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Veterinary and sanitary evaluation of egg containers and egg disinfection

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Abstract Based on the results of the microbiological evaluation of cardboard containers, the level of contamination was determined. With an increase in the period of its use, contamination with several microorganisms is observed. Regardless of the type of eggs, micromycetes (*Aspergillus* spp., *Cladosporium* spp., *Penicillium* spp., *Enterobacter agglomerans*, *Corynebacterium* spp., *Escherichia coli*) were induced from the transport container during its repeated (≥ 10) use at an average level of contamination (< 10^6-10^8 CFU/cm^2). It has been proven that reusable cardboard containers can be an additional source of egg contamination. To determine the microbial contamination, 620 poultry eggs were used in the experiments, including quail (n = 120), turkey (n = 80), duck (n = 95), goose (n = 85), and chicken (n = 240). It has been proven that poultry eggs, regardless of their species, are contaminated (< 10^3 CFU/cm^2) with *Corynebacterium* spp. and *Bacillus* spp. It was also found that waterfowl eggs were contaminated with *Enterobacter* spp. and turkey eggs with *Lactobacillus* spp. The Ufotek 2 unit was used to disinfect the test eggs. It produces both ozone and UV radiation. Increasing exposure to physical disinfectants showed a gradual increase in the bactericidal effect. It was found that the combined effect of ozone (100 mg/h) and UV irradiation (253.7 nm) for five minutes causes disinfection of poultry eggshells, regardless of their species.

Keywords: Eggs, Ozone, UV irradiation, Cardboard containers, Microorganisms

Introduction

Providing high-quality animal products to the population is the main task of professionals in the livestock and processing industries. Meat, milk, and eggs are the most popular food products, and the demand for them by the general population is steadily increasing every year (Nanka *et al*., 2018; Prasad and Kothari, 2021; Aliiev *et al*., 2022). Today, the most dynamically developing livestock sector is poultry farming, which is explained by the annual increase in poultry population, the creation of additional jobs, and the increase in production volume (Vissers *et al*., 2019; Gautron *et al*.,

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2021). Poultry eggs are valuable food products as well as raw materials for the production of various products (Lordelo *et al*., 2017; Kowalska *et al*., 2021).

The main aspect of producing high-quality commercial eggs is observing all necessary technological, veterinary, and sanitary measures (Gogo *et al*., 2021; Orobchenko *et al*., 2022). The content of a freshly laid egg obtained from a healthy bird with a normal physiological state is sterile, i.e. it does not contain microorganisms. The sterility of the egg is explained by the active phagocytic reaction in the oviducts of healthy birds, peristaltic contractions that mechanically remove microbes, and the bactericidal effect of lysozyme (Du *et al*., 2020; van der Klein *et al*., 2020).

Endogenous contamination of the egg contents occurs during its formation in the ovary and oviduct of a sick bird or bacterial carrier in a variety of infectious diseases, including salmonellosis, tuberculosis, ornithosis, Q fever, pasteurellosis, infectious bronchitis, mycoplasmosis, leukemia, and others (Roberts *et al*., 2011; Zhao *et al*., 2017). The presence of the pathogen in eggs obtained from sick poultry has been documented (Hassan and Abdul-Careem, 2020). The prevalence of infected eggs obtained from bacteria-carrying birds ranges from 10% to 95%. The period of increased egg laying, which is associated with the weakening of the bird's body and increased virulence of the pathogen, has been observed to be the time when the highest number of infected eggs is recorded (Betancor *et al*., 2010; Hewson *et al*., 2014).

Foodborne toxic infections in humans are often associated with consuming eggs and egg products contaminated with *Salmonella* (Lutful Kabir, 2010). Endogenous contamination of eggs with viruses is also observed during the immunization of poultry with live virus vaccines used in industrial poultry production (Swayne *et al*., 2012; Bello *et al*., 2018). In addition, endogenous contamination of eggs with microorganisms is possible in the presence of vitamin A deficiency in poultry and ovarian and oviduct diseases of various etiologies. In this case, in addition to the pathogen, eggs often contain *Staphylococcus aureus*, *Pseudomonas aeruginosa*, fluorescent bacteria, bacteria of the *Proteus genus*, bacteria of the *E. coli* group, and other microorganisms (Spitzer, 2016).

