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Microstructure of the kidneys and liver of naked neck chickens under the influence of silver nanoparticle preparation

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ABSTRACT

The ban or restriction on the use of antibiotics in livestock, particularly in poultry farming, has prompted the search for alternatives with bactericidal activity and enhanced productivity. Such means include preparations obtained using nanotechnology, in particular, nanosilver. To determine the effectiveness of nanosilver in chicken farming, two groups of 25 day-old Naked Neck chickens were formed. The nanosilver preparation was administered to the chickens in the experimental group at a rate of 0.4 mg/l in water for 14 days of rearing. It was found that the nanosilver preparation increased the body weight of the chickens by 19.8%, the kidney weight by 10.3%, and the length by 4.6%. No significant effect of nanosilver on the structure of nephrocytes and nephron tubules and morphometric indicators of the kidneys was detected, but in some cases, signs of a slight increase in the lumen of the renal corpuscle capsule and desquamation of epithelial cells of the renal tubules were recorded. In some areas, cystic dilatation of the renal parenchymal tubules and moderate vascular filling were observed. Under the influence of nanosilver, the absolute mass of a chicken liver increased by 8.7%, the volume of hepatocytes by 11.8%, and the nucleocytoplasmic ratio of hepatocytes by 8.4%. The cytoplasm of hepatocytes of the experimental group of chickens had a looser consistency than that of the control birds, and the sinusoidal capillaries were dilated. In some cases, focal infiltration with polymorphic cells, vessel dilation, and blood filling of the vessels, forming hepatic triads, were observed around the vessels. The regenerative function of the liver in chickens under the influence of nanosilver was characterised by the presence of both mononuclear and binuclear cells. The results of the studies show that feeding Naked Neck dual-purpose chickens nanosilver preparation at a dose of 0.4 mg/l did not cause a pronounced toxic effect on the microstructure of the liver and kidneys, and increased body weight. This could provide a rationale for further studies on the quality and safety of poultry slaughter products when nanosilver is used as a productivity enhancer and bactericidal agent.

Keywords: silver nanocompound, chickens, liver, kidneys, microstructure.

INTRODUCTION

Chicken is among the most consumed meats worldwide due to its affordability and nutritional properties [1]. Therefore, not only are crosses of broiler chickens used for their production, but also chickens for meat and egg production [2]. Large and small producers of chicken and table eggs have faced, and continue to face, the challenge of protecting livestock from infectious and invasive pathogens that cause high mortality and significant economic losses [3], and [4]. This is facilitated by intensive poultry production systems, in which birds are exposed to negative impacts from technological, feed, transport, and post-vaccination stresses [5], and [6]. In addition, the large concentration of poultry in a limited area contributes to the spread of many infectious

and invasive diseases that suppress the level of natural resistance of their body, which leads to various pathologies, reduced productivity and efficiency of the industry as a whole [7], [8].

The use of antibiotics at subtherapeutic doses for this purpose has long been used to prevent disease and improve chicken productivity; however, this practice has led to the emergence of resistance among pathogenic and conditionally pathogenic microorganisms to a wide range of antimicrobial drugs. For this reason, the widespread use of antibiotics as growth promoters, particularly in poultry, was banned by the European Union in 2003 [9]. This prompted the search for alternatives to antibiotics, which, along with probiotics [10], [11], [12], have led to increased interest in nanotechnology products, in particular the use of silver nanoparticles (AgNPs), which have antimicrobial activity [13], [14], and [15].

Against the background of the growing threat of antimicrobial resistance in foodborne pathogens that cause dangerous human toxicoinfections, such as *Escherichia coli*, *Salmonella enterica*, and *Listeria monocytogenes*, there is a need for a detailed and in-depth analysis of the impact of nanosilver on the body of animals, especially birds [16], [17].

The use of AgNPs involves various routes of administration in poultry, including via drinking water and feed, and is considered not only an alternative to antimicrobial drugs but also a performance enhancer [18], [19], [20]. This effect has been demonstrated in numerous studies across various productive poultry crosses. Thus, in the study [21], it was shown that silver-doped silica nanoparticles (SiO_2 and AgNPs), when added to the poultry diet at doses of 2, 4, and 8 mg/kg, were safe for the body. Evaluation of growth indicators, haematological, biochemical, and antioxidant characteristics of body tissues showed that they can be considered as a promising and safe nanostimulant for broiler growth when added to the poultry diet. These studies indicate that silver nanoparticles exhibit strong antimicrobial activity against pathogenic microorganisms and can be used as disinfectants on poultry farms. Several studies have demonstrated the antimicrobial efficacy of nanosilver against pathogenic agents in poultry. This is evidenced by the reduction in colonisation of the poultry intestine by pathogenic *Escherichia coli*, which reduced chicken mortality [22]. In other studies, the antiparasitic effect of nanopreparations against *Haemonchus contortus* larvae has been confirmed [23].

Given that nanomaterials, such as silver, can accumulate in the meat, eggs, and internal organs of productive poultry when ingested, a risk assessment of poultry products for consumers is provided [24]. Silver is a heavy metal, and therefore the toxicity of its nanoparticles in the poultry body depends on various chemical and physical factors, especially the composition of the feed ration. The changes to which silver nanoparticles are subjected in the digestive system of poultry include agglomeration/aggregation under the influence of gastric juice, dissolution under acidic pH conditions, and the presence of oxygen in the environment, as well as sulfuration due to interaction with sulfur-containing compounds. On the other hand, reports indicate that the presence of silver traces in chicken meat and organs does not pose a danger to the human body [25].

At the same time, the introduction of new nanoadditives into poultry feed requires appropriate studies to objectively assess the impact of nanomaterials not only on productive animals but also on the quality and safety of food products [26], [27], and [28]. For predicting the safety of nanoparticles of different elements, the European Food Safety Authority (EFSA) [29] has proposed a structured approach to assess the safety of nanomaterials in food and feed for human and animal health. One of the important steps outlined in this document is the *in vivo* study, which assesses nanomaterial absorption, distribution, accumulation, and excretion from the body, as well as toxicity [30], [31]. Most studies on the toxicity of silver nanocompounds focus on determining their residues in chicken meat and eggs, which helps assess their entry into the human body.

However, the functional state of internal organs is often overlooked by researchers, thereby impeding an objective assessment of the benefits and safety of silver nanocompounds for productive poultry. One of the criteria for assessing the safety of silver nanocompounds for poultry is the study of the microstructure of vital organs involved in detoxifying harmful feed components, particularly the liver and kidneys. This will enable objective assessment of the risks associated with the use of nanosilver in broiler chicken rearing for meat production, as well as substantiation of the application regime and dosages that are safe for poultry and slaughter products. Therefore, our study aimed to examine the microstructure of the liver and kidneys in Naked Neck chickens following administration of a nanosilver solution in water.

Scientific Hypothesis

Feeding Naked Neck dual-purpose chickens nanosilver as a growth stimulant during the 14 days of life will increase productivity and preserve the microstructure of the liver and kidneys during growth.

Objectives

To determine changes in the microstructure of the liver and kidneys of chickens of the Naked Neck dual-purpose poultry breed in the first 14 days of growing under the influence of nanosilver. Auxiliary tasks: to

analyse body weight and the main macro- and microscopic characteristics of the morphometry of structural components of the liver and kidneys after feeding chickens a nanosilver preparation.

MATERIAL AND METHODS

Samples

Samples description: The liver and kidneys of Naked Neck dual-purpose chickens were examined in the experiment. To study the microstructure of the liver and kidneys of Naked Neck chickens following oral administration of a nanosilver preparation, ten birds from both the control and experimental groups were euthanised after 14 days of the experiment.

Samples collection: For microscopic research, the liver and kidneys were separated from the thoracoabdominal cavities immediately after slaughtering the control and experimental chicken groups (10 chickens each) on day 14 of the research.

Samples preparation: For histological studies, pieces of liver and kidneys up to 1 cm³ in size were selected, fixed in a 10–12% solution of neutral formalin, then placed in sealed containers and sent to the laboratory for histostructural studies.

Number of samples analysed: A total of 10 livers and 10 pairs of kidneys from chickens in the control group, and 10 livers and 10 pairs of kidneys from chickens in the experimental group, were used.

Chemicals

Neutral formalin (Khimlaborreaktyv LLC, Ukraine) Ethyl alcohol (Khimlaborreaktyv LLC, Ukraine) Hematoxylin (Diapath, Italy, 2020)

Eosin (Leica Geosystems, Germany, 2020) Paraffin (Khimlaborreaktyv LLC, Ukraine)

Animals, Plants and Biological Materials

The study used 50 Naked Neck dual-purpose chickens, divided into two groups according to the principle of analogy: control and experimental, with 25 in each.

Instruments

Zetasizer particle distribution analyser Nano ZS (Malvern Instruments, United Kingdom, 2015) CAM V–200 video camera (InterMed, PRC, 2017);

Microscope Micros MC-50 (InterMed, PRC, 2017) microscope MBS-10 (Micromed, Ukraine, 1998);

Sledge microtome MS-2 (PP "StandartPribor", Ukraine, 2015).

Laboratory Methods

Synthesis of silver nanoparticles. Silver nanoparticles were synthesised using the classical citrate method. For this purpose, 0.0425 g of AgNO₃ (1 mM) was dissolved in 250 ml of distilled water, and the solution was brought to a boil on a hot plate with a magnetic stirrer. After boiling, 5 ml of a 1% (w/v) sodium citrate solution was rapidly added to the hot solution, and the mixture was boiled for 30 minutes until a stable yellow-brown colour developed. The suspension was cooled to room temperature and stored in a dark glass at 4°C. The particle size and morphology were monitored using a Zetasizer Nano ZS particle distribution analyser. The nanosilver preparation was characterised by a nanoparticle diameter of 84.78 ± 1.54 nm, a polydispersity index of 0.23 ± 0.015, and a zeta potential of 22.03 mV, indicating high stability of the colloidal system [32].

Microstructural analysis of chicken liver and kidney: For macroscopic and organometric analysis, the liver and kidneys of the chickens were dissected from the thoraco-abdominal cavity, and their linear dimensions, absolute and relative masses, both overall and of their components, were determined [33].

