



Veterinary and Sanitary Parameters and Histological Characteristics of Muscle Tissue and Internal Organs of Broilers when using the Shungikus Non-Traditional Feed Additive

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ABSTRACT

The aim was to evaluate the effect of the Shungikus non-traditional feed additive based on locally produced native Shungite on the veterinary and sanitary parameters of meat and internal organs of broiler chicken, including physicochemical, microbiological, and morphophysiological characteristics. Experimental work was carried out on Arbor Acres broilers. The birds were divided into a control group, which received a basic diet, and experimental groups, in which Shungikus was introduced at 3 and 5% of the basic diet. The study analyzed organoleptic, physicochemical, and histomorphohistological analysis of muscle tissue and internal organs. The pH, concentration of heavy metals, and total bacterial contamination were determined. Shungikus did not hurt the meat's organoleptic and physicochemical parameters. On the contrary, a decrease in pH was noted, which improved the product's technological characteristics. A significant reduction in the concentration of heavy metals in muscle tissue was established, confirming the sorption properties of Shungite. Microbiological analysis did not reveal an excess of the standard values, and morphohistological studies showed uniform development of muscle fibers and internal organs in the birds receiving the additive. Using Shungikus is a promising way to improve the quality and safety of broiler meat. Shungite helps reduce the accumulation of toxic elements without adversely affecting organoleptic and structural characteristics. It is recommended that 5% of it be added to the basic diet, which ensures an optimal effect on poultry productivity and meat quality.

Keywords: Meat, Veterinary and sanitary indicators, Histological structures, Shungite, Heavy metals, Bacterial purity.

INTRODUCTION

Modern industrial poultry farming places increased demands on the quality and safety of meat, associated with stricter veterinary and sanitary standards and the growing consumer demand for environmentally friendly products (Barbut and Leishman 2022; Nasiyev et al. 2025). In the context of industry intensification, special attention is paid to introducing effective feed additives (FAs) that increase poultry productivity (Adedokun and Olojede 2019;

Vorobyov et al. 2025) and improve the sanitary and hygienic characteristics of the final product (Prytkov et al. 2022). One promising area is using natural sorbents with pronounced detoxification and antimicrobial properties (Ossowski et al. 2021).

Using natural minerals in poultry feeding is becoming particularly important as global safety standards for animal products become stricter. According to the World Health Organization (WHO), a significant part of the diseases associated with meat consumption are caused by its

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contamination with pathogenic microorganisms, mycotoxins, and heavy metals (Vaisberg et al. 2019; Al Mashhadany 2021; Bachinskaya et al. 2022). The high bacterial contamination and accumulation of toxic elements in products threaten consumers' health and reduce their export potential (Mohammad et al. 2018).

Previous studies have shown that introducing natural sorbents into the poultry diet helps reduce the content of heavy metals in organs and tissues. In particular, zeolites and bentonites effectively bind cadmium, lead, and mercury, preventing their accumulation in meat (Sulaimanova 2021; Othman et al. 2024). In addition, they normalize the intestinal microbiota and reduce the level of ammonia and hydrogen sulfide, which helps to improve the sanitary condition of production (Kochish et al. 2022). A growing body of evidence also highlights the importance of modulating the intestinal environment to support overall health. For instance, the introduction of *Bacillus subtilis* spores into broiler diets has been shown to stimulate the development of goblet cells, enhancing mucosal barrier function and intestinal resilience (Prosekova et al. 2022).

Furthermore, the use of encapsulated plant-based and bioactive compounds has demonstrated the potential to stabilize gut morphology and promote favorable physiological responses in broilers, supporting long-term health and productivity (Idowu et al. 2025). These developments underscore the relevance of integrated dietary strategies not only for detoxification but also for maintaining gut homeostasis and reducing systemic toxic load. Moreover, such approaches align with broader principles of rational and sustainable resource use, particularly when evaluating the environmental footprint of poultry farming and the safe integration of local mineral resources (Kuandykova et al. 2024).

However, the effect of locally produced Shungite as an FA in poultry farming has not been sufficiently studied. No comprehensive studies assess its impact on the microbiological and histological parameters of broilers' internal organs and meat's sanitary and veterinary characteristics, especially concerning raw materials of Kazakhstan origin.

For the first time, this paper investigates the effect of native Kazakhstan Shungite presented as a specialized non-traditional FA (NTFA) called Shungikus on the quality and safety of broiler meat.

During the experiments, the following studies were carried out: a survey of veterinary and sanitary indicators of meat quality and organoleptic analysis of poultry meat; bacteriological studies for the content of pathogenic and conditionally pathogenic microflora; chemical analysis for the content of heavy metals and toxic elements; and histological studies of the structure of internal organs and muscle tissue of broilers of the control and experimental groups.

MATERIALS AND METHODS

Study design

The experimental economic study was conducted from December 2023 to March 2024 at the TSS Group LLP commercial poultry farm, Ornek village, Enbekshikazakh district, Almaty region, Kazakhstan. A total of 180 Arbor Acres broilers (average live weight 3.6 kg, age 5 months) were randomly assigned to three groups

(n=60 each). The control group received a standard basal diet (BD), while experimental groups were supplemented with Shungikus at 3% and 5% of the BD, respectively (Table 1). Housing, feeding, and management conditions were identical for all groups and followed the recommendations for Arbor Acres broilers.

