Egg quality of Banat Naked Neck hens during storage

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Original scientific paper

Abstract: In less intensive production systems, native poultry breeds can be used in order to diversify the products and achieve self-sustainability of these breeds through production. Given the missing data on the sustainability of quality of eggs obtained from indigenous, native hens, during storage, the aim of the study was to determine the most important parameters of egg quality of indigenous breed of Banat Naked Neck hens during a four-week period in different storage conditions. The design of the experiment was two-factorial with 4 levels of storage time factors (fresh eggs - 0, 1, 2, 3 and 4 weeks of storage) and 2 levels of temperature storage condition factors (room temperature and refrigerator). The room temperature was on average 21.3°C and the refrigerator temperature 8°C. Quality analysis was performed on a total of 200 eggs, and it included following parameters: egg weight, egg weight loss, weight and proportion of structural components: shell, yolk and albumen, albumen height, yolk colour, Haugh Units and albumen pH. The storage time had a significant effect on all properties of egg quality, except for the yolk colour, which was under the impact of the interaction of storage time and temperature. Storage temperature influenced egg weight loss (<0.001), shell weight (<0.05), albumen height (<0.0001), Haugh Units (<0.0001) and albumen pH (<0.0001). By storing in the refrigerator, changes in internal quality were significantly slowed down. After 28 days of storage in the refrigerator, the values of albumen and Haugh Units were higher than the same parameters of eggs stored for only 7 days at room temperature.

Key words: egg quality, Banat Naked Neck, storage time, storage temperature
Introduction

Egg is a nutritionally high quality product, with low energy values and high digestibility, which makes it a desirable food product. The specific structure of the egg, thanks to the shell and membranes, has a protective role in terms of biosafety and quality, which in certain conditions of farm management and storage can be maintained for different periods of time.

It is known that the table egg has the best internal quality at the time of oviposition. Subsequently, depending on the handling on the farm, the storage duration and conditions, the quality of the eggs decreases at different rates. In addition to the storage time, factors recognized as important for the sustainability of egg quality are temperature and relative humidity (Jin et al., 2011; Chung and Lee, 2014; Kopacz and Drazbo, 2018). According to Feddern et al. (2017), Kopacz and Drazbo (2018), storing eggs at room temperature leads to a decrease in quality, which is measured by unusability after 4 weeks. Egg quality parameters that change significantly under the influence of storage conditions and time are the height of the thick albumen, the egg weight, Haugh Units, the pH values of the albumen and egg yolk.

The effect of storage time in some studies is related to the effect of age and genotype of hens, breeding systems and nutrition on egg quality (Jin et al., 2011; Jones et al., 2014; Feddern et al., 2017; Perić et al., 2018; Vlčkova et al., 2019; Santos et al., 2019). In most studies, egg quality during storage is tested on eggs from hybrid hens, which are the basis of commercial egg production.

With the development of various forms of alternative egg production in which, in addition to different hybrids and pure breeds, native hens can be used, the question of sustainability of egg quality of these hens during storage is opened. Earlier research by Škrbić et al. (2020) points out the possibility of using Banat Naked Neck hens in alternative egg production systems, in order to diversify the products and achieve self-sustainability of this breed, whose population according to FAO (2020) is increasing, but still very vulnerable. In addition, the results of the research by Škrbić et al. (2011) show that the initial quality, as well as changes in egg quality traits during the laying period, differ significantly between the indigenous breed of the Banat Naked Neck from traditional, extensive production and hybrid laying hens in the cage breeding system.

Considering the missing data on the sustainability of the egg quality of autochthonous, native hens during storage, the aim of the research is to determine the most important parameters of egg quality of the autochthonous breed of Banat Naked Neck hens during a four – week period in different storage conditions.
Material and Methods

The experimental part of the research involving storage time influence, temperature conditions and their interaction on egg quality was conducted in a two-factor experiment, with 4 levels of storage time factors (fresh eggs - 0, 1, 2, 3 and 4 weeks of storage) and 2 levels of temperature storage condition factors (room temperature and refrigerator temperature). The room temperature was on average 21.3°C and 8°C in the refrigerator. Eggs of native Banat Naked Neck hens in the second production cycle were analyzed. The Banat Naked Neck hens were reared in a floor system divided into boxes, with a space of 3 hens per m². The diet was a complete mixture with 16.25% CP, 11.7 MJ/kg ME, 4.1% Ca, 0.33% P. The duration of daylight was 15 hours.