The absolute mass (AM) of the organs was determined by weighing. The relative mass (RM) was calculated using the formula (1):

$$RM = AM/MT*100\% \quad (1)$$

Where AM is the absolute mass (AM) of the organ; MT – mass of the chicken.

The linear parameters of the organ (length, width) were determined by direct measurement. The liver development index (LDI) was determined by the ratio of its total length to its width using the following formula (2):

$$LDI = LL/LW*100 \quad (2)$$

Where LL – organ length; LW is the width of the organ.

For microscopic analysis, tissue samples of up to 1 cm³ in volume were collected, fixed in a 10–12% neutral formalin solution, and subsequently embedded in paraffin [33]. After fixation and washing, the liver and kidney slices were passed through alcohols of increasing strength (40°, 60°, 70°, 80°, 96° and 100°) and xylene and

embedded in paraffin. Subsequently, histological sections were cut from the paraffin blocks using a sledge microtome MS-2 at a thickness of 8–10 μm .

For the study of morphology at the tissue and cellular levels, as well as for histo- and cytometric analysis, the histological sections were 283eparaffinized and stained with haematoxylin and eosin [33]. Microscopy of sections and histometric studies of structural elements of tissues were performed using a Micros MC-50 microscope.

The volume of renal corpuscles, hepatocytes, and their nuclei was determined using the formula (3):

$$V = \pi/6 * A * B^2 \quad (3)$$

Where: V is the volume of a hepatocyte (nuclei), π – 3.14, A – length hepatocyte (nuclei), B is the width of the hepatocyte (nucleus).

Nucleocytoplasmic ratio was determined using the following formula (4):

$$\text{N:C ratio} = \text{Nuclear volume} / (\text{Cell volume} - \text{Nuclear volume}) \quad (4)$$

Feed conversion was also calculated during the rearing of chicks. This parameter was determined for the experimental period using the following formula (5):

$$C = M/P \quad (5)$$

C – feed conversion ratio; M – mass of feed consumed during a specific rearing period, kg; P – body weight gain of chicks during the same rearing period, kg.

Description of the Experiment

Study flow: Naked Neck chickens were formed according to the principle of analogues: control and experimental - 25 chickens in each group (Table 1).

Growing and keeping chickens was carried out on litter. From day one, the chickens were fed a basal diet that met their nutritional and biologically active nutrient requirements. Access to drinking water was not limited (drinking was carried out using cup drinkers).

Chickens in the experimental group were given a nanosilver preparation at a dose of 0.4 mg/l in their drinking water daily starting from day 14 of the rearing period.

Table 1 Study design.

Groups of chickens	n	Experimental conditions (14 days)
Control	25	Basal diet
Experimental	25	Basal diet + nanosilver preparation with drinking water at a dose of 0.4 mg/l

To study the macro- and histological architecture of the organs under the influence of the nanosilver preparation, 10 chickens from the control and experimental groups were slaughtered slaughtered at 14 days following oral administration via drinking water. Before slaughter, the chickens were stunned with an electric current using a Le Reve poultry stunning device (FAF, France).

Quality Assurance

Number of repeated analyses: Ten samples were used in each analysis.

Number of experiment replication: 1.

Reference materials: -

Calibration: Each instrument was calibrated prior to each experiment, and calibration checks were performed regularly to ensure measurement accuracy. Each instrument was calibrated before each experiment, and calibration checks were performed periodically to maintain measurement accuracy.

Laboratory accreditation: The experiments were conducted based on the Ukrainian Laboratory of Quality and Safety of Agricultural Products, which is managed through the implementation of a management system built (since 2007) following the requirements of DSTU EN ISO/IEC 17025:2019 ((EN ISO/IEC 17025:2017, IDT; ISO/IEC) 17025:2017, IDT) and confirmed by the Accreditation Certificate of the National Accreditation Agency of Ukraine.

Data Access

The data supporting the findings of this study are not publicly available

Statistical Analysis

The obtained digital data were analysed using variational statistical methods, including one-way analysis of variance, in Microsoft Excel 2021. The normality of the sample distribution and the homogeneity of variances were assessed using the XLSTAT software, applying the Shapiro–Wilk test (Addinsoft, Paris, France, 2017). The morphometric results for the chicken liver and kidneys were compared between groups. The data in the tables

are presented as $x \pm SD$ (mean \pm standard deviation). The difference between the options was considered significant at $p < 0.05$.

Reporting and transparency statement

Samples were randomly allocated to experimental groups. No blinding was applied. The sample size was based on previous studies. No data were excluded.

RESULTS AND DISCUSSION

In accordance with EFSA requirements [29], the use of nanomaterials in food-producing animals requires evaluation of their toxicity following oral exposure. The calculated dose of nanosilver administered via drinking water to chicks averaged 0.08 mg/kg body weight during the first week of supplementation (Table 2). At the same time, feed intake in the experimental group of chicks exceeded that of the control group by 13.1%. One key criterion for evaluating the effectiveness of biologically active additives in poultry diets is body weight. Administration of nanosilver at a dose of 0.4 mg/L of drinking water to Naked Neck chicks promoted a 19.8% increase in body weight on day 14 of the experiment compared with the control group (Table 2).

Table 2 Productivity performance indicators of dual-purpose Naked Neck chicks under the influence of nanosilver administration.

Indicator	Group of chickens	
	control	experimental
Body weight of one-day-old chicks, g	46.2 \pm 1.1	45.8 \pm 0.9
Body weight of chicks on day 14 of the experiment, g	338 \pm 44	405 \pm 42*
Nanosilver intake via drinking water in chicks, mg/kg body weight:	-	0.08
Total feed consumed during the rearing period, g per bird	543 \pm 22	614 \pm 18*
Feed conversion	1.86	1.71

Note: * $p \leq 0.05$ compared with the control, $x \pm SD$, $n = 25$.

In this study, feed conversion was improved in the experimental group of chicks receiving nanosilver in drinking water compared with chicks fed the basal diet. However, these results need to be verified under industrial rearing conditions, where a more representative sample size would be used. The results of this study are consistent with those of other authors who evaluated the effects of different levels of silver nanoparticles (AgNPs) in the diet on the performance of Ross cross-broiler chickens. Feeding broiler chickens a basal diet containing AgNPs at 2.5, 5, 10, and 20 mg/kg feed increased final body weight, weight gain, and feed conversion ratio. At the same time, the best indicators of broiler chicken performance and relative organ weights were observed at an AgNP dose of 2.5 mg/kg feed [34]. In another experiment, the administration of bio-AgNP at 25 and 51 mg/kg feed to broiler chickens also increased body weight [35]. It is assumed that nanosilver's mechanism of action is its ability to increase cellular oxygen consumption, thereby stimulating metabolic processes in tissues. In addition, silver nanoparticles can affect fibroblast growth factor gene expression, enhance immune cell activity, and stimulate chicken growth [36].

In accordance with the increase in body weight, kidney weight also increased in the experimental group on the 14th day of experiment. According to the organometric studies we conducted, the absolute weight of the kidneys of chickens that were given a nanosilver preparation at a dose of 0.4 mg/l for 14 days exceeded the similar value in the control by 10.3%, which occurred due to an increase in kidney length by 4.6% compared to the control (Table 3).

Table 3 Macroscopic morphometric parameters of the kidneys of Naked Neck chickens under the influence of nanosilver preparation.

Indicator	Group of chickens	
	control	experimental
Absolute kidney mass, g	3.38 ± 0.13	3.73 ± 0.04*
Relative kidney weight, %	1.02 ± 0.11	1.06 ± 0.10
Kidney length, mm	34.61 ± 0.75	36.20 ± 0.68*
Kidney width, mm	7.22 ± 0.37	7.83 ± 0.73

Note: * p ≤ 0.05 compared to control, x ± SD, n = 20.

The microstructure of the kidneys of the experimental group of chickens did not differ significantly from the control group in terms of capsule thickness, renal lobule area, diameter and average volume of the renal corpuscle, as well as the diameter of the proximal and distal tubules (Table 4).

Table 4 Microscopic morphometric parameters of the kidneys of Naked Neck chickens under the influence of nanosilver preparation.

Indicator	Group of chickens	
	control	experimental
Capsule thickness, µm	0.34 ± 0.09	0.35 ± 0.13
Renal lobule area, mm ²	2.07 ± 0.24	2.16 ± 0.31
Renal corpuscle diameter, µm	43.6 ± 7.7	45.2 ± 6.73
Average renal corpuscle volume, thousand µm ³	36.14 ± 4.34	37.44 ± 5.02
Proximal tubule diameter, µm	34.10 ± 8.25	34.7 ± 5.21
Distal tubule diameter, µm	21.7 ± 5.5	23.05 ± 8.22

Note: x ± SD, n = 20

Analysis of histological sections showed that the kidneys in the control group of birds are covered with a rather thin and dense fibrous connective tissue capsule (Figure 1). From the renal capsule, weakly expressed, very thin connective tissue layers branch into the organ, dividing the kidney parenchyma into separate lobules. That is why in young poultry, due to the small amount of interlobular connective tissue, the boundaries between the kidney lobules are smoothed. The kidney lobules on the cross section have an oval or rounded shape (Figure 2).

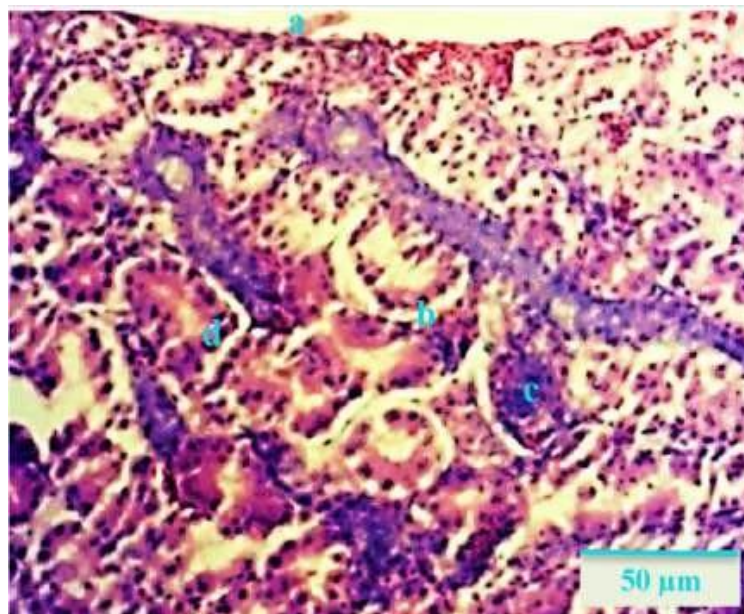


Figure 1 Fragment of the microscopic structure of a kidney lobule of Naked Neck chickens of the control group.

