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The Effect of Nanosilver-Based Preparation Added to Litter on Silver and Antagonistic Elements Content in Broiler Tissues and Organs

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Abstract: Nanosilver has a wide range of applications in many industries, including animal production. Its beneficial effects resulting from, among others, antibacterial properties are known; however, some concerns remain related to safety of its use and possible accumulation in the environment and all its components. This study aimed to analyze the content of silver and antagonistic elements (copper, selenium, zinc) in breast and thigh muscles, abdomen skin, liver, lung and blood serum of broilers exposed to a nanosilver-based preparation added to litter. Ross 308 broilers were divided into three groups: control and two experimental (84 birds in each) differing by the amount of preparation addition. The study lasted 24 days, after which the birds were slaughtered and the mentioned samples were collected for laboratory analyses using atomic absorption spectroscopy. An application of preparation based on nanosilver caused an increase in Ag content in chickens' liver, lung and abdomen skin. Cu content decreased in all experimental groups, except for abdomen skin, while Zn content increased in most cases compared to the control. No clear decrease of the increasing tendency was found for Se. It did not demonstrate an antagonism with respect to the content of Cu, Se and Zn in examined samples.

Keywords: silver nanoparticles; vermiculite; chicken meat; accumulation

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1. Introduction

Animal production, including poultry farming, is inextricably linked to environmental impact in the form of odor or microbial pollution, so numerous studies are being conducted on the application of various types of measures that can reduce emerging environmental problems [1,2]. Among the entire pool of available disinfectant and fumigant formulations, silver nanoparticles occupy an important role. Silver in the ionic form and as nanoparticles has been widely used for years as an antimicrobial and disinfecting agent in a wide range of applications, including animal production [3,4].

According to Mahmoud [5], there may be a few reasons that silver nanoparticle applications are used in the poultry industry. The first reason results from antibacterial properties of nanosilver. The author suggests that nanosilver may contribute to oxygen demand, thus leading to an increase in metabolism rate; silver nanoparticles may also affect fibroblast growth factor gene expression; and they can enhance cells' immune activity [5].

Despite proven effectiveness of silver nanoparticles, studies on the safety of its application are still inconclusive and may raise some doubts. The issue with use of nanoparticles is related to their entrance to the environment as well as animal bodies and food. Regardless

of the mode of exposure, whether by inhalation or ingestion, the nanoparticles will be transported to tissues and organs and can cause toxic effects, e.g., to kidneys, spleen, liver or brain [6].

The effect of the nanosilver-based preparation presented in this paper on reduction of ammonia emissions from the broiler house was also demonstrated in our previous paper [7]. Here, we also aim to examine the degree of accumulation of silver and its antagonistic elements, i.e., copper, selenium and zinc, in various tissues and organs of broiler chickens following an application of a nanosilver and mineral carrier preparation to the litter. We hypothesized that despite its beneficial disinfecting effect, the application of nanosilver directly to the litter may carry the risk of excessive accumulation of this element in the chickens' bodies.

2. Materials and Methods

The preparation used in the study was obtained by spraying aqueous nanosilver suspension (Amepox, Łódź, Poland) on mineral sorbent, which was expanded vermiculite (Rominco, Krakow, Poland). An addition of 5% humodetrynite was applied in order to increase sorptive capacity of the preparation. Aqueous nanosilver suspension at a concentration of 1000 mg/kg was sprayed on sorbent in an amount of 100 mL/1 L of sorbent (v/v) at room temperature. This allowed us to obtain a friable preparation of a solid consistency that is easy to use as a litter additive. Detailed characteristics of the preparation are presented in another study [8].

The experimental material consisted of broiler chickens of the Ross 308 line at the age of 2 weeks. The experiment lasted 24 days, i.e., until 38th days of broilers' life. Three research groups were formed, randomly assigning 84 broilers to each group (252 birds in total). Stocking density was about 17 birds per m^2. The chickens were maintained on straw and sawdust bedding with a 1:1 ratio of individual components, while the method of preparation administration was varied in each group:

Control group (C)—without preparation addition;

Group I—addition of the preparation in the amount of 3.7 kg (15 L) under the surface of the litter—once at the beginning of the experiment (1st day);

Group II—addition of the preparation in the amount of 3.7 kg (15 L) mixed with the litter and then added at each supplementation of straw and sawdust, i.e., on the 3rd, 10th, 17th and 24th day of the experiment.

