Research Article

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Silver (Ag) in tissues and eggshells, biochemical parameters and oxidative stress in chickens

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Abstract: This study aimed to assess levels of silver nanoparticle residues in eggshells and tissues as well as the levels of selected biochemical parameters and oxidative stress indices in chickens hatched from nanosilver disinfected eggs. The samples included 40 Greenleg Partridge chicks allocated into two groups. The experimental group (group D) consisted of chickens hatched from eggs disinfected with a nanosilver preparation prior to incubation, while the control group (group C) included chickens whose eggs were exposed to UV radiation for disinfection. The eggshells and kidney sections obtained from group D chickens showed a significantly higher silver level compared to group C. For the biochemical parameters, only the uric acid content was higher in group D compared to group C. Analysis of the antioxidative stress biomarkers (superoxide dismutase and catalase), showed a significant increase in group D in relation to group C.

Keywords: nanosilver, chicken, oxidative stress, liver, kidney

1 Introduction

Poultry and egg production is one of the most important sectors of Polish agricultural production. Poland is an important and still growing exporter of poultry meat to the European market. Care for the highest possible quality of final product requires the maintenance of high standards of microbiological hygiene by poultry producers. It is absolutely essential to observe a strict hygiene regime in the stages of egg incubation and chicken hatching as it determines the hatching yields. Additionally, eggshell contamination favors the transmission of pathogens inducing respiratory and alimentary diseases in chickens [1]. To maintain high biological safety and hygiene, the disinfection of hatchers and eggs meant for hatching is indispensable. Eggshell disinfection is designed to eliminate microorganisms with minimal interference in embryonic growth. The most common disinfectant used is formaldehyde, a relatively low cost biocide with high effectiveness. However, this agent displays high toxicity, carcinogenicity and irritating activity and, therefore, alternative disinfection procedures that are environmentally friendly and safe are being investigated [2].

Nanometals (Au, Ag, Ti), especially nanometric silver particles, have aroused great hope. The antimicrobial properties of silver combined with nanomaterial properties have given rise to a new generation of disinfectants. According to the studies by Li et al. [3] silver nanoparticles sized above 30 nm showed antimicrobial activity towards Gram-negative and Gram-positive bacteria as well as prevented the development of harmful microorganisms on the tested surfaces.

Currently, with an increase in bacteria resistant organisms and other microorganisms to conventional the disinfecting agents require novel solutions in this field. Effective disinfection procedures and the prevention of harmful microflora growth are the focus of the health service, as well as the agriculture and the food industries.
In animal production, nanosilver is applied for animal disinfection, i.e. claws, hooves and udders. The strong antibacterial, fungicidal and deodorizing properties of nanoscale silver make it effective for the disinfection and the prevention of contamination in animal breeding facilities, especially for bedding, litter and excrement [4]. A study by Gholami-Ahangaran and Zia-Jahromi [5] showed that a nanomaterial-supplemented diet reduces the toxic activity of aflatoxin-contaminated feeds. In the work of Sawosz et al. [6] and Fondevila et al. [7] the application of silver nanoparticles is investigated as a likely alternative to feed additives for stimulating bird growth.

A number of authors have emphasized that nanosilver is a disinfecting agent that is relatively safe for health [2,7,19]. However, its potential use in poultry production needs more thorough studies on its possible toxic effects. Notably, many reports have been published indicating the toxicity of this nanomaterial not only toward bacteria but also mammalian cells, and in humans [8,9].

The unique spatial arrangement and dimension of nanoscale silver contributes to its high bioavailability [3,10]. Nanoparticles are able to readily penetrate biological membranes, which makes them a powerful weapon against microorganisms, yet at the same time, poses a hazard to higher organism cells when they enter and cause serious damage to cellular structures [3]. Silver nanoparticles can induce chromosomal aberrations, mitosis disturbances and changes to cell morphology. The mechanism of the antimicrobial action of nanoscale silver consists of large amounts of ROS (reactive oxygen species) [8,11,12]. Studies on gene expression have also shown that, in cultured human cells, protein coding genes associated with cell defense against oxidative stress are activated by nanostructured silver. This is recognized as one of the crucial mechanisms for silver nanoparticle toxicity [8,13]. Any disturbances in redox homeostasis can lead to serious biological effects, i.e. the initiation of cell type-specific pathways of the inflammatory response and apoptotic death [7,12].

The objective of the present research was to assess levels of silver (Ag) residues in eggshells, tissues and selected biochemical indices and oxidative stress parameters in chicks hatched from nanosilver disinfected eggs.

