

The effect of nanosilver in carriers based on polymer/inorganic hybrids on the quality and safety of edible chicken eggs

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One of the modern antibacterial agents that are an alternative to antibiotics are nanoparticles of noble metals, including silver. To reduce their toxicity, cumulative effect and prolong the effect in animals, there is ongoing work on development and improvement of the methods for their synthesis using various carriers, including those based on polymer/inorganic hybrids. In this study, the quality and safety of edible eggs were determined on Hy-Line laying hens using W36 solutions of nanosilver in carriers based on polymer/inorganic hybrids (AgNPs/SPH) in the concentration of 0.0, 1.0 and 2.0 mg/L of water (0.0, 0.2 and 0.4 mg per hen per day) three times with 10 day interval. We determined that one-, two- and three-time feeding of nanosilver in doses of 0.0, 0.2 and 0.4 mg per hen per day did not affect water consumption, feed, egg productivity, as well as dry matter content, crude protein, fat, ash, and calcium and phosphorus in eggs for 30 days. Contamination of the surface of the shell and yolks of eggs with mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) did not depend on the dose and duration of consumption of the nanosilver drug by laying hens. The nanosilver drug in doses of 0.0, 0.2 and 0.4 mg per hen per day did not affect the contamination of the egg shell surface with microorganisms of genera *Citrobacter*, *Klebsiella*, as well as *Escherichia coli*, *Proteus mirabilis*, *Salmonella* spp., *Staphylococcus aureus* and *S. epidermidis*. When administered orally, nanosilver in the dose of 0.2 mg per hen per day did not change the number of symbiotic microorganisms of genera *Bifidobacterium* and *Lactobacillus*, while and the dose of 0.4 mg per hen daily slightly reduced the number of microorganisms of genus *Lactobacillus* in the hens' manure. The obtained data can be used for further research to determine the effective dose and interval of application of nanosilver preparations to poultry for preventive and therapeutic measures, taking into account the preservation of the microbiome of the digestive system of hens.

Keywords: chemical composition of eggs; MAFAnM; lactobacilli; bifidobacteria; AgNPs/SPH.

Introduction

The ban on the use of antibiotics in industrial livestock, especially in poultry breeding, has prompted scientists to find new drugs having bactericidal, virucidal and fungicidal effects (Długosz et al., 2021). The rapid development of nanotechnology has led to the use of nanosilver drugs as antimicrobial agents in medical, textile, food, and veterinary practice (Motelica et al., 2020; Pulić-Prociak et al., 2020). A number of studies have shown that silver nanoparticles have a positive effect on animal productivity, stimulate immunogenesis (Anwar et al., 2019), may be effective in prevention and treatment of infectious diseases (Gopinath et al., 2016), and be efficient anticancer agents (Hamed et al., 2017; Salem et al., 2020). The study of the effectiveness of nanosilver preparations in industrial poultry farming is especially relevant, as the production, transportation, sale and storage of eggs are associated with the risk of contamination of the shell surface with opportunistic and pathogenic microflora that poses a threat to the consumers. According to Stępień-Pyśniak et al. (2010), the shell of chicken eggs during sale and storage is most often contaminated with two serotypes of bacteria of *Salmonella* genus: *S. enteritidis* and *S. arizonae*, as well as bacteria of Enterobacteriaceae family: *Enterobacter* spp., *Klebsiella* spp. and *Citrobacter freundii*. Qualitative analysis of the bacterial microflora of eggs also showed the presence of other Gram-negative bacteria, including *Acinetobacter* spp., *Pseudomonas* spp., *Tatumella ptyseos*, *Providencia stuartii*, *Serratia liquefaciens*, *Flavimonas oryzihabitans*, *Vibrio metschnikovii*, *Leclercia adecarboxylata*, *Kluverera* spp., *Rahnella aquatilis*, *Proteus mirabilis* and *Achromobacter* spp. The

use of nanosilver preparations to reduce microbial contamination of eggs is justified by the ability of AgNPs in 6.25–100.00 ppm concentrations to show antibacterial activity against a wide range of pathogens, including *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhimurium* (Hamed et al., 2017; Salem et al., 2020). In most cases, nanosilver is administered to poultry orally, further accumulating in various organs and tissues, which, along with reduction of the risk of infectious diseases of poultry and contamination of eggs for consumption with dangerous infectious agents, may damage the intestinal microbiome; today it is considered as an integral metabolic organ with numerous physiological functions (Zorraquín-Peña et al., 2020). The symbiotic ecosystem, consisting of more than 100 trillion bacteria, is essential for intestinal maturation, local angiogenesis, regulation of enterocyte gene expression, vitamin synthesis, support of homeostasis, innate and adaptive immunity.