Not all egg components are equally resistant to microbes. The dense protein is the most resistant to decomposition and microbial contamination, which is explained by its high amount of lysozyme (Carrillo *et al*., 2014; Shimazaki and Takahashi, 2018). It is higher in chicken egg whites (5.71 mg/mL) and much lower in ducks (1.80 mg/mL) and geese (0.38 mg/mL) (Sun *et al.*, 2017; Quan and Benjakul, 2019).

Exogenous contamination of eggs occurs during collection, storage, and transport as a result of penetration of saprophytic, opportunistic, and pathogenic microorganisms through the pores of the shell and sub-shell membranes (Gast *et al*., 2021). Exogenous contamination of eggs with

microbes is facilitated by contamination of the shell with bird droppings, soil, feathers, litter, transportation in dirty containers, etc. (Moyle *et al*., 2016; Trudeau *et al.,* 2020). Depending on the contamination of the shell, the number of microorganisms on the shell varies greatly (Lee *et al*., 2016). A standard 1.0 cm^2 of the shell surface of clean eggs typically contains tens, hundreds, and, in rare cases, thousands of microbial cells. In contrast, contaminated eggs generally have an estimated number of microbial cells in the tens of thousands or even millions. The degree of eggshell contamination with microorganisms is largely influenced by the conditions of poultry housing and feeding (Holt, 2021).

The most effective method of significantly reducing microbial contamination of eggs is their sanitization with antimicrobial agents. For this purpose, both chemical and physical disinfectants are used (Carrique-Mas *et al*., 2009; Paliy, 2018; Kukhtyn *et al*., 2024). Chemical compounds used include hydrogen peroxide, alcohol, organic acids, essential oils, chlorine agents, and formaldehyde (Chung *et al*., 2018; Tebrün *et al*., 2020; Motola *et al*., 2023; Bordunova *et al*., 2024).

However, previous studies have shown that the current egg disinfection technologies used in the industry cannot eliminate bacteria from the surface of the eggshell, and therefore alternative technologies that may be more effective and environmentally friendly should be investigated (Al-Ajeeli *et al*., 2016; Hudson *et al*., 2016).

The research finding aimed to study the level of contamination of eggs of different poultry species and egg containers and to determine the effectiveness of disinfecting them with ozone and UV radiation.

Materials and methods

Ethics

No laboratory or farm animals were used in the study.

Samples

A total of 620 poultry eggs were examined: quails - 120 eggs, turkeys - 80 eggs, ducks - 95 eggs, geese - 85 eggs, and chickens - 240 eggs.

In the experiments, cardboard containers were used (n = 45), which were used once (transportation of eggs over a distance of up to 500 km) and repeatedly (10 or more times).

Location of the experiment

Bacteriological examination of eggs and egg containers, as well as determination of the effectiveness of ozone and UV disinfection of the

experimental objects, were carried out in the specialized laboratories of the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine" of the National Academy of Agrarian Sciences of Ukraine.

Eggs from poultry were obtained directly from farms of different ownership and productivity levels located in the Kharkiv, Sumy, and Poltava regions of Ukraine. Quail and hen eggs were obtained from cage housing and taken directly from the commercial flocks. Eggs from turkeys, geese, and ducks were obtained from free-ranging poultry.

Equipment and facilities

To disinfect the experimental objects, we used the Ufotek 2 unit, which produces both ozone and UV radiation. Technical characteristics of the Ufotek 2 unit (Ukraine): UV radiation power at a wavelength of 253.7 nm - 30×2/60×2 W; ozone production power - 35 W; air circulation system capacity - 25 m^3/h; UV lamp life - 8000 hours; operating temperature range - 2-50°C; short-term humidity, up to 95% during operation; power supply ~220 V, 50 Hz; power consumption - 150/200 W.