Straw and sawdust were also bedded at the same times in the control and group I—without the addition of the preparation.

At the beginning and end of the experiment (1st and 24th day, respectively), the body weights of the broilers were determined, while throughout the experiment, feed consumption was studied and the number of bird falls was determined. Based on the collected data, standard performance indicators were calculated, i.e., weight gain, feed conversion ratio (FCR) and percentage of dead animals. In addition, for the entire rearing period, the European Performance Efficiency Factor (EPEF) was calculated according to the following formula:

$$\text{EPEF} = [(\text{survival rate (\%)} \times \text{body weight (kg)})/(\text{study period (days)} \times \text{FCR})] \times 100$$

Broilers were fed according to the recommendations of the Poultry Nutrition Standards [9] with GROWER and then FINISHER type complete feed mixes (pellet form) from PROVIMI POLSKA Ltd. (Warsaw, Poland). According to manufacturer data, crude protein level in the mixtures was min. 18% for GROWER, and min. 17.7% for FINISHER mixtures while metabolizable energy level was min. 3000 kcal for both. GROWER mixture was applied until the 33rd day of the birds' life, followed by FINISHER.

The research was conducted with the approval of the 2nd Local Ethical Committee for animal experiments in Wroclaw (Resolution No. 115/2007, dated 22 October 2007).

On the last day of the experiment, 2 mL of blood was collected from the brachial wing vein from eight birds in each group after 12 h fasting. Blood was collected by 2 people, one

immobilized the bird on its back with an upright wing to provide access to the vein; the other collected the blood with a 1.1 mm needle, with the blood dripped freely into a tube. After the serum was centrifuged (MPW-54 laboratory centrifuge (MPW Med. Instruments, Warsaw, Poland, 10 min at 3500 rpm), the contents of silver, selenium, copper and zinc were determined. After the birds were decapitated (following stunning), breast muscles, thigh muscles, lung, abdomen skin and liver were collected for chemical analysis for the content of the same elements. Samples were collected in sterile plastic bags and frozen at −70 °C until analysis.

Samples of collected muscles, organs and blood serum were wet mineralized with nitric acid (spectrally pure, from MERCK Life Science Ltd., Poznań, Poland) in a high-pressure, closed microwave oven MARS-5 (CEM, Matthews, NC, USA) in a 3-stage mineralization process [10]. After cooling to room temperature, the mineralizes were filtered on 0.45 µM Whatman 1 filters and transferred to test tubes and diluted with distilled water to 25 cm^3 and subsequently analyzed for the content of silver, zinc and copper using ETAAS (Spectra AA-110/220, Varian, Australia).

Selenium concentration was determined by hydride-generated atomic absorption spectrophotometry (HG AAS) on the same apparatus, according to the methodology described by Diaz-Alarcon et al. [11] in a closed system.

The above analyses were performed in the laboratory of the Department of Hydrobiology and Aquaculture of the Wrocław University of Environmental and Life Sciences.

The results were statistically analyzed using Statistica 13.0 software package (StatSoft, Kraków, Poland). Mean values of particular element content and standard deviations were calculated. Normality was verified using Shapiro–Wilk test, and the significance of differences between groups was determined using Duncan's test at the significance level of $p < 0.05$ and $p < 0.01$. The correlation between the levels of the analyzed elements, i.e., silver, copper, zinc and selenium in selected tissues and organs of chickens was determined using Pearson's simple correlation.