Currently, there is a considerable amount of conflicting data on the effects of silver nanoparticles on the intestinal microbiome of different species of animals and humans, which are manifested by dysbacteriosis and other disorders (Lamas et al., 2020), and the use of nanosilver preparations in poultry farming still require studies determining the level of risk of microbial contamination of eggs for consumption and their chemical composition. The development of the new generation of nanosilver preparations, obtained and stabilized in aqueous solutions of synthetic and natural polymers (Rozenberg et al., 2008; Natsuki et al., 2015; Lee et al., 2019), and in the recent years in solutions of biocompatible and biodegradable polymer/inorganic hybrids (Zheltonozhskaya et al., 2021), makes

it possible to reduce their doses and toxicity and increase the efficiency of their use due to production of very small silver nanoparticles in such carriers and increasing the duration of their action in a living organism. In this context, the objective of our research was the effect of different doses of nanosilver in carriers based on polymer/inorganic hybrids on the quality and safety of chicken eggs, as well as the symbiotic microorganisms of the intestines of laying hens.

Materials and methods

A grafted polymer/inorganic hybrid carrier (PIHC) based on silica nanoparticles and polyacrylamide chains was obtained by radical polymerization of acrylamide (AAm) from the surface of silica nanoparticles in accordance with the methodology developed in the study (Zheltonozhskaya et al., 2021). In this synthesis, we used samples of Aerosil A-175 from "Orisil" (Ukraine), cerium (IV) ammonium nitrate (CAN) from "Aldrich" (USA) and AAm from "Merck" (Germany), as well as weight ratios $[\text{CeIV}]/[\text{SiO}_2] = 0.2$ and $[\text{CeIV}]/[\text{AAm}] = 7.72 \cdot 10^{-3}$, with the concentration of $\text{CSiO}_2 = 1.35 \text{ g/L}$. The procedure was carried out at 23°C in an inert (argon) atmosphere with stirring for 24 hours. The gelled product was diluted with deionized water, re-precipitated with acetone, then re-dissolved in water and freeze-dried. The main characteristics of PIHC were determined according to the study (Zheltonozhskaya et al., 2021) using static light scattering, elemental analysis, dynamic thermogravimetric analysis, viscometry, and transmission electron microscopy (TEM) (Table 1).

Table 1

Main parameters of polymer/inorganic hybrid carrier and the obtained silver preparation

| Sample | Pure carrier | | | Preparation | | |
|--------|---------------------------|---------------------------|-------|--------------------------|---------------------------|--------------------------------|
| | $R_{\text{SiO}_2}^a$, nm | M_{PAAm}^b , kDa | N^c | d_{PIHC}^d , nm | d_{AgNPs}^d , nm | $d_{\text{AgNPs/PIHC}}^e$, nm |
| PIHC | 7.7 | 1513 | 8 | 20–45 | 1.2–9.6 | ~31–47 |

Note: ^a – average radius of silica nanoparticles determined by static light scattering; ^b – viscosity-average molecular weight of PAAm grafted chains; ^c – average number of PAAm grafts per SiO_2 particle; ^d – diameters of spherical particles of pure hybrid and silver found by TEM; ^e – effective diameters of hybrid particles with silver nanoparticles.

Then a preparation of nanosilver was obtained according to the previously developed method (Zheltonozhskaya et al., 2021) by in situ reduction of silver nitrate with sodium borohydride in an aqueous solution of a hybrid. For this synthesis, the concentrations of CPIHC = 1.0 g/L and $\text{CAgNO}_3 = 36.4 \text{ mg/L}$ and an eight-fold molar excess of NaBH_4 with respect to the silver salt were used. According to the standard protocol, the hybrid aqueous solution was mixed with AgNO_3 at room temperature and stored for 30 min in a dark box; then a reducing agent was added. After three minutes, a yellow colour appeared, indicating the appearance of a dispersion of AgNPs in water. The formation of silver nanoparticles was also confirmed by the manifestation of a surface plasmon resonance band with $\lambda_{\text{max}} = 388 \text{ nm}$ in the UV-Vis spectrum (Fig. 1).

