

# THE IMPACT OF STORAGE TEMPERATURE AND DURATION ON THE MICROBIOLOGICAL SAFETY, QUALITY, AND SHELF-LIFE OF BANAT NAKED NECK HEN EGGS BEFORE EXPIRATION

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**Abstract:** Temperature is a major determinant of egg quality loss, but data for Banat Naked Neck hens are limited. This study evaluated 240 freshly laid eggs stored for 28 days at room temperature (RT, 20–22°C) or under refrigeration (RF, 4–6°C). Egg mass, shell deformation and breaking strength, shell weight and thickness, albumen height, Haugh units (HU), yolk colour, and albumen and yolk pH were measured on days 1, 7, 14, 21, and 28; preliminary screening for determine the total bacterial count, *Salmonella* spp. and *Listeria monocytogenes* was also performed. The egg mass remained stable. Albumen height significantly dropped by 35.18% under RF and by 57.58% at RT when compared to the first and last day of the four-week storage period, while HU declined from 80 to 62 under RF and from 81 to 46 at RT ( $p < 0.05$ ). Shell breaking, shell thickness and yolk colour showed no significant variation. Albumen pH increased to 9.14 under RF and 9.37 at RT ( $p < 0.05$ ), and yolk pH increased more slowly yet followed the same temperature-dependent trend ( $p < 0.05$ ). No target pathogens have been detected in either treatment to date. A gradual increase in total bacterial count was observed during the storage period, with a significant difference ( $p < 0.05$ ) observed on day 28. Our results show the need for the perseverance of the intact cold chain and proper storage within the recommended shelf life to preserve the optimal quality and safety of eggs.

**Key words:** microbiological safety, egg quality, storage temperature, sustainability, Banat Naked Neck hens

## Introduction

Global egg production and consumption have been steadily increasing. According to a report by The Business Research Company (2024), the global egg market grew in 2024 compared to the previous year, and it is projected to continue growing until 2028. These data show a steady increase in egg production and consumption, suggesting that comparable trends may exist in other regions, including Serbia. According to the Statistical Office of the Republic of Serbia, the turnover of agricultural products at markets in Serbia increased by 4.5% in the first quarter of 2025 compared to the same period last year. Poultry and eggs accounted for 16.7% of the total turnover, making them one of the most significant categories alongside vegetables, milk, and fruit (<https://www.stat.gov.rs>)

Eggs are a valuable source of animal-derived nutrients, and with rising global demand, increased sales of this product are also expected. Their quality largely depends on the basic features—the shell, yolk, and albumen—which possess distinct nutritional and functional properties (Razi et al., 2023). Egg weight, shell strength, albumen height, yolk colour, HU, and moisture content serve as standard measures for assessing egg freshness and quality (Wengerska et al., 2023, Rho and Cho, 2024). Storage conditions greatly affect these characteristics, especially temperature and storage period. Higher temperatures and prolonged storage negatively impact quality, which is reflected in lowered albumen height and lower Haugh unit values (Kim et al., 2024). Nowaczewski et al. (2022) have reported similar results, emphasizing that extended storage leads to increased weight loss, elevated pH values of the yolk and albumen, enlargement of the air cell, and decreased albumen clarity. Obianwuna et al. (2022) also confirm these findings, highlighting that a rise in albumen pH leads to structural breakdown and reduced viscosity.

Most studies on egg quality during storage focus on eggs from hybrid laying hens, which dominate commercial production. With the development of alternative egg production systems, the question arises of how to preserve the quality of eggs from native breeds, such as the Banat Naked Neck (Škrbić et al., 2021). Previous studies have shown that this breed has potential for use in extensive and semi-extensive production systems, which can help preserve genetic diversity (Škrbić et al., 2020). For this reason, there is an increasing need to study the egg quality of such breeds in more detail, especially during storage.