3. Results

Indicators for the rearing of broiler chickens, i.e., initial and final body weight, mortality, average feed consumption and EPEF index, are shown in Table 1. Initial body weight was slightly higher in chickens of group II compared to the other groups. Body weight gain throughout the experiment was highest in group I, while it was lowest in the control group. Mortality, on the other hand, was at the same level, i.e., 4.8%, in the control group and group II, while it was lowest, at 3.6%, in group I. The average feed consumption was similar in all groups and amounted to 119, 121 and 120 g/head/day, respectively. The EPEF index in the control group was 319.2, while in groups I and II it was at the level of 362.1 and 361.7, respectively. There was a tendency towards better feed utilization by chickens from the experimental groups, which was reflected in a higher EPEF index in these groups.

Table 1. Broiler chicken rearing indicators (average values) [7].

Parameter	Group		
	Control (C)	I	II
Initial number of chickens	84	84	84
Final number of chickens	80	81	80
Mortality (%)	4.8	3.6	4.8
Initial body weight (g)	400.0	399.0	420.0
Final body weight (g)	1730.0	1830.0	1842.0
Weight gain (g)	1330.0	1431.0	1422.0

Table 1. *Cont.*

Parameter	Group		
	Control (C)	I	II
Feed consumption (g/bird)	2856	2904	2880
Feed conversion ratio (kg/kg body weight gain)	2.15	2.03	2.02
EPEF	319.2	362.1	361.7

Tables 2–5 summarize the results showing the content of silver and antagonistic elements, i.e., copper, zinc and selenium, in breast, thigh muscles, lung, liver, abdomen skin and blood serum.

Table 2. Silver content (mg/kg) in tissues and organs of broiler chickens.

Parameter		Ag		
		Control Group (C)	Group I	Group II
Breast muscle	$\overline{X}$	0.178	0.180	0.183
	SD	0.010	0.021	0.018
Thigh muscle	$\overline{X}$	0.198	0.173	0.181
	SD	0.023	0.009	0.045
Lung	$\overline{X}$	0.198 ^{a}	0.201 ^{a}	0.225 ^{b}
	SD	0.007	0.013	0.034
Abdomen skin	$\overline{X}$	0.201 ^{a}	0.213	0.234 ^{b}
	SD	0.035	0.025	0.010
Liver	$\overline{X}$	0.034 ^{A,a}	0.112 ^{a}	0.209 ^{B,b}
	SD	0.011	0.137	0.085
Blood serum	$\overline{X}$	0.021	0.023	0.025
	SD	0.007	0.011	0.011

^{A,B}—values in rows marked with different letters differ at the significance level of $p < 0.01$; ^{a,b}—values in rows marked with different letters differ at the significance level of $p < 0.05$.

The silver content of the tissues and organs of broiler chickens is shown in Table 2. In general, in all cases, with the exception of the thigh muscles, an increase in silver concentration was observed with increasing amounts of nanosilver preparation added to the litter.

The highest content of this element was found in skin taken from the abdominal region, where values ranged from 0.201 ± 0.035 mg/kg in the control group to 0.234 ± 0.010 mg/kg in group II. The differences between the groups were statistically significant ($p < 0.05$). The values observed in the lung were also at similar levels, i.e., from 0.198 ± 0.007 mg/kg in group C to 0.225 ± 0.034 mg/kg in group II. Statistically significant differences ($p < 0.05$) were observed between groups C and I and group II. The largest increase in silver concentration with the amount of nanotechnology-mineral preparation added to the litter was observed in the liver, where the value increased from 0.034 ± 0.011 mg/kg of the control group to 0.209 ± 0.085 mg/kg in group II. Statistically significant differences ($p < 0.05$) were observed between groups C and I, and group II, and between group C and group II ($p < 0.01$). The lowest silver content, on the other hand, was found in chicken serum, ranging from 0.021 ± 0.007 mg/kg in group C to 0.025 ± 0.011 mg/kg in group II. The values observed in the breast muscles were in the range of 0.178 ± 0.010 to 0.183 ± 0.018 mg/kg in the control and group II, respectively. In the thigh muscles, the silver content showed

a decreasing trend in the groups with the addition of nanosilver, i.e., from a value of 0.198 ± 0.023 mg/kg in group C to 0.173 ± 0.009 mg/kg in group I.