Extinction spectra were recorded in the range of 200–1,000 nm using a Cary 50 Scan UV-Vis spectrometer manufactured by Varian (USA). The obtained nanosilver preparation was re-precipitated with ethanol, centrifuged at 6,000 rpm, and re-dissolved in deionized water to remove by-products of the reduction reaction. For subsequent biological experiments, this basic nanosilver preparation with $\text{CAgNPs} = 24 \text{ mg/L}$ was diluted to the required concentrations ($\text{CAgNPs} = 1$ and 2 mg/L).

Experiments on laying hens were conducted at the Faculty of Veterinary Medicine of the National University of Life and Environmental Sciences of Ukraine in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes adopted in 1986 and the Law of Ukraine "On protection of animals from abuse" of 21.02.2006 No. 3447-IV as amended on 04.08.2017.

For the experiment, we used 45 38-week-old Hy-Line W36 laying hens. Before the experiment, they were divided into three groups (15 individuals in each). The birds were given a solution of the nanosilver preparation in carriers based on polymer/inorganic hybrids in the concentrations of 0 mg/L (control), 1.0 and 2.0 mg/L of water (respectively, 0.0,

0.2 and 0.4 mg per hen per day) three times with an interval of 10 days. During the experiment, hens of all groups were fed with commercial compound feed, balanced according to energy, nutrients and biologically active substances. Laying hens were fed according to the Management Guide W-36 commercial layers. The composition of the complete feed included the following components (%): corn – 50.08, soybean meal – 17.90, sunflower meal – 9.60, wheat – 9.00, limestone – 1.14, salt – 0.23, sorbent, mineral and vitamin complexes, and probiotic Proaktivo. One kg of Proaktivo contains: bacteria *Bacillus licheniformis* and *B. subtilis*, no less than $1 \cdot 10^{12}$ CFU, bacteria *Enterococcus faecium*, no less than $5 \cdot 10^{10}$ CFU, fermentation products of *Bacillus subtilis*, *Lactococcus lactis*, *Bacillus licheniformis* 100 g, xylanase 600,000 units, cellulase 20,000 units, protease 500 units, milk thistle meal 20 g, acidity regulator 10 g, betaine 10 g, chitosan 0.1 g, and yeast cell walls (mannan-oligosaccharides) 100 g, natural aluminosilicates – up to 1 kg.

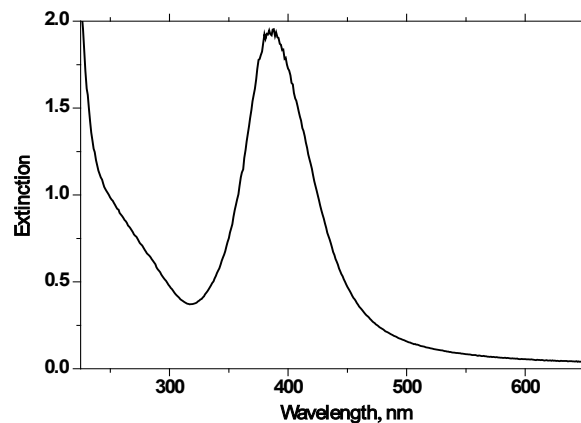


Fig. 1. Extinction spectrum of the $\text{AgNO}_3/\text{PIHC}$ mixture 13.3 min after the addition of the reducing agent: CPIHC = 1 g/L; $\text{CAgNO}_3 = 36.4 \text{ mg/L}$; $T = 20^\circ\text{C}$

All groups of pullets had free access to water throughout the experiment. Drinking bowls with a graduated cylinder were used for watering the birds. During the experiment, the amounts of consumed water and the solution of the nanosilver preparation were monitored daily. The birds were kept in cages with egg collectors and group trough feeders, 5 individuals in each. In the room, hens of all experimental groups were provided with proper temperature-humidity and light regimes (16 hours a day (30 lux illumination), 8 hours of darkness).

The consumption of feed, water, egg productivity and the condition of hens in the groups were monitored throughout the experiment (30 days). On the 10th day after each feeding of the solution of the nanosilver preparation, in the morning before feeding, we collected 5 eggs and 5 samples of manure from each group of hens in sterile bags.