To measure the ozone concentration, we used the NTIKO-1 device. Technical characteristics of the NTIKO-1 meter (Ukraine): overall dimensions of the monoblock - 50×100×180 mm; overall dimensions of the sensor - 30×60×95 mm; form of information provision - liquid crystal display; measurement limits - 0...600 mg/m^3; resolution - 0.8 mg/m^3; error ± 20%; sampling method - diffusion; power supply ~ 9 V; measurement discreteness - 1-60 s; memory - 32000 points; weight - 0.4 kg.

Methods used in the research

Bacteriological studies included determining the presence of bacteria from the *E. coli* group, *Proteus, Salmonella, Staphylococcus aureus*, *Bacillus* spp. and *Lactobacillus* spp. and micromycetes according to generally accepted methods (Pondit *et al*., 2018; El Ftouhy *et al*., 2022).

The main research methods

Total bacterial contamination of the egg surface was determined by inoculating a specific volume of swabs or their 10-fold dilution into Petri dishes with meat peptone agar (MPA). Cultures were incubated in a thermostat at 30.0 ± 0.5°C for 72 hours, after which all grown surface and deep colonies were counted, the arithmetic mean of the number of colonies in two Petri dishes of the same dilution was determined, multiplied by the dilution value and divided by the surface area of the egg (Motola *et al*., 2023).

Data analysis

The statistical processing of the results was performed using the R language program code in the open development environment R Studio and the licensed software STATISTICA 10.0 (StatSoft) for Windows.

Results

According to the results of the experiments, the level of microbial contamination of eggs of different poultry species after their laying was determined (Table 1).

Table 1. Level of microbial contamination of eggs of different poultry species

Poultry eggs	Contamination level	
	Microorganisms	CFU/cm^2
quail	*Corynebacterium* spp.	< 10^3
	Bacillus spp.	< 10^3
turkey	*Corynebacterium* spp.	< 10^3
	Bacillus spp.	< 10^3
	Lactobacillus spp.	< 10^3
duck	*Corynebacterium* spp.	< 10^3
	Bacillus spp.	< 10^3
	Enterobacter spp.	< 10^3
goose	*Corynebacterium* spp.	< 10^3
	Bacillus spp.	< 10^3
	Enterobacter spp.	< 10^3
chicken	*Corynebacterium* spp.	< 10^3
	Bacillus spp.	< 10^3
	Lactobacillus spp.	< 10^3
	Enterobacter spp.	< 10^3

According to the results of the studies, it was found that eggs from poultry, regardless of the species, after laying were contaminated with *Corynebacterium* spp. and *Bacillus* spp. at a level of < 10^3 CFU/cm^2. The contamination of waterfowl eggs with *Enterobacter* spp. microorganisms was also found, and *Lactobacillus* spp. was isolated from turkey eggs, the level of which also did not exceed 10^3 CFU/cm^2.

At the next research stage, the bacterial contamination of cardboard containers after a single use during the transportation of eggs of different poultry species was assessed (Table 2).

Table 2. Level of microbial contamination of cardboard containers during a single use

Poultry eggs	Contamination level	
	Microorganisms	**CFU/cm²**
quail	*Corynebacterium* spp.	$1.2 \pm 0.1 \times 10^3$
	Enterobacter agglomerans	$2.3 \pm 0.3 \times 10^3$
turkey	*Corynebacterium* spp.	$3.8 \pm 0.2 \times 10^4$
	Enterobacter agglomerans	$3.3 \pm 0.2 \times 10^4$
duck	*Enterobacter agglomerans*	$1.2 \pm 0.1 \times 10^4$
goose	*Corynebacterium* spp.	$3.4 \pm 0.2 \times 10^3$
	Enterobacter agglomerans	$2.8 \pm 0.1 \times 10^4$
chicken	*Corynebacterium* spp.	$3.1 \pm 0.2 \times 10^4$
	Enterobacter agglomerans	$2.7 \pm 0.3 \times 10^4$
	Staphylococcus spp.	$5.3 \pm 0.3 \times 10^2$