Table 3. Copper content (mg/kg) in tissues and organs of broiler chickens.

Parameter		Cu		
		Control Group (C)	Group I	Group II
Breast muscle	$\overline{X}$	0.875	0.934 ^{a}	0.780 ^{b}
	SD	0.088	0.064	0.163
Thigh muscle	$\overline{X}$	1.236	1.103	1.060
	SD	0.286	0.059	0.243
Lung	$\overline{X}$	1.308 ^{a}	1.135 ^{b}	1.298 ^{a}
	SD	0.084	0.173	0.078
Abdomen skin	$\overline{X}$	0.452 ^{A,a}	1.321 ^{B,b}	1.338 ^{B,b}
	SD	0.055	0.103	0.103
Liver	$\overline{X}$	2.640 ^{A,a}	1.980 ^{B,b}	2.348 ^{A,a}
	SD	0.263	0.452	0.422
Blood serum	$\overline{X}$	0.347 ^{A,a}	0.303 ^{A,a}	0.252 ^{B,b}
	SD	0.080	0.050	0.052

^{A,B}—values in rows marked with different letters differ at the significance level of $p < 0.01$; ^{a,b}—values in rows marked with different letters differ at the significance level of $p < 0.05$.

Copper levels ranged from 0.303 ± 0.050 mg/kg in serum in group I to 2.640 ± 0.263 mg/kg in the liver of control birds. In most cases, a decrease in copper concentration was observed in the experimental groups compared to the control. The exception was the abdomen skin, where copper levels were more than three times higher in groups I and II compared to the control group (0.452, 1.321 and 1.338 mg/kg, respectively). The differences between the groups were statistically significant.

Table 4. Selenium content (μg/kg) in tissues and organs of broiler chickens.

Parameter		Se		
		Control Group (C)	Group I	Group II
Breast muscle	$\overline{X}$	34.77 ^{A,a}	73.97 ^{B,b}	68.31 ^{B,b}
	SD	21.93	3.29	9.28
Thigh muscle	$\overline{X}$	84.54 ^{A,a}	88.98 ^{A,a}	54.30 ^{B,b}
	SD	9.94	6.77	13.79
Lung	$\overline{X}$	238.22	188.84	222.67
	SD	63.84	28.71	56.15
Abdomen skin	$\overline{X}$	120.85 ^{A,a}	159.68 ^{B,b}	147.08
	SD	33.76	21.43	20.31
Liver	$\overline{X}$	392.36 ^{A,a}	266.27 ^{B,b}	249.84 ^{B,b}
	SD	57.38	71.58	54.86
Blood serum	$\overline{X}$	178.01	194.33	193.57
	SD	44.05	49.10	53.43

^{A,B}—values in rows marked with different letters differ at the significance level of $p < 0.01$; ^{a,b}—values in rows marked with different letters differ at the significance level of $p < 0.05$.

Table 5. Zinc content (mg/kg) in tissues and organs of broiler chickens.

Parameter		Zn		
		Control Group (C)	Group I	Group II
Breast muscle	$\overline{X}$	4.52 [a]	4.32 [a]	5.27 [b]
	SD	0.62	0.44	0.97
Thigh muscle	$\overline{X}$	7.89	7.46	9.37
	SD	2.94	0.89	2.07
Lung	$\overline{X}$	7.94	8.02	8.57
	SD	0.82	2.81	0.86
Abdomen skin	$\overline{X}$	7.20	7.60	7.72
	SD	1.17	1.09	1.00
Liver	$\overline{X}$	23.48	22.68 [a]	28.42 [b]
	SD	4.91	7.60	4.90
Blood serum	$\overline{X}$	1.17	1.40	1.49
	SD	0.33	0.40	0.20

[a,b]—values in rows marked with different letters differ at the significance level of $p < 0.05$.

In the case of the breast muscles, the copper content of the control group was lower compared to group I, however, higher than in group II. The difference between group I and group II was statistically significant at $p < 0.05$. Statistically significant differences were observed for all tissues and organs, with the exception of thigh muscles.

