the egg

Basic statistical evaluation of the influence of laying hen age on shell thickness is shown in Table 4. The average value of eggshell thickness in the determined egg locations examined ranged from 0.347±0.038 mm (equatorial plane 3) to 0.350±0.037 mm (equatorial plane 1) or to 0.351±0.037 mm (equatorial plane 2). The average eggshell thickness reached 0.355±0.059 mm at the sharp end of the egg and 0.344±0.051 mm at the blunt end of the egg. The effect between laying hen age was recorded statistically significant (P<0.001, P<0.01) in eggshell thickness. Studies show that eggshell thickness decreases with advancing age **(Bozkurt & Tekerli, 2009; Park & Sohn, 2018; Benavides-Reyes *et al.*, 2021)**. Higher age of laying hens has a negative effect on the thickness and strength of the eggshell, which causes high economic losses **(Ketta & Tůmová, 2016)**. **Tůmova & Ledvinka (2009)** reported thicker eggshell 0.372 mm at the age of laying hens from 56 to 60 wk in comparison with 0.354 mm at age laying hens from 20 to24 wk.

The results of another study show that eggshell thickness, including the membrane, was affected by age. Its values decreased from 0.392 ± 0.0039 mm (the 29th wk of laying hens) to 0.380±0.005 mm (the 49th wk of laying hens), statistically insignificant, up to 0.373±0.009 mm (the 70th wk of laying hens) statistically significant. These results show that modern laying hens, represented by Lohmann Selected Leghorn and Lohmann Brown, produce eggs with reduced shell quality relatively early in the laying period, but that they can maintain this quality at least until the 70th wk of age **(Wistedt et al., 2019)**. **Bain et al. (2016)** pointed out that this pattern has not yet been established for longer laying cycles.

Table 4 Basic statistical evaluation of the shell thickness of the influence of the laying hen age

Indicator	Eggshell thickness (mm)		
	n	Mean ± SD	Statistical significance *F* value
Equatorial plane 1	80	0.350±0.037	<0.001
Equatorial plane 2	80	0.351±0.037	<0.001
Equatorial plane 3	80	0.347±0.038	<0.001
Sharp end	80	0.355±0.059	0.002
Blunt end	80	0.344±0.051	0.004

Abbreviations: P≤0.001, P≤0.01- statistically significant difference

Basic statistical evaluation of the eggshell thickness of the influence of laying hen age to eggshell sample's location on the egg is shown Table 6. The average thickness of the eggshell was found from 0.33±0.04 mm to 0.35±0.04 mm in the equatorial plane 1 of laid eggs by laying hens at the age of 61 wk. The minimum values of the measured shell thickness ranged from 0.25 to 0.29 mm and the maximum values in the range of 0.39 to 0.44 mm in the equatorial plane 1. Eggs laid by laying hens at the age of 104 wk reached a statistically significant (P≤0.05) a higher shell thickness of 0.39±0.03 mm in equatorial plane 1 with a minimum measured value of 0.31 mm and a maximum measured value of 0.43 mm.

The average eggshell thickness was found from 0.33±0.04 mm to 0.35±0.04 mm in the equatorial plane 2 of eggs laid by laying hens at the age of 61 wk. Eggs laid by laying hens at the age of 104 wk reached a statistically significant (P≤0.05) a higher shell thickness of 0.39±0.03 mm in equatorial plane 2 with a minimum measured value of 0.32 mm and a maximum measured value of 0.43 mm.

The average eggshell thickness was found from 0.33±0.04 mm to 0.35±0.04 mm in the equatorial plane 3 of eggs laid by laying hens at the age of 61 wk. Eggs laid by laying hens at the age of 104 wk reached a statistically significant (P≤0.05) a higher shell thickness of 0.38±0.03 mm in the equatorial plane 3 with a minimum measured value of 0.31 mm and a maximum measured value of 0.43 mm.

Average eggshell thickness was found to range from 0.33±0.06 mm to 0.36±0.06 mm at the sharp end of eggs laid by hens at 61 wk of age. The shell thickness of eggs laid by hens at the age of 104 wk reached a higher average value of 0.38±0.03 mm at the sharp end of the egg, statistically significantly (P≤0.05) compared to the shell thickness at the sharp end of eggs laid by hens in aged 61 wk in the small-breedings 1 and 2 and statistically insignificantly (P>0.05) compared to the shell thickness at the sharp end of eggs laid by hens aged 61 wk in the small-breeding 3.