A total of 200 eggs were analysed and collected in two weeks. Eggs were collected in the morning and immediately the initial quality was examined on a sample of 20 eggs. From the remaining eggs, groups of 20 eggs were formed and stored for one, two, three and four weeks at room temperature, and in the refrigerator. Egg quality analysis was performed based on following parameters: egg weight, egg shell weight (without drying), albumen height (tripod micrometer), yolk weight, yolk colour (Roche Color Fan) and albumen pH (pH meter Consort C830). Haugh units (HU) were calculated based on egg weight and albumen height (Haugh, 1937). The weight of the albumen was calculated based on the difference between the weight of the egg, the weight of the shell and the weight of the yolk. For each structural part of the egg, the share in the weight of the egg was calculated. By measuring the weight on a sample of 20 eggs stored at room temperature, and in the refrigerator, the weight loss of the egg was determined at weekly intervals.

The test results were statistically analysed by two-way ANOVA analysis of variance in the Statistica software package (StatSoft Inc., 2012). Significance of differences was assessed at the probability level p≤0.05 by LSD post hoc test.

Results and Discussion

The effects of storage temperature and time on egg weight, egg weight loss and shell weight are shown in Table 1. The results show that longer storage time at room temperature affects (p<0.05) the reduction in egg weight. A significant difference in the weight of stored eggs in relation to fresh eggs was determined after 14 days of storage. The difference was maintained at the same level of significance in relation to the fresh eggs until the end of the examined storage period (28 days). The weight of eggs stored in the refrigerator was not affected (p>0.05) by storage time.
A more accurate indicator of the effect of storage time and temperature on egg weight is the loss of egg weight during storage. Egg weight loss was significantly influenced by storage temperature (p<0.001), storage time (p <0.001), as well as the interaction of temperature and storage time (p<0.001). With a longer storage time, the weight loss of the egg was higher at both storage temperatures. Storage temperature determined the level of egg weight loss during storage (graph. 1). After 4 weeks of storage at room temperature, eggs lost 5.68% of their weight, or an average of 3.45 g, and eggs stored in the refrigerator lost 2.66% of their weight, or an average of 1.59 g.

Similar to the egg weight, the egg shell weight stored at room temperature was also influenced (p<0.001) by the time of storage. Significant differences were found after 14 and 28 days of storage at room temperature compared to fresh eggs. The absence of difference after 21 days of storage is probably a consequence of the more difficult separation of albumen from the shell during the measurement of the shell, which was performed without drying. Observed as a percentage, as a share in the egg weight, the weight of the shell did not show the significance of differences in relation to the time of storage. The weight and proportion of the shell in eggs stored in the refrigerator were not affected (p> 0.05) by storage time. Chung and Lee (2014) find no significant changes in shell weight under the influence of storage temperature and time, while Kopacz and Drazbo (2018) find a significantly higher share of shell in eggs stored at room temperature compared to fresh eggs without significant changes in other egg shell quality properties.

The egg begins the aging process from the moment it is laid. The basis of this process is the evaporation of water through the pores on the shell, which results in a reduction in the weight of the egg. The results of our study show that by storing eggs in the refrigerator, it is possible to maintain the weight of the eggs without a significant difference during 4 weeks of storage. Significantly lower egg weight compared to fresh eggs was recorded after 14 days of storage at room temperature (21°C). Jin et al. (2011) report a significant reduction in egg weight after 10 days of storage at 29°C but not at 5°C or 21°C. A better indicator of the changes in the egg that occur during storage, regardless of temperature, is the loss of egg weight. According to Grashorn (2016), higher egg weight loss indicates faster changes in the internal quality of eggs. Storage at lower temperatures reduces egg weight loss (Chung and Lee, 2014; Feddern et al., 2017; Kopacz and Drazbo, 2018), which is confirmed by our results. According to Lewko and Gornowicz (2011), water loss is faster in smaller eggs due to the unfavourable surface-to-egg ratio, which explains the higher weight loss of native hen eggs in our study compared to the results of other studies conducted on hybrid hen eggs (Jin et al., 2011; Perić et al., 2017; Đukić Stojčić and Perić, 2018).
Table 1. Effects of storage temperature and time on egg weight and egg shell weight

| Storage temperature | Storage time, day | Egg Weight, g | Loss, % | Egg shell Weight, g | % |
|---------------------|------------------|---------------|---------|---------------------|---|
| Room temperature    | 0                | 61.79<sup>ab</sup> | -       | 8.01<sup>a</sup>   | 12.98 |
|                     | 7                | 62.24<sup>a</sup> | 1.56<sup>d</sup> | 7.67<sup>a</sup>   | 12.31 |
|                     | 14               | 58.16<sup>c</sup> | 2.91<sup>c</sup> | 6.88<sup>b</sup>   | 11.83 |
|                     | 21               | 58.74<sup>a</sup> | 4.15<sup>b</sup> | 7.40<sup>ab</sup>  | 12.60 |
|                     | 28               | 57.26<sup>c</sup> | 5.68<sup>a</sup> | 6.91<sup>b</sup>   | 12.06 |
| SEM                 | 0.57             | 0.22          |         | 0.12               | 0.15 |
| p                   | 0.012            | <0.001        |         | 0.004              | 0.105 |