The highest selenium concentration was observed in the liver of the control group chickens (392.36 ± 57.38 μg/kg), while the lowest was observed in the breast muscles of the control group (34.77 ± 21.93 μg/kg). The few-fold lower selenium content was found in the breast and thigh muscle samples compared to the other samples. The most pronounced changes within the groups were observed in the breast muscles (in the range of 34.77–73.97 μg/kg), while the lowest variation in selenium content was in the blood serum (178.01–194.33 μg/kg). Statistically significant differences between groups were observed in all samples except lung and blood serum.

For breast muscle, abdomen skin and blood serum, selenium concentrations were higher in the experimental groups compared to the control group (Table 4). In samples taken from the lung, selenium concentrations decreased in the experimental groups compared to the control group, but were lowest in group I, where a smaller amount of nanosilver preparation was applied.

Zn concentrations ranged from 1.17 ± 0.33 mg/kg in serum in the control group to 28.42 ± 4.90 mg/kg in the liver of group II birds. In most cases, an increase in zinc concentration was observed in the experimental groups compared to the control. The exceptions were breast muscle, thigh muscle and liver samples in group I, where zinc concentrations were lower than in the control group, while they were highest in group II.

The next table shows the correlations between the content of the analyzed elements, i.e., silver, copper, selenium and zinc, in selected tissues and organs of broiler chickens (Table 6).

An antagonistic effect against silver was observed only for selenium in the liver, where the correlation coefficient was −0.6227 ($p < 0.05$).

Table 6. Pearson correlations between the content of individual elements in selected tissues and organs of chickens.

		Breast Muscles		
	Ag	Cu	Se	Zn
Ag	1.0000	0.3833	0.2574	0.0482
Cu		1.0000	0.1403	−0.3668
Se			1.0000	0.0332
Zn				1.0000
		Thigh Muscles		
	Ag	Cu	Se	Zn
Ag	1.0000	0.3782	0.0291	−0.0636
Cu		1.0000	0.3797	0.2991
Se			1.0000	−0.0546
Zn				1.0000
		Lung		
	Ag	Cu	Se	Zn
Ag	1.0000	0.3709	0.0953	0.1217
Cu		1.0000	0.3441	0.5754 *
Se			1.0000	0.1852
Zn				1.0000
		Abdomen Skin		
	Ag	Cu	Se	Zn
Ag	1.0000	0.4401 *	0.4248 *	0.0548
Cu		1.0000	0.5736 *	0.3280
Se			1.0000	0.4719 *
Zn				1.0000
		Liver		
	Ag	Cu	Se	Zn
Ag	1.0000	−0.2942	−0.6227 *	0.2102
Cu		1.0000	0.6836 *	0.1969
Se			1.0000	0.0047
Zn				1.0000
		Blood Serum		
	Ag	Cu	Se	Zn
Ag	1.0000	0.0219	0.0306	0.1738
Cu		1.0000	0.0087	−0.5342 *
Se			1.0000	0.3509
Zn				1.0000

* Correlation coefficients are significant at the $p < 0.05$.

4. Discussion

There is little data in the available literature on the silver content of the tissues and organs of broiler chickens analyzed in this study.

Sharma et al. [12] analyzed the effect of ingesting various forms of silver with food on the content of selected chemical elements in chickens' tissues. The silver content in blood

was 0.05–1.2 µg/g *w*/*w*, while that in liver ranged from 0.19 to 1.9 µg/g, depending on the form of silver taken in. Nabinejad et al. [6] examined silver residue accumulation in broilers after nanosilver application, and the values recorded by them were as follows: breast muscles—168 µg/kg, thigh muscles—172 µg/kg, liver—185 µg/kg, skin—194 µg/kg, and lung—160 µg/kg. In turn, after the use of colloidal silver instead of antibiotics, silver content in thigh muscles was about 0.06 µg/g, in breast muscle it was about 0.07 µg/g, while in liver about 0.065 µg/g [13]. This study demonstrated that silver level following colloidal silver application instead of antibiotics was comparable in control and experimental groups, except for chest muscle where it was three-fold higher [13]. Another study demonstrated silver content in breast muscle at a level of 0.048 mg/kg, in thigh muscle at 0.008 mg/kg and in liver at 0.012 mg/kg [14]. Silver migration from silver-based materials used for packaging to chicken meat was also confirmed by das Neves et al. [15].