Note: a – renal capsule; b – cortical substance; c – renal corpuscle; d – renal tubules. Hematoxylin and eosin.

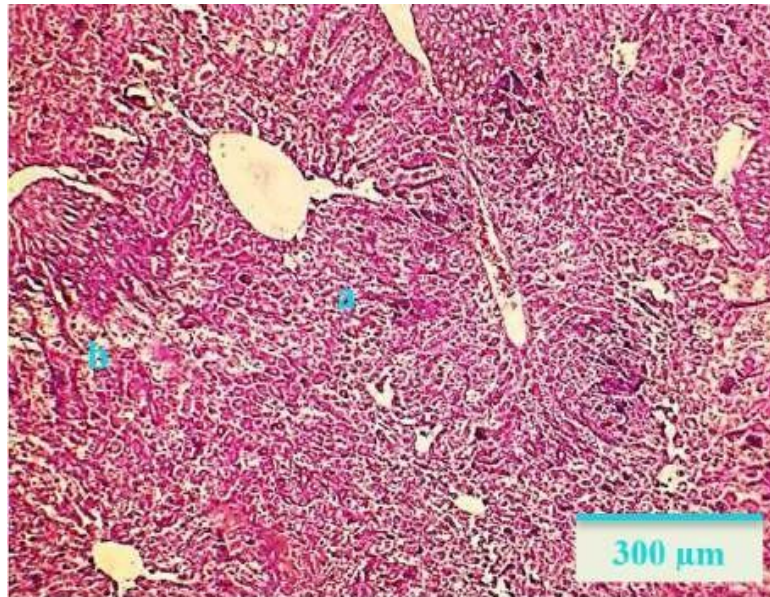


Figure 2 Fragment of the microscopic structure of the kidney of Naked Neck chickens of the control group. Note: a – kidney lobule; b – interlobular connective tissue. Hematoxylin and eosin.

On the cross-section of each renal lobule, the cortical substance is clearly differentiated, which occupies the peripheral part of the organ. In the central part of the renal lobules is the medulla (Figure 3). In the cortical substance of each lobule are the renal corpuscles (Figure 4) and the convoluted renal tubules (Figure 5), which form the renal labyrinth. The renal corpuscles have an ambiguous size (large, medium, small), which is a feature of the histoarchitectonics of the kidneys for birds. The medulla is formed by collecting tubules, which are combined into interlobular collecting tubules. Between the renal corpuscles and the renal tubules lies the kidney's connective tissue stroma, composed of loose connective tissue.

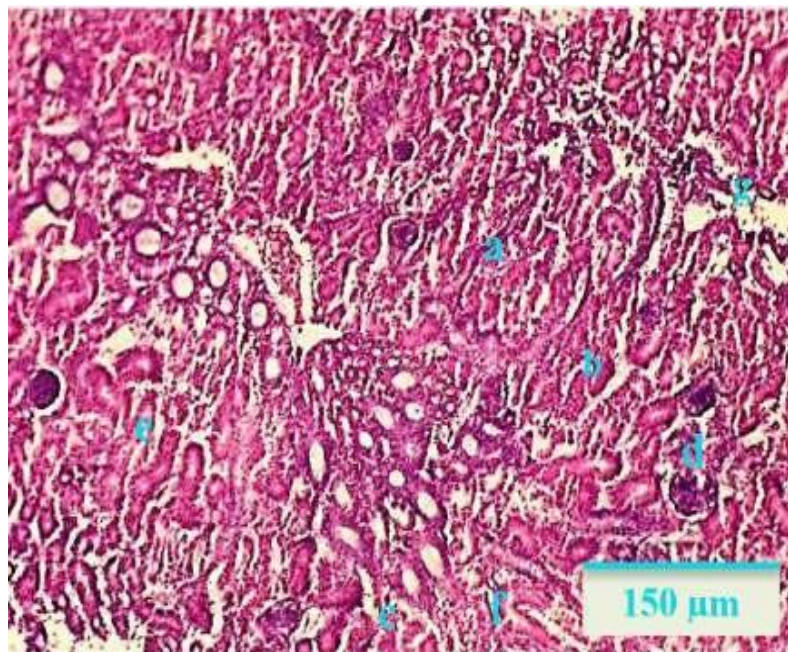


Figure 3 Fragment of the microscopic structure of the kidney of Naked Neck chickens of the control group. Note: a – kidney lobule; b – cortical substance; c – medulla; d – renal corpuscles; e – convoluted renal tubules; f – straight renal tubules; g – interlobular connective tissue. Hematoxylin and eosin.

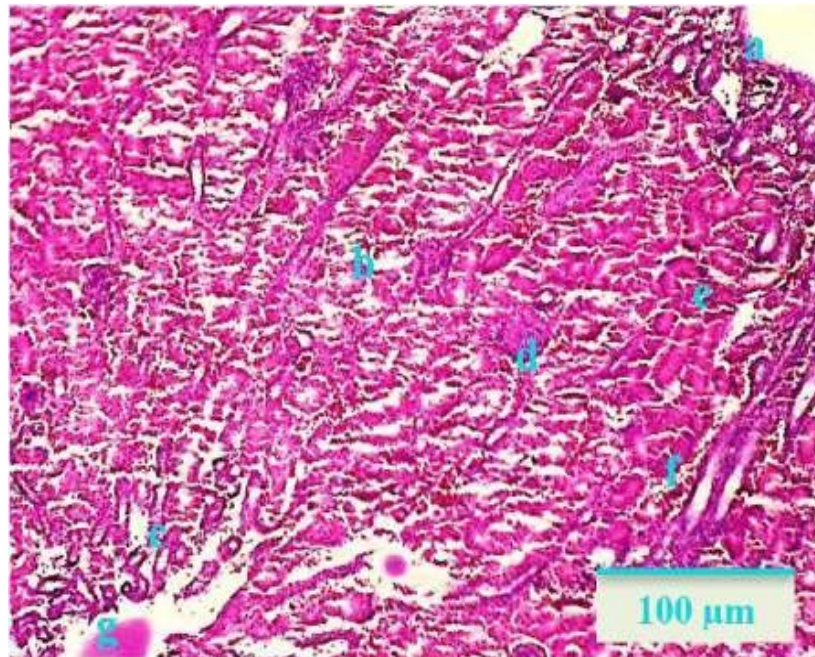


Figure 4 Fragment of the microscopic structure of a kidney lobule of Naked Neck chickens of the control group. Note: a – renal capsule; b – cortical substance; c – medulla; d – renal corpuscles; e – convoluted renal tubules; f – straight renal tubules; g – renal calyx. Hematoxylin and eosin.

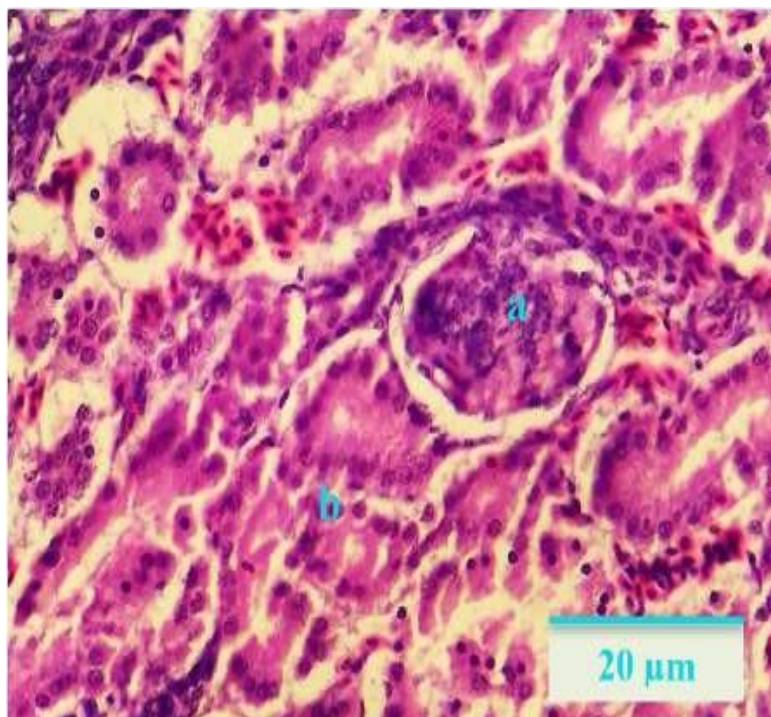


Figure 5 Fragment of the microscopic structure of the cortical substance of the kidney lobule of Naked Neck chickens of the control group. Note: a – renal corpuscles; b – convoluted renal tubules. Hematoxylin and eosin.

The renal corpuscles are formed by a glomerulus, around which is a capsule of the renal corpuscle (Figure 6). The glomerulus passes into the tubular system, which is quite noticeable on a longitudinal section of histopreparations stained with hematoxylin and eosin. In chickens, unlike mammals, there are two types of nephrons. Some of them do not go beyond the cortical lobules - cortical nephrons (intracortical), others descend into the medulla - medulla nephrons (juxtamedullary) (Figure 7). The wall of the renal tubules is formed by a single-layered cubic epithelium, the epithelial cells of which contain an intensely stained nucleus of a rounded shape (Figure 8).