The contents of water, dry matter, crude ash, crude protein, crude fat, calcium and phosphorus were determined in the eggs. The mass fraction of moisture was determined by successive weighing and drying of the egg samples in an oven. Dry matter in egg samples was calculated on the basis of data of mass moisture content. The mass nitrogen content in eggs was determined using the Kjeldahl method, mineralization of samples was performed using a digester for wet mineralization DK 6 (Velp Scientifica, Italy), and the distillation of ammonia was carried out on a semi-automatic apparatus for steam distillation according to Kjeldahl UDK 129 (Velp Scientifica, Italy). The mass protein content was calculated after determining nitrogen in the egg samples. The mass ash content in eggs was determined by burning samples in a muffle furnace Nabertherm L15 (Germany). The mass fat content was determined by degreasing the egg samples with diethyl ether using the Soxhlet method with an automatic extraction device SER 148 (Velp Scientifica, Italy). The content of calcium and phosphorus was determined in crude ash after mineralization of eggs by colorimetric method. To determine the number of mesophilic aerobic and mesophilic anaerobic microorganisms (MAFAnM), bacteria of the genera *Citrobacter*, *Klebsiella*, *Salmonella* spp., as well as *E. coli*, *P. mirabilis*, *S. aureus* and *S. epidermidis*, we used eggshell surface rinses.

The number of MAFAnM was determined in the yolks of freshly laid chicken eggs. The number of bacteria of genera *Bifidobacterium* and *Lactobacillus* was determined in the manure of hens. For this purpose, we prepared subsequent nine-fold dilutions in sterile saline. Analyses of the microflora in the manure, on the shells and in egg yolks were performed simultaneously. Bacteriological studies used medium and diagnostic tests manufactured by HiMedia (India).

Platecountagar M091 medium was used to determine the amount of MAFAnM on the surface of the shells and in the egg yolks. MacConkey Broth M007 and Endo Agar M029 media were used to isolate and calculate bacteria of the genera *Citrobacter*, *Klebsiella*, *Bifidobacterium* Agar M 1396 for *Bifidobacterium*; *Lactobacillus* MRS Agar M641 for *Lactobacillus*. To isolate pathogenic and non-pathogenic staphylococci, we used Baird Parker Agar M043; for *P. mirabilis* – Xylose Lysine Deoxycholate Agar M031; for *Salmonella* – Bismuth Sulphite Agar M027 and Xylose Lysine Deoxycholate Agar M031. The number of microorganisms was expressed in colony-forming units (CFU), the results were expressed in lg CFU/cm² of egg shell surface and lg CFU/g of yolk or

manure. Genus and species identification of isolated microorganisms were performed according to current methods.

Statistical processing of the obtained results was performed using the ANOVA software. The data in the tables are presented in the form of $\bar{x} \pm SD$ (mean \pm standard deviation). The difference between the groups was considered significant using the Tukey test at $P < 0.05$ (taking into account the Bonferroni correction).

Results

Feeding laying hens with the solution of nanosilver in carriers based on polymer/inorganic hybrids in the doses of 0.2 and 0.4 mg per hen per day did not significantly affect water and compound feed consumption compared to the control during the entire period of the experiment (Table 2). Also, there was no significant difference between egg production by hens that consumed solutions of the nanosilver preparations in carriers based on polymer/inorganic hybrids in the doses of 0.0, 0.2 and 0.4 mg per hen per day.

Table 2

Consumption of feed, water, and egg productivity of laying hens consuming the preparations of nanosilver in carriers based on polymer/inorganic hybrids ($\bar{x} \pm SD$, n = 15)

| Indicator | The dose of AgNPs/SPH, mg/hen | | |
|---|-------------------------------|-----------------|-----------------|
| | 0.0 | 0.2 | 0.4 |
| Single administration of AgNPs/SPH | | | |
| Consumption of compound feed, g/hen per day | 97.0 \pm 0.0 | 97.0 \pm 0.0 | 97.0 \pm 0.0 |
| Water consumption, mL/hen per day | 205.2 \pm 2.0 | 202.8 \pm 2.0 | 203.6 \pm 1.9 |
| Average daily egg production, eggs | 14.5 \pm 0.2 | 14.8 \pm 0.1 | 14.6 \pm 0.2 |
| Two administrations of AgNPs/SPH | | | |
| Consumption of compound feed, g/hen per day | 97.0 \pm 0.0 | 97.0 \pm 0.0 | 97.0 \pm 0.0 |
| Water consumption, mL/hen per day | 202.1 \pm 1.9 | 202.6 \pm 1.8 | 201.8 \pm 2.1 |
| Average daily egg production, eggs | 14.6 \pm 0.2 | 14.8 \pm 0.1 | 14.9 \pm 0.1 |
| Three administrations of AgNPs/SPH | | | |
| Consumption of compound feed, g/hen per day | 97.0 \pm 0.0 | 97.0 \pm 0.0 | 97.0 \pm 0.0 |
| Water consumption, mL/hen per day | 202.9 \pm 2.3 | 203.9 \pm 2.2 | 205.2 \pm 2.0 |
| Average daily egg production, eggs | 14.7 \pm 0.2 | 14.8 \pm 0.1 | 14.9 \pm 0.1 |