The results obtained in the present study on the content of silver in the breast muscles, thigh muscles, lung, abdomen skin, liver and blood serum of broiler chickens proved that the use of the addition of a nanotechnology-mineral preparation based on an aqueous suspension of nanosilver caused an increase in the concentration of silver in the tissues and organs of the chickens studied. This is not a large increase; however, in the case of the liver the difference was significant, which may indicate the need for further research in this area to determine the safe limits of nanosilver application as an effective and safe disinfectant in animal husbandry. Literature reports also suggest that main sites of silver accumulation may be liver, lung and blood [12,16,17].

In the available literature, one can find far more work on the copper content of animal tissues and organs than was possible with silver. In a study conducted by Kalisinska et al. [18] on wild ducks living in the northwestern part of Poland, copper content in liver was observed to range from 20.04 to 34.14 µg/g *w*/*w*, depending on the age of the ducks and their habitat. The concentration of this element in the breast muscles, on the other hand, ranged from 4.04 to 6.23 µg/g. Szymczyk and Zalewski [19], in turn, observed copper concentrations in the liver and breast muscles of wild birds from the Warmia and Mazury region at levels of 5.937–9.899 mg/kg and 4.128–7.327 mg/kg *w*/*w*, respectively. The serum copper content of chickens given various copper salts was 0.27–0.52 µg/mL [20]. Copper concentrations recorded by Benito et al. [21] in the blood of wild birds from the vicinity of the Doňana National Park in Spain located in the area of influence of metal ore mines ranged from 0.133–0.586 mg/L. The copper content in the blood of chickens observed by Sharma et al. [12] was 0.2–1.1 µg/g *w*/*w*, while in the liver it was 1.8–3.0 µg/g *w*/*w* and clearly decreased with increasing silver concentration, confirming the antagonistic effect of both elements. The concentration of copper in the blood serum of laying hens ranged from 3.4–5.3 µmol/L, depending on the housing system [22]. Dmoch and Polonis [23], on the other hand, studied the effect of copper biocomplex on the content of selected minerals in the blood of broiler chickens and observed copper concentrations of 0.0053–0.005 µmol/L. A study by Kołacz et al. [24] showed that the concentration of copper in the muscles of hens in the Copper Belt region averaged 0.90 mg/kg, while in the liver it was 5.62 mg/kg *w*/*w*.

The copper content observed in the present study was within the ranges reported by other authors. The antagonism of silver to this element has not been conclusively proven.

Selenium is an element that has received a lot of attention in science, among others, due to its role in nutrition. Numerous authors have analyzed the effects of various factors on selenium levels in the tissues and organs of animals.

Payne and Southern [25] conducted a study to determine selenium concentrations in the tissues of broilers given various selenium supplements. They observed selenium levels in plasma ranging from 0.019 to 0.129 mg/kg, in breast muscle between 0.25 and 1.2 mg/kg, while selenium concentrations in liver ranged from 0.4 to 2.3 mg/kg. In contrast, Zoidis et al. [26], administering selenium in organic form to broilers, recorded the content of this element in the livers of birds at 491–3271 ng/g, while in blood the concentration ranged from 88–1249 ng/g. Wang and Xu [27], on the other hand, analyzed the effects of different sources of selenium (sodium selenite and selenium yeast) on broiler chickens.