Table 1: Design of the experiment on broiler feeding

No. Groups	Feeding conditions	Number of broilers
1 Control	100% BD	60
2 Experiment 1	97% BD+3% Shungikus	60
3 Experiment 2	95% BD+5% Shungikus	60

Broilers of all groups were kept in one poultry house, which consisted of one room with a cage system for keeping poultry in an ecologically clean room with free access to water and in three-level cages with adjustable ventilation and lighting (length of light: 16 hours). The keeping conditions in all three groups were identical and corresponded to the technological parameters adopted for the keeping of broilers of this breed.

Production technology and characteristics of shungikus

The additives were introduced into the BD as powder by step-by-step mixing. The boilers' BD corresponded to the commercial recommendations for maintaining and feeding the Arbor Acres breed (Table 2).

Table 2: The composition of the BD

Ingredients	Quantity, %	Ingredients	Quantity, %
Corn	35.3	Bone meal	0.6
Wheat	20	Chalk	3
Barley	10	Shell	3.7
Sunflower meal	13	Table salt	0.4
Feed yeast	3	P1-2 premix without methionine	1
Fish meal	5	Lysine per 100 kg	0.7
Grass meal	4	Methionine per 100 kg	0.7

Metabolizable energy: 291.3 kcal/100 g

Shungikus was obtained in a specialized form based on native Kazakhstan Shungite from the Bakyrchik deposit in the Zharma district, East Kazakhstan. During preparation, Shungite is crushed, activated, and finely ground (50-150µm), which increases its sorption properties and bioavailability. Elemental composition: carbon (C): 17.7%, sulfur (S): 0.2%, nitrogen (N): 0.3%, hydrogen (H): 0.02%. Silicon dioxide (SiO₂) is found in this mineral in the most significant proportion (52.0%), followed by aluminum oxide (Al₂O₃) (16.3%), iron oxide (Fe₂O₃ + FeO) (4.2%), calcium oxide (CaO) (2.8%), potassium oxide (K₂O) (2.3%), magnesium oxide (MgO) (2.2%), sodium oxide (Na₂O) (1.4%) and titanium oxide (TiO₂) (0.7%). The phosphorus (P) content is 0.1% (Efremov et al. 2013).

Post-slaughter veterinary and sanitary inspection of bird carcasses

At the end of the trial, 10 birds per group were slaughtered for veterinary and sanitary assessment. Carcasses and organs were examined following GOST R 52702-2006 (Federal Agency for Technical Regulation and Metrology 2006) and GOST 31467-2012 standards (Interstate Council for Standardization, Metrology and Certification 2012a). Organoleptic properties of meat and

broth (appearance, odor, elasticity, broth clarity) were assessed according to GOST 7269-79 and GOST R 51944-2002. Meat quality studies were conducted in the Department of Veterinary and Sanitary Expertise and Hygiene laboratory of the Kazakh National Agrarian Research University (KazNAIU).

Organoleptic examination of meat and broth

The organoleptic assessment of broiler meat's quality and freshness was carried out per GOST 7269-79 (The USSR State Committee for Standards 1979) and GOST R 51944-2002 (Gosstandart of Russia 2002). To assess the organoleptic characteristics of poultry meat, the following indicators were noted: the appearance and colors of the meat were determined by cutting and evaluated in deep layers of muscle tissue. Stickiness and moisture were checked on the cut surface using filter paper. A clean knife was used to make an incision to determine the odor, and the odor was immediately assessed in the deep layers of the muscle tissue, especially near the bone. The consistency and elasticity were determined by lightly pressing a finger on the meat and observing how the tissue was restored after pressure.

To determine the soup's transparency and taste, 70g of muscle tissue was cut from a poultry carcass and ground in a meat grinder. The resulting minced meat was thoroughly mixed. Then, 20g of minced meat was placed in a flask, and 60mL of distilled water was added. The mixture was cooled in the refrigerator for several minutes. After cooling, the mixture was stirred, covered with a watch glass, and placed in a water bath to maintain the temperature for 10min.

The broth's odor was determined by heating it to 80-85°C and sensing the aroma of vapors from the flask. The transparency of the broth was visually checked by pouring 20mL of broth into a measuring cylinder. Physicochemical studies were carried out following GOST 31470-2012 (Interstate Council for Standardization, Metrology and Certification 2012c).

Reaction to ammonia and ammonium salts

To prepare the meat extract, pieces of meat were cut from the superficial and deep layers of the femoral muscle after removing fat and connective tissue. The meat was chopped with a knife until a homogeneous mass was obtained. The resulting mass (5g) was weighed and placed in a conical flask. Distilled water (20mL) was added and infused for 10-15min. After boiling, the extract was thoroughly mixed and filtered through a double paper filter to remove meat residues and obtain a pure extract.

To determine the ammonia content, 1mL of the prepared extract was added to the test tube. Then, 10 drops of Nessler's reagent were added. The contents of the tube were shaken well. A change in the color and transparency of the extract was observed, indicating the ammonia content.