Note: no differences were found between the consumption of hen compound feed by hen, as its daily norm was consumed completely without residues.

Table 3

Chemical composition of chicken eggs produced by hens consuming nanosilver preparations in carriers based on polymer/inorganic hybrids ($\bar{x} \pm SD$, n = 5)

| Indicator | The dose of AgNPs/SPH, mg/hen | | |
|------------------------------------|-------------------------------|-------------------|-------------------|
| | 0.0 | 0.2 | 0.4 |
| Single administration of AgNPs/SPH | | | |
| Moisture | 76.12 \pm 0.12 | 76.07 \pm 0.15 | 74.65 \pm 0.62 |
| Dry matter | 23.88 \pm 0.11 | 23.93 \pm 0.15 | 23.35 \pm 0.62 |
| Crude protein | 13.61 \pm 0.31 | 13.78 \pm 0.24 | 12.87 \pm 0.48 |
| Crude fat | 9.52 \pm 0.29 | 11.74 \pm 0.92 | 9.58 \pm 0.58 |
| Crude ash | 0.99 \pm 0.10 | 0.99 \pm 0.03 | 1.06 \pm 0.05 |
| Ca | 0.055 \pm 0.004 | 0.061 \pm 0.001 | 0.059 \pm 0.001 |
| P | 0.141 \pm 0.007 | 0.190 \pm 0.012 | 0.170 \pm 0.010 |
| Two administrations of AgNPs/SPH | | | |
| Moisture | 75.68 \pm 0.39 | 75.42 \pm 1.96 | 75.39 \pm 0.57 |
| Dry matter | 24.32 \pm 0.39 | 24.58 \pm 1.96 | 24.61 \pm 0.57 |
| Crude protein | 13.90 \pm 0.41 | 13.83 \pm 0.17 | 13.04 \pm 0.31 |
| Crude fat | 9.55 \pm 0.48 | 10.04 \pm 0.55 | 9.80 \pm 0.22 |
| Crude ash | 1.03 \pm 0.04 | 1.05 \pm 0.04 | 0.93 \pm 0.05 |
| Ca | 0.051 \pm 0.006 | 0.054 \pm 0.006 | 0.057 \pm 0.004 |
| P | 0.166 \pm 0.022 | 0.160 \pm 0.022 | 0.170 \pm 0.014 |
| Three administrations of AgNPs/SPH | | | |
| Moisture | 75.56 \pm 0.73 | 75.93 \pm 1.10 | 75.44 \pm 0.75 |
| Dry matter | 24.44 \pm 0.73 | 24.07 \pm 1.10 | 24.56 \pm 0.75 |
| Crude protein | 14.49 \pm 0.53 | 13.99 \pm 0.96 | 14.39 \pm 0.31 |
| Crude fat | 10.16 \pm 0.76 | 10.21 \pm 0.41 | 10.61 \pm 0.55 |
| Crude ash | 1.03 \pm 0.03 | 1.11 \pm 0.08 | 1.06 \pm 0.06 |
| Ca | 0.058 \pm 0.003 | 0.052 \pm 0.002 | 0.060 \pm 0.001 |
| P | 0.181 \pm 0.013 | 0.184 \pm 0.019 | 0.203 \pm 0.004 |

Analysis of the chemical composition of eggs obtained from hens of the experimental groups showed no effect of nanosilver on moisture, dry

matter, crude protein, crude fat, crude ash, as well as calcium and phosphorus compared with the control (Table 3). Throughout the experiment, during the use of nanosilver in polymer-based/inorganic hybrid carriers at doses of 0.0, 0.2 and 0.4 mg per hen per day, we found no colonies of microorganisms of the genus *Citrobacter*, *Klebsiella*, also *E. coli* and *P. mirabilis*, which belong to the opportunistic microflora, on the shell surface of freshly laid eggs. Furthermore, no colonies of pathogenic *Salmonella* spp., *S. aureus* and *S. epidermidis* were found on the surfaces of eggshells of eggs from chicken of the experimental groups throughout the experiment.