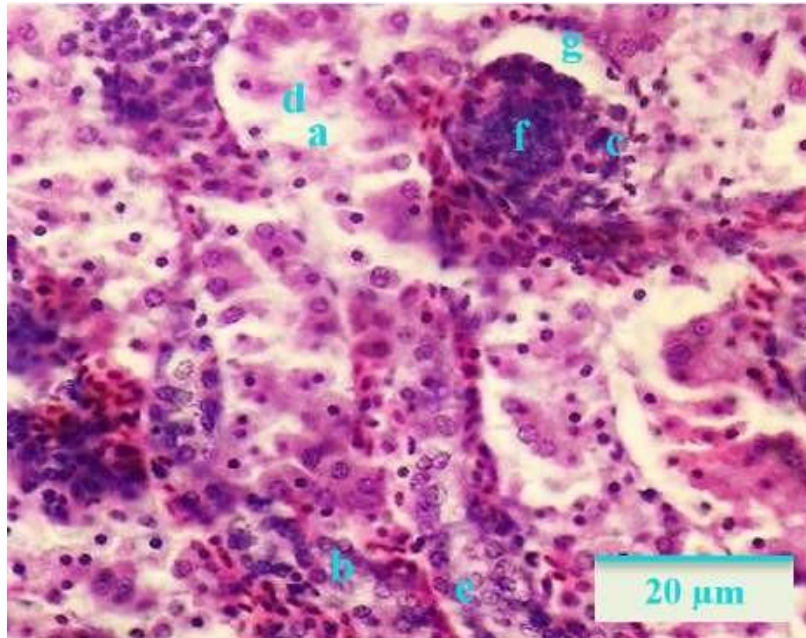


Figure 6 Fragment of the microscopic structure of a kidney lobule of Naked Neck chickens of the control group. Note: a – cortical substance; b – medulla; c – renal corpuscle; d – convoluted renal tubules; e – straight renal tubules; f – vascular glomerulus of the renal corpuscle; g – renal corpuscle capsule. Hematoxylin and eosin.

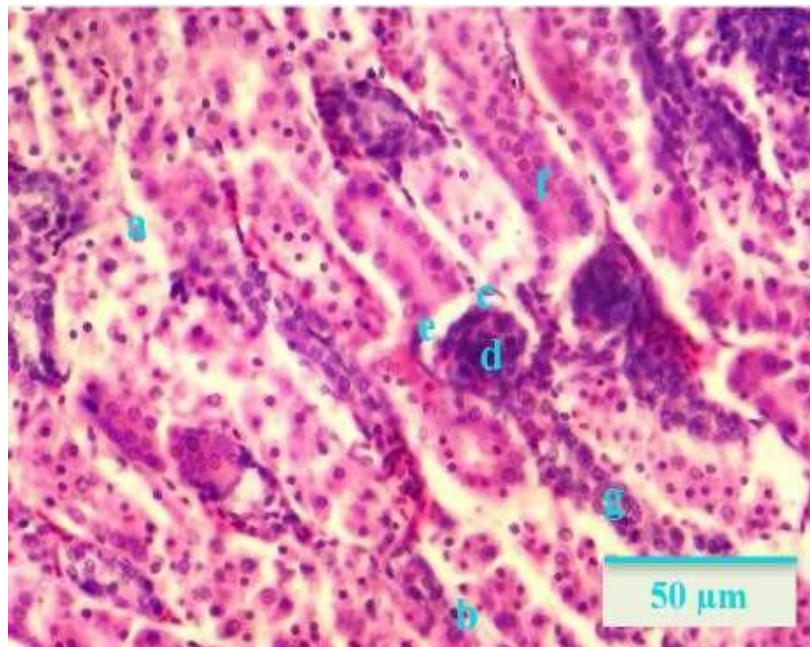


Figure 7 Fragment of the microscopic structure of a kidney lobule of Naked Neck chickens of the control group. Note: a – cortical substance; b – medulla; c – renal corpuscle; d – vascular glomerulus of the renal corpuscle; e – capsule of the renal corpuscle; f – direct renal tubules of cortical nephrons (intracortical); g – direct renal tubules of medulla nephrons (juxtamedullary). Hematoxylin and eosin.

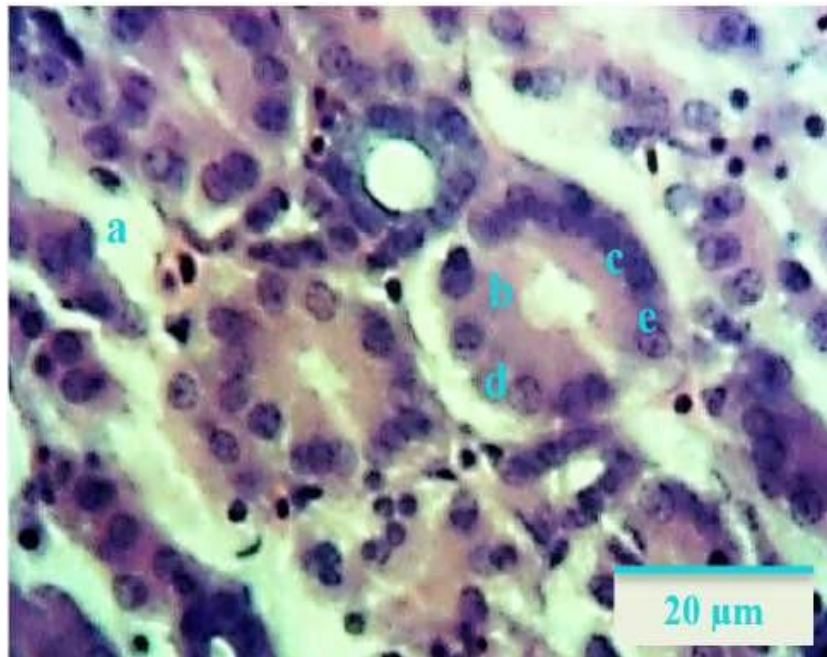


Figure 8 Fragment of the microscopic structure of a kidney lobule of Naked Neck chickens of the control group. Note: a – cortical substance; b – renal tubules; c – renal tubule wall; d – single-layered cubic epithelium; e – epithelial cell nuclei. Hematoxylin and eosin.

The kidneys of the experimental group of chickens had similar morphoarchitectonics and morphotopography compared to the control: they were characterised by a lobular structure typical of birds, where each lobule is formed by cortical (renal corpuscles and convoluted tubules of nephrons are visible) and medullary (formed by collecting tubules that combine into interlobular collecting tubules, which are components of nephrons) substances (Figure 9, 10).

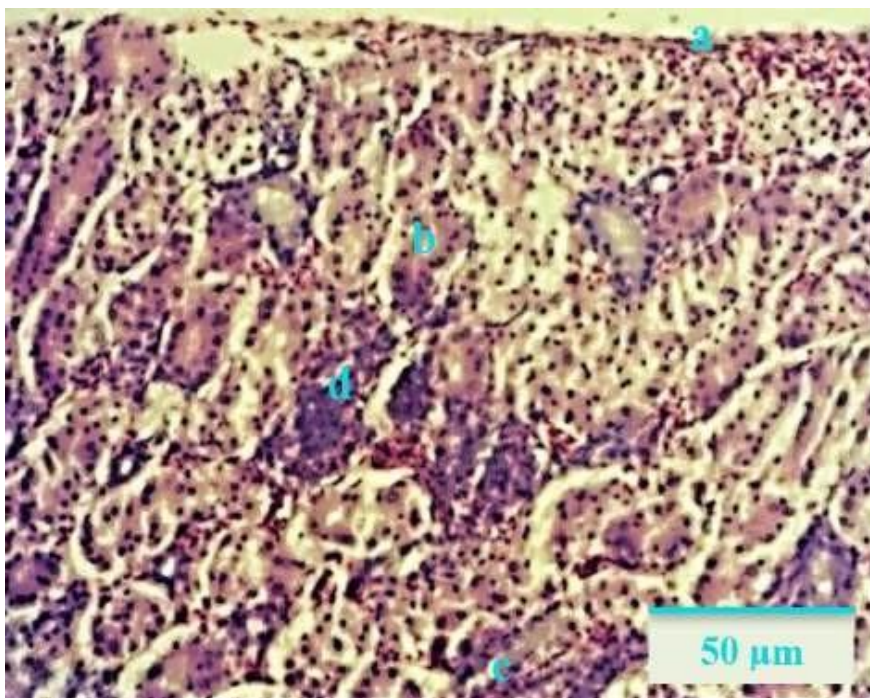


Figure 9 Fragment of the microscopic structure of the kidney lobule of Naked Neck chickens of the experimental group. Note: a – kidney capsule; b – cortical substance; c – medulla; d – renal corpuscle. Hematoxylin and eosin.

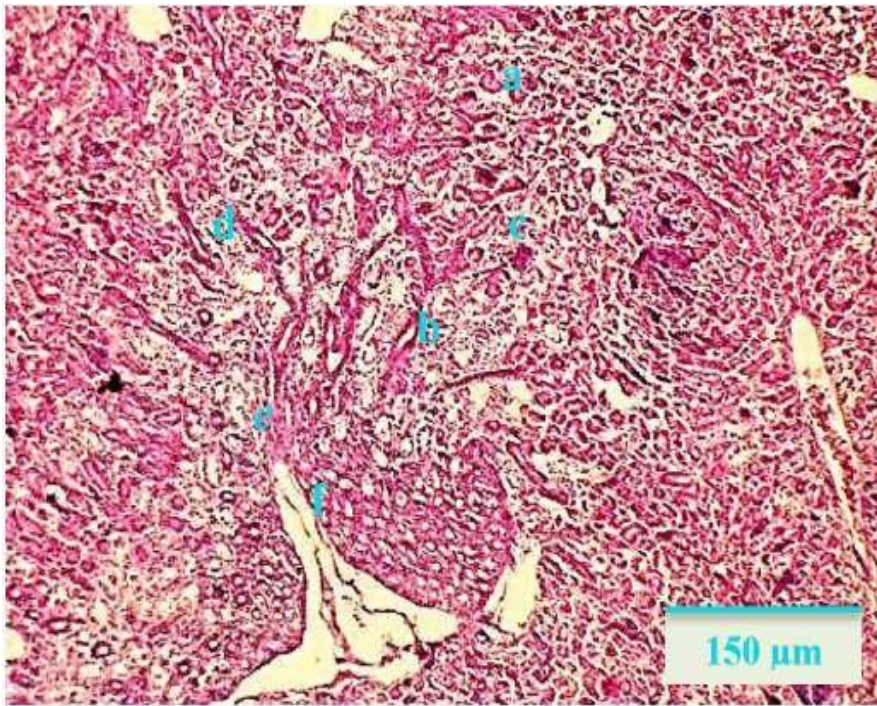


Figure 10 Fragment of the microscopic structure of a kidney lobule of Naked Neck chickens of the experimental group.

