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Impact of Shungite-Based Minerals Additives Into Water as A Measure to Control Campylobacter Jejuni Counts in Poultry

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ABSTRACT:

Campylobacter jejuni, a gram-negative bacterium, is a common bacterial cause of gastrointestinal diseases in poultry. This paper aimed to study the antimicrobial activity of the shungite mineral against *Campylobacter jejuni* infection in chickens. Sirtila Pro shungite-based mineral supplement containing probiotic cultures and Mustala mineral digestion activator were the studied samples. Chickens produced from specific pathogen-free (SPF) Valo embryos at the age of 1-44 days were used for the experiments. *Campylobacter jejuni* strain was used to infect the chickens. At the end of the 10-day observation period, all chickens were weighed, slaughtered, and necropsied. The results of histological examinations indicated that the studied chickens died as a result of the acute course of the infection caused by *Campylobacter jejuni*. Recently, scientists have been searching for the *Campylobacter jejuni* infection prevention and control methods in chickens and, subsequently, humans.

Keywords. Campylobacter jejuni; morbidity; Chickens; Shungite; Human foodborne diseases.

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1. Introduction

Campylobacter jejuni, one of the most important foodborne pathogens, is considered the most common causative agent of human gastroenteritis worldwide (Kaakoush *et al*., 2015; Facciola *et al*., 2017; Igwaran and Okoh, 2020). Moreover, the chronic effects of *C. jejuni* infection can lead to human autoimmune dis-eases such as Guillain-Barré syndrome and reactive arthritis (Babich *et al*., 2020; Umaraw *et al*., 2017; Ivankin *et al*., 2020). Although these diseases are usually mild, they can be fatal for young children, the elderly, and those with weakened immune systems (Sibanda *et al*., 2018, Johnson *et al*., 2017; Hansson *et al*., 2018). Poultry is considered the primary carrier of *C. jejuni*. Consumption of raw poultry meat is considered a major risk factor for human campylobacteriosis (Hermans *et al*., 2011; Altekruse *et al*., 1999; Blaser, 1997). It is known that poultry products cause about 60–80% of cases of campylobacteriosis globally (Sahin *et al*., 2003). *C. jejuni* colonization in the chicken intestine can reach 106–1010 CFU/g (Fancher *et al*., 2020). To effectively control the incidence of the disease in humans, it is important to reduce the level of *C. jejuni* colonization in poultry (Ivankin *et al*., 2020; Sibanda *et al*., 2018). However, due to the complex interactions between

C. jejuni and its host, only limited progress has been made in developing effective strategies against *C. jejuni* in both poultry and humans (Von Wintersdor *et al*., 2016; Shen *et al*., 2018).
There are currently no effective control measures to prevent Campylobacter colonization in commercial broiler herds (Wieczorek *et al*., 2018; Cui *et al*., 2020; Poly *et al*., 2019; Zeng and Lin, 2017). A study (Cui *et al*., 2020) showed that there is no commercial vaccine against *C. jejuni*. Although several studies have investigated the immunity of poultry against Campylobacter colonization (Liu et al., 2019; Adams et al., 2019; Wang et al., 2019; Wang et al., 2020; Carver, 2018), the nature of the protective immune responses against *C. jejuni* colonization in chickens is still unknown.
There is a hypothesis based on the structure and sorption, bactericidal, catalytic, reducing properties of shungite, that using a mineral supplement with shungite might be effective as *C. jejuni* preventive measure in chickens. Shungite (Shunga, Republic of Karelia, Russia) is a mineral of a new generation natural mineral sorbents, an intermediate product between amorphous carbon and crystalline graphite, containing carbon (30 wt%), quartz (45 wt%), and silicate mica (about 20 wt%). According to the latest data, schungite carbon is a fossilized substance of highly carbonized organic bottom sediments with the presence of fullerene-like regular structures from 0.0001 to 0.001 wt%. Due to its unique properties, shungite is used in many industries. There are several commercial shungite mineral supplements that are of interest for research.
This study aimed to investigate the antimicrobial activity of shungite against *Campylobacter jejuni* infection in chickens.