While extended storage affects the physicochemical characteristics of eggs, it can also increase the risk of microbiological contamination. Keeping eggs microbiologically safe is essential for consumer health because eggs, though nutritious, become high-risk foods if they are handled or stored poorly. Harmful bacteria such as *Salmonella*, *Listeria*, and *Escherichia coli* can contaminate eggs during production, handling, transport, or storage, and thus pose a serious

public-health problem (Chousalkar et al., 2021). Contaminated eggs lose quality—odor, taste, and texture changes render them unfit for consumption, and could pose particular danger (Holt et al., 2011; Hayder and Amer, 2024).

The way eggs are handled, especially washing, further impacts microbiological safety. Although egg washing is practiced in some countries for hygienic reasons, studies have shown that it can damage the shell cuticle and thus increase the risk of contamination (Wellman-Labadie et al., 2008; Gole et al., 2014). For this reason, the European Union prohibits the washing of Class A eggs (European Commission, 2008). This regulation is based on research indicating that washing may increase spoilage and reduce microbiological stability during storage (Hutchinson et al., 2004).

Various scientific findings suggest that cleaning practices and handling methods have a significant impact on egg safety, which becomes especially important in the context of intensive global trade and increasing consumption (Zhi et al., 2016, Jones et al., 2018, Mahmoud et al., 2022). Contaminated eggs in supply chains can contribute to the spread of infections among consumers. Contamination risk varies with the product's origin, the environment in which it is produced, and how it is handled, shipped, and stored (Yamak et al., 2021). Eggs from farms, particularly in high-density and poorly ventilated environments, are at greater risk of bacterial contamination (Solis et al., 2023). Eggs stored at lower temperatures tend to have a lower contamination rate than those kept at higher temperatures (Chousalkar et al., 2021).

The performance of the safety analysis of eggs enables the detection of the critical control points in the production and distribution chain. It serves as the basis for defining measures that contribute to reducing consumer health risks (Crivei et al., 2025). Since various egg origin and their production and storage conditions can significantly affect their microbiological profile and quality, monitoring the microbiological safety of eggs from diverse sources is vital for ensuring food safety and protecting consumer health (Holt et al., 2011).

Given the limited research on preserving egg quality in autochthonous Serbian hen breeds during extended storage, this study aimed to assess how different storage conditions—simulating common household environments—affect key egg quality parameters. Similarly to other research (Gogo et al., 2021; Kim et al., 2024), selected temperatures reflect typical household refrigerator and room temperatures, which is especially relevant for consumers in moderate climates like Serbia. Particular attention was paid to microbiological safety, physicochemical properties, and indicators of freshness. These research findings were intended to contribute to a better understanding of the factors that could affect the egg quality of Banat Naked Neck hens and to advance the necessary steps that need to be taken in order to further preserve their quality over time.

## Materials and Methods

The study was conducted using eggs obtained from 63-week-old Banat Naked Neck laying hens reared in a floor system. A total of 240 eggs were collected, of which 120 were allocated for quality assessment and 120 for microbiological analysis. The eggs were stored under two temperature conditions: room temperature (20–22°C) and refrigeration (4–6°C), with 60 eggs assigned to each treatment group. The refrigerated range of 4–6°C was selected to reflect typical household refrigerator temperatures. Although the Egg Quality Regulation (2019) recommends storing eggs above +5°C in commercial settings, this study focused on home storage practices, where temperatures may occasionally fall slightly below this threshold. These storage conditions were used to evaluate the impact of temperature on egg quality and microbiological safety over time. The investigation was carried out over a four-week period, with analyses performed on day 1 (fresh eggs) and at weekly intervals thereafter (days 7, 14, 21, and 28). At each time point, 12 eggs from each treatment group (i.e., 24 eggs in total per time point) were evaluated for external and internal egg quality characteristics. The same sampling schedule was used for microbiological analysis, with 12 eggs per treatment group analysed at each time point (a total of 24 eggs per time point).