Note: a – cortical substance; b – medulla; c – renal corpuscles; d – renal tubules; e – collecting tubules; f – interlobular collecting tubules. Hematoxylin and eosin.

The renal corpuscles are formed by the glomerulus and the renal corpuscle capsule. The latter is formed by two sheets - the parietal (outer) and the visceral (inner), between which there is a cavity (lumen) in the form of a slit. The visceral sheet of the renal corpuscle capsule tightly covers the glomerulus from the outside, and the parietal sheet passes into the wall of the proximal convoluted tubule of the nephron.

In general, the morphology of nephrocytes and nephron tubules in chickens of the experimental group at the microscopic level was without signs of structural disorganisation and alteration. However, in some places of the histopreparation, a slight increase in the lumen of the renal corpuscle capsule and desquamation of epithelial cells of the walls of the renal tubules were detected (Figure 11). The vessels of the renal parenchyma were moderately filled with blood, and in some birds of the experimental group, blood filling of the vessels was detected in combination with the expansion of their lumen. Cystic dilatation of the renal parenchyma tubules was also observed (Figure 12, Figure 13), indicating a certain toxicity of the silver nanopreparation to the chicken body.

To assess the toxicity of nanosilver following oral administration to chicks, the scheme developed by [37] for evaluating stages of progressive chronic nephropathy was used. Based on the number, severity, and frequency of pathological changes in the kidneys of chicks receiving nanosilver solution, most were classified as grade 0, indicating absence of pathological alterations. In a few kidneys, minor pathological changes corresponding to minimal severity (grade 1) were observed. Administration of nanosilver to chicks for 14 days did not induce pathological changes characteristic of moderate or severe grades (Table 5).

The pathological changes that nanosilver can cause in the bird's body have been reported in several studies by other scientists. In experiment, it was found that the inclusion of AgNPs in the diet of Ross 308 crossbred broiler chickens at doses of 2.5, 5, 10, and 20 mg/kg feed caused dose-dependent lesions in the liver, kidney, spleen, and duodenum tissues, including degeneration, necrosis, mononuclear infiltration, and focal aggregation of inflammatory cells [34]. In this study, a nanosilver dose of 0.4 mg/L of drinking water was used for 14 days of chick rearing, which is considerably lower than the aforementioned parameters. Consequently, the severity of toxic effects on internal organs, particularly the kidneys, was markedly lower (Table 5). In contrast to these studies, the *in ovo* administration of inorganic and organic synthesised silver nanoparticles conjugated with L-arginine into chicken embryos showed that nanosilver promoted the survival and hatching of chickens. Serum immunoglobulin (IgM) levels and the expression of proteins related to muscle growth (myoD and myogenin) improved significantly under the influence of nanosilver [38]. It has also been confirmed in Japanese quails that the addition of 10 mg/kg Ag-NPs to the feed is a safe and effective feed additive for improving both productive

and reproductive performance [39]. The selective effects of nanoparticles of different metals on the functional state of the chicken intestine were noted in one study [40]. Given that silver exhibits cumulative properties, at the current stage of research, it is advisable to focus on findings indicating the potential risks of toxic damage to vital organs in poultry. This necessitates further assessment of the safety of poultry slaughter products, in particular meat and edible by-products, with regard to residual silver content.

Table 5 Semi-quantitative pathological scoring scale for kidney lesions in chicks following nanosilver administration.

Severity of lesions	Grade	Description of changes	Number of cases observed
No lesions detected	0	Absence	16
Minimal	1	5 or fewer focal lesions (including single tubules or hyaline casts) in 2 (left and right) kidney sections	4
Moderate	2	From 6 to 15 focal lesions	-
Low-moderate	3	From 16 to 30 focal lesions	-
Moderate-intermediate	4	From 31 to 50 focal lesions	-
High-moderate	5	Focal lesions and hyaline casts too numerous to count	-
Low-severe	6	Adjacent foci begin to coalesce, eventually forming an interconnected network of affected CPN tissue in the cortex; the OSOM remains relatively intact.	-
Moderate-severe	7	The cortex at the level of the kidney curvature is almost completely affected (with less involvement at the outer edges); the OSOM is disrupted by CPN tubules and dilated hyaline casts.	-
End stage	8	Normal parenchyma is absent or remains only in small, scattered islands; kidney regions are noticeably enlarged; the kidney surface is uneven due to wedge-shaped protruding tubules alternating with atrophic tissue	-

Note: n = 20; abbreviations: CPN – chronic progressive nephropathy; OSOM – outer stripe of the outer medulla.

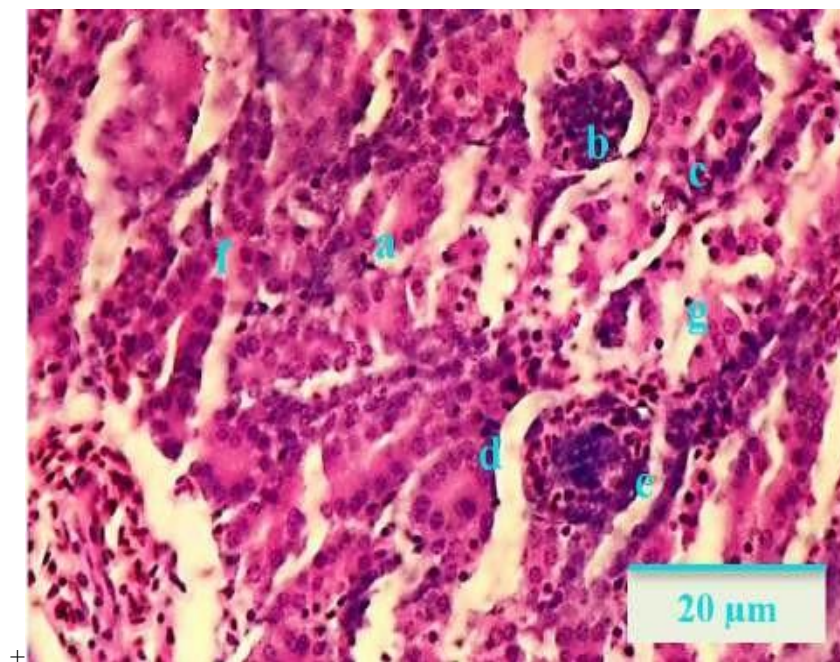


Figure 11 Fragment of the microscopic structure of a kidney lobule of Naked Neck chickens of the experimental group.

Note: a – cortical substance; b – renal corpuscles; c – renal tubules; d – parietal leaf of the renal corpuscle

capsule; e – visceral leaf of the renal corpuscle capsule; f – enlarged cavity of the renal corpuscle capsule; g – desquamation of convoluted tubule epithelial cells. Hematoxylin and eosin.

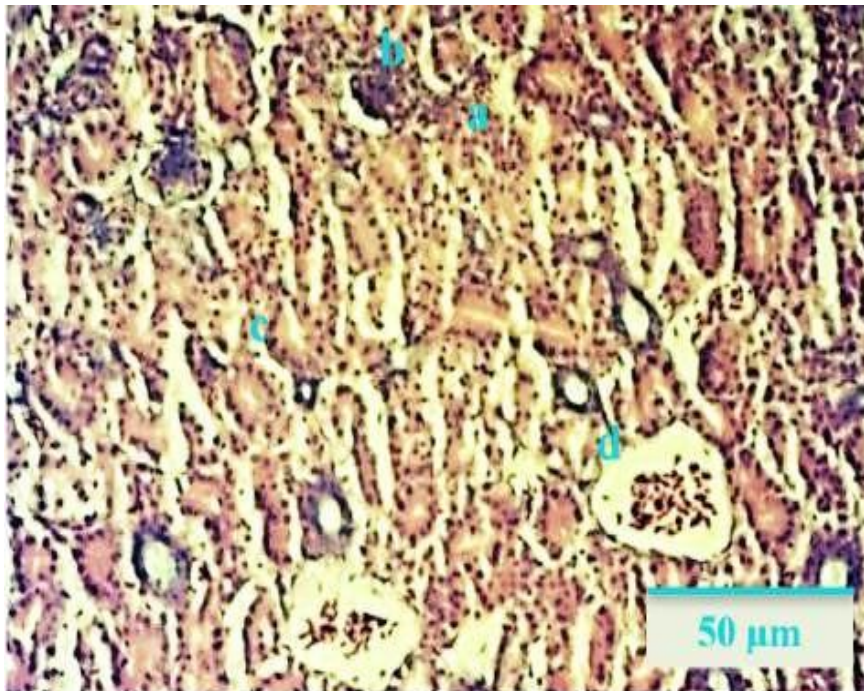


Figure 12 Fragment of the microscopic structure of a kidney lobule of Naked Neck chickens of the experimental group.

Note: a – cortical substance; b – renal corpuscle; c – renal tubules; d – vessel (transverse section). Hematoxylin and eosin.