Refrigeration

| 0 | 62.06 | - | 8.20 | 13.20 |
| 7 | 62.80 | 0.72<sup>d</sup> | 7.93 | 12.65 |
| 14 | 60.67 | 1.20<sup>b</sup> | 7.39 | 12.19 |
| 21 | 60.16 | 1.65<sup>b</sup> | 7.43 | 12.38 |
| 28 | 59.10 | 2.66<sup>a</sup> | 7.54 | 12.77 |
| SEM | 0.53 | 0.11 | 0.11 | 0.16 |
| p | 0.181 | <0.001 | 0.1 | 0.336 |

Source of variation

| Storage temperature | Storage time | p-value |
|---------------------|--------------|---------|
| 0.08                | <0.001       | 0.037   | 0.192 |
| 0.001               | <0.001       | <0.001  | 0.041 |
| 0.877               | <0.001       | 0.740   | 0.743 |

<sup>a-c</sup> Different letters indicate significant differences among the means in each column with the same storage temperature

Graph. 1. Egg weight loss(g) during the storage period in different temperature conditions

Changes in albumen weight, egg yolk weight and egg yolk colour under the influence of storage time and temperature are shown in Table 2. Storage temperature did not have a significant effect on the weight and proportion of albumen and yolk in the egg. Changes in these structural parts of the egg occurred
under the influence of storage time (p<0.05). In eggs stored at room temperature, the percentage of albumen was at the limit of significance (p = 0.054) of the storage time effect. The percentage of albumen in eggs stored in the refrigerator decreased significantly (p<0.05) only after 4 weeks of storage compared to fresh eggs.

In both storage temperatures, an increase in the percentage of yolks was recorded with a longer period of egg storage. In eggs at room temperature, a significantly higher percentage of egg yolk was found after 14 days of storage, and in eggs stored in the refrigerator, after 28 days.

The yolk colour was not significantly influenced by temperature and storage time, but the significance of the interaction effect of the examined factors was established. Colour changes in egg yolks Santos et al. (2019) explain as the degradation of carotenoids by oxidative processes that occur as a consequence of water diffusion from albumen into yolks under conditions of longer storage periods and higher storage temperatures.

Table 2. Effects of storage temperature and time on the albumen weight, yolk weight and yolk colour

| Storage temperature | Storage time, day | Albumen Weight, g | % | Yolk Weight, g | % | Yolk color, Roche |
|---------------------|-------------------|-------------------|---|---------------|---|------------------|
| Room temperature    | 0                 | 34.15<sup>a</sup> | 55.09 | 19.63 | 32.94<sup>c</sup> | 13.33 |
|                     | 7                 | 33.94<sup>ab</sup> | 54.37 | 20.63 | 33.32<sup>abc</sup> | 13.07 |
|                     | 14                | 30.89<sup>c</sup> | 53.03 | 20.39 | 35.13<sup>abc</sup> | 13.07 |
|                     | 21                | 31.22<sup>bc</sup> | 53.03 | 20.12 | 34.36<sup>ab</sup> | 12.93 |
|                     | 28                | 29.96<sup>c</sup> | 52.23 | 20.39 | 35.71<sup>a</sup> | 13.67 |
| SEM                 |                   | 0.47              | 0.34 | 0.17 | 0.36 | 0.09 |
| p                   |                   | 0.009             | 0.054 | 0.429 | 0.006 | 0.082 |
| Refrigeration       | 0                 | 34.10             | 54.67<sup>a</sup> | 19.76<sup>c</sup> | 32.13<sup>b</sup> | 13.20 |
|                     | 7                 | 33.90             | 53.89<sup>a</sup> | 20.97<sup>ab</sup> | 33.45<sup>b</sup> | 13.80 |
|                     | 14                | 33.45             | 55.03<sup>a</sup> | 19.84<sup>bc</sup> | 32.77<sup>b</sup> | 13.13 |
|                     | 21                | 32.17             | 53.43<sup>ab</sup> | 20.56<sup>abc</sup> | 34.20<sup>ab</sup> | 13.20 |
|                     | 28                | 30.37             | 51.22<sup>b</sup> | 21.19<sup>a</sup> | 36.01<sup>a</sup> | 13.07 |
| SEM                 |                   | 0.48              | 0.42 | 0.19 | 0.38 | 0.10 |
| p                   |                   | 0.078             | 0.031 | 0.05 | 0.01 | 0.117 |