### Egg quality

Egg quality was evaluated immediately after collection, providing information on the initial quality of table eggs. External and internal quality traits parameters were analysed for each egg. Egg weight was determined using an electronic balance with an accuracy of  $\pm 0.02$  g. Egg shell quality testing included measurements of deformation, breaking strength, weight, and thickness of the shell. Shell weight (with membranes) was determined using an electronic balance with precision  $\pm 0.02$  g. Shell thickness was measured at the equator using a micrometric screw (SOMET), following membrane removal. Shell deformation was evaluated using a Marius device by applying a 500 g load to the equatorial area of the egg; results were expressed in micrometres ( $\mu\text{m}$ ), as the mean of three measurements. Shell breaking strength was determined using a 15 kg spring at a loading speed of 70 mm/min. The obtained value was multiplied by the corresponding coefficient ( $k = 0.150$ ) to express the breaking strength in kilograms. The height of the thick albumen was measured using a tripod micrometre (AMES company) at the midpoint between the yolk edge and the thick albumen, with a measurement precision of 0.1 mm. Yolk colour was assessed by visual comparison with the "Roche Yolk Colour Fan 1969", with intensity scored from 1 to 15. Egg freshness was objectively assessed by calculating HU, which combine egg weight and albumen height into a single quality index. HU were calculated employing the subsequent formula (Obianwuna et al., 2022):

$$HU = 100 \log_{10}(H - 1.7W^{0.37} + 7.6)$$

where, H represents the height of the thick albumen (in mm), and W is the egg weight (in grams).

The pH value of the albumen and yolk was measured using a pH meter (PH meter Consort C830).

### Microbiological quality of eggs

Microbiological analyses were performed under aseptic conditions to minimize external contamination. For microbiological analysis, egg contents were examined. To avoid shell surface contamination, each eggshell was disinfected with 70% ethanol according to SRPS EN ISO 6887-4:2017, dried under sterile conditions, and then aseptically cracked with sterile instruments. The contents were homogenized in a 1:10 ratio with buffered peptone water prior to analysis. Total Bacterial Count (TBC) was assessed by pour plate method on Plate Count Agar and incubated at 30°C for 72 ± 3 h according to SRPS EN ISO 4833-2:2017. Upon completion of incubation, colonies were counted and expressed as CFU/mL. A representative sample was considered valid if it contained 15 to fewer than 300 colonies. The CFU/mL was calculated according to the relevant ISO standard.

Detection of *Listeria monocytogenes* was carried out according to SRPS EN ISO 11290-1:2017, which includes primary enrichment in half Fraser (LABM, England) broth (24 h at 30°C), followed by secondary enrichment in Fraser broth (24 h at 37°C), and plating onto two selective agars - ALOA (Agar *Listeria* according to Ottaviani and Agosti) (LABM, England) and Oxford (Biolife Italiana, Italy) agar.

To detect the presence of *Salmonella* spp., the method specified in SRPS EN ISO 6579-1:2017 was employed. Samples were first pre-enriched in non-selective medium - buffered peptone water (BPW, Biolife Italiana, Italy) for 18h at 37 °C, followed by selective enrichment in Rappaport-Vassiliadis Soya (RVS, LABM, England) broth (24 h at 41.5°C) and Müller-Kauffmann tetrathionate novobiocin (MKTTn, Biolife Italiana, Italy) broth (24 h at 37°C), and subsequently plated onto Xylose Lysine Deoxycholate (XLD, LABM, England) and Hektoen Enteric (HE, Biolife Italiana, Italy) agar at 37°C for 24 h.

### Statistical analysis

The software package STATISTICA, version 12 (StatSoft Inc.), was used for statistical analysis. Two-way ANOVA analysed the results, and the significance of differences between groups was assessed at the probability level of  $p < 0.05$  using the Tukey post hoc test.

