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# THE EFFECT OF CHEMICALLY-SYNTHESIZED SILVER NANOPARTICLES ON PERFORMANCE AND THE HISTOLOGY AND MICROBIOLOGICAL PROFILE OF THE JEJUNUM IN CHICKENS*

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**Abstract**

**The aim of the study was to analyse how *per os* application of hydrocolloids of silver nanoparticles (22 nm) and lipid-coated nanosilver hydrocolloids (5 nm) affect the microbiological status and morphology of the jejunum of broiler chickens and their growth performance. The experiment was conducted on 60 chickens. The first group was the control. The chickens in group II received a silver nanoparticle hydrocolloid (Ag-nano) at a dose of 5 mg/kg b.w./day. The chickens in group III received a lipid-coated nanosilver hydrocolloid (AgL-nano) at a dose of 5 mg/kg b.w./day. Samples of digesta were taken from the jejunum during dissection and the total numbers of fungi, aerobic bacteria and bacteria of the coli group were determined in the samples. Samples of the jejunum were also collected during dissection to determine the length of the villi and depth of the crypts. The silver nanoparticles had no effect on growth performance or the histological picture of the jejunum. An increase was noted in the total number of aerobic mesophilic bacteria and a decrease in the number of coli group bacteria, which are facultative anaerobes, which indicates that the nanosilver had a selective effect on the microflora of the digestive tract in the chickens.**

**Key words: chickens, nanoparticles, performance, jejunum**

The antimicrobial properties of silver and its compounds have been known and exploited for millennia, serving this function until the invention of antibiotics. Re-

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cent years have seen a return to the use of silver as a microbicidal agent in the form of solutions, suspensions and/or nanoparticles. Owing to their characteristic construction resulting from fragmentation, particles in the nanoscale (1–100 nm), such as the noble metals silver, platinum, gold and palladium, acquire new properties that significantly differentiate them from macrostructures constituting the same chemical compound (Nel et al., 2006). This is due to the large number of atoms on the surface of the particle having direct contact with the external environment, which has a significant effect on absorption properties and antibacterial activity. Aqueous solutions containing silver nanoparticles deposited on different media, e.g. silica or polymers, are currently regarded as one of the most effective disinfectants. They have been shown to exhibit activity against numerous pathogenic bacterial strains, even antibiotic-resistant ones, both Gram-negative, including *Acinetobacter*, *Escherichia*, *Pseudomonas*, *Salmonella* and *Vibrio*, and Gram-positive, such as *Bacillus*, *Clostridium*, *Enterococcus*, *Listeria*, *Staphylococcus* and *Streptococcus* (Sawosz et al., 2007; Egger et al., 2009; Prabhu and Poulose, 2012). Silver nanoparticles play a very important role in agriculture and animal production. Nanosilver-based products are used to sterilize tools, equipment in livestock buildings, and packaging and storage places, both for food and for animal waste. Due to their unique antimicrobial, anti-inflammatory and immunostimulatory properties (Małaczewska, 2014), silver nanoparticles could also find application as additives to poultry feed. They are expected to improve the health condition of birds and to increase growth performance. However, unfavourable effects should be expected as well, resulting from their high reactivity and the possibility of inducing oxidative stress, which is essentially the body's inherent reaction to application of nanosilver. The reactions of the organism may differ depending on the magnitude of the oxidative stress generated. It may be a mild antioxidant response, inflammation, or DNA damage and neoplastic changes (Małaczewska, 2010). Few experiments have been conducted on the application of nanosilver in rearing chickens (Sawosz et al., 2007; Pineda et al., 2012 a, b; Ahmadi et al., 2013); moreover, the results of these studies are still inconsistent and do not definitively demonstrate whether silver nanoparticles can be safely applied in poultry. The differing results may be due to the use of nanoparticles with different properties in these studies. Each type of metal nanoparticles, whether produced by electrical, electrochemical or chemical methods, will have different properties depending on the size and the medium.

Therefore it was considered expedient to analyse the manner in which *per os* application of hydrocolloids of silver nanoparticles (22 nm) and lipid-coated nanosilver hydrocolloids (5 nm) affect the microbiological status and morphological picture of the jejunum of broiler chickens and their growth performance.