Determination of pH, acid and peroxide of fat

Clarified fat was prepared in advance to determine the acid number of the fat. The acid exponent of the fat was determined by titrating it with an alkaline solution. The samples were considered fresh if the acid exponent of the fat in the chilled carcass did not exceed 1. The fat peroxide value was determined by titrating fat samples with a solution of thiosulfate and iodine. The peroxide value of the fat of a chilled carcass in fresh meat should not exceed 0.01.

To determine the pH of the meat, 5g of the prepared minced meat sample was weighed, and 50mL of distilled water was added. The mixture was stirred for 30 minutes. After infusion, the mixture was filtered through a double paper filter. The concentration of hydrogen ions was measured using a laboratory pH meter. The acidity of poultry meat was determined using an I-500 ionomer pH meter (NV-Lab, Russia) following the requirements of GOST R 51478-99 (State Committee of the Russian Federation for Standardization and Metrology 1999).

Volatile Fatty Acids (VFA) determination

We determined the amount of VFA in broiler meat according to GOST 33819-2016 "Meat and meat products. Determination of the composition of volatile fatty acids by gas chromatography." For this purpose, broiler meat samples (100g) were frozen at -18°C, then thawed at 4°C for 12 hours and ground. The VFAs were extracted with sulfuric acid, distilled at 100°C, and collected in a sodium hydroxide solution. The samples were purified with diethyl ether, then methylated with methanol and sulfuric acid. The analysis was performed on a Crystallux-4000M gas chromatograph (Meta-chrome, Russia) with a flame ionization detector. The quantitative content was expressed in mg per 100g of meat.

Microbiological examination of poultry meat

Microscopic analysis (microscopy of smear prints from the surface and deep layers of muscles) was performed according to GOST 31931-2012 (Interstate Council for Standardization, Metrology and Certification 2012e). To prepare swabs from the deep layers of the thigh muscles, samples were taken, and swabs were prepared on slides. To perform Gram staining on the smear, it was covered with filter paper, coated with a solution of crystalline violet, and then left for 1min. The filter paper was removed, and the smear was rinsed under running water. A prepared Lugol solution was applied for several seconds. The excess solution was washed off. The smears were discolored with alcohol. The smears were washed under running water. A neutral red solution was applied for 3min. Then the smears were allowed to dry. The dried smears were examined under a microscope. The average number of microorganisms in one field of view of the microscope was calculated.

The number of mesophilic aerobic and facultative anaerobic microorganisms was determined according to GOST 7702.2.1-2017 (Interstate Council for Standardization, Metrology and Certification 2017) and the presence of pathogenic microorganisms was determined according to GOST 31747-2012 (Interstate Council for Standardization, Metrology and Certification 2012d) and GOST 31468-2012 "Poultry meat, by-products, and semi-finished products from poultry meat. Salmonella detection method" (Interstate Council for Standardization, Metrology and Certification 2012b).

Determination of heavy and toxic metals in meat

Determining residual amounts of heavy metals was done according to GOST 30178-96 (Interstate Council for Standardization, Metrology and Certification 1996). The meat was dried at a temperature of 105°C to a constant

weight. The dried samples were thoroughly crushed and homogenized to ensure uniformity of the sample composition. The bulk of the meat (about 0.5-1.0g) was placed in a special vessel for decomposition. A 3:1 mixture of concentrated nitric acid (HNO₃) and hydrogen peroxide (H₂O₂) was added 3.3. The mixture was heated on a hot stove until the organic substances were destroyed. After decomposition, the solution was cooled, filtered, and adjusted to the desired volume with distilled water. The measurement was performed at the appropriate analytical wavelengths to determine the metals.

Histomorphological examination of muscles and internal organs

Histological studies of muscle tissue, intestines, liver, and kidneys of broilers were conducted in the Laboratory of Pharmacology and Pathology of Animals, KazNAIU. The procedure followed the requirements of the interstate standard GOST 19496-93 (Interstate Council for Standardization, Metrology and Certification, 1993).

Preparation of slides and staining reagents

For optimal adhesion and staining quality, histological slides were prepared using classical protocols. A mixture of egg white and glycerin was used as an adhesive medium: fresh egg whites were separated, whipped, and filtered through moistened filter paper, after which glycerin was added in a 2:1 ratio. To prevent microbial growth, 0.1 g of camphor was incorporated. The solution was applied evenly to clean slides with gauze swabs and dried under controlled heat until stable.

Staining solutions were prepared per standard histological methods. A 1% eosin solution was obtained by dissolving 1g of eosin in 100mL of distilled water with thorough mixing until complete dissolution. Ehrlich's hematoxylin was prepared by combining 20mL of a 10% alcohol solution of hematoxylin, 80 mL of 96% ethanol, 100mL of glycerin, 100mL of distilled water, 10mL of glacial acetic acid, and 3g of alum. The mixture was exposed to light for four weeks for maturation and subsequently filtered before use.