Source of variation

| Source of variation | p-value |
|---------------------|---------|
| Storage temperature | 0.236   |
| Storage time        | 0.001   |
| Time x temperature  | 0.69    |

<sup>a-c</sup> Different letters indicate significant differences among the means in each column with the same storage temperature
During storage of eggs, their structural components, albumen and yolks, change their percentage (Table 2). The basis of the process of egg freshness loss is the diffusion of water and gases that occurs between the egg and the environment but also inside the egg (Lewko and Gornowicz, 2011). Chung and Lee (2014) find a significant decrease in albumen weight under the influence of egg storage and storage time. Egg yolk weight increased with longer storage time. Similar results are reported by Jin et al. (2011). Khan et al. (2013) report a significant increase in yolk weight during storage, while a significant effect of storage time on albumen weight is absent, in accordance with our results for eggs stored at room temperature. In another study, Khan et al. (2014) find that prolonged storage time leads to a decrease in albumen weight and egg yolk weight. The absence of changes in the weight of albumen and yolks during storage of eggs at temperatures of 4°C and 20°C is determined by Akyiurek and Okur (2009). A slower albumen weight decrease is reported by Feddern et al. (2017) for eggs stored in the refrigerator. In our study, we found that storing at a lower temperature slowed down the change in the percentage of yolks. The inconsistency of the stated research results is probably a consequence of the inconsistency of the examined factors in different studies, as well as the initial quality of the eggs that were stored.

Table 3. Effects of storage temperature and time on albumen quality, Haugh Units and albumen pH

| Storage temperature | Storage time, day | Albumen height, 0.1 mm | Haugh Units | Albumen pH |
|---------------------|------------------|------------------------|-------------|------------|
| Room temperature    |                  |                        |             |
| 0                   | 61.60<sup>a</sup> | 75.93<sup>a</sup>      | 8.46<sup>c</sup> |
| 7                   | 45.80<sup>b</sup> | 61.87<sup>b</sup>      | 9.27<sup>b</sup> |
| 14                  | 40.80<sup>c</sup> | 59.07<sup>a</sup>      | 9.36<sup>a</sup> |
| 21                  | 33.07<sup>e</sup> | 48.93<sup>c</sup>      | 9.34<sup>a</sup> |
| 28                  | 24.60<sup>d</sup> | 38.00<sup>d</sup>      | 9.31<sup>ab</sup> |
| SEM                 | 1.79             | 1.82                   | 0.04        |
| p                   | <0.001           | <0.001                 | <0.001      |
| Refrigeration       |                  |                        |             |
| 0                   | 61.33<sup>a</sup> | 75.47                  | 8.48<sup>c</sup> |
| 7                   | 56.93<sup>a</sup> | 71.93                  | 8.85<sup>b</sup> |
| 14                  | 55.67<sup>ab</sup>| 71.53                  | 9.00<sup>a</sup> |
| 21                  | 54.13<sup>ab</sup>| 69.67                  | 9.06<sup>a</sup> |
| 28                  | 47.20<sup>b</sup>| 64.47                  | 9.07<sup>a</sup> |
| SEM                 | 1.45             | 1.24                   | 0.03        |
| p                   | 0.035            | 0.073                  | <0.001      |

Source of variation

| Storage temperature | p-value          |
|---------------------|------------------|
| Storage time         | <0.0001          |
| Time x temperature   | <0.0001          |

<sup>a-d</sup> Different letters indicate significant differences among the means in each column with the same storage temperature
The impact of storage temperature and time on albumen height, Haugh Units and albumen pH is shown in Table 3. The presented results confirm a highly significant (p<0.0001) influence of storage temperature, storage time and their interaction on internal egg quality. Height of albumen stored at room temperature was significantly reduced after only 7 days of storage and after that the trend continued to a value of only 24.6 (0.1mm). In concordance with these results are the values of Haugh Units, which decreased from the initial 75.93 to 38. Storage of eggs in the refrigerator contributed to a moderate (slower) decrease in albumen height during storage. In relation to the initial value of fresh eggs, a significant difference was found after 28 days of storage. In regard to the Haugh Units, there were no significant differences in refrigerated eggs during the four-week storage period. The pH value of the albumen increased significantly with the storage time of the eggs, both at room temperature and in the refrigerator.