## Material and methods

### Nanoparticle synthesis

The silver nanoparticle hydrocolloid (Ag-nano) was synthesized according to the method given by Pyatenko (Pyatenko et al., 2007). In this method silver ions

were reduced with trisodium citrate at a temperature of about 100°C, which at the same time served as a stabilizer of the nanoparticles produced. The hydrocolloid of lipid-coated silver nanoparticles (AgL-nano) was obtained in two stages. First the nanoparticles were synthesized according to the method described by Oliveira (Oliveira et al., 2005). This method involves reduction of silver ions by sodium borohydride. Binding of dodecanethiol to the surface of the nanoparticles allows them to be suspended in the organic phase. In order to transfer them to water they were incorporated in hydrophobic phase of single bilayer liposomes, that were prepared by means of Batzri and Korn method (Batzri and Korn, 1973). Injecting an ethanolic solution of phospholipid admixed with dodecanethiol coated nanoparticles into water results in formation of water soluble lipid-silver hybrid nanoparticles (AgL-nano). In the second stage, DPPC (2 mg/ml) was added to nanoparticles (Ag at a concentration of 2 mg/ml) suspended in anhydrous ethanol. Next, the nanoparticles with the lipid were injected into water heated to 50°C, constantly mixed on a magnetic stirrer. The final concentration of silver in the hydrocolloids was 0.1 mg/ml. To test the stability of the hydrocolloids, their absorption was measured immediately after synthesis and 24, 48 and 72 hours later (Figure 1 and 2). Spectra were measured on a Cary 50 UV-Vis spectrophotometer (Varian, Australia). On the basis of photographs taken with a transmission electron microscope (FEI Tecnai G2 T20 X-TWIN and Zeiss LEO 912AB) the average size of the Ag-nano and AgL-nano silver nanoparticles was estimated at 22 nm (Figure 1) and about 5 nm (Figure 2).

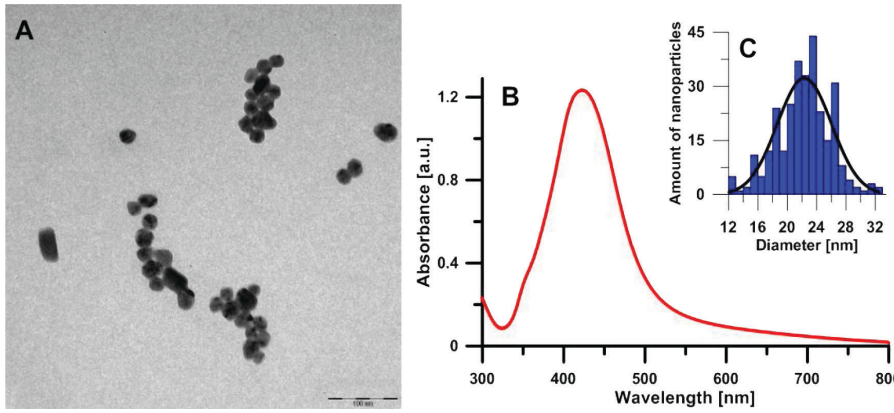


Figure 1. Hydrocolloid of silver nanoparticles (Ag-nano). TEM photo (A). Absorption spectrum (B). Size distribution (C)

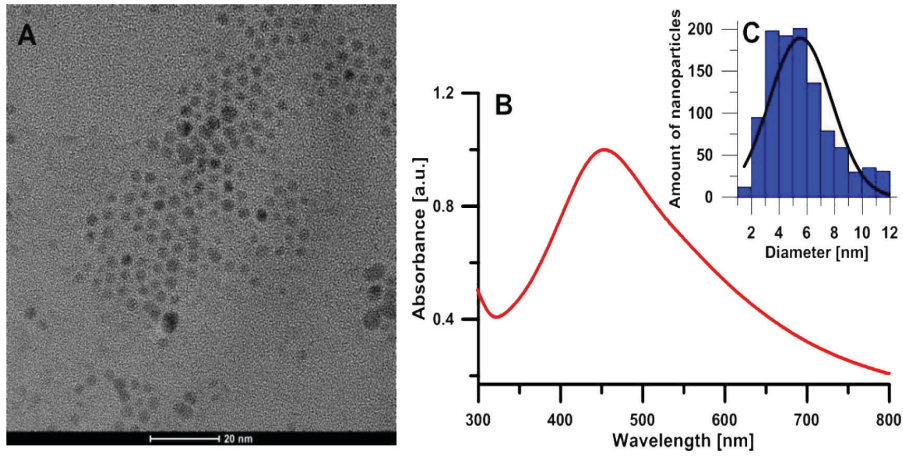


Figure 2. Hydrocolloid of lipid-coated silver nanoparticles (AgL-nano). TEM photo (A). Absorption spectrum (B). Size distribution (C)