Following fixation in 10% neutral buffered formalin, tissues were rinsed in running water for 15m, cut into fragments (<3 cm³), and sequentially infiltrated with 12.5% gelatin (6h) and 25% gelatin (24 h at 37°C). Blocks were stored at 4°C, then stabilized in 20% formalin for 12h before sectioning. Tissue blocks (15 × 15 × 4mm) containing both surface and deeper layers were trimmed and sectioned at 10–30µm on a microtome. Sections were transferred into water baths and mounted on pretreated adhesive slides. After gentle drying under filter paper pressure, the slides were ready for staining.

Prepared sections were stained using Ehrlich's hematoxylin (3min), rinsed, differentiated in 1% HCl solution, neutralized in ammonia water, and counterstained with 1% eosin for 1min. After final rinsing, the sections were mounted under coverslips for microscopic analysis.

Section staining

Paraffin sections were stained according to standard histological protocols. Briefly, sections were immersed in Ehrlich's hematoxylin for 3min, rinsed in running water for 2min, differentiated in 1% hydrochloric acid until light

pink, and neutralized in ammonia water until blue. After a second rinse, sections were counterstained with 1% eosin for 1 minute, rinsed again, and mounted under cover slips.

Tissue slicing was performed with an ERM 3100 microtome (MedTechnikPoint, Russia), and processing was conducted using a Leica ST 4040 tissue processor (Leica Microsystems, Austria). Digital micrographs were obtained with a Carl Zeiss light microscope (Carl Zeiss AG, Germany) equipped with a high-resolution camera.

Statistical analysis

Data was processed using Microsoft Excel 2019. Results are presented as mean±standard error of the mean (SEM). Statistical significance was determined using Student's t-test, with P≤0.05 considered significant.

RESULTS

Veterinary and organoleptic assessment

Using the organoleptic method of investigation, the following indicators were determined: the odor, appearance and color of the carcass surface, subcutaneous and internal adipose tissue, and the serous membrane of the abdominal cavity; the consistency and condition of the muscles on the incision; and the quality of the broth. Each sample was analyzed separately. Post 24 hours of slaughter, the chilled meat of the broilers of the control and experimental groups remained whitish yellow with a pink tinge, with a specific odor characteristic of fresh meat. The femoral muscles were reddish; the pectoral muscles were pale pink. Subcutaneous and internal fat were pale yellow, with a specific odor characteristic of fresh poultry meat. The serous membrane of the abdominal cavity was moist and shiny. The muscles were dense, elastic, and when pressed with a finger, the resulting dimple was leveled within a few seconds. The muscles on the incision were pale pink, slightly moist, but did not leave a wet spot on the filter paper. After a veterinary examination of the bird carcass, the internal examination was conducted. The study began with the intestines and mesentery, and after the viscera were removed entirely, the liver, ovaries, testes, stomach, spleen, heart, kidneys, and lungs were examined. The mesentery and intestines were examined for hemorrhages, inflammation, fibrin, parasites, and helminths characteristic of infectious diseases such as plague, cholera, paratyphoid, tuberculosis, and leukemia. A veterinary and hygienic assessment of the birds' insides showed that all organs were proportional in size, had a color characteristic of each organ, and did not contain hemorrhages, spots, ulcers, or neoplasms.

After the boiling test, the transparency and aroma of the broth were determined. The broth from the broiler meat samples from all groups was clear and fragrant. According to organoleptic parameters, the samples of the 3rd group of broilers had signs characteristic of fresh meat obtained from healthy poultry.

The next stage was to conduct physicochemical studies, the results of which are presented in Table 3. Determination of the concentration of hydrogen ions in meat indicates the physiological state of poultry before slaughter (concentration of hydrogen ions and glycogen in muscles). The pH level of the muscle tissue of birds in the control group was significantly higher than that of the 2nd

and 3rd groups. This may indicate a low glycogen content in the muscle tissue of birds and a low activity of intramuscular enzymes involved in the meat maturation process, which means that the decrease in the pH of the muscle tissue of birds of the 1st group was not as intense as in the muscle tissue of the experimental groups. The benzidine peroxidase test was positive for the broiler meat from all groups. The meat extract acquired a blue-green color, turning into a reddish brown color within 1-2min.

The amount of VFAs in broiler meat was 3.9-4.0mg KOH/g. The acid and peroxide numbers did not exceed the indicators of biological control of meat.

The content of amino-ammonia nitrogen in the meat of the control and experimental groups was between 1.28 and 1.29mg, which proves the freshness of the meat under study. The physicochemical parameters of the meat of the control and experimental groups had no significant differences compared with the indicators of biological control. Also, they corresponded to the signs of good-quality meat, which indicates the positive effect of native Shungite on preserving the qualities of poultry meat.

The study of microbiological parameters of broiler meat is also important in the veterinary and sanitary assessment of food. When examining smear prints from the surface layers of muscles, single bacteria were detected. Bacterial microflora was not detected in smears from the other experimental groups' deep layers of muscle tissue.

The results of the microbiological examination of poultry muscles are presented in Table 4. The number of mesophilic aerobic and facultatively anaerobic microorganisms (NMAFAnM) in the samples of all groups was in the range of 1.8×10^2 - 1.7×10^2 colony-forming units (CFU)/g. No pathogenic organisms, including *Salmonella*, were found in any broiler meat sample (Table 4).