The results of the examination showed a high level of bacterial contamination of cardboard containers even after a single use. The lowest level of contamination of transport containers with microorganisms was found during the transportation of duck eggs, while the highest level was found during the transportation of chicken eggs. In all cases, the presence of *Enterobacter agglomerans* microorganisms was found at an average contamination level of $2.5 \pm 0.2 \times 10^4$ CFU/cm^2. Microorganisms *Corynebacterium* spp. were found on the containers after transportation of quail, turkey, goose, and chicken eggs at an average contamination level of $2.9 \pm 0.2 \times 10^4$ CFU/cm^2. In addition, *Staphylococcus* spp. was isolated from the containers when chicken eggs came into contact with them.

In the case of repeated (≥ 10) use of containers, the degree of microbial contamination increases significantly (Table 3).

With the increase in the term of use of cardboard containers, their contamination with many microorganisms is observed. Regardless of the type of eggs, micromycetes (*Aspergillus* spp., *Cladosporium* spp., *Penicillium* spp.) were induced from the transport container in all cases at an average contamination level of $3.2 \pm 0.2 \times 10^6$ CFU/cm^2. An increase in the presence of *Enterobacter agglomerans* and *Corynebacterium* spp. was found compared to the single use of cardboard containers, while the repeated use revealed its contamination with *Escherichia coli* at an average level of $3.5 \pm 0.2 \times 10^8$ CFU/cm^2.

Analyzing the results, it is clear that cardboard containers used to transport eggs of different poultry species can be an additional source of contamination, which negatively affects the further use and sale of poultry products.

Table 3. The level of microbial contamination of cardboard containers during repeated use

Poultry eggs	Contamination level	
	Microorganisms	**CFU/cm²**
quail	*Corynebacterium* spp.	$7.6 \pm 0.3 \times 10^7$
	Enterobacter agglomerans	$3.1 \pm 0.2 \times 10^6$
	micromycetes	$1.7 \pm 0.1 \times 10^6$
turkey	*Corynebacterium* spp.	$6.7 \pm 0.3 \times 10^6$
	Enterobacter agglomerans	$7.1 \pm 0.3 \times 10^5$
	Staphylococcus spp.	$8.4 \pm 0.5 \times 10^4$
	micromycetes	$3.9 \pm 0.2 \times 10^5$
duck	*Enterobacter agglomerans*	$6.6 \pm 0.3 \times 10^7$
	Enterococcus faecalis	$6.2 \pm 0.3 \times 10^6$
	Proteus vulgaris	$7.4 \pm 0.4 \times 10^6$
	Staphylococcus saprophyticus	$4.5 \pm 0.2 \times 10^5$
	Escherichia coli	$3.7 \pm 0.2 \times 10^8$
	micromycetes	$5.8 \pm 0.5 \times 10^5$
goose	*Enterobacter agglomerans*	$4.8 \pm 0.3 \times 10^6$
	Corynebacterium spp.	$4.6 \pm 0.3 \times 10^7$
	micromycetes	$2.7 \pm 0.2 \times 10^6$
chicken	*Corynebacterium* spp.	$5.2 \pm 0.4 \times 10^6$
	Enterobacter agglomerans	$4.4 \pm 0.3 \times 10^7$
	Escherichia coli	$3.2 \pm 0.2 \times 10^8$
	Staphylococcus spp.	$4.1 \pm 0.3 \times 10^5$
	Citrobacter freundii	$1.5 \pm 0.1 \times 10^6$
	micromycetes	$1.9 \pm 0.2 \times 10^7$

To minimize microbiological risks, it is necessary to perform routine egg disinfection. In our studies, physical disinfectants (ozone + ultraviolet irradiation) were used for this purpose (Table 4).

According to the results of the conducted experiments, the level of bactericidal effect of ozone (100 mg/h) and UV irradiation (253.7 nm) on the microflora of poultry eggs was determined. With increasing exposure to physical disinfectants, their bactericidal effect gradually increases. Thus, during the operation of the Ufotek 2 unit for one minute, the bactericidal effect was absent, and microflora was released from the shells of treated eggs in an amount equal to the initial indicator after laying.