The average eggshell thickness was found from 0.32±0.05 mm to 0.34±0.06 mm at the blunt end of eggs laid by laying hens at the age of 61 wk. The shell thickness of eggs laid by laying hens at the age of 104 wk reached a higher average value of 0.38±0.03 mm at the blunt end of the eggs, statistically significantly (P≤0.05), compared to the shell thickness at the blunt end of eggs laid by laying hens at the age of 61 wk in small-breeding 1 and 2 and statistically no significant (P>0.05), compared to the shell thickness at the blunt end of eggs laid by laying hens at age of 61 wk in small-breeding 3.

Statistical significance in the thickness of the influence of laying hen age to eggshell sample's location on the egg is shown in Table 7. A statistically significant (P≤0.05) difference in the shell thickness was found at eggs laid by laying hens between the age 61 and 104 wk in the equatorial plane 1 in the small-breeding's 1, 2 and 3, in the equatorial plane 2 in the small-breeding's 1, 2 and 3, in the equatorial plane 3 in the small-breeding's 1, 2 and 3, at the sharp end of the egg in small-breeding 2 and 3 and at the blunt end of the egg in the small-breeding

2 and 3. A statistically no significant (P>0.05) difference in shell thickness was found at eggs laid by laying hens between the age 61 and 104 wk at the sharp and blunt ends of the eggs in the small-breeding 1. Depending on the age of the hens, the shell thickness was measured 0.387±0.018 mm at 33 wk, 0.372±0.017 mm at 45 wk, and 0.363±0.022 mm at 67 wk. Differences in shell thickness were statistically significant between the examined ages of laying hens **(Benavides-Reyes et al., 2021)**. In our experiment, the effect of the later age of laying hens on the eggshell thickness was investigated in three locations of the equatorial plane and at the sharp and blunt ends of the eggs. We can conclude that the late age of laying hens Dominant has an influence on the eggshell thickness parameter as part of the eggshell quality.

Table 5 Statistical significance in thickness of the influence of the eggshell sample's location on the egg

Indicator	**Age 61 wk[1]**					**Age 61 wk[2]**					**Age 61 wk[3]**					**Age 104 wk**				
	SE	BE	EP1	EP2	EP3	SE	BE	EP1	EP2	EP3	SE	BE	EP1	EP2	EP3	SE	BE	EP1	EP2	EP3
Age 61 wk[1]																				
SE	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
BE		P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
EP1			P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
EP2				P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
EP3					P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
Age 61 wk[2]																				
SE						P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
BE							P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
EP1								P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
EP2									P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
EP3										P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
Age 61 wk[3]																				
SE											P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
BE												P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
EP1													P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
EP2														P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
EP3															P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05

Abbreviations: age 61 wk[1, 2, 3], small-breeding 1, 2, 3 SE - sharp end; BE - blunt end; EP - equatorial plane Marking P>0.05 - statistically no significant difference

Correlations between eggshell sample's location on the egg at shell thickness

Correlation relationship between eggshell locations on the egg and eggshell thickness due to the laying hen age is shown in Table 8. By evaluating the correlation relationship in the thickness of the egg shell in three positions of the equatorial plane, at the sharp and blunt end of the egg according to the age of the laying hens in four small-breeding's, a linear positive trivial power was found between the two variables (r = up to 0.1) , through linear negative weak (r = -0.1 to -0.3), or positive linear medium (r = 0.3 to 0.5), to linear positive strong (r = above 0.5), but with a different level of statistical significance (P≤0.001, P<0.01, P<0.05), or without statistical significance (P>0.05).A strong linear positive relationship in thickness was found between the two variables for all examined eggshell locations on the egg from r = 0.709 (between the sharp end of the egg and equatorial plane 1) to r = 0.993 (between equatorial plane 2 and equatorial plane 3), statistically significant P<0.001, at eggs laid by laying hens aged 61 wk in the small-breeding 1.

A strong linear positive correlation in thickness was found between the two variables for all investigated eggshell locations on the egg from r = 0.594 (between the sharp end of the egg and equatorial plane 1, statistically significant P < 0.01), to r = 0.989 (between equatorial plane 2 and equatorial plane 3, statistically significant P<0.001), on the eggs laid by laying hens at the age of 61 wk in the small-breeding 2. The strong linear positive correlation in eggshell thickness was found statistically significant (P≤0.001, P<0.01), between the two variables at the other investigated eggshell locations on the eggs laid by laying hens aged 61 wk in the small-breeding 2.