**Animals**

The material for the study consisted of day-old Ross 308 chicks raised until their 6th week of life. The study was carried out with the consent of the Local Ethics Commission (2014). The birds were kept in pens on straw litter and reared in standard hygiene conditions in a building with regulated temperature and humidity. The chicks had permanent access to drinking water and received *ad libitum* complete feed mixes appropriate for the rearing period according to the Nutrient Requirements for Poultry (2005). The experiment was carried out on 60 chicks assigned to three experimental groups of 20 each (5 × 4 repetitions). The first group was the control (K), which did not receive silver nanoparticles. The chicks from control group received distilled water via a tube into the crop. The chicks belonging to group II received an aqueous solution of Ag-nano at a dose of 5 mg/kg b.w./day via a tube into the crop. The chicks in group III received an aqueous solution of lipid-coated AgL-nano at a dose of 5 mg/kg b.w./day via a tube into the crop. The Ag-nano and AgL-nano were administered to the chicks on the first three days of their 2nd, 4th and 6th weeks of life, i.e. on days 8–10, 22–24 and 36–38 of life.

**Performance**

During the experiment, the body weight of the broiler chickens was monitored at the end of each week of rearing (all birds were also weighed at the start of the experiment). The Production Number (PN) was calculated on the basis of productive performance according to the formula given by Euribrid (1994):

PN = average liveweight × % survivability/days (duration of fattening) × Feed Conversion Ratio : 10

Feed intake and mortality were monitored during the entire growth period. After the rearing period (6 weeks) 8 chickens from each experimental group were slaugh-

tered according to a procedure approved by the Local Ethics Commission (all individuals in the group/subgroup were weighed and on the basis of the average individuals – 2.35 kg, were selected for further experimental procedures). The slaughtered birds were dissected and the carcasses were analysed during dissection.

**Laboratory analysis**

During dissection of the chicks samples of the digesta were collected from the jejunum into sterile containers for microbiological analysis. In this material the total number of aerobic bacteria was determined on nutrient agar, the total number of yeast and moulds on DG18 medium, incubated for 5–7 days at 25°C, the total number of coli group bacteria on VRBL medium, for 24 hours at 37°C, and the number of *Escherichia coli* on mFC medium, for 18–24 hours at 44°C. Following incubation the colonies were counted and the number was converted to CFU/g. For identification of microorganisms the colonies were evaluated macro- and microscopically and Gram staining was performed. Final identification was carried out using API tests (bioMérieux Polska). All tests were performed according to PN-ISO 4832, PN-EN ISO 7218 and PN ISO 4832. During dissection samples of the jejunum were collected for histological analysis. The samples were cut in two lengthwise and fixed for 24 h in 5% formalin, pH = 7.2. Within 24 hours the fixed tissue fragments were passed through increasing concentrations of alcohol solutions, acetone and xylene into paraffin blocks in a tissue processor (Leica TP-20). Paraffin-embedded microscope sections 5 µm thick were stained with hematoxylin and eosin (HE staining). Morphometric evaluation of the length of the villi and depth of the crypts was carried out using a computer-assisted microscopic image analysis system. The system includes a light microscope (Nikon Eclipse E600) with a digital camera (Nikon DS-Fi1) and a PC with image-analysis software (NIS-Elements BR-2.20, Laboratory Imaging). In each jejunum tissue slide 20 villi cut in two lengthwise and 20 crypts were measured. The length of the villi was measured from the tip to the base.

**Statistics**

Numerical data was processed with analysis of variance (ANOVA) and the results were presented as mean values for groups and standard error (StatSoft Inc., 2009).

## Results

The results presented in Table 1 show that in the period from weeks 14 to 21 of rearing, the chicks in group II receiving Ag-nano gained significantly ($P \leq 0.05$) more weight than the chicks in the remaining groups and attained considerably higher body weights. In the 35 week, however, the rate of growth in this group decreased and the body weight of Ag-nano and AgL-nano was slightly lower than in the control. No significant differences were noted between groups in the average weight gain calculated for the entire fattening period. Optimal rearing conditions ensured a 100% survival rate in the birds, both in the control and in the experimental groups.