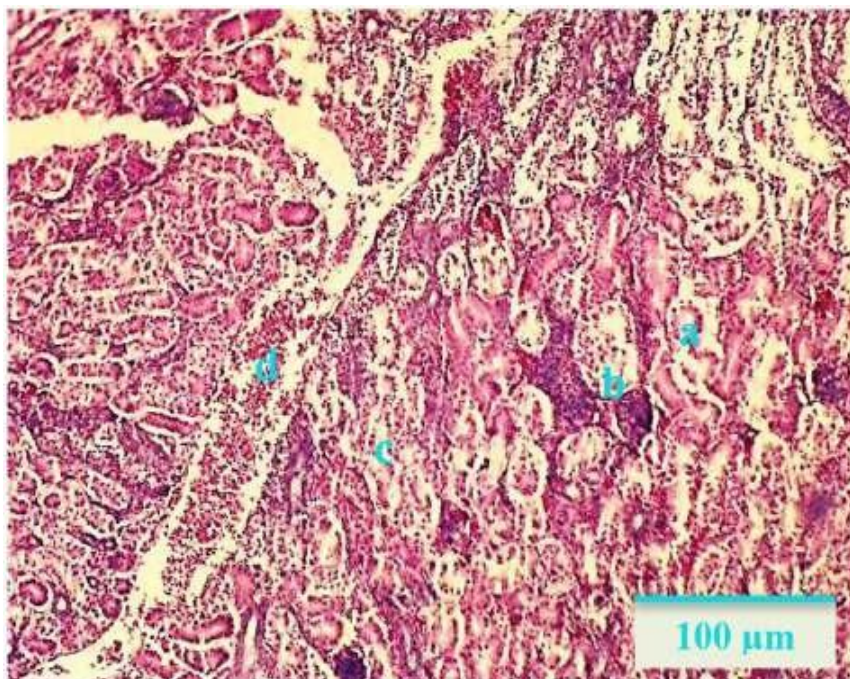


Figure 13 Fragment of the microscopic structure of a kidney lobule of Naked Neck chickens of the experimental group.

Note: a – cortical substance; b – renal corpuscle; c – cystic dilatation of the tubules; d – vessel (longitudinal section). Hematoxylin and eosin

All beneficial and toxic components absorbed in the intestine enter the bloodstream and are transported to the liver; therefore, its functional state determines the efficiency of digestion, nutrient assimilation, and detoxification of harmful substances. Nanosilver ingested orally also enters the liver and may affect its metabolic

and detoxification capacity. According to the results of our studies, the livers of the control and experimental groups of 14-day-old Naked Neck breed chickens had a red-brown colour and did not differ in appearance between the groups. At the same time, the absolute mass of the liver of the experimental group chickens exceeded that of the control by 8.7%, but the relative mass of the liver of the experimental group chickens was inferior to that of the control by 10.9%. In terms of length and width, the livers of the experimental group chickens did not differ significantly from those of the control group, but there was a tendency toward increased measurements. With these indicators, the liver development index did not differ between the 14-day-old chicken groups (Table 6).

Table 6 Macroscopic morphometric parameters of the liver of Naked Neck chickens under the influence of nanosilver.

Indicator	Group of chickens	
	control	experimental
Absolute liver mass, g	12.86 ±0.49	13.98 ±0.36*
Relative liver mass, %	3.84 ±0.12	3.42 ±0.06*
Liver length, mm	50.01 ±1.45	52.60 ± 0.97
Liver width, mm	47.43 ±1.25	49.52 ±1.21
Liver development index, %	105.5	106.0

Note: * $p \leq 0.05$ compared to control, $x \pm SD$, $n = 10$.

Such changes in the morphometric characteristics of the liver of chickens in the experimental group may be associated with the cumulative ability of nanosilver. This assumption is consistent with the results of studies [41], where it was found that when AgNPs were orally administered to chickens at a dose of 6 mg/kg for 22 days with feed or with water at a dose of 2.5–10 mg/l for 35 days, silver accumulated in the liver in the range of 141–269 µg/kg. The use of nanosilver at a dose of 2.5–20 mg/kg for 42 days caused its accumulation in the pectoral and thigh muscles, while at concentrations of 4, 8, 12 and 15 mg/l of water for 40–42 days, silver accumulated in the femurs and liver of chickens. At doses of 0.1, 0.04 and 0.08 mg/kg, silver accumulation was observed in the kidneys and liver of birds. Similar data on silver accumulation were found in the liver, lungs and abdominal skin of Ross 308 crossbred chickens treated with silver nanoparticles in the litter [42]. Elevated levels of silver in tissues have a toxic effect on both macro- and microorganisms, especially beneficial bacteria. In the case of *Lactobacillus reuteri*, it has been shown that silver nanoparticles adsorb onto the cell surface and thereby cause cell wall destruction, which is accompanied by significant leakage of cellular protein and oxidative damage to components [43]. By analogy, silver nanoparticles bioaccumulate and migrate through the tissues of birds and their parasites. Using the example of the nematode *Heterakis dispar*, which parasitises geese, the ability of silver nanoparticles (Ag NPs) at a dose of 100 µg/ml to bioaccumulate in the parasite's tissues and cause pathological changes has been proven. This can undoubtedly be regarded as a positive effect. However, the ability of nanosilver to penetrate the vessels of the intestinal submucosa and enter the liver and striated muscles of birds was revealed. The latter was accompanied by the development of various pathological changes in the intestinal tissue, liver, and skeletal striated muscles of birds [44]. The observed decrease in relative liver weight in chicks during this experiment may be partly associated with their increased age and body weight under nanosilver administration. This is likely attributable to a relative increase in the mass of muscle tissue, intestines, and other organs compared with the liver. Similar patterns have been reported by other researchers during the rearing of meat-type poultry crossbreeds. [45]. Therefore, despite the potentially positive effect of nanosilver preparations on poultry productivity, one should also consider its toxicity to the organs and tissues of the digestive system, especially the liver. In this regard, nanosilver, as a bactericidal drug and at the same time a heavy metal, is not an exception to the rule, although it is significantly inferior to silver nitrate and antibiotics in terms of the strength and severity of its toxic effect on the liver and other tissues [46].

More detailed information about the effect of nanosilver on the histostructure of the liver can be obtained through microscopic analyses. Studies of the liver of chickens of the control group showed that the histoarchitectonics of their liver is characterised by a lobular structure, in the center or eccentrically of which the central vein is located (Figure 14). The liver lobules on the cross-section have a multifaceted (prismatic) shape, and between them, there is interlobular connective tissue, which is poorly developed in young poultry. That is why the boundaries between the liver lobules are smoothed and poorly differentiated on histopreparations stained with hematoxylin and eosin for analysis of the microscopic structure of the organ (Figure 14). At the same time, interlobular connective tissue is found only in the form of rather thin layers, which is better developed in the areas of the portal tracts - at the contact border of the hepatic lobules, where the

hepatic (arteries, veins, bile duct) triads are located (Figure 15).

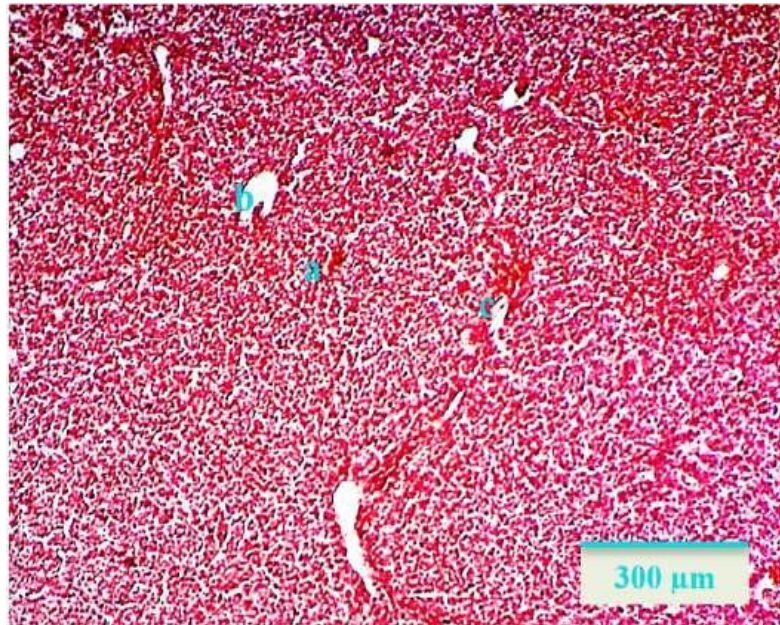


Figure 14 Fragment of the microscopic structure of the liver of Naked Neck chickens control group: a – liver lobule; b – central vein; c – interlobular connective tissue. Hematoxylin and eosin.

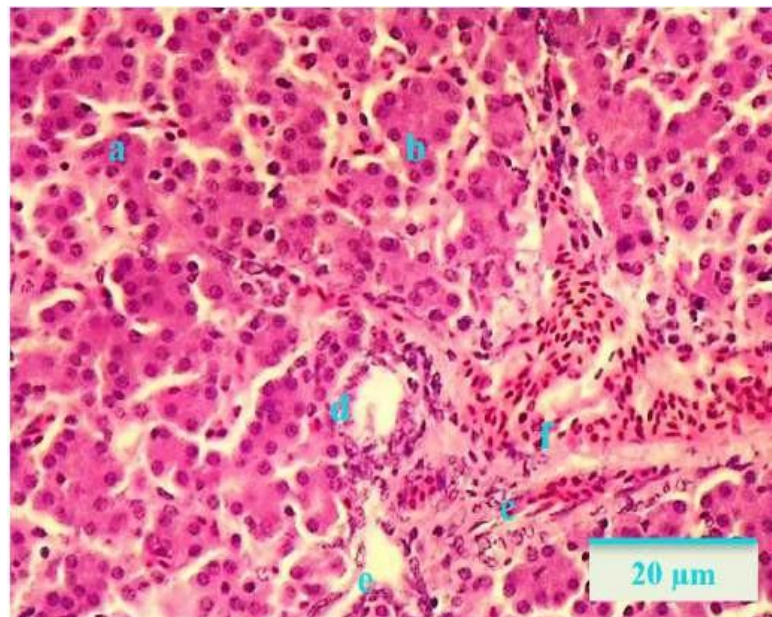


Figure 15 Fragment of the microscopic structure of the liver of Naked Neck chickens from the control group: a – particle liver; b – hepatocytes; c – interlobular connective tissue; d – artery; e – vein; f – bile duct. Hematoxylin and eosin.

Each liver lobule is composed of hepatic cords (formed by hepatocytes and arranged radially towards the central vein), which, by contacting one another, form slit-like structures known as sinusoidal capillaries. In chickens of this age group, these capillaries were moderately blood-filled, narrow in diameter, and exhibited variable sizes in different regions of the liver parenchyma (Figure 16).