Thus, the meat of broilers that received Shungikus along with the main diet did not differ from the meat of the control group regarding microbiological parameters,

confirming compliance with regulatory requirements. This allows the meat to be used for human consumption without restrictions.

Heavy metals with shungikus

The study found that the cadmium and lead content in the control and experimental groups did not exceed the maximum permissible concentration (MPC) (Table 5). The average cadmium content in poultry meat of the control group was 0.007mg/kg, and in groups 2 and 3, 0.0005mg/kg. The use of Shungikus in the diets of broilers led to a significant decrease in the cadmium content in meat by 28.5% ($P \geq 0.05$).

The average lead content in the experimental groups was also significantly lower ($P \geq 0.05$) than in the control group. In the 3rd group, where Shungikus was used at 5% of the BD, it was 0.009mg/kg. The MPC for lead in meat is 0.5mg/kg. Arsenic and mercury were not detected in meat samples from either the control or experimental groups.

Data analysis shows that adding Shungikus helps reduce the content of heavy metals in the birds. These results may be due to the adsorption and ion exchange properties of Shungite. The analysis showed that the heavy metal concentrations in poultry meat were within the established norms. Therefore, adding 5% Shungikus to the BD can significantly reduce lead and cadmium content in poultry meat.

Muscle and liver histology

Examination of broiler muscle tissue

The histological picture of the muscle tissue of the control group of broilers consists of muscle fibers, with the sarcolemma, nucleus, and cytoplasm visible. The nuclei of the muscle fiber are located under the sarcolemma. The shape of the nuclei is oval; the chromatin is dispersed into small grains. Transverse striation is detected in the muscle fibers, and eosin uniformly colors them red (Fig. 1).

Table 3: Physicochemical parameters of broiler meat when using Shungikus in the diet

Indicators	Groups (n=5)		
	1 (Control)	2 (Experimental)	3 (Experimental)
pH	5.72±0.1*	5.12±0.3*	5.13±0.1*
Acid number of fat, mg KOH/g	0.71±0.03	0.76±0.02*	0.82±0.05*
Fat peroxide value, % iodine	0.008±0.01*	0.009±0.01	0.009±0.01*
VFA amount, mg KOH/g	3.9±0.03	4.0±0.02	3.9±0.02*
Amino-ammonia nitrogen, mg	1.28±0.02	1.28±0.01*	1.29±0.01
Bacterioscopy	3-4, no breakdown of muscle fibers		

*Significant difference compared with control ($P \leq 0.05$).

Table 4: Microbiological indicators of broiler meat of the control and experimental groups

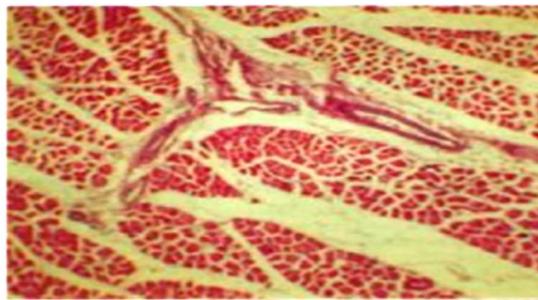
Indicators	Acceptable values	Groups			Regulatory documentation (RD) for the test method
		1 (Control)	2 (Experimental)	3 (Experimental)	
NMAFAnM, CFU/g	1.0×10^3	1.8×10^2	1.7×10^2	1.8×10^2	GOST 10444.15-94
Coliform bacteria (CB) in 0.01g	not allowed	not detected			GOST 31747-2012
Pathogenic microorganisms, including <i>Salmonella</i> , in 25 g	not allowed	not detected			GOST 31468-2012

*No statistically significant differences were observed among groups ($P \geq 0.05$)

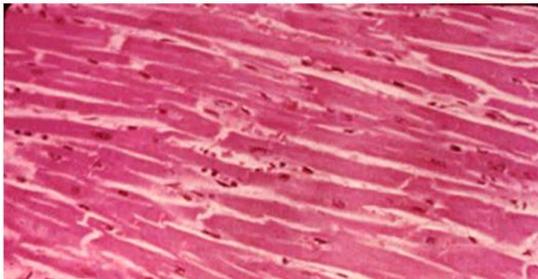
Table 5: Heavy metal content in broiler meat of the control and experimental groups

Indicator name, mg/kg	Groups (n=3)			MPC
	1 (Control)	2	3	
Mercury (<i>Hg</i>)	not detected	not detected	not detected	0.03
Arsenic (<i>As</i>)	not detected	not detected	not detected	0.1
Cadmium (<i>Cd</i>)	0.007±0.0001	0.005±0.00002*	0.005±0.00001*	0.05
Lead (<i>Pb</i>)	0.011±0.0001	0.011±0.0001	0.009±0.0002*	0.5

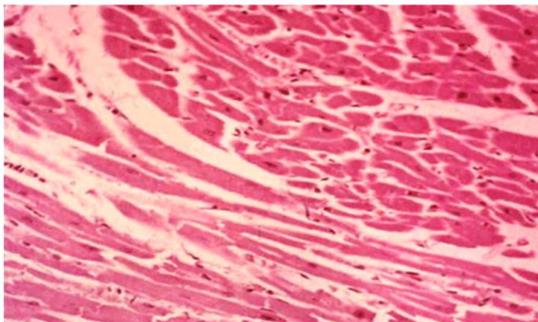
*Significant difference compared with control ($P \leq 0.05$).