There was also no significant differentiation between groups in daily feed intake or the feed conversion ratio in particular weeks of rearing.

Table 1. Performance of the broiler chickens

| Age/Period/Days | I control | II Ag-nano | III AgL-nano | SEM | P-value |
|---|---|---|---|---|---|
| Body weight (kg) | | | | | |
| 1 | 0.045 | 0.045 | 0.045 | 0.0002 | 0.531 |
| 7 | 0.149 | 0.150 | 0.150 | 0.0004 | 0.784 |
| 14 | 0.390 b | 0.400 a | 0.388 b | 0.001 | 0.006 |
| 21 | 0.802 b | 0.818 a | 0.799 b | 0.002 | 0.0004 |
| 28 | 1.298 ab | 1.281 b | 1.302 a | 0.003 | 0.038 |
| 35 | 1.831 | 1.797 | 1.825 | 0.007 | 0.154 |
| 42 | 2.421 | 2.369 | 2.413 | 0.014 | 0.316 |
| Body weight gain (kg/bird) | | | | | |
| 1–21 | 0.756 b | 0.772 a | 0.754 b | 0.002 | 0.0003 |
| 22–35 | 0.533 | 0.516 | 0.522 | 0.006 | 0.557 |
| 36–42 | 0.589 | 0.572 | 0.588 | 0.013 | 0.859 |
| 1–42 | 2.375 | 2.324 | 2.368 | 0.014 | 0.313 |
| Feed intake (kg/bird/day) | | | | | |
| 1–21 | 0.054 | 0.053 | 0.054 | 0.0004 | 0.674 |
| 22–35 | 0.073 | 0.073 | 0.072 | 0.0008 | 0.864 |
| 36–42 | 0.173 | 0.169 | 0.171 | 0.002 | 0.791 |
| 1–42 | 0.099 | 0.097 | 0.098 | 0.004 | 0.464 |
| Feed conversion ratio (FCR) (kg/kg) | | | | | |
| 1–21 | 1.515 | 1.456 | 1.518 | 0.015 | 0.884 |
| 22–35 | 1.940 | 1.983 | 1.946 | 0.025 | 0.191 |
| 36–42 | 2.067 | 2.080 | 2.051 | 0.036 | 0.782 |
| 1–42 | 1.75 | 1.76 | 1.74 | 0.013 | 0.955 |
| Mortality (%) | 0 | 0 | 0 | - | - |
| Survival rate (%) | 100 | 100 | 100 | - | - |
| PN – production number | 329.7 | 319.9 | 329.3 | 4.243 | 0.605 |

a, b – values in rows marked with different letters differ significantly at $P \leq 0.01$; $P \leq 0.05$.

As in the case of growth performance, the introduction of nanosilver in the diet of the chicks did not significantly differentiate the carcass parameters analysed (Table 2). A slightly higher dressing percentage (by about 3.5%) was noted in the control in comparison to the groups receiving silver nanoparticles. The size, position and macroscopic picture of the internal organs, such as the stomach, heart and liver, were similar to macroscopic picture of organs of control group.

The results of the microbiological analyses presented in Table 3 show that the additives affect the abundance of fungi in the digesta of the jejunum of the chicks. Also, the total number of aerobic bacteria in both groups of broilers receiving an aqueous solution of silver nanoparticles was considerably higher than in the control. The use of nanosilver, especially AgL-nano, also caused a slight decrease in the number of coli group bacteria.