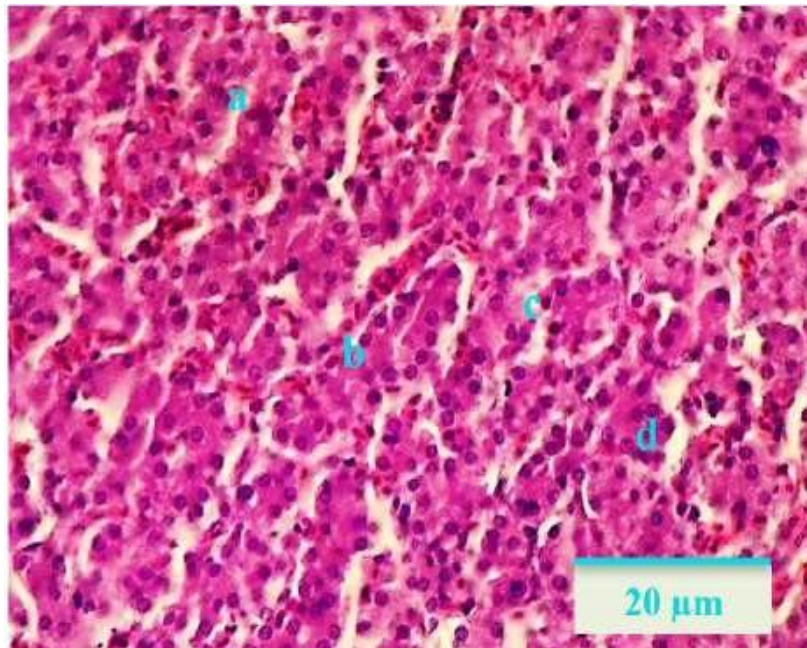


Figure 16 Fragment of microscopic structures slices liver of Naked Neck chickens of the control group: a – parenchyma liver; b – hepatic tubules; c – hepatocytes; d – sinusoidal capillaries. Hematoxylin and eosin.

According to histological studies, the cytoplasm of hepatocytes, when stained with hematoxylin and eosin, was of high optical density. Hyperchromic nuclei of hepatocytes were located in the centre of the cytoplasm or eccentrically. In the karyoplasm of such cells, nucleoli and nuclear chromatin were detected (Figure 17).

As a result of mitotic cell division in the liver parenchyma of chickens (especially in the experimental group), binuclear hepatocytes were detected simultaneously with mononuclear ones (Figure 17), which indicated the activity of physiological liver regeneration - the constant restoration of a multicellular organism under the influence of the nanosilver preparation.

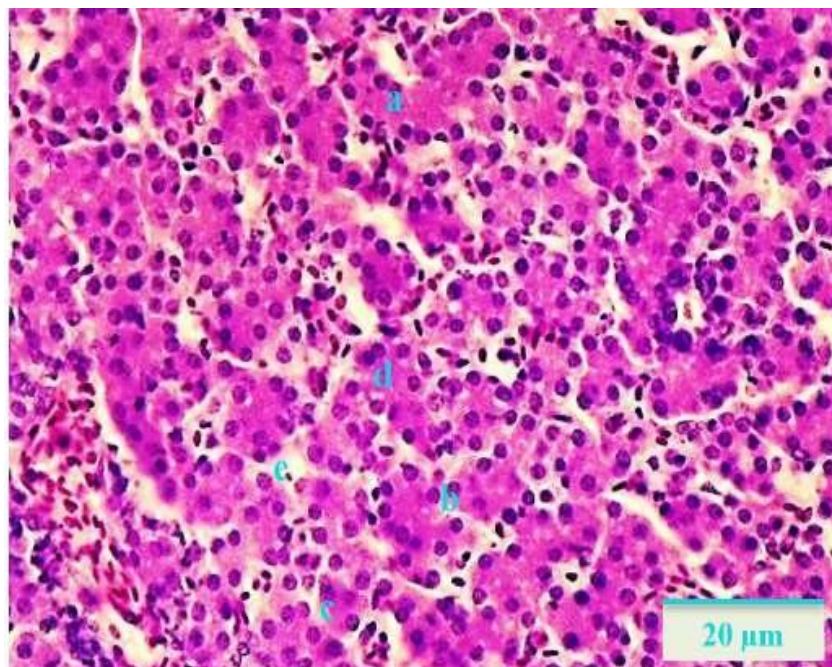


Figure 17 Fragment of the microscopic structure of a liver lobule of Naked Neck chickens of the experimental group: a – liver parenchyma; b – hepatic tubules; c – mononuclear hepatocytes; d – binuclear hepatocytes; e – sinusoidal capillaries. Hematoxylin and eosin.

In addition, in the chickens of the experimental group, the hepatic tubules of the liver parenchyma did not fit tightly together (Figure 18). With such a structure, the sinusoidal capillaries (their space) of the liver were dilated (Figure 19), the cytoplasm of hepatocytes had a looser consistency, and, in some cases, focal infiltration by polymorphic cells was observed around the vessels (Figure 20). In some areas, dilation and blood filling of the vessels were detected, which formed hepatic triads (Figure 21). Such changes in the liver tissue may be associated with the small size and high physicochemical reactivity of silver nanoparticles. This provides them with easy penetration through biological membranes, which contributes to their accumulation in the liver, kidneys and reproductive organs of the bird [47]. It has been reported that AgNPs can bioaccumulate in various tissues [48]. Feeding chickens with silver nanoparticle hydrocolloid or lipid-coated silver nanoparticle hydrocolloid at a dose of 5 mg/kg body weight per day increased phagocytosis and metabolic activity of leukocytes, which may indicate the development of an inflammatory state in the body [49]. Nanosilver is also believed to significantly increase heterophilic extracellular trap levels in serum, thereby causing liver and kidney damage in poultry. Therefore, the authors of this study emphasise the moderate use of nanosilver, which can strengthen immunity and, where feasible, minimise the negative consequences of its side effects on poultry [50]. The analysis of the microstructure of internal organs under the influence of nanosilver can be considered a preliminary assessment of its impact on the quality and safety of slaughter products, as well as on the risk of microbial contamination and on their suitability for storage and consumption. This study also enables comparison of the benefits and potential adverse effects of nanosilver in chickens when selecting an antimicrobial agent. These findings indicate the need for strict control of the dosage and application regimen of silver nanopreparations in animals, particularly poultry [51], [52], and [53].

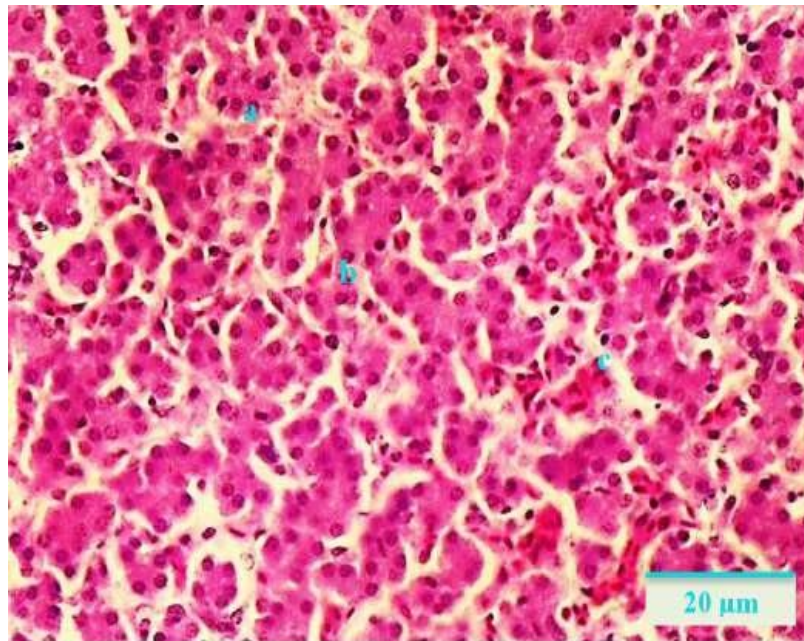


Figure 18 Fragment of the microscopic structure of a liver lobule of Naked Neck chickens of the experimental group.

Note: a – liver parenchyma; b – hepatic tubules; c – sinusoidal capillaries. Hematoxylin and eosin.

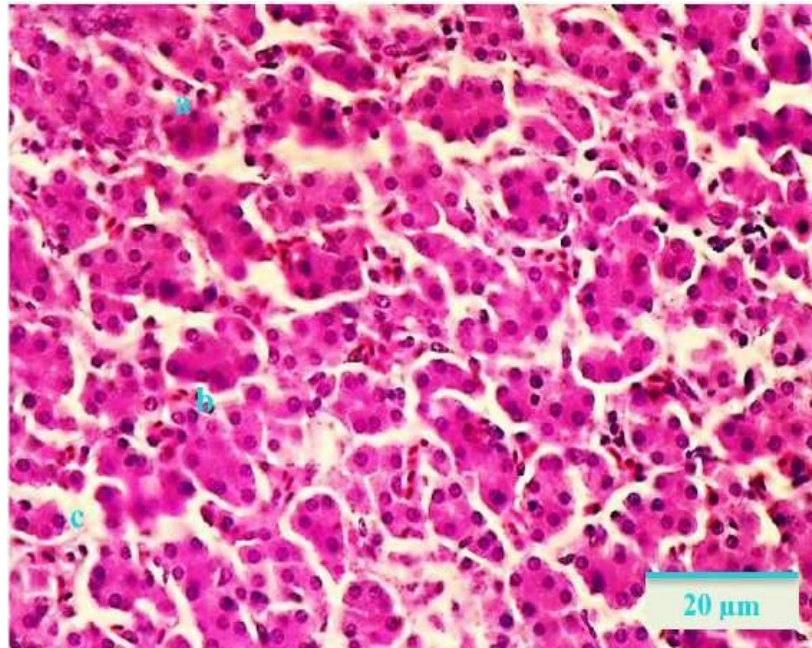


Figure 19 Fragment of the microscopic structure of the liver lobule of Naked Neck chickens of the experimental group.