The authors observed a selenium content of 0.19–0.30 μg/g in muscle, while in liver the concentration ranged from 0.34 to 0.68 μg/g, depending on the source of selenium in the diet. A study on the effect of different levels of selenium in the diet of broiler chickens on the content of this element in organs was conducted by Mihaljev et al. [28]. The selenium concentration in the liver was in a wide range from 9.0 to as much as 570.8 μg/kg, in the blood from 8.5 to 430.3 μg/kg, while in the meat the selenium content was the lowest and did not exceed 160.0 μg/kg. Studying the dependence of serum concentrations of selected elements in laying hens on the housing system, Pavlik et al. [22] recorded selenium levels in the range of 1.65–3.25 μmol/L. In contrast, Leeson et al. [29] analyzed the effect of organic selenium supplementation to broiler diets on the content of this element in animal tissues. The selenium concentration they observed was 0.156–0.173 μg/mL for blood serum, 0.44–0.48 μg/g for liver and 0.162–0.174 μg/g for breast muscle.

Therefore, based on data in the literature, it can be concluded that the results obtained in the presented work regarding selenium content in tissues and organs of broiler chickens are within the ranges reported by other authors. In addition, it should be emphasized that no antagonistic effect of silver on selenium concentration was found.

The literature presents a wide range of zinc concentrations in tissues and organs of various animal species.

In ducks from the region of northwestern Poland, the concentration of zinc in the liver ranged from 34.88–49.78 μg/g *w*/*w*, while the content of this element in the breast muscles ranged from 11.99 to 12.97 μg/g *w*/*w* [18]. Szymczyk and Zalewski [19], on the other hand, observed zinc concentrations in the liver of wild birds of 15.393–27.426 mg/kg *w*/*w*, while in the breast muscles they ranged from 8.739 to 20.093 mg/kg *w*/*w* The serum zinc content of broiler chickens whose diets were supplemented with phytate ranged from 1.09 to 2.06 mg/L, depending on the type and dose of supplement [26,30]. Kaya et al. [31] conducted a study on laying hens whose diets were supplemented with zinc and vitamin A. They observed liver zinc concentrations ranging from 49.55 to 69.04 μg/g *w*/*w* depending on the type and dose of supplementation. Mondal et al. [20] observed serum zinc concentrations in chickens fed diets supplemented with copper salts at 2.00 to 2.40 μg/mL. In the muscles of hens from the Copper Belt, the average concentration of zinc was 10.45 mg/kg, while in the liver the value was 41.97 mg/kg [24]. Cao et al. [32] recorded the concentration of zinc in the liver of chickens given zinc supplements at 5.1–14.4 μg/g, depending on the type of supplement. Zinc concentrations in the blood serum of wild birds from an area exposed to metal ore mines ranged from 0.9 mg/L to 4.4 mg/L [21]. The concentration of zinc in the blood of chickens observed by Sharma et al. [12] ranged from 1.5–7.2 μg/g *w*/*w*, while in the liver it ranged from 9.0–18.6 μg/g. The concentration of zinc in the serum of laying hens was determined to be about 50–83 μmol/L. The zinc content in the blood of broiler chickens observed by Dmoch and Polonis [23] was in the range of 0.036 to 0.04 μmol/L. Herzig et al. [33] analyzed the degree of zinc accumulation in the tissues of broiler chickens as a result of feeding them humic acid supplements. Their results indicated Zn content in the thigh muscles at 40.1–62.1 mg/kg, in the liver in the range of 72.2 up to 430.5 mg/kg while in the blood serum in the range of 1.04–4.37%. Bartlett and Smith [34] administering different levels of zinc supplement to broiler chickens observed serum levels of this element in the range of 177.33–220.49 μd/dL. According to Stef and Gergen [35], the main site for Zn deposition in chicken meat is legs and breast. Also, liver is known as a site of trace minerals, including Zn, deposition in chickens [36].

The values for zinc content in broiler tissues and organs obtained in the present study are within the limits reported by other authors. As in the case of selenium and copper, no antagonism of silver to this element was demonstrated.

5. Conclusions

In summary, an application of preparation based on nanosilver suspension to broiler litter caused an increase in Ag content in chickens' liver, lungs and abdominal skin. It did not demonstrate an antagonism with respect to the content of Cu, Se and Zn in examined

samples. Despite the known bactericidal and disinfectant properties of nanosilver, it is important to keep in mind the possibility of this element's accumulation in living organisms and its possible antagonistic effect against other elements. Further research in this aspect are needed.

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