The reduction in albumen height during storage is an expected consequence of the egg aging process during which CO₂ is released from the albumen. thinning of the thick albumen leads to a decrease in the number of Haugh Units. Under refrigerated storage conditions, these processes are less intense than at room temperature. Preservation of internal quality, based on Haugh Units, was better with eggs from the refrigerator thanks to a more moderate reduction in albumen height during storage. In relation to the initial value of fresh eggs, a significant difference was found after 28 days of storage. In regard to the Haugh Units, there were no significant differences in refrigerated eggs during the four-week storage period. The pH value of the albumen increased significantly with the storage time of the eggs, both at room temperature and in the refrigerator.

Since Haugh Units are considered a biased quality parameter (Silversides and Scott, 2001) according to Jin et al. (2011), the pH of the albumen objectively shows the freshness of the egg. A rapid increase in pH was recorded after only two days of storage, regardless of temperature conditions, however, the largest increase occurred during the first 5 days of storage. In our study, the increase in albumen alkalinity was intense during the first 14 days at both storage temperatures, subsequently the processes were more moderate and the pH maintained at values that did not differ statistically from the 14th to the 28th day of storage. Similar tendencies are reported by Feddern et al. (2017) but only for refrigerated eggs. Lower storage temperatures contributed to a slower rate of increase of albumen pH value.

**Conclusion**

The eggs of the native Banat Naked Neck hen, in the second year of production, were of satisfactory initial quality, which significantly decreased under the influence of storage time. Storage temperature affected egg weight loss (<0.001), shell weight (<0.05), albumen height (<0.0001), Haugh Units (<0.0001) and albumen pH (<0.0001). When stored in the refrigerator, changes in internal quality were significantly slowed down. After 28 days of storage in the refrigerator,
the values of albumen height and Haugh Units were higher than the same parameters in eggs stored for only 7 days at room temperature. The obtained results indicate the speed of changes in the internal quality of Banat Naked Neck eggs at room temperature, which indicate the necessity of storing these eggs in the refrigerator, regardless of the duration of storage.

Kvalitet jaja Banatske gološijanke tokom skladištenja

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Rezime

U manje intenzivnim sistemima proizvodnje nativne rase živine se mogu koristiti u cilju diverzifikacije proizvoda i samooodrživosti ovih rasa kroz proizvodnju. S obzirom na nedostajuće podatke o održivosti kvaliteta jaja autohtonih, nativnih kokoši tokom skladištenja, cilj istraživanja je bio da se tokom četvoronedelnog perioda u različitim temperaturnim uslovima skladištenja utvrde najvažniji parametri kvaliteta jaja autohtone rase kokoši banatska Naked Neck. Dizajn ogleda je bio dvofaktorijalni sa 4 nivoa faktora dužina skladištenja (sveža jaja - 0, 1, 2, 3 i 4 nedelje skladištenja) i 2 nivoa faktora temperaturni uslovi skladištenja (sobna temperatura i frižider). Sobna temperatura je bila prosečno 21.3°C i u frižideru 8°C. Analiza kvaliteta je izvršena na ukupno 200 jaja, obuhvatajući parametre: težina jajeta, gubitak težine jajeta, težina i udeo strukturnih komponenti: ljuska, žumance i belance, visina belanca, boja žumanca, Haugh Units i pH belanca. Dužina skladištenja je imala signifikantan efekat na sve osobine kvaliteta jaja, osim na boju žumanca koja je bila pod interakcijskim uticajem vremena i temperature skladištenja. Temperatura skladištenja je uticala na gubitak mase jajeta, težinu ljuske, visinu belanca, Haugh Units i pH belanca. Skladištenjem u frižideru, promene u unutrašnjem kvalitetu se značajno usporavaju. Nakon 28 dana skladištenja u frižideru vrednosti visine belanca, Haugh Units i pH belanca su bile veće, odnosno bolje u odnosu na iste parametre jaja skladištenih samo 7 dana na sobnoj temperaturi.

Ključne reči: kvalitet jaja, Banatska gološijanka, dužina skladištenja, temperatura skladištenja
Acknowledgments

The results of the research presented in this paper were financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia, on the basis of the Agreement on the realization and financing of scientific research work of SRO in 2021 no. 451-03-9/2021-14/200022

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Received 2 June 2021; accepted for publication 19 June 2021