With an increase in exposure to two minutes, the amount of isolated microflora decreased by one order and amounted to 10^2 CFU/cm^2. When the unit was operated for three minutes, a corresponding decrease in the growth of microflora was noted, which amounted to 10^1 CFU/cm^2. A significant bactericidal effect was observed when exposed to ozone and UV irradiation for four minutes, and when the unit was operated for five minutes, no growth of microflora from treated eggs was observed.

Table 4. Determination of the disinfecting effect of ozone (100 mg/h) and UV irradiation (253.7 nm) on egg surfaces, x±SE

Poultry eggs	Microorganisms	Growth of microorganisms (CFU/cm^2) under exposure, min.				
		1	2	3	4	5
quail	*Corynebacterium* spp.	$6.4 \pm 0.2 \times 10^3$	$4.4 \pm 0.3 \times 10^2$	$2.2 \pm 0.1 \times 10^1$	4.1 ± 0.1	–
	Bacillus spp.	$8.9 \pm 0.3 \times 10^3$	$5.1 \pm 0.1 \times 10^2$	$3.7 \pm 0.1 \times 10^1$	5.3 ± 0.2	–
turkey	*Corynebacterium* spp.	$7.1 \pm 0.2 \times 10^3$	$5.2 \pm 0.1 \times 10^2$	$4.1 \pm 0.2 \times 10^1$	3.4 ± 0.1	–
	Bacillus spp.	$6.5 \pm 0.2 \times 10^3$	$5.3 \pm 0.1 \times 10^2$	$3.3 \pm 0.2 \times 10^1$	6.6 ± 0.3	–
	Lactobacillus spp.	$2.4 \pm 0.2 \times 10^3$	$3.3 \pm 0.1 \times 10^2$	$4.2 \pm 0.2 \times 10^1$	1.8 ± 0.1	–
duck	*Corynebacterium* spp.	$8.9 \pm 0.1 \times 10^3$	$4.8 \pm 0.2 \times 10^2$	$3.1 \pm 0.1 \times 10^1$	2.8 ± 0.1	–
	Bacillus spp.	$6.1 \pm 0.3 \times 10^3$	$4.8 \pm 0.2 \times 10^2$	$4.0 \pm 0.2 \times 10^1$	4.8 ± 0.2	–
	Enterobacter spp.	$8.8 \pm 0.2 \times 10^3$	$2.7 \pm 0.1 \times 10^2$	$1.8 \pm 0.1 \times 10^1$	2.4 ± 0.1	–
goose	*Corynebacterium* spp.	$8.4 \pm 0.2 \times 10^3$	$2.2 \pm 0.3 \times 10^2$	$3.8 \pm 0.3 \times 10^1$	5.2 ± 0.1	–
	Bacillus spp.	$8.3 \pm 0.3 \times 10^3$	$3.5 \pm 0.2 \times 10^2$	$4.1 \pm 0.3 \times 10^1$	7.1 ± 0.3	–
	Enterobacter spp.	$7.6 \pm 0.2 \times 10^3$	$4.1 \pm 0.1 \times 10^2$	$2.1 \pm 0.1 \times 10^1$	2.9 ± 0.2	–
chicken	*Corynebacterium* spp.	$5.8 \pm 0.1 \times 10^3$	$4.6 \pm 0.2 \times 10^2$	$3.9 \pm 0.2 \times 10^1$	4.4 ± 0.1	–
	Bacillus spp.	$5.3 \pm 0.2 \times 10^3$	$2.6 \pm 0.3 \times 10^2$	$4.4 \pm 0.3 \times 10^1$	9.1 ± 0.2	–
	Lactobacillus spp.	$3.2 \pm 0.2 \times 10^3$	$2.2 \pm 0.1 \times 10^2$	$3.5 \pm 0.1 \times 10^1$	6.3 ± 0.1	–
	Enterobacter spp.	$4.7 \pm 0.1 \times 10^3$	$5.2 \pm 0.1 \times 10^2$	$1.7 \pm 0.1 \times 10^1$	3.5 ± 0.2	–

Note: $p > 0.05$, before the biocide effect.