## Results and Discussion

The external egg quality parameters, as influenced by storage temperature and duration, are summarized in Table 1. The table is structured to present results at three levels: (1) average values by temperature (RF and RT) to illustrate the main effect of storage temperature; (2) average values by storage day (1, 7, 14, etc.), regardless of temperature, to show the effect of storage duration; and (3) individual values for each treatment group and time point (e.g., RF-day 1, RT-day 1, etc.), which represent the interaction between storage temperature and duration.

The examined external quality parameters did not significantly change under the influence of temperature, storage duration, or their interaction. The egg mass did not show a significant change throughout the experiment, and a gradual decline was observed in eggs stored at both temperatures until day 28, which can be attributed to water loss through the shell pores.

**Table 1.** Changes in external egg quality parameters as affected by storage temperature, storage duration, and their interaction

ST (°C)	SD (day)	Egg weight (g)	Shell deformation ( $\mu$ m)	Breaking strength (kg)	Shell weight (g)	Shell thickness (0.01 mm)
		$\bar{x}\pm Sd$	$\bar{x}\pm Sd$	$\bar{x}\pm Sd$	$\bar{x}\pm Sd$	$\bar{x}\pm Sd$
RF		55.70 $\pm$ 4.68	25.80 $\pm$ 3.44	3.85 $\pm$ 0.41	7.18 $\pm$ 0.65	30.50 $\pm$ 1.50
RT		55.67 $\pm$ 3.73	25.77 $\pm$ 2.84	3.78 $\pm$ 0.41	7.07 $\pm$ 0.73	29.60 $\pm$ 2.30
	1	56.39 $\pm$ 3.07	24.33 $\pm$ 2.46	3.94 $\pm$ 0.39	7.29 $\pm$ 0.45	30.67 $\pm$ 1.23
	7	56.28 $\pm$ 5.53	27.42 $\pm$ 2.50	3.77 $\pm$ 0.47	7.10 $\pm$ 0.62	29.67 $\pm$ 2.31
	14	55.77 $\pm$ 4.99	25.00 $\pm$ 4.49	3.90 $\pm$ 0.38	7.11 $\pm$ 1.13	30.00 $\pm$ 2.70
	21	55.46 $\pm$ 3.93	25.50 $\pm$ 2.81	3.77 $\pm$ 0.36	7.18 $\pm$ 0.53	30.08 $\pm$ 1.98
	28	54.53 $\pm$ 3.38	26.67 $\pm$ 2.27	3.69 $\pm$ 0.47	6.93 $\pm$ 0.56	29.83 $\pm$ 1.53
	1	56.38 $\pm$ 3.11	23.83 $\pm$ 1.33	3.92 $\pm$ 0.38	7.37 $\pm$ 0.19	30.17 $\pm$ 1.17
	7	56.00 $\pm$ 7.40	27.50 $\pm$ 2.17	3.71 $\pm$ 0.56	7.17 $\pm$ 0.73	29.83 $\pm$ 1.33
RF	14	55.68 $\pm$ 6.87	26.33 $\pm$ 5.85	3.75 $\pm$ 0.42	7.13 $\pm$ 1.12	30.00 $\pm$ 2.37
	21	55.63 $\pm$ 3.06	24.67 $\pm$ 3.27	3.92 $\pm$ 0.44	7.32 $\pm$ 0.56	31.67 $\pm$ 0.52
	28	54.78 $\pm$ 2.02	26.67 $\pm$ 2.50	3.96 $\pm$ 0.33	6.90 $\pm$ 0.40	30.83 $\pm$ 1.17
	1	56.40 $\pm$ 3.32	24.83 $\pm$ 3.31	3.96 $\pm$ 0.43	7.22 $\pm$ 0.64	31.17 $\pm$ 1.17
	7	56.57 $\pm$ 3.50	27.33 $\pm$ 3.01	3.83 $\pm$ 0.41	7.03 $\pm$ 0.55	29.50 $\pm$ 3.15
RT	14	55.85 $\pm$ 2.76	23.67 $\pm$ 2.42	4.04 $\pm$ 0.29	7.08 $\pm$ 1.24	30.00 $\pm$ 3.22
	21	55.28 $\pm$ 4.95	26.33 $\pm$ 2.25	3.63 $\pm$ 0.21	7.03 $\pm$ 0.52	28.50 $\pm$ 1.52
	28	54.27 $\pm$ 4.58	26.67 $\pm$ 2.25	3.42 $\pm$ 0.44	6.97 $\pm$ 0.73	28.83 $\pm$ 1.17
p value						
ST		0.984	0.967	0.474	0.563	0.071
SD		0.852	0.109	0.543	0.821	0.746
ST x SD		0.998	0.487	0.092	0.984	0.069