A linear correlation in the thickness was found between the two variables in the investigated eggshell locations on the egg, positive trivial from r = 0.005 (between the blunt end of the egg and the equatorial plane 2, statistically insignificant P>0.05), to r = 0.084 (between blunt end of egg and equatorial plane 1, statistically insignificant P>0.05), weak negative r = -0.201 (between blunt end of egg and sharp end of egg, statistically insignificant P>0.05), medium positive from r = 0.474 (between sharp end of the egg and equatorial plane 3, statistically significant P<0.05), to r = 0.485 (between the sharp end of the egg equatorial plane 1, statistically significant P<0.05), and a strong positive from r = 0.948 (between equatorial plane 2 and equatorial plane 1, statistically significant P<0.001), to r = 0.969, respectively r = 0.967 (between equatorial plane 3 and equatorial plane 2, respectively between equatorial plane 3 and equatorial plane 1 statistically, statistically significant P<0.001), at eggs laid by laying hens aged 61 weeks in small-breeding 3.

A linear relationship in thickness was found between the two variables at the examined eggshell locations on the egg, positive trivial r = 0.071 (between the blunt end of the egg and the sharp end of the egg, statistically no significant P>0.05), positive medium from r = 0.375 (between the sharp end of the egg and equatorial plane 1, statistically no significant P>0.05), to r = 0.481 (between the sharp end of the egg and equatorial plane 3, statistically significant P<0.05), and strong positive from r = 0.963 (between equatorial plane 2 and equatorial plane 1, statistically significant P<0.001), to r = 0.986, respectively r = 0.974 (between equatorial plane 3 and equatorial plane 2, respectively between equatorial plane 3 and equatorial plane 1, statistically significant P<0.001), at eggs laid by laying hens aged 104 weeks in small-breeding 4.

Correlation relationship between eggshell locations on the egg at eggshell thickness is shown in Table 9. A strong linear positive correlation in eggshell thickness was found between the two variables at all sampled locations on the egg from r = 0.536 (between the blunt end of the egg and the sharp end of the egg) to r = 0.988 (between equatorial plane 3 and the equatorial plane), statistically significant P<0.001, at eggs laid by laying hens Dominant regardless of age.The result of correlation analysis showed that eggshell thickness was positively correlated with breaking strength **(Amini et al., 2022)**. Based on these results, it can be concluded that eggs with better uniformity will have a stronger shell **(Sun et al., 2012)**.

Table 6 Basic statistical evaluation of the eggshell thickness of the influence of laying hen age to eggshell sample's location on the egg

Indicator	n	Eggshell thicknes (mm) Age 61 wk[1] Mean ± SD	Min Max	Age 61 wk[2] Mean ± SD	Min Max	Age 61 wk[3] Mean ± SD	Min Max	Age 104 wk Mean ± SD	Min Max
Equatorial plane 1	80	0.35[a]±0.04	0.29	0.33[a]±0.04	0.25	0.33[a]±0.04	0.26	0.39[b]±0.03	0.31
Equatorial plane 2	80	0.35[a]±0.04	0.44	0.33[a]±0.04	0.40	0.34[a]±0.03	0.39	0.39[b]±0.03	0.43
Equatorial plane 3	80	0.35[a]±0.04	0.28	0.33[a]±0.04	0.23	0.33[a]±0.04	0.27	0.38[b]±0.03	0.32
Sharp end	80	0.36[ac]±0.06	0.44	0.34[a]±0.07	0.39	0.33[a]±0.06	0.40	0.40[bc]±0.04	0.43
Blunt end	80	0.34[ac]±0.06	0.28	0.32[a]±0.05	0.23	0.33[a]±0.06	0.26	0.38[bc]±0.03	0.31
			0.44		0.39		0.40		0.43
			0.27		0.18		0.21		0.34
			0.47		0.46		0.42		0.49
			0.23		0.23		0.24		0.31
			0.49		0.40		0.50		0.43

Abbreviations: age 61 weeks[1, 2, 3], small-breeding 1, 2, 3
Marking with a different letter at the average value in a row means statistically significant difference (P≤0.05)
Marking with a different letter at the average value in a row means statistically no significant difference (P>0.05)

Table 7 Statistical significance in the thickness of the influence of laying hen age to eggshell sample's location on the egg

Indicator	Equatorial plane 1 61[2]	61[3]	104	Equatorial plane 2 61[2]	61[3]	104	Equatorial plane 3 61[2]	61[3]	104	Sharp end 61[2]	61[3]	104	Blunt end 61[2]	61[3]	104
Equatorial plane 1 61[1]	P>0.05	P>0.05	P≤0.05	P>0.05	P>0.05	P≤0.05	P>0.05	P>0.05	P≤0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
Equatorial plane 2		P>0.05	P≤0.05		P>0.05	P≤0.05		P>0.05	P≤0.05		P>0.05	P≤0.05		P>0.05	P≤0.05
61[2]			P≤0.05									P≤0.05			P≤0.05
Equatorial plane 3 61[3]				P≤0.05			P≤0.05								