Note: a – liver parenchyma; b – hepatic tubules; c – expansion of sinusoidal capillary spaces. Hematoxylin and eosin.

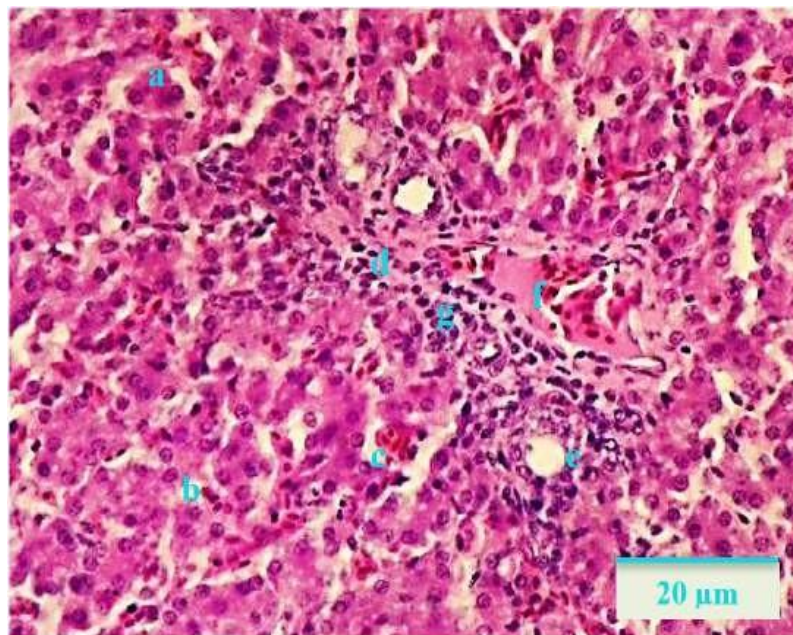


Figure 20 Fragment of the microscopic structure of a liver lobule of Naked Neck chickens of the experimental group.

Note: a – liver parenchyma; b – hepatic tubules; c – sinusoidal capillaries; d – interlobular connective tissue; e – artery; f – bile duct; g – polymorphic cells. Hematoxylin and eosin.

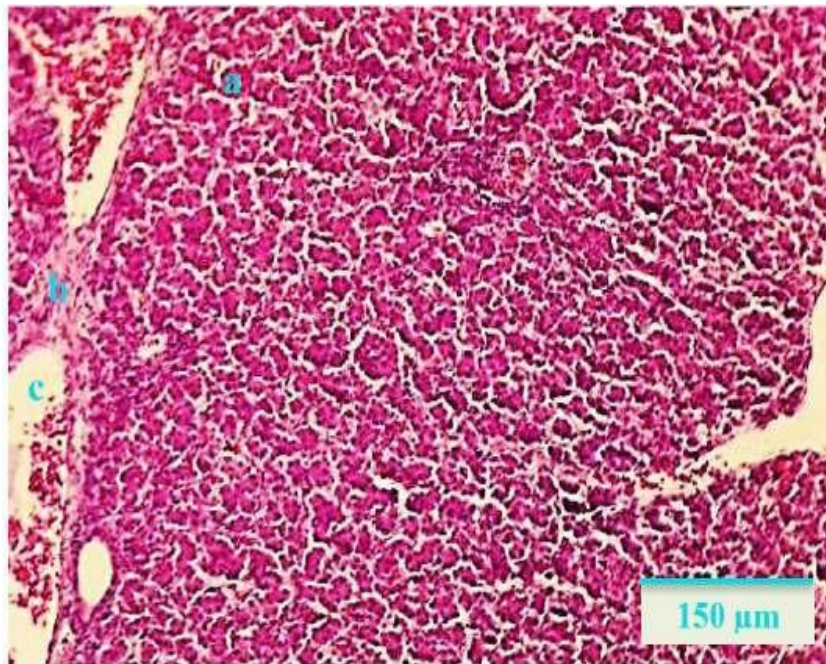


Figure 21 Fragment of the microscopic structure of a liver lobule of Naked Neck chickens of the experimental group.
 Note: a – liver parenchyma; b – interlobular connective tissue; c – dilated (blood-filled) vessels. Hematoxylin and eosin.

According to the results of histo- and cytometry, the diameter of the hepatic tubules and the volume of the hepatocyte nuclei of the liver parenchyma of the chickens of the experimental group did not significantly differ from the control (Table 7). However, the number of hepatocytes in the experimental group exceeded that in the control by 11.8%. As a result, the N:C ratio of hepatocytes of the chickens of the experimental group was 8.4% higher than in the control, indicating a higher morphofunctional activity of liver cells in the chickens of the experimental group, compared to the control, which may, to some extent, indicate the manifestation of stress in hepatocytes.

Table 7 Microscopic morphometric parameters of the liver of Naked Neck chickens under the influence of the nanosilver preparation.

Indicator	Group of chickens	
	control	experimental
Diameter of hepatic tubules, μm	26.04 ± 0.42	27.16 ± 0.54
Volume hepatocytes, μm ³	255.93 ± 18.17	286.02 ± 19.84*
Hepatocyte nuclear volume, μm ³	24.32 ± 1.64	25.11 ± 2.01
N:C ratio of hepatocytes	0.1050 ± 0.0069	0.0962 ± 0.0052*

Note: * $p \leq 0.05$ compared to control, $x \pm SD$, $n = 10$.

The semi-quantitative assessment of liver lesion severity, which considered the intensity of tissue damage [54], showed that changes in the majority of chicks in the experimental group were minor or minimal, corresponding to a grade of 1. In some chicks, slight or a few pathological alterations were observed, corresponding to a severity grade of 2 (Table 8).

Table 8 Semi-quantitative pathological scoring scale for liver lesions in chicks following nanosilver administration.

Severity	Affected portion of the liver	Grade	Quantifiable findings	Number of cases observed
Mild or minimal	Very few	1	1–2 foci	7
Mild or few	Few	2	3–6 foci	3
Moderate or a few	Many	3	7–12 foci	-
Severe or numerous	Many	4	>12 foci	-
Severe	Very large number	5	Diffuse	-

Note: n = 10.

However, to obtain more detailed information on the extent of liver damage caused by nanosilver, it is necessary to determine its cumulative potential in the internal organs of poultry that have reached slaughter weight and age. Thus, our studies showed the absence of a pronounced toxic effect of the nanosilver preparation when used at a dose of 0.4 mg/l of water for 14 days at the level of macroscopic and microscopic characteristics of the kidneys and liver and a positive effect on the productivity of Naked Neck chickens. Similar results were obtained in experiments with broiler chickens fed nanosilver at 4 mg/kg for 35 days, as evidenced by improved growth performance, liver function, silver absorption, and reduced abdominal fat content [55]. Thus, even the administration of nanosilver to chicks at a dose nearly 10 times higher than that used in our experiment, and for a period 2.5 times longer, did not result in any hazardous effects on the organism. A more pronounced bactericidal effect of the use of nanosilver was manifested at a dose of 150 µg/bird infected with *C. perfringens*. Nanosilver reduced intestinal colonisation by *C. perfringens*, severity of clinical manifestations of infection, and mortality compared to infected, untreated birds. The use of nanosilver reduced the severity of intestinal and hepatic pathological lesions in chickens. The authors concluded that silver nanoparticles have a positive effect on intestinal health, without affecting immune organs, but can accumulate in muscles [56] and eggshells [57].

Limitations

The main limitation of this study is the relatively small sample size, which may reduce the statistical power to detect minor differences between treatment groups. Although significant effects were observed for the primary outcomes, the results should be interpreted with caution when extrapolating to larger populations. Another limitation of this study is the short duration of nanosilver administration to Naked Neck chicks, which lasted only 14 days, thereby limiting the assessment of its effects on poultry productivity as well as the quality and safety of slaughter products.

This study should be regarded as preliminary, as it allows consideration of the risk level and the extent of microstructural damage to the liver and kidneys of productive poultry, particularly Naked Neck chickens, when nanosilver is administered via drinking water. To obtain a definitive conclusion regarding the appropriate dosage of the nanosilver preparation and the watering regimen for chickens, further studies are required, taking into account the safety indicators of poultry slaughter products in terms of residual silver content, as well as microbiological and physicochemical parameters.

CONCLUSION

Administration of a nanosilver preparation to Naked Neck chickens at a dose of 0.4 mg/L of drinking water for 14 days resulted in an increase in kidney weight by 10.3% and kidney length by 4.6%. The increase in body weight of chickens receiving nanosilver via drinking water may be considered promising in terms of improving poultry productivity; however, the obtained results require verification under production conditions using a representative sample. The structure of nephrocytes and nephron tubules at the microscopic level under the influence of nanosilver was without signs of structural disorganisation and alteration. In some cases, a slight increase in the lumen of the renal corpuscle capsule and desquamation of renal tubular epithelial cells were detected. In some places, cystic dilatation of the renal parenchyma tubules and moderate vascularisation were observed. A significant effect of nanosilver on the thickness of the capsule, the area of the renal lobule, the diameter and average volume of the renal corpuscle, as well as the diameters of the proximal and distal tubules, was not observed.

An increase in the absolute weight of the liver of chickens given a nanosilver preparation for 14 days by 8.7% was established against the background of a decrease in its relative weight by 10.9%. The length and width of the liver of chickens given nanosilver tended to increase.

The diameter of hepatic tubules and the volume of hepatocyte nuclei in the chicken liver parenchyma did not change under the influence of nanosilver, but the volume of hepatocytes increased by 11.8% and the N:C ratio of hepatocytes by 8.4%. The cytoplasm of hepatocytes of chickens of the experimental group was characterised by a looser consistency than in the control bird, sinusoidal capillaries (their space) were dilated. In some cases, focal infiltration by polymorphic cells and dilation and blood filling of vessels that formed hepatic triads were observed around the vessels. Along with mononuclear cells, binucleated hepatocytes were also detected in the experimental chickens, which indicates the need to apply additional criteria for assessing the risks associated with the use of nanosilver during poultry rearing.

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