Group 1 (control)



Group 2 (experimental)



Group 3 (experimental)

Fig. 1: Histological patterns of superficial pectoral muscle tissue of broilers. Hematoxylin-eosin staining. Magnification×100; scale bar = 50 μm.

The muscle tissue of the wet group was evenly colored, its thickness was the same and the contours were even. The striation of the muscle fibers was well expressed. The bundles of muscle fibers of group 3 were well defined and closely adjacent. Locally, areas of fibrillation of muscle fibers were noted. Eosin uniformly colored the cytoplasm of muscle tissue red, and the nuclei' tinctorial properties ranged from weakly basophilic to intensely basophilic. The nuclei occupied a peripheral position, lying almost under the sarcolemma. The intermuscular connective tissue contained a small amount of adipose tissue.

Data on the structure of the superficial musculature of the large pectoral muscle of birds are presented in Table 6.

The cross-sectional area of the muscle fiber is an

important indicator of muscle hypertrophy and directly affects broilers' productive qualities. This indicator was 3,512.2±2.11mm² in the control group, while in the experimental groups, it slightly increased (3,526.3±1.24mm² and 3,544.4±1.61mm², respectively). Although the differences between the groups are statistically unreliable (P≥0.05), the upward trend may indicate a positive effect of the FA on bird muscular development. The thickness of the endomysium in the control group was 6.61±1.42μm (P≥0.05), whereas in the experimental groups, this indicator was lower (5.81±0.12μm and 5.84±1.41μm). A decrease in the thickness of the endomysium may indicate a reduction in the amount of connective tissue, which increases tenderness and improves the meat's organoleptic properties.

The thickness of the inner perimysium, which separates the bundles of muscle fibers, also decreased in the experimental groups: 15.22±1.52μm in the control group versus 14.81±1.12μm and 14.65±1.88μm in the experimental groups (P≥0.05). The thickness of the epimysium, which forms the outer shell of the muscle, was found to be comparable in all groups. In the control group, the indicator was 36.06±1.63μm, in group 2, 36.05±1.54μm, and in group 3, there was a slight decrease (34.85±1.52μm). The diameter of the muscle cell nucleus is an indicator of metabolic activity and tissue regeneration. In the control group, this indicator was 5.06±0.12μm, in group 2, 5.18±0.16μm and group 3, 5.32±0.14μm.

An analysis of the morphometric characteristics of broiler muscle tissue showed that positive changes were observed in the experimental groups, expressed in an increase in the area of muscle fibers and a decrease in the thickness of the endomysium and inner perimysium. These trends indicate that Shungikus optimizes the processes of muscle tissue growth and development.

Examination of the intestines of the control and experimental groups of broilers

Histological examination of the intestines of the broilers of the control and experimental groups showed that the main structural components of the organ were preserved (Fig. 2). The mucous membrane was represented by a single-layered cylindrical bordered epithelium, including bordered, goblet-shaped, and enterochromaffin cells. The epithelial layer, which consists of loose connective tissue, was located on the lamina propria of the mucous membrane. The villous apparatus of the intestine was well developed. The villi had a typical structure and were covered with fringed epithelium. Tubular crypts performing secretory and regenerative functions were observed at the base of the villi. Moderate lymphocyte infiltration was noted in the lamina propria of the mucous membrane, which was not accompanied by pronounced inflammatory changes, indicating a normal immunological status of the intestine.

Table 6: Indicators of muscle tissue structures of the superficial pectoral muscle of broilers

Indicators	Group, n=3		
	1 (Control)	2 (Experimental)	3 (Experimental)
Cross-sectional area of the muscle fiber, mm ²	3,512.2±2.11	3,526.3±1.24	3,544.4±1.61
Endomysium thickness, μm	6.61±1.42	5.81±0.12	5.84±1.41
Thickness of the inner perimysium, μm	15.22±1.52	14.81±1.12	14.65±1.88
Thickness of the epimysium, μm	36.06±1.63	36.05±1.54	34.85±1.52
Nucleus diameter, μm	5.06±0.12	5.18±0.16	5.32±0.14

*No statistically significant differences were observed among groups (P≥0.05)

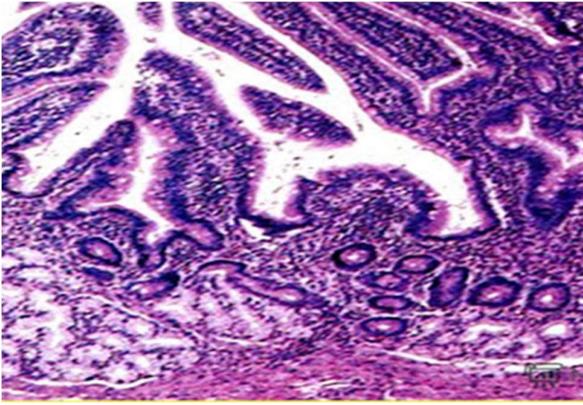


Fig. 2: The small intestine in group 3. Stained with hematoxylin-eosin. Magnification $\times 200$; scale bar = 50 μm .