The number of MAFAnM on the surface of the shell and in the yolks of eggs at single, two and three consumptions of solution of nanosilver in carriers based on polymer/inorganic hybrids by the hens significantly did not depend on its dose and expiration date (Table 4).

Table 4

Contamination of MAFAnM chicken eggs with the use of nanosilver in carriers based on polymer/inorganic hybrids ($\bar{x} \pm SD$, n = 5)

| The frequency of application of AgNPs/SPH to hens | The dose of AgNPs/SPH, mg/hen | | |
|---|-------------------------------|----------------|----------------|
| | 0.0 | 0.2 | 0.4 |
| Egg shell surface, lg CFU/cm ² | | | |
| Single | 3.2 \pm 0.1 | 3.3 \pm 0.1 | 3.4 \pm 0.1 |
| Two | 3.0 \pm 0.2 | 3.1 \pm 0.1 | 3.0 \pm 0.1 |
| Three | 3.1 \pm 0.1 | 3.1 \pm 0.1 | 2.9 \pm 0.03 |
| Yolk, lg CFU/g | | | |
| Single | 2.6 \pm 0.2 | 2.8 \pm 0.05 | 2.8 \pm 0.1 |
| Two | 2.4 \pm 0.1 | 2.7 \pm 0.1 | 2.6 \pm 0.1 |
| Three | 2.5 \pm 0.2 | 2.5 \pm 0.1 | 2.4 \pm 0.2 |

Analysis of the amount of symbiotic microflora in chicken manure showed that single, two and three admissions of nanosilver solution in carriers based on polymer/inorganic hybrids in the doses of 0.0, 0.2 and 0.4 mg per hen a day did not affect the number of colonies of microorga-

nisms of genus *Bifidobacterium*. The number of bacteria of genus *Lactobacillus* in the manure of hens of the experimental groups did not change after application of the drug nanosilver in the dose of 0.2 mg, nonetheless the dose of 0.4 mg per hen per day slightly decreased their number ($P < 0.05$) after two and three applications compared with control (Table 5).

Table 5

The number of bacteria of *Lactobacillus* and *Bifidobacterium* genera in the manure of laying hens that consumed the nanosilver preparation in carriers based on polymer/inorganic hybrids (lg CFU/g, $x \pm SD$, $n = 5$)

| Genus of bacteria | The dose of AgNPs/SPH, mg/hen | | |
|------------------------------------|-------------------------------|---------------------------|--------------------------|
| | 0.0 | 0.2 | 0.4 |
| Single administration of AgNPs/SPH | | | |
| <i>Bifidobacterium</i> | 7.82 ± 0.01 ^a | 7.89 ± 0.03 ^a | 7.82 ± 0.02 ^a |
| <i>Lactobacillus</i> | 7.86 ± 0.05 ^a | 7.77 ± 0.01 ^a | 7.72 ± 0.02 ^a |
| Two administrations of AgNPs/SPH | | | |
| <i>Bifidobacterium</i> | 7.80 ± 0.01 ^a | 7.88 ± 0.02 ^a | 7.80 ± 0.03 ^a |
| <i>Lactobacillus</i> | 7.81 ± 0.01 ^a | 7.76 ± 0.02 ^a | 7.69 ± 0.01 ^b |
| Three administrations of AgNPs/SPH | | | |
| <i>Bifidobacterium</i> | 7.91 ± 0.17 ^a | 7.78 ± 0.02 ^a | 7.74 ± 0.03 ^a |
| <i>Lactobacillus</i> | 7.81 ± 0.01 ^a | 7.65 ± 0.07 ^{ab} | 7.50 ± 0.03 ^b |

Note: different uppercase letters ^a, ^b indicate values that probably differed in one row of the table ($P < 0.05$) by comparison using Tukey test with Bonferroni correction.