ST - Storage temperature; SD - Storage duration; RF - refrigeration temperature (4–6°C); RT - room temperature (20–22°C);  $\bar{x}$  - Average mean; Sd - Standard deviation; p>0.05 not significant

These results are consistent with the findings of Akter et al. (2014), Kumari et al. (2020), and Škrbić et al. (2021), who indicate that water evaporation increases with prolonged storage, especially at higher temperatures, leading to a reduction in egg mass.

Shell deformation changed over the storage period, but these changes seemed to leave overall egg quality unchanged. On the other hand, the shell breaking strength recorded a decrease over time, especially in eggs stored at room temperature, indicating reduced elasticity and shell strength due to moisture loss. Hagan and Eichie (2019) reported comparable results, observing that extended storage at higher temperatures weakens the shell's mechanical properties. The results of our research show that the eggshell mass did not significantly differ depending on the temperature. In contrast to our study, some authors report that storage duration at room temperature significantly affects the reduction of eggshell weight (Škrbić et al., 2021). Kanasuah et al. (2025) suggest that storage at room temperature did not significantly affect the external qualities of eggs from Naked Neck hens. The use of appropriate storage methods, such as oil coatings on the eggshell, can dramatically reduce weight loss and preserve shell thickness, thereby better-maintaining egg quality.

The internal quality of the eggs was assessed by measuring albumen height, HU, yolk color, and the pH of both albumen and yolk (Table 2). The structure of Table 2 follows the same format as Table 1, presenting results according to the effects of storage temperature, storage duration, and their interaction. The height of the thick albumen significantly decreased ( $p < 0.05$ ) during storage in both groups of eggs, with the decline being more pronounced and statistically significant ( $p < 0.05$ ) at room temperature, indicating protein structure degradation. A similar trend was observed for HU, which followed the decline in albumen height. A statistically significant difference ( $p < 0.05$ ) in HU values was found under the influence of both factors. HU values decreased during storage, with a more pronounced decline at room temperature. The results of Kanasuah et al. (2025) are consistent with our findings regarding the internal egg quality of the Naked Neck hens. Kaya et al. (2024) showed that higher storage temperatures cut both HU and albumen height, with the steepest quality loss at 30 °C. Kim et al. (2024) confirmed this trend, noting similar drops in the same measures. The study by An et al. (2023) shows that subsequent storage at low temperatures can mitigate the unfavorable effects of prior heat exposure, partially preserving HU. As a reliable indicator of albumen quality, HU tend to decrease during storage, which is supported by the results of Obianwuna et al. (2022). Lordelo et al. (2020) state that eggs from indigenous breeds can match the quality of commercial eggs and that breeding these birds supports genetic diversity and local farmers, which is crucial for sustainable agriculture and biodiversity. Similarly, Romero et al. (2024) note that indigenous breeds have competitive egg quality compared to commercial eggs.