Table 2. Results of carcass analysis of the broiler chickens

| Item | I control | II Ag-nano | III AgL-nano | SEM | P-value |
|---|---|---|---|---|---|
| Weight (g) | | | | | |
| body before slaughter | 2421 | 2369 | 2413 | 0.014 | 0.316 |
| carcass after refrigeration | 1798 a | 1636 b | 1738 ab | 0.016 | 0.019 |
| breast muscles | 381.3 | 375.9 | 386.5 | 2.448 | 0.219 |
| thigh muscles | 208.8 | 206.6 | 205.8 | 2.013 | 0.841 |
| shank muscles | 151.8 | 146.5 | 153.9 | 2.284 | 0.428 |
| stomach | 34.9 | 35.5 | 35.3 | 0.386 | 0.820 |
| liver | 46.3 | 49.0 | 46.8 | 0.715 | 0.272 |
| heart | 10.1 | 10.4 | 10.5 | 0.158 | 0.641 |
| adipose fat | 51.1 | 49.0 | 51.9 | 1.450 | 0.738 |
| Dressing percentage (%) | 74.3 | 71.6 | 72.0 | 0.562 | 0.104 |
| breast muscles | 21.4 | 21.1 | 21.7 | 0.137 | 0.219 |
| thigh muscles | 11.7 | 11.6 | 11.4 | 0.122 | 0.262 |
| shank muscles | 8.54 | 8.24 | 8.66 | 0.128 | 0.428 |
| liver | 2.60 | 2.75 | 2.63 | 0.040 | 0.272 |
| stomach | 1.96 | 2.0 | 1.98 | 0.021 | 0.820 |
| heart | 0.57 | 0.58 | 0.59 | 0.008 | 0.641 |
| adipose fat | 2.87 | 2.76 | 2.92 | 0.081 | 0.738 |

a, b – values in rows marked with different letters differ significantly at $P \leq 0.01$; $P \leq 0.05$.

Table 3. Microbiological analysis of the contents of the jejunum of the broiler chickens (CFU/g)

| Parameter | I control | II Ag-nano | III AgL-nano |
|---|---|---|---|
| Total number of fungi | $4.2 \times 10^1$ | $2.7 \times 10^1$ | $2.3 \times 10^1$ |
| Total number of aerobic bacteria | $2.1 \times 10^5$ | $2.6 \times 10^6$ | $2.8 \times 10^6$ |
| Total number of coli group bacteria | $3.5 \times 10^5$ | $2.3 \times 10^5$ | $1.4 \times 10^5$ |

Table 4. Measurements of the villi and crypts of the jejunum of the broiler chickens (μm)

| Parameter | I control | II Ag-nano | III AgL-nano | SEM | P-value |
|---|---|---|---|---|---|
| Mean length of villi of the jejunum | 1029.10 b | 1003.07 b | 1143.70 a | 42.95 | 0.041 |
| Mean depth of crypt of the jejunum | 278.94 b | 284.40 ab | 299.91 a | 9.87 | 0.042 |

a, b – values in rows marked with different letters differ significantly at $P \leq 0.01$; $P \leq 0.05$.

The histological examination of the jejunum samples (Table 4) showed that in group III, which received an aqueous solution of lipid-coated AgL-nano, the mean length of the villi and the mean depth of the crypts were greater than in the control ($P \leq 0.05$). In contrast, in group II, which received an Ag-nano, the villi were slightly shorter than in the control. The differences were small but significant. The shortening of the villi (by about 2%) entailed compensatory growth of the crypts, whose average depth increased (also by about 2%).

## Discussion

The results of the experiment showed that administration of an aqueous solution of silver nanoparticles (with or without a lipid coating) did not affect feed intake or weight gain in the chicks. This is consistent with the observations of other authors, which indicate that the use of silver nanoparticles in birds reared under optimal conditions neither inhibits nor stimulates growth (Sawosz, 2007; Pineda, 2012 b). Ahmadi et al. (2013) administered nanosilver in different concentrations (300, 600 and 900 ppm) to chicks and found that the highest concentration of nanoparticles significantly stimulated growth in the birds while substantially decreasing the feed conversion factor. Partially different results, i.e. a significant decrease in weight gain, were obtained by Pineda et al. (2012 a) in an experiment in which nanosilver (at doses of 10 mg/kg and 20 mg/kg) was administered *in ovo* and during the post-hatch period. The decrease in weight gain was linked to the decrease noted in feed intake in the experimental groups, but the feed conversion ratio was similar to the control. In another study, Ahmadi and Rahimi (2011) reported that birds fed a diet supplemented with a powder of silver nanoparticles had significantly decreased performance compared to the control. The discrepant results obtained by different authors may be due to differences in dosage, the size of the nanoparticles, the method of synthesizing them, or the means of administration. According to Pineda et al. (2012 a), colloidal nanosilver administered in drinking water is less durable than in powdered form. It is also supposed that the beneficial effect of nanosilver as a factor regulating the microbial population in the digestive tract is manifested in the stress conditions prevailing on large farms rather than in optimal conditions, where the quantity of pathogenic microbes is small (Fondevila, 2009). Administration of silver nanoparticles with or without a lipid coating did not significantly affect the carcass characteristics of the chicks, although the dressing percentage in the experimental groups was found to be slightly lower than in the control, possibly due to increased weight of inedible parts, such as the small intestine. It is worth emphasizing here that in the group of birds receiving AgL-nano there was a slight increase in the length of the villi and the depth of the crypts, which unquestionably increased the weight of the jejunum. The differences were small but significant. The available literature contains few data on the effect of nanosilver on carcass parameters of birds, but in a study by Ahmadi et al. (2013) a significant increase in the weight of the liver and the small intestine was observed in broilers that received feed enriched with silver nanoparticles, while the weight of the heart, stomach and pancreas did not differ significantly from the control. According to the authors, the increase in the size of the liver may have been linked to the accumulation of nanosilver in this organ; following absorption into the bloodstream it is known to penetrate various organs, particularly the liver and kidneys (Savolainen et al., 2010). Ahmadi and Rahimi (2011) found silver levels in the edible parts of broilers, such as the breast, thigh and liver, to be about 0.1 mg/kg. The results of the present study found no negative effect of silver nanoparticles on the size and macroscopic appearance of the liver and other internal organs.