2 Experimental Procedure

2.1. Animals, experimental design, methodology

This study included 40 Greenleg Partridge chicks allocated into two groups. The experimental group (D) consisted of chicks hatched from the eggs disinfected with nanosilver preparation prior to incubation, while the control (group C) included birds whose eggs were exposed to UV radiation for disinfection for 30 min. The eggs of the control and experimental cells were incubated in two separate incubators. The hatched chickens of the two groups were euthanized by cervical dislocation and decapitation (approved by the Local Ethics Committee on animal experimentation, No. 52/2012). Blood was collected before the chickens were euthanized and organ and tissue sections were taken postmortem (kidneys, liver, pectoral muscles and gastrointestinal tract). The Ag content in chick tissues and whole eggshells was determined by inductively coupled plasma mass spectrometry with excitation (ICP-MS; Varian 820-MS, Mulgrave, Australia). The instrumental conditions for Ag determination by ICP-MS are included in Table 1. Mineralization of the sample solution was performed using a mixture of acids with microwave energy. Drying and burning in the furnace and the ash sample dissolved in nitric acid (V) was performed according to the method accredited CLA/ASA/11/2012 version 3 of 05.11.2012.

Blood plasma was examined using Cormay monotests (Cormay S.A., Lomianki, Poland) to estimate the concentrations of uric acid (UA), urea (UREA), bilirubin (BIL) and creatinine (CREAT). As biomarkers of the antioxidant status, the activity of superoxide dismutase (SOD) and catalase (CAT) was evaluated spectrophotometrically. The SOD activity was assayed using the adrenaline method modified by Misra [14] at a wavelength of 320 nm. The determinations of biomarkers of the antioxidant status were performed using spectrophotometer (UNICAM 939 AA Spectrometer, Labexchange Burladingen, Germany).

The results were analyzed statistically, and the arithmetic mean and standard deviation were calculated (M±SD). The statistical analysis was performed using Student’s t-test at the 5% and 1% significance level (p ≤ 0.05 and p ≤ 0.01).
The eggs were sprayed with colloidal silver at a concentration of 50 ppm. The suspension was obtained using a liquid phase chemical reduction process. The source of silver ions was silver nitrate (AgNO₃), and deionized water was applied to prepare the solutions. The reduction process was conducted in a 4525 PARR pressure reactor at 70–80°C and an elevated pressure. Using sodium pyrophosphate as a stabilizer and glucose as a reducing agent, the silver nanoparticle suspensions were obtained. The suspension contained monodisperse polyhedral nanostructured silver with an average size of 10 nm and an electrokinetic potential ζ = -31.5 mV [15].

3 Results and Discussion

The spectrophotometric studies showed a substantial concentration of silver ions on the eggshells disinfected with nanosilver (p < 0.01) (Tab. 2). As compared to the control group, a higher silver level was also detected in the kidneys of the chicks hatched from these eggs (p < 0.01). In both groups, slight contamination was observed in the pectoral muscle and gastrointestinal tract. There was no influence of the nanosilver preparation on silver bioaccumulation in the liver (p > 0.05).

The biochemical studies performed in this study demonstrated that, of the evaluated biochemical parameters (the concentrations of UREA, BIL and CREAT) were higher in the control group compared to the experimental one (p < 0.01) (Table 3). Additionally, the content of uric acid (UA), which is the major end product of nitrogen metabolism in birds, was also elevated in the experimental group (by 42.5%).

Owing to a lack of analogous studies, these results can be compared only to experiments on the toxicity of oral delivery or injection of nanosilver. A study by Sharma et al. [16] investigating the effects of oral ingestion of different forms of silver on the content of essential elements in chick tissues showed a marked rise in the silver content in the liver, kidneys, spleen as well as in the blood of chickens fed nanosilver-supplemented feed, irrespective of the form of silver supplied. In their research on rats, Kim et al. [8], observed the accumulation of this metal in all the studied tissues, i.e. testicles, kidneys, liver, brain, lungs, stomach and blood; notably, the liver proved to be the main target organ for nanosilver accumulation. Sung et al. [17] also pointed to the liver, as well as the lungs, as the target organs for nanoscale silver. A five-week long experiment by Fondevila et al. [7] conducted on broiler chicks showed substantial bioaccumulation of silver nanoparticles not only in the liver but in muscle tissue as well; the concentration was dependent on the exposure level. In all the studies the authors noted the presence of silver ions also in tissues control animals. This demonstrates that increased use of nanosilver may lead to increased environmental contamination and level of exposure.

The retention of silver in the liver, lungs, and kidneys, after a single intravenous dose of nanosilver has also been studied [18]. For three days, there was a rapid decline

### Table 1: ICP-MS operating conditions.

| Parameters            | Values          |
|-----------------------|-----------------|
| Plasma                | Argon plasma    |
| Plasma flow           | 18.0 L min⁻¹    |
| Auxiliary flow        | 1.1 L min⁻¹     |
| Sheath gas            | 0.2 L min⁻¹     |
| Nebulizer flow        | 0.98 L min⁻¹    |
| Sampling depth        | 6.0 mm          |
| RF power              | 1.45 kW         |
| Pump rate             | 5 rpm           |
| Stabilization delay   | 45 s            |
| First extraction lens | 20 V            |
| Second extraction lens| 187 V           |
| Third extraction lens | 220 V           |
| Corner lens           | 225 V           |
| Mirror lens left      | 35 V            |
| Mirror lens right     | 23 V            |
| Mirror lens bottom    | 50 V            |
| Reference material    | NIST 1577c - Bovine Liver with a silver content of 5.9 µg kg⁻¹ |