Thus, it can be concluded that the combined effect of ozone (100 mg/h) and UV irradiation (253.7 nm) for five minutes causes the disinfection of eggshells of poultry, regardless of their type.

A three-factor analysis of variance ANOVA was performed using a linear regression model, which included the type of egg, type of microorganism, and exposure time as factors (Table 5).

Table 5. Results of a three-factor analysis of variance using a linear regression model

Source of variation	Df (degrees of freedom)	A sum of squares (Sum Sq)	Mean Square (Mean Sq)	F value	p-value
Poultry eggs	4	8124273	2031068	2.1538	0.08817
Exposition	3	464280458	154760153	164.1147	< 2e-16 ****
Microorganisms	3	4070593	1356864	1.4389	0.24282

Note: Signs of reliability: **** - 0, *** - 0.001, ** - 0.01, * - 0.05.

Thus, F and P-values are used to determine whether the difference between groups is significant. The higher the F-value, the more evidence there is that the factor affects the dependent variable. If the P-value is < 0.05, then the impact is significant. Based on the results, the duration of ozone and UV exposure does have a statistically significant effect on microbial growth, while the type of egg and the type of microorganism did not have a statistically significant effect on microbial growth.

Discussion

The results of the study showed that reusable cardboard containers are an additional source of egg contamination. The contamination of used packaging increases significantly with each subsequent stage of use. Therefore, it is necessary to use cardboard containers only once or to use other packaging materials that are subject to high-quality sanitization.

Under appropriate sorting, packaging, and storage conditions, eggs can remain sterile for a long time despite the presence of pores in the shell (Demyanenko *et al*., 2021; Kulshreshtha *et al*., 2021). This is because the egg is a living germ cell with natural immunity. The protection of the egg against the penetration and growth of microbes in it is provided by the shell, sub-shell membranes, and bactericidal properties of the egg white (Javůrková *et al*., 2019). When an egg is laid, a layer of mucus is deposited on the surface of the shell, which dries to form an over-shell film - the cuticle. The cuticle contains lysozyme, which has a bactericidal effect. The cuticle is easily damaged, so it is not recommended to wash eggs intended for storage. The sub-shells also contain lysozyme (Wilson, 2017; Kulshreshtha *et al*., 2018; Lewko *et al*., 2021).

With a decrease in the bactericidal activity of the shell and sub-shell membranes, microorganisms on the surface of the egg penetrate the shell and sub-shell membranes into the egg contents. Bacteria penetrate through the pores of the shell and multiply on the outer sub-shell, forming small colonies. Under the influence of proteolytic enzymes, the sub-shells dissolve, the bacteria penetrate the egg contents, and actively grow and multiply in the egg yolk (Gantois *et al*., 2009; Fonseca *et al*., 2014). In addition to putrefactive bacteria, molds, and actinomycetes often multiply in eggs (Tomczyk *et al*., 2018). At low above-zero temperatures and high humidity, fungal spores germinate and penetrate the pores of the shell and then the sub-shells. They find the most favorable conditions near the air chamber. Ovoscopy of the affected eggs reveals dark spots - fungal colonies. Subsequently, fungal hyphae penetrate the white, form a branched network, and liquefy it under the influence of enzymes. Among the fungi, the most common are fungi of the genera *Penicillium, Aspergillus*, and *Cladosporium*, which produce several mycotoxins (Wang *et al*., 2018). Other researchers have reported that the shell microbiota consists mainly of the genera *Firmicutes, Actinobacteria, Proteobacteria*, and *Bacteroidete* (Trudeau *et al.*, 2020).

When putrefactive bacteria, fungi, actinomycetes, and other microorganisms multiply in an egg, the yolk turns light yellow under the influence of the enzymes they secrete. Due to the destruction of the yolk shell, the white is mixed with the yolk, and a homogeneous cloudy liquid mass is formed (Fardows and Shamsuzzaman, 2015; Kang *et al*., 2021).