**Table 2.** Changes in internal egg quality parameters as affected by storage temperature, storage duration, and their interaction

ST (°C)	SD (day)	Albumen height (mm)	Albumen pH	Haugh units	Yolk colour (Roche)	Yolk pH
		$\bar{x}\pm Sd$	$\bar{x}\pm Sd$	$\bar{x}\pm Sd$	$\bar{x}\pm Sd$	$\bar{x}\pm Sd$
RF		5.25 <sup>a</sup> ±1.13	8.97 <sup>b</sup> ±0.27	71.39 <sup>a</sup> ±9.88	13.30±0.47	6.19 <sup>b</sup> ±0.11
RT		4.57 <sup>b</sup> ±1.44	9.24 <sup>a</sup> ±0.29	64.36 <sup>b</sup> ±13.86	13.29±0.39	6.24 <sup>a</sup> ±0.14
	1	6.54 <sup>a</sup> ±0.75	8.63 <sup>c</sup> ±0.35	81.35 <sup>a</sup> ±5.53	13.00±0.60	6.08 <sup>b</sup> ±0.07
	7	5.50 <sup>b</sup> ±0.96	9.08 <sup>ab</sup> ±0.13	73.51 <sup>b</sup> ±8.51	13.08±0.51	6.11 <sup>b</sup> ±0.06
	14	4.76 <sup>bc</sup> ±0.70	9.12 <sup>b</sup> ±0.14	67.64 <sup>ab</sup> ±7.14	13.75±0.71	6.27 <sup>a</sup> ±0.07
	21	4.24 <sup>cd</sup> ±0.80	9.23 <sup>a</sup> ±0.12	62.59 <sup>cd</sup> ±8.68	13.25±0.45	6.28 <sup>a</sup> ±0.09
	28	3.50 <sup>cb</sup> ±0.97	9.25 <sup>a</sup> ±0.14	54.29 <sup>d</sup> ±11.71	13.42±0.51	6.34 <sup>a</sup> ±0.08
	1	6.48±0.84	8.57±0.38	80.88±6.42	13.17±0.41	6.09±0.08
	7	5.72±1.06	9.00±0.11	75.34±8.79	13.33±0.52	6.11±0.09
RF	14	5.05±0.83	9.01±0.07	70.15±8.68	13.31±0.53	6.21±0.05
	21	4.80±0.70	9.12±0.06	68.06±7.50	13.32±0.51	6.24±0.11
	28	4.20±0.87	9.14±0.12	62.51±8.92	13.33±0.52	6.29±0.03
	1	6.60±0.72	8.69±0.35	81.81±5.05	12.83±0.75	6.06±0.06
	7	5.28±0.89	9.15±0.09	72.68±6.60	12.84±0.41	6.11±0.03
RT	14	4.46±0.43	9.23±0.08	65.12±4.65	14.17±0.40	6.32±0.05
	21	3.68±0.42	9.33±0.05	57.13±6.14	13.17±0.41	6.30±0.06
	28	2.80±0.35	9.37±0.04	46.07±7.75	13.50±0.55	6.38±0.11
		p value				
ST		<0.001	<0.001	<0.001	1.000	<0.013
SD		<0.001	<0.001	<0.001	0.282	<0.001
ST x SD		0.124	0.937	0.055	0.408	0.115

ST - Storage temperature; SD - Storage duration; RF - refrigeration temperature (4–6°C); RT - room temperature (20–22°C);  $\bar{x}$  - Average mean; Sd - Standard deviation; p<0.05 significant; p>0.05 not significant; <sup>a, b, c, d</sup> Different letters indicate significant differences among the means in each column with the same storage temperature

The yolk color mainly remained stable during storage, and had a slightly higher value in eggs stored at lower temperatures. Islam et al. (2001) recorded a higher yolk colour value in Naked Neck chickens, which is in agreement with the findings of Kanasuah et al. (2025). Yolk colour is believed to be primarily influenced by diet rather than genetics (Grashorn, 2016), which could explain the inconsistent results reported in the literature. Barbosa et al. (2011) showed that the color intensity, assessed visually, remained stable during 35 days of storage in the refrigerator, while at room temperature, color reduction was observed only after 28 days. However, the same authors report that spectrophotometric analysis revealed a gradual decline in carotenoid concentration in the yolk regardless of storage temperature, indicating that the visual appearance of color may remain unchanged despite chemical changes. The stability of yolk color has also been confirmed by recent studies, with modern methods such as fluorescent spectroscopy showing potential for detecting changes in pigmentation and yolk freshness in a nondestructive manner (Atwa et al., 2024). Gu et al. (2024) highlight that

fluorescent spectroscopy detects chemical changes before visible color changes, allowing for a more precise assessment of egg freshness and quality during storage.