The mechanism of the antimicrobial action of silver is linked to its capacity to absorb oxygen and catalyse oxidation reactions (Choi and Hu, 2008). Atomic oxygen

absorbed on the surface of silver ions reacts with active SH (thiol) groups of amino acids in the cell wall of bacteria or viruses, thereby causing sulphur atoms to form -S-S- bonds. Bacteria lose their capacity for respiration because the electron transport 'channels' (running across the cell membrane), i.e. the respiratory chain, are closed. This leads to the death of the bacteria. Owing to the catalytic properties of silver and the production of active oxygen, the genetic material of the bacterial cell also undergoes oxidation, making it incapable of dividing (Choi and Hu, 2008). The results of the present study indicate that silver nanoparticles (Ag-nano and AgL-nano) strongly stimulated the growth of aerobic bacteria living in the jejunum of the chicks. This is unsurprising when we consider that nanosilver is a carrier of oxygen absorbed on its surface. By changing the aerobic conditions prevailing in the lumen of the digestive tract of the chicks, the increase in obligately anaerobic bacteria, and to a lesser degree facultatively anaerobic bacteria, which include *E. coli*, could be slightly inhibited. As no tests were performed in anaerobic conditions, we do not know whether the silver nanoparticles affected other anaerobic bacteria colonizing the jejunum of the chicks, such as *Clostridium*, but it is likely. Lacking more precise microbiological analyses, it is not possible to state whether the nanosilver (Ag-nano and AgL-nano) at the rate applied inhibited growth of useful lactic acid bacteria, which require lower oxygen levels for growth. This possibility is suggested by a study by Pineda et al. (2012 b), in which the antimicrobial activity of silver nanoparticles administered to chicks (at doses of 10 mg/kg b.w. and 20 mg/kg b.w.) was limited to a few species of bacteria, i.e. lactic acid bacteria and lactose-negative enterobacteria, but did not affect caecal numbers of anaerobic, coliform bacteria, enterococci or *C. perfringens*. Another study, by Sawosz et al. (2007), showed that administration of an aqueous solution of silver nanoparticles to quails at doses of 5 and 15 mg/kg b.w. did not substantially affect the microflora of the intestines, although a slight inhibition of the development of *E. coli*, *Enterobacter* and *Streptococcus bovis* bacteria was observed. In contrast, administration of the solution at a dose of 25 mg/kg b.w. strongly stimulated growth of lactic acid bacteria (*Lactobacillus salivarius* and *Lactobacillus fermentum*), but had no effect on the growth of *E. coli*. Thus dosage is seen to play a significant role. *In vitro* studies have shown that even at low concentrations nanosilver inhibits the growth of many bacterial strains, both Gram-positive and Gram-negative, such as *Escherichia*, *Pseudomonas*, *Salmonella*, *Vibri*, *Bacillus*, *Clostridium*, *Enterococcus*, *Listeria*, *Staphylococcus*, *Streptococcus*, methicillin-resistant and vancomycin-resistant *Staphylococcus aureus*, and *Enterococcus faecium* (Sawosz et al., 2007). *In vitro* tests, however, are not always confirmed *in vivo*. It should be kept in mind that bacteria in the digestive tract of animals generally do not occur in planktonic form but in the form of a biofilm, i.e. a spacious, organized structure containing bacteria surrounded by a matrix constructed mainly of polymers of sugars and proteins (extracellular polymeric substances – EPS). Biofilm formation is a natural characteristic of all bacteria making up the microflora of skin and mucous membranes. Pathogenic bacteria penetrating the organism in planktonic form, after the initial stage of adhesion to the host cells, also form a biofilm in the portal of entry (Furowicz et al., 2010). The components of the biofilm matrix protect bacteria against resistance mechanisms (e.g. phagocytosis) and penetration by chemotherapeutics (e.g.