Abbreviations: 61, 104, weeks laying hen age; age 61 weeks[1, 2, 3], small-breeding 1, 2, 3
P≤0.05, statistically significant difference. P>0.05, statistically no significant difference

Table 8 Correlation relationship between eggshell locations on the egg and eggshell thickness due to the laying hen age

	Equatorial plane 2	Equatorial plane 3 r *P* value	Sharp end	Blunt end
Age 61 wk[1]				
Equatorial plane 1	0.971	0.986	0.709	0.738
	<0.001	<0.001	<0.001	<0.001
Equatorial plane 2		0.993	0.711	0.724
		<0.001	<0.001	<0.001
Equatorial plane 3			0.721	0.724
			<0.001	<0.001
Sharp end				0.864
				<0.001
Age 61 wk[2]				
Equatorial plane 1	0.968	0.986	0.594	0.766
	<0.001	<0.001	0.006	<0.001
Equatorial plane 2		0.989	0.683	0.809
		<0.001	<0.001	<0.001
Equatorial plane 3			0.636	0.766
			<0.003	<0.001
Sharp end				0.843
				<0.001
Age 61 wk[3]				
Equatorial plane 1	0.948	0.967	0.485	0.084
	<0.001	<0.001	0.030	0.724
Equatorial plane 2		0.969	0.477	0.005
		<0.001	0.033	0.984
Equatorial plane 3			0.474	0.016
			0.034	0.947
Sharp end				-0.201
				0.395
Age 104 wk				
Equatorial plane 1	0.963	0.974	0.375	0.651
	<0.001	<0.001	0.103	0.002
Equatorial plane 2		0.986	0.469	0.675
		<0.001	0.037	0.001
Equatorial plane 3			0.481	0.622
			0.032	0.003
Sharp end				0.071
				0.765

Abbreviations: r - the numerical value of the correlation coefficient
age 61 weeks[1,2,3], small-breeding 1, 2, 3; P≤0.001, P<0.01, P<0.05, statistically significant correlation relationship; P>0.05, statistically no significant correlation relationship

Table 9 Correlation relationship between eggshell locations on the egg at eggshell thickness

Indicator	Equatorial plane 2	Equatorial plane 3	Sharp end	Blunt end
		r *P* value		
Equatorial plane 1	0.972 <0.001	0.984 <0.001	0.644 <0.001	0.642 <0.001
Equatorial plane 2		0.988 <0.001	0.680 <0.001	0.641 <0.001
Equatorial plane 3			0.664 <0.001	0.617 <0.001
Sharp end				0.536 <0.001

Abbreviations: r - the numerical value of the correlation coefficient
P<0.001 - statistically significant correlation relationship

CONCLUSION

Four small-breeding and laying hens Dominant were selected for the study solution. The object of the study was the eggshell thickness of the influence of the sample taken on the egg in the equatorial plane 1, 2, 3, at the sharp end and the blunt end of the egg and a laying hens aged 61 wk (3 small-breeding) and 104 wk (1 small-breeding). *From the evaluated measured results of eggshell thicknesses, the following conclusion emerged about the influence of the location of the shell sample taken on the egg and laying hen age:*

- Eggshell thickness measured in 3 locations of the equatorial plane, at the sharp and blunt end of the egg was comparable, where the influence of the location of the shell sample on the egg was not statistically confirmed.
- On eggshell thickness, the effect of laying hen age of samples taken in equatorial plane 1, equatorial plane 2, equatorial plane 3, at the sharp end of the egg and at the blunt end of the egg was statistically confirmed, except for shell thickness at the sharp and blunt end of the egg in one small-breeding, which was comparable.
- The influence of laying hen age was statistically confirmed by a higher eggshell thickness at 104 wk compared to 61 wk.
- A strong linear positive correlation in eggshell thickness was found between the two variables at all investigated sample locations on eggs laid by laying hens Dominant regardless of age.

The overall results of eggshell thickness indicate that its evaluation is a complex process as an indicator of eggshell quality due to multifactorial external and internal influence. Considering the trend of lengthening the laying cycle in laying hens for environmental reasons, it is important to address the influence of age, housing system and breed suitability in relation to optimizing the uniformity of the thickness as part of the shell quality of the eggs.

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