The pH of the albumen showed a highly statistically significant ( $p < 0.05$ ) increase during storage under the influence of both temperatures, resulting from carbon dioxide loss and increased acidity of the albumen. The differences in albumen pH values between day 21 and day 28 were not statistically significant, indicating that the pH remained stable during this period. Additionally, the pH of the yolk showed statistically significant ( $p < 0.05$ ) differences depending on temperature and significant ( $p < 0.05$ ) differences due to storage duration. Over time, the pH of the yolk increased at both temperatures. The differences in yolk pH values between day 1 and day 7 were not statistically significant. However, a statistically significant increase in this parameter was observed with further storage. These results are consistent with the findings of An et al. (2023), who report that the pH of the albumen increased until the sixth day of storage and stabilized until the 15th day, depending on the storage temperature. Jin et al. (2010) also report a sharp increase in the pH of the albumen within 5 days, reaching 9.0, after which the level remained stable during further storage. Kaya et al. (2024) and Kim et al. (2024) confirm that storing eggs at higher temperatures increases the pH values of both albumen and yolk.

Microbiological quality is a key indicator of egg safety during storage and distribution. In the present study, although no pathogenic bacteria such as *Salmonella* spp. or *Listeria monocytogenes* were detected in either storage condition, variations in TBC were observed over the 28-day storage period. Table 3 summarizes the changes in total bacterial count (TBC) in eggs during storage. As with Tables 1 and 2, the results are structured to reflect the individual and combined effects of storage temperature and duration. These variations support the hypothesis that storage temperature plays a key role in bacterial proliferation, even in the absence of detectable pathogens. Our results demonstrated that eggs stored at both temperatures exhibited a gradual increase in total bacterial count, with a significant ( $p < 0.05$ ) difference observed on day 28.

These modest yet microbiologically relevant changes agreed with previous findings, which reported faster microbial proliferation at ambient temperatures and stressed that keeping eggs below 10°C inhibits bacterial growth, metabolism, and reproduction and helps preserve internal quality (Messens et al., 2006). De Reu et al. (2005) similarly found that eggshell bacterial contamination was significantly higher when eggs were kept at room temperature, particularly in systems with more exposure to environmental contaminants. Although our study handled the eggs under sterile conditions, the native shell microbiota present on the shell and in the environment could still have contributed to the overall microbial load, especially at higher temperatures. Similarly, Tan et al. (2023) found that cold chain maintenance in post-washing and storage stages contributes to reduced microbial risks and extended egg shelf life. Even though the absence of *Salmonella* and *Listeria*

monocytogenes is favorable, an increase in total bacterial load at room temperature underlines the need for effective refrigeration practices. Therefore, based on our findings and consistent with literature data, it is evident that refrigerated storage remains a fundamental factor in maintaining the microbiological safety of eggs. These results further support regulatory guidelines promoting the preservation of the cold chain throughout egg handling and distribution.

**Table 3.** Total Bacterial Count (TBC) of eggs depending on storage temperature, storage duration, and their interaction

ST (°C)	SD (day)	Total Bacterial Count (log CFU/mL)
		$\bar{x} \pm Sd$
RF		1.26 ± 0.30
RT		1.30 ± 0.18
	1	1.20 <sup>b</sup> ± 0.13
	7	1.23 <sup>b</sup> ± 0.13
	14	1.25 <sup>b</sup> ± 0.13
	21	1.27 <sup>b</sup> ± 0.14
	28	1.44 <sup>a</sup> ± 0.16
	1	1.20 ± 0.12
	7	1.20 ± 0.11
RF	14	1.24 ± 0.15
	21	1.25 ± 0.12
	28	1.37 ± 0.13
	1	1.21 ± 0.15
	7	1.25 ± 0.16
RT	14	1.26 ± 0.11
	21	1.28 ± 0.17
	28	1.50 ± 0.18
		p value
	ST	0.243
	SD	0.002
	ST x SD	0.801