In our experiments, poultry eggs at the initial stage were contaminated with *Corynebacterium* spp, *Bacillus* spp, *Lactobacillus* spp, *Enterobacter* spp. Other studies have identified *Enterobacter agglomerans, Klebsiella* spp, *Enterobacter cloacae, E. coli, Serratia* spp, *Pseudomonas aeruginosa, Shigella* spp., *Salmonella enteritidis, Proteus* spp., *Enterobacter sakazakii, Rahnella aquatilis*, and *Staphylococcus aureus* (El Ftouhy *et al*., 2022).

Thus, it has been demonstrated that eggs can be contaminated with several types of bacteria that pose a potential health risk to consumers.

The rate of microbial penetration into the egg is influenced by temperature, humidity, egg freshness, lysozyme inactivation and biological characteristics of the microbes. Contamination of the shell with pathogenic and opportunistic microorganisms most often occurs in the floor-based system of poultry housing in poultry houses with poorly equipped nests, poor quality bedding, and microclimatic disturbances (Kone *et al*., 2013; van Staaveren *et al*., 2018).

Salmonella spp. (Solís *et al*., 2023) and *Staphylococcus* spp. (Pondit *et al*., 2018) are common pathogens, but in our studies they were not isolated from the shells of the experimental eggs.

At a temperature of 20°C and relative humidity of 80-85%, bacteria of the genera *Pseudomonas* and *Proteus* penetrate from the shell surface into the egg on days 2-5, *S. typhimurium* on days 8-11, *E. coli* on days 13-15, and *Aspergillus* spp. on days 5-9. The rate of penetration of mesophilic microbes slows down to 11 weeks at temperatures below 15°C and humidity of 60-65%, and it almost stops at temperatures below 10°C. Psychrophilic microbes of the *Pseudomonas* group and molds penetrate the shell's pores at zero degrees (Zagaevsky, 1969).

During storage, the physicochemical properties of the egg content gradually change (it dries out, and the pH of the white increases); the antimicrobial effect of the white, shell, and sub-shell membranes is weakened, as lysozyme and other bactericidal substances are inactivated; the pores of the shell become more permeable. All this creates favorable conditions for microorganisms to enter and multiply inside the egg. To slow down the natural biochemical changes in eggs and preserve the protective properties of the shell, membranes, and protein, storing eggs in cool, dry places at temperatures between minus 2 and 0°C and relative humidity not exceeding 85% is necessary. High temperatures and high humidity accelerate the inactivation of bactericidal substances in eggs (Jones *et al*., 2018; da S Oliveira *et al*., 2020; Gogo *et al*., 2021).

Gamma irradiation, lyophilization, hot air, hot water, infrared radiation, atmospheric steam, microwave and radio frequency heating are various decontamination methods currently being considered to produce microbiologically safe eggs. However, each decontamination process has a different effect on the properties and components of the egg. Pasteurization processes are the most common and well-understood; however, they affect the coagulation, foaming, and emulsifying properties of the egg. Further research is needed to explore combinations of different decontamination methods to produce safe eggs without affecting white structure and usability (Keerthirathne *et al*., 2017). The use of H_2O_2+UV treatment for shell eggs is an emerging technology that may have important implications for egg quality and preservation (Al-Ajeeli *et al*., 2016). In our studies, we have obtained positive results when combining ozone and UV radiation to disinfect eggshells of different poultry species. For example, the microflora is completely destroyed in 5 minutes of operation of the Ufotek 2 unit.

Research shows that the appropriate use of antimicrobials in agricultural environments does not contribute to antibiotic resistance and does not reduce the susceptibility of microbiota to disinfectants (Maertens *et al*., 2019; Chan *et al.,* 2021).

It should be noted that the quality of commercial and hatching eggs directly depends on the housing conditions, hygiene, feeding of productive poultry and their epizootic status (Bogach *et al*., 2020; Paliy *et al*., 2021; Solís *et al.*, 2023).

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Conflicts of interest

The authors declare no conflict of interest.

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