Discussion

As can be seen from our research results, the preparation of nanosilver in carriers based on polymer/inorganic hybrids did not affect the average daily consumption of feed, water and egg production of hens. That is consistent with data obtained on broiler chickens by Vadalasetty et al. (2018) for 8.26 mg/day doses of AgNPs and Kulak et al. (2018) for 2.87 and 12.25 mg per bird doses. In addition, in another study, administration of 15 nm nanosilver in the concentration of 50 mg/L of water increased feed intake and body weight of domestic chickens (Kumar & Bhattacharya, 2019). In contrast to these studies, Farzinpour et al. (2013) found a negative effect of nanosilver in the doses of 0, 4, 8 and 12 ppm in feed, which was manifested by a decrease in egg yolk mass and egg production of Japanese quails.

According to our studies, the nanosilver preparation in carriers based on polymer/inorganic hybrids in the doses of 0.2 and 0.4 mg per hen per day did not affect the chemical composition of chicken eggs, which is consistent with the level of feed and water consumption by poultry throughout the experiment. Our results are difficult to compare with other data due to their lack in the available literature.

One of the parameters of egg safety is the level of contamination of their shell with dangerous microflora (Stepień-Pyśniak, 2010). The microflora of the surface of the egg shell in birds is largely determined by the species composition and its ratio in the intestinal contents, especially the colon (Maki et al., 2020). In contrast to the effect of antibiotics on the intestinal microflora, the mechanism of action of nanosilver drugs on the microbiome of different parts of the intestine of hens has not been studied. In birds, the peculiarity of egg production is the presence of contact of the mucous membrane of the cloaca with the surface of the egg shell, which provides a vertical path of transmission of microflora. We found no contamination of the surface of the shell of freshly laid eggs with opportunistic and pathogenic microorganisms of genera *Citrobacter*, *Klebsiella*, as well as *E. coli*, *P. mirabilis*, *Salmonella* spp., *S. aureus* and *S. epidermidis* both at different doses of nanosilver and in hens of the control group. This is due to the bactericidal properties of the cuticle of the egg shell (Ohshima et al., 2015; Chen et al., 2019).

The number of mesophilic aerobic and mesophilic anaerobic microorganisms on the shell surface of eggs from hens that consumed nanosilver drug in carriers based on polymer/inorganic hybrids in our experiment did not differ between groups of experimental hens. That suggests that the main microorganisms belonging to MAFAnM on the surface of the egg-shell of hens in our case were bacteria that formed the basis of the Proactivo probiotic used in feed, as well as bacteria of genera *Bifidobacterium* and *Lactobacillus*, the development of which in the intestines of hens was likely promoted by this probiotic (Elshaghabe et al., 2017). That is con-

sistent with the number of bacteria of genera *Bifidobacterium* and *Lactobacillus* in the manure of hens that received the preparation of nanosilver in carriers based on polymer/inorganic hybrids with water in doses of 0.0, 0.2 and 0.4 mg per hen a day. Moreover, in our studies we did not note the effect of this drug on the number of bacteria of genus *Bifidobacterium*, while the number of bacteria of genus *Lactobacillus* in hen manure decreased slightly at the dose of 0.4 mg per hen per day with increasing frequency of drug administration. Another experiment revealed no effect of AgNPs on the intestinal microflora in birds against the background of weight gain with a decrease in the relative weight of the bursa and spleen, accompanied by a decrease in plasma IgG and IgM (Vadalasetty et al., 2018).

The effect of nanosilver preparations on the intestinal microbiome of hens has been studied insufficiently, while a significant number of studies have been performed on laboratory animals showing that oral nanoparticles of silver (10, 75 and 110 nm) and nanosilver acetate in Sprague-Dawley rats in the doses of AgNPs equaling 9, 18 and 36 mg/kg body weight per day caused shifts in the intestinal microbiota towards increase in the proportion of Gram-negative bacteria and decrease in the populations of Firmicutes phyla and genus *Lactobacillus* (Williams et al., 2015). By contrast, the data obtained after oral repeated administration of AgNPs to mice (20 and 110 nm) in the dose of 10 mg/kg body weight per day indicated no changes in the composition, structure and diversity of the intestinal microbiome. Wilding et al. (2016) conclude that by contrast to the effects of broad-spectrum antibiotics, repeated dosing of AgNPs does not affect the intestinal microbiome of mice and can be considered as an alternative to broad-spectrum antibiotics. Conversely, the study by van den Brule et al. (2015) points out that AgNPs can cause changes in the microbial composition of the intestine of mice, characteristic of obesity and inflammatory diseases, as well as the appointment of antibiotics. Disorders of the intestinal microbiome under the influence of AgNPs in mice in the study of Chen et al. (2017) was associated with disruption of microvilli structure and dense connections in the intestinal epithelium, as well as regulation of proinflammatory cytokine synthesis. Gene analysis by ¹⁶S rRNA sequencing showed that oral administration of AgNPs to mice caused changes in the number and ratio of intra- and interphylous bacteria and firmicutes, decreased the ratio of Firmicutes/Bacteroidetes, increased the number of rare bacteria and reduced the number of probiotic bacteria of genus *Lactobacillus*.