antibiotic substances). The effective therapeutic concentration of some antibiotics has been shown to be over 100 times higher for bacteria enclosed in a biofilm than for planktonic bacteria (Czaczyk and Wojciechowska, 2003). The selective effect of nanosilver on particular bacteria can be supposed to result from differences in their more peripheral or more central position within the biofilm. Researchers point out that the antimicrobial activity of nanosilver is especially manifested in unfavourable animal care conditions rather than optimal ones (Sawosz et al., 2007; Pineda et al., 2012 a). This theory is also confirmed by the number of fungi determined in the jejunum of the chicks studied, which decreased only slightly under the influence of the nanosilver additive. The antifungal properties of nanosilver resulting from its destructive effect on the cell walls of fungi are shown by results obtained by various authors (Gajbhiye et al., 2009).

Modification of materials in the nanoscale, apart from beneficial effects, can also pose new toxicological risks. This is because nanoparticles have much greater chemical, biological and catalytic reactivity than larger particles with the same chemical composition (Nel et al., 2006). It is much easier for them to penetrate biological membranes and reach cells (even cell nuclei), tissues and organs, where they can cause significant damage (Małaczewska, 2010, 2014). There have been reports that high doses of nanosilver are highly toxic not only for bacteria, but also for mammalian cells. Silver nanoparticles have been shown to cause damage to brain cells (Hussain et al., 2006), liver cells (Hussain et al., 2005) and haemocytoblasts (Braydich-Stolle et al., 2005). The authors explain the mechanism of the toxic action of nanosilver by its capacity to generate reactive oxygen species (Hussain et al., 2005) and cause DNA damage in mammalian cells (Braydich-Stolle et al., 2005). *In vivo* tests on rats found no bone marrow damage in the case of long-term (28 days) administration of high doses of silver nanoparticles, but symptoms of moderate liver damage were observed. In a study by Ahmadi et al. (2009) on the effect of silver nanoparticles applied in feed on changes within the liver of broiler chickens, the authors observed slight necrotic changes in the group that received the highest concentration of nanosilver. No pathology, however, was observed with regard to the structure of the liver tissue, fibrosis of the parenchyma or inflammatory cell infiltration. In the present study, histological analysis of the tissue samples from the jejunum of the chicks receiving Ag-nano and AgL-nano showed no deviations from the norm. The villi were slender, finger-shaped and regular, which indicates that the silver nanoparticles had no negative effect on the histological picture of the jejunum. In addition, in the group of birds receiving AgL-nano an increase of about 11% in the mean length of the villi was observed in comparison to the control, as well as an increase of about 7% in the mean depth of the crypts. In a study by Sawosz et al. (2007), administration of a solution of silver nanoparticles to quails in their drinking water had no destructive effect on the intestinal villi. According to the authors, nanosilver can affect the first, outer layer of cells of the intestinal wall, inducing their exfoliation, but without causing destruction of the tissue itself. Here it is worth recalling the presence of the bacterial biofilm, which may offer protection against the destructive effect of various agents, including silver nanoparticles.

## Conclusions

Silver nanoparticles administered *per os*, differing in size and in the presence or absence of a lipid coating, had no effect on the growth performance or carcass parameters of the chicks. Also no negative effect of the nanoparticles was found on the histological picture of the jejunum. The increase noted in the total number of aerobic bacteria and the decrease in the number of coli group bacteria may indicate a selective effect of nanosilver on the microflora of the digestive tract of the chicks. The present findings are only relevant for the nanoparticles used in this study and cannot be generalized. Further research is needed to precisely verify the results obtained.

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