Morphofunctional changes include the preservation and non-deformity of villi, moderate hyperemia of blood vessels, and mild desquamative-proliferative changes in the integumentary epithelium of the small intestine. An increase in the number of goblet-shaped cells containing a large amount of secretions and in the size of the glands was revealed. There was a slight increase in the number of cellular elements in the lamina propria of the mucous membrane, which may indicate adaptive processes in the intestine.

Histological examination of the liver and kidney of broilers

Histological examination of the livers of the broilers of the control and experimental groups showed that their morphological structure was preserved without marked deviations from the norm (Fig. 3). The liver's architecture was expressed: the hepatic tubules, consisting of interconnected hepatocytes, were visible. The structure of the tubules remained unchanged; they were arranged radially around the central vein. Hepatocytes had a regular, rounded-oval shape; their cytoplasm was uniformly colored pink by eosin and contained granular inclusions. The cell nuclei were large, rounded, and intensely purple. They had clearly defined nucleoli and chromatin lumps, indicating the preservation of active metabolic processes. The bile ducts contained a moderate amount of bile, with no signs of stagnation or destructive changes. The connective tissue structures of the liver, including the interlobular connective tissue septa, had not undergone significant changes. Their slight infiltration by cellular elements was noted, which may be a variant of an adaptive reaction. The liver's vascular network was represented by moderately blood-filled central veins, which indicated the preservation of regular blood supply to the organ.

Histological examination of the kidneys of the broilers of the control and experimental groups showed that the main structural elements of the organ were preserved, indicating the absence of significant morphological changes (Fig. 4). The kidneys' tubular apparatus had a typical structure: the epithelial cells of the proximal and distal convoluted tubules were distinguishable, evenly colored, and without signs of degenerative changes. The boundaries of the nephrocytes were clear, and the cell nuclei were well defined, indicating the renal epithelium's regular metabolic activity.

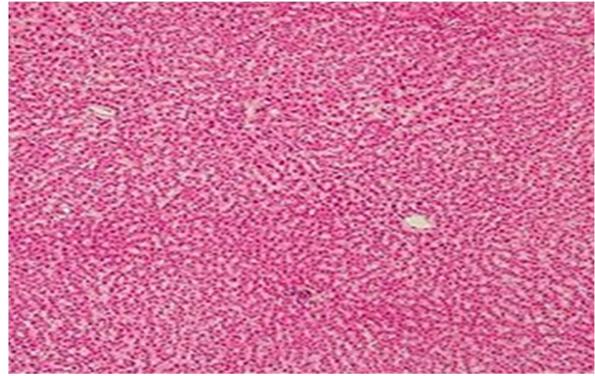


Fig. 3: Histological picture of the liver in group 3. Stained with hematoxylin-eosin. Magnification $\times 200$; scale bar = 50 μm .

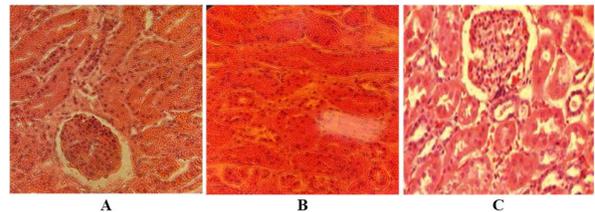


Fig. 4: Histological picture of broiler kidneys (group *a*: group 1 (control), group *b*: group 2 (experimental), group *c*: group 3 (experimental)) with hematoxylin-eosin staining. Magnification $\times 400$; scale bar = 20 μm .

The tubule lumen was preserved in most cases, but it was moderately narrowed in some places. In isolated instances, a fine-grained protein mass stained red with eosin was detected in the tubule lumen, which may indicate a physiological load on the organ but is not a pathological change. The renal corpuscles had a preserved structure: the well-defined glomeruli and the moderately blood-filled capillaries. There were no signs of disrupted protein and fat metabolism in the tubular epithelium.

Thus, the morphological organization of the kidneys of the control and experimental broiler groups remained unchanged, which confirms their normal functioning without signs of a pathological process.

DISCUSSION

We performed a comprehensive veterinary and sanitary assessment of broilers that had received Shungikus based on the local native Shungite as part of the diet. The data indicate that this additive does not adversely affect meat's organoleptic, physicochemical, and microbiological properties, which is consistent with the results of several studies on the use of sorption minerals in poultry feeding (Zasorin et al. 2019). Recent evidence from a systematic review shows that probiotics can enhance chicken gut microbiota, immunity, behavior, productive performance, and aspects of meat quality via microbiome-mediated mechanisms (Idowu et al. 2025). In contrast, our Shungikus (Shungite) approach attained comparable end-points primarily through adsorption/ion-exchange-driven detoxification rather than microbiota modulation.