A study by Javurek et al. (2017) confirmed that the effect of nanosilver nanoparticles on the intestinal microbiome depends on their shape and causes histopathological changes in the gastrointestinal tract and brain. In male Sprague-Dawley rats, the two-week consumption of cubic AgNPs reduced the numbers of *Clostridium* spp., *Bacteroides uniformis*, Christensenellaceae, and *Coproccoccus eutactus*, whereas the spherical shape of nanoparticles of nanosilver reduced the number of *Oscillospira* spp., *Dehalobacterium* spp., Peptococcaceae, *Corynebacterium* spp., and *Aggregatibacter pneumotropica*.

Although the antibacterial properties of nanosilver nanoparticles have been demonstrated throughout the spectrum of bacterial pathogens, the effect of AgNPs on beneficial bacteria is studied poorly. Comparison of the antibacterial activity of AgNPs against two beneficial lactobacilli (*Lactobacillus delbrueckii* subsp. *Bulgaricus* and *Lactobacillus casei*) and two common opportunistic pathogens (*Escherichia coli* and *Staphylococcus aureus*) showed that lactobacilli are more susceptible to AgP, which is coherent with our data (Table 5). The acidic environment formed by lactobacilli enhances the bactericidal action of AgNPs by increasing their dissolution and the formation of hydroxyl radicals (-OH). In addition, the increase in the concentration of nanosilver (Ag⁺) and -OH ions depletes the pool of glutathione inside the cell of the microorganism, which is associated with an increase in the content of intracellular reactive oxygen species. High levels of reactive oxygen species in the environment can cause DNA damage and lead to further cell death (Tian et al., 2018). These results also indicate that oral administration of AgNPs alters the mucosal-associated microbiota and modulates the intestinal immune response and overall intestinal homeostasis in animals. Nanosilver is an effective bactericidal agent against Gram-negative and Gram-positive strains, and its activity depends on the thickness of the peptidoglycan layer of the bacterial wall. Nanosilver ions can bind to the negatively charged surface of bac-

teria and disrupt its integrity. AgNPs and ions are also able to enter bacterial cells and interfere with the functioning of the respiratory chain and the absorption of phosphates. Disorders of DNA replication or protein modification, as well as strong interactions with cytoplasmic and membrane proteins containing thiol, may also enhance the bactericidal effects of AgNPs (Jung et al., 2008; Völker et al., 2013).

Consumption of nanosilver in carriers based on polymer/inorganic hybrids may be considered a way to reduce the accumulation of residues of antibiotics and other antibacterial drugs in food, including eggs and poultry meat (Bayer et al., 2017), which may to some extent solve not only medical but also environmental problems.

Conclusions

One-, two- and three administrations of nanosilver based on polymer/inorganic hybrids to hens in doses of 0.2 and 0.4 mg per hen per day together with drinking water did not affect water consumption, feed, egg productivity of hens and chemical composition of eggs which characterizes their quality. The nanosilver preparation in carriers based on polymer/inorganic hybrids did not affect the contamination of the surface of the shell and yolks of chicken eggs with MAFnM. The intake of nanosilver in carriers based on polymer/inorganic hybrids by laying hens in the doses of 0.0, 0.2 and 0.4 mg per hen per day caused no accumulation of opportunistic and pathogenic microorganisms of genera *Citrobacter*, *Klebsiella*, and *E. coli*, *P. mirabilis*, *Salmonella* spp., *S. aureus* and *S. epidermidis* on the surface of the shell. In the dose of 0.4 mg per hen per day, the nanosilver preparation in polymer/inorganic hybrid carriers did not affect the number of microorganisms of genus *Bifidobacterium* and caused no significant reduction of the number of bacteria of genus *Lactobacillus* in the manure of hens. The results of the research indicate the safety of the nanosilver drug in carriers based on polymer/inorganic hybrids for laying hens and can be used to determine the effective regime of using nanosilver drugs in poultry farming.

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