ST - Storage temperature; SD - Storage duration; RF - refrigeration temperature (4–6°C); RT - room temperature (20–22°C);  $\bar{x}$  - Average mean; Sd - Standard deviation; p<0.05 significant; p>0.05 not significant; <sup>a,b</sup> Different letters indicate significant differences among the means in each column with the same storage temperature

## Conclusion

During the four-week trial, temperature emerged as the dominant driver of quality change in Banat Naked Neck eggs. Storage of eggs at a lower temperature (4–6°C) significantly slowed the decline in internal quality parameters (albumen height and HU), as well as the increase in pH of the albumen and yolk, compared to storage at room temperature (20–22°C). No significant differences were

observed in egg weight, shell quality characteristics, and microbiological safety of the eggs between the two storage conditions. Storage time significantly affected on the reduction of albumen height and HU, the increase in the pH of albumen and yolk, and the increase in the total bacterial count. The obtained results highlight the importance of implementing a cold chain in maintaining the quality and safety of eggs during prolonged storage.

In addition, by using eggs from Banat Naked Neck hens, a native Serbian breed, this research supports the recognition and preservation of local poultry genetic resources. Further studying the quality and storage characteristics of eggs from native breeds provides scientific evidence that can help promote their use in sustainable farming systems and in markets that value biodiversity and the authenticity of traditional products.

## **Uticaj temperature i dužine skladištenja na mikrobiološku ispravnost, kvalitet i održivost jaja kokoši Banatskih gološijana pre isteka roka trajanja**

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### **Rezime**

Temperatura je ključni činilac degradacije kvaliteta jaja, međutim, podaci o jajima kokoški rase Banatski gološijan i dalje su ograničeni. Ovo istraživanje obuhvatilo je 240 svežih jaja skladištenih tokom 28 dana na sobnoj temperaturi (RT, 20–22°C) i na temperaturi frižidera (RF, 4–6°C). Na 1., 7., 14., 21. i 28. dan određivani su: masa jajeta, deformacija i čvrstoća ljuske, masa i debljina ljuske, visina belanca, Haugh-ove jedinice (HU), boja žumanca, pH belanca i žumanca; sproveden je i preliminarni mikrobiološki skrining na ukupan broj bakterija, *Salmonella* spp. i *Listeria monocytogenes*. Masa jaja je ostala stabilna. Visina belanca se značajno smanjila za 35,18 % u RF, odnosno 57,58 % na RT, kada se uporede prvi i poslednji dan četvoronedelnog perioda skladištenja, dok su se HU smanjile sa 80 na 62 u RF, odnosno, sa 81 na 46 na RT ( $p < 0,05$ ). Čvrstoća ljuske, debljina ljuske i boja žumanca ostale su statistički nepromenjene. pH belanca porastao je na 9,14 u RF i 9,37 na RT ( $p < 0,05$ ), a pH žumanca postupno je rastao prateći isti temperaturno zavisani trend ( $p < 0,05$ ). Ciljani patogeni nisu detektovani ni u jednom tretmanu. Tokom perioda skladištenja zabeleženo je postupno povećanje ukupnog broja bakterija, pri čemu je statistički veoma značajna razlika ( $p < 0,05$ ) uočena 28. dana. Rezultati ovog rada pokazuju važnost održavanja intaktnog lanca i pravilnog skladištenja u roku trajanja za očuvanje optimalnog kvaliteta i bezbednosti jaja.

**Ključne reči:** mikrobiološka ispravnost, kvalitet jaja, uslovi skladištenja, održivost, Banatski gološijan

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### Conflict of interest

The authors declare that they have no conflict of interest.

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