The analysis of organoleptic characteristics showed that the poultry meat from the studied groups had a fresh

smell, characteristic color, and elastic consistency. The transparency and aroma of the broth also met the standards of good-quality meat, which indicates the absence of protein decomposition and accumulation of products of undesirable microbiological metabolism. These indicators are fundamentally important since they determine consumer attractiveness. Similar data were obtained by Buryakov et al. (2023), who studied the effect of mineral sorbents on meat quality and noted an improvement in organoleptic characteristics due to a decrease in bacterial contamination. Comparable results were reported by Bagno et al. (2021), who found that plant-based supplements like milk thistle extract helped maintain freshness and sensory attributes of broiler meat by stabilizing metabolic functions and reducing oxidative degradation. Moreover, Shokhan et al. (2025) reported that probiotic supplementation enhanced gut health and nutrient absorption, indirectly improving the sensory quality and safety profile of poultry meat.

Physicochemical analysis showed that the pH level of muscle tissue in the experimental groups was lower than in the control group, which may indicate more intensive glycolysis processes and improved meat maturation. This trend is consistent with findings by Sultana et al. (2025), who observed probiotic-enriched diets, such as yogurt supplementation, promoted post-mortem acidification, enhancing meat tenderness and shelf life. Similarly, Taechanan et al. (2024) reported that autolyzed yeast supplementation contributed to favorable pH shifts in broiler meat, reflecting healthier muscle metabolism and better post-slaughter quality outcomes. A decrease in pH after slaughter is typical for high-quality meat and is associated with the accumulation of lactic acid in the muscles. This fact is confirmed by Macháček et al. (2010), who showed that mineral additives with adsorbing properties could affect carbohydrate metabolism and energy metabolism in poultry, which leads to more stable physicochemical characteristics of the final product. Similar observations were reported by Othman et al. (2024), who demonstrated that fermented palm kernel cake improved nutrient availability and metabolic efficiency in broiler diets, leading to enhanced meat quality.

The analysis of the residual content of heavy metals in muscle tissue deserves special attention. It was found that the use of Shungikus contributed to a significant decrease in the concentration of cadmium and lead in broiler meat. The average cadmium content decreased by 28.5% ($P \geq 0.05$) and the lead level was lower than that of the control group. This confirms the sorption properties of Shungite, its capacity to bind and eliminate toxic elements, such as heavy metals, from the body through adsorption and ion-exchange mechanisms. Similar results were obtained by Kadikov et al. (2020), who showed that mineral sorbents such as vermiculite, zeolites, and Shungite contributed to reducing the level of heavy metals in the tissues of farm animals due to their high ion exchange capacity (Shevchenko et al. 2023).

Morphohistological examination of the muscle tissue showed that the structure of the muscle fibers in the broilers receiving the FA remained unchanged, with well-defined striation and a characteristic arrangement of the nuclei. These findings are consistent with the results of Ogbuewu

et al. (2024), who noted that diets enriched with fermented plant components preserved normal muscle histoarchitecture and supported cellular integrity in broiler chickens. Similarly, Shokhan et al. (2025) demonstrated that probiotic-supplemented feed maintained muscle tissue structure while enhancing the physiological resilience of the birds. A slight increase in the cross-sectional area of muscle fiber in the experimental groups indicates a tendency to improve muscle mass growth, which may be due to the positive effect of Shungite on metabolic processes, particularly protein metabolism (Bachinskaya et al. 2021). This corresponds to the data obtained by Kundryukova et al. (2017), who noted that the use of natural minerals in the diet of poultry contributed to an increase in metabolic rate, which resulted in an increase in body weight gain and an improvement in the structure of muscle tissue.

Thus, the inclusion of Shungikus in broilers' diets not only has a positive effect on the quality of meat but also helps to reduce the content of heavy metals and improve the maturation of muscle tissue. Our data make it possible to recommend this additive for industrial poultry farming to enhance product safety and quality.

Conclusion

The study allowed us to provide a comprehensive veterinary and sanitary assessment of meat from broilers that received Shungikus based on native Shungite from local production. The results obtained indicate that including this additive in the broiler diet in the amount of 3-5% of the BD does not adversely affect the organoleptic, physicochemical, and microbiological parameters of meat. On the contrary, it contributes to its improvement according to several criteria. A significant decrease in the content of heavy metals (cadmium and lead) in broilers' muscle tissue was found, confirming the sorption properties of Shungite and its ability to reduce the toxic load on the poultry body. This makes using this FA a promising direction for improving the safety of poultry products.

Thus, including Shungikus based on native Shungite in broilers' diets can be considered an effective tool for improving the quality and safety of meat.

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Data Availability: The data will be available upon request from the corresponding author.

Ethics Statement: The study was approved by the Bioethics Commission of the KazNARU (Protocol No. 156/7 dated 13 October 2023) and complies with the professional Code of Ethics of veterinarians and ethical standards for animal research established by the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes.

Author's Contribution: NS: conceptualization of the study, experimental design; MK: laboratory analyses, microbiological and histological evaluations, interpretation of results; TA: corresponding author, manuscript drafting, statistical analysis; BL: methodological support on toxicological evaluation, interpretation of data; SY: development and characterization of the Shungikur additive, materials preparation and quality control; PI: histological studies of muscle and organ tissues, image acquisition and data visualization.

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