### Table 2: Silver content (Ag/µg kg⁻¹) in tissues and egg shells.

| Tissue          | Group D n=20 | Group C n=20 | Significance level |
|-----------------|--------------|--------------|--------------------|
| Kidneys         | 13.02±2.20   | 4.52±2.99    | xx                 |
| Liver           | 0.07±0.01    | 0.07±0.02    | -                  |
| Pectoral muscle | 0.22±0.08    | 0.40±0.23    | -                  |
| Gastrointestinal tract | 0.06±0.03 | 0.18±0.25    | -                  |
| Egg shell       | 0.90±0.27    | 0.05±0.01    | xx                 |

xx –significance at p≤ 0.01
in silver content in liver and lung tissue, but not in the kidneys.

Previous studies have shown that silver nanoparticles are absorbed and metabolized by soft tissues where they display toxic activity and produce macro- and/or microscopic changes [19,20].

However, Korani et al. [21] stated that even high doses of nanosilver (up to 10000 µg mL\(^{-1}\), applied topically) caused neither animal death nor macroscopic lesions in the internal organs. Prolonged dermal exposure to nanoscale particles triggered an inflammatory response manifested by an increased count of Langerhans cells. Additionally, the authors noted a reduced thickness of the reticular dermis together with an elevated collagen level. Nanosilver particles were found to induce microscopic changes within the liver and spleen. A close correlation between dermal exposure and tissue levels of Ag NPs was found (p < 0.05) and tissue uptake happened in a dose dependent manner with the following ranking: kidney > muscle > bone > skin > liver > heart > spleen [22]. In histopathological studies, severe proximal convoluted tubule degeneration and distal convoluted tubule were seen in the kidneys of the middle and high-dose animals.

Studies investigating the effect of applying different levels of silver nanoparticles in feed on hepatic changes in broiler chickens were conducted by Ahmadi et al. [23]. The authors did not observe any significant differences between the control and the experimental groups in relation to the liver tissue structure, fibrosis in the parenchyma, inflammatory cell infiltration or sinusoid congestion. However, they noted that some liver cells showed features of slight necrosis in the group that received the highest silver nanoscale dose (900 mg kg\(^{-1}\)). In another study it was demonstrated that the administration of nanosilver caused depression and necrosis in liver cells [24]. In fact, free radicals from the nanosilver particles have attacked hepatocytes and therefore the urea cycle in liver may be negatively affected; therefore the ammonia produced from proteins catabolism in body cannot convert to urea. Hence the decrease urea concentration in our study may be justified. Bilirubin is a breakdown product of hemoglobin in red blood cells and cleared by the liver where it is taken up into hepatocytes, conjugated and secreted into the bile, which is excreted into the intestine.

Serum uric acid constitutes a valuable source of information on the intensity of protein catabolic reactions and has been frequently mentioned as a marker of oxidative stress in an organism [25]. Increased serum levels of uric acid may result from the adaptive response triggered by an organism to oxidative stress conditions and the production of ROS [26]. It has been estimated that UA is responsible for 60% of the serum total antioxidant status (TAS) [27]. Moreover, the presence of uric acid in serum (at physiological concentrations) prevents the inactivation of extracellular superoxide dismutase by hydrogen peroxide [14,28].

One of the most undesirable effects of nanoscale silver is its non-specific induction of oxidative damage. In the case of enhanced production of factors inducing oxidative stress, an organism uses its physiological defense systems, encompassing endogenous enzyme systems such as superoxide dismutase and catalase [29]. The present study showed a significant rise in the activity of superoxide dismutase and catalase in the experimental group as compared to the control group. Similar trends were observed by Haase et al. [30] in their studies on human cells and by Ahamed et al. [31] who investigated Drosophila melanogaster larvae. In both cases, the authors showed a marked increase in superoxide dismutase and catalase activity in organisms exposed to nanosilver particles. The SOD expression level was dependent on the concentration and exposure time.

### 4 Conclusions

Nanoscale silver exhibits highly potent antimicrobial activity and thus it is commonly applied in daily life, in medicine as well as in animal breeding. The consequences of such exposure, both deliberate and inadvertent, to large populations are currently debated, with little current consensus on the risks, toxicities, risk management and exposure. The toxic mechanism of nanoparticles to organism and the repercussions are still unclear. The present work is another attempt to provide insight into
the influence of nanostructured silver on chicken embryos exposed to nano-Ag disinfection of eggshells. This study has clearly showed that nanosilver stimulate the oxidative stress condition in chicks hatched from nanosilver disinfected eggs. Very intensive development of embryos makes them sensitive to even very small amounts of toxic substances. Disinfection procedures based on this type of preparation are quite effective, yet the effects of nanoscale silver on living organisms still needs thorough studies and evaluation. This study recommended the use of nanosilver as antimicrobial agent in poultry industry with caution and that necessary conditions should be observed.

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