2. Materials and methods

Supplements under study

The main studied samples in this research were shungite-based additives. Sirtila Pro (Nadvoitskiy zavod TDM, Nadvoitsy, Segezhsky district, Republic of Karelia, Russia) is a supplement based on the burnt shungite rock (Skrypnik *et al*., 2021). Mustala Mineral Digestion Activator (Nadvoitskiy zavod TDM, Nadvoitsy, Segezhsky district, Republic of Karelia, Russia) is a feed supplement

Formulation of feed mixtures

Chickens of all groups received a basic ration in the form of a PK-5 "Sytnyy broiler start" compound feed for 1-3-week broilers by Agro-Ekspert (Shuya, Ivanovo region, Russia) water, without restrictions during the entire period.
The shungite-based Sirtila Pro supplement was prepared as follows: a weighed portion of the supplement was diluted with water at room temperature, stirred, and left for 1 hour, after which it was thoroughly shaken and placed in drinkers. The diluted in water supplement was used within 24 h. For this experiment, 4.5 g of Sirtila Pro was dissolved in 0.5-1.0 L of water, incubated for 1-2 h, and then added to drinking water during the day.
The shungite-based Mustala supplement was prepared as follows: shungite was crushed to a particle size of 0.2 - 0.8 mm and mixed with the basic diet in an amount of 1% of the feed weight. For this experiment, Mustala was fed at a rate of 0.15 g per 1 kg (or 0.15 mg per 1 g) of chicken body weight, which were mixed with the main diet (MD) feed. MD was based on a commercial formulation for feeding chickens of each study age. PK-5 "Sytnyy broiler start" (Agro-Expert, Shuya, Ivanovo Region, Russia) compound feed was used for 0-14 days-old broiler chickens (Agro-Expert, Shuya, Ivanovo Region, Russia), PK-5 "Sytnyy broiler rost" (Agro-Expert, Shuya, Ivanovo Region, Russia) was used for 15-32 days-old chickens, and PK-5 "Sytnyy broiler finish" (Agro-Expert, Shuya, Ivanovo Region, Russia) was used for 33-55 days-old chickens.

Exprimental animals
Chickens from SPF Valo certified embryos (VALO-SPF, Cuxhaven, Germany), which were kept in an isolation box of the vivarium dedicated to handling infectious agents from the time of hatching, were used. All birds were clinically healthy at the time of infection. At each stage there was a control group of uninfected chickens. SPF embryos are essentially sterile. For the first stage, 49 chickens with the hatching date 27.12.2022 were used. For the second stage, 146 chickens with the hatch-ing date 05.02-06.02.2023 were used. All isolators and cages in which the chickens were kept were provided with labels with indelible inscriptions containing information about the group number in the experiment, the timing of the main stages of the study, with explanatory basic experimental characteristics. The studies were approved by the Bioethical Commission of the Federal State-financed Institution «Federal Centre for Animal Health» (protocol No. 13/01.12.2022)

Experimental design
The experiment was divided into two stages. The first stage was to develop a design for exposure to infection. 30 days-old chickens were infected. The aim was to select the optimal infective dose.
There was 6 groups. One control group. Each group had 10 chickens. Water was administered to the control instead of infections. The observation was conducted for 10 days.
The design of the stage 1 of the experiment on selection of the infective dose of *C. jejuni* is presented in Table 1.

Table 1: Selection of the infective dose for the stage 1 of the experiment

Group	Infective dose	Number of chickens, heads
Control	Water 0.5 mL	10
Experimental 1	100 CFU	10
Experimental 2	500 CFU	10
Experimental 3	1000 CFU	10
Experimental 4	5000 CFU	10
Experimental 5	10000 CFU	10

Laboratory research procedure
Affected organs of dead and euthanized birds of different ages, swabs from the trachea of clinically healthy individuals, group samples of excrements, blood, washings from carcasses, and water samples from cooling baths of slaughterhouses were the research material. The organs were collected in sufficient quantities, the most affected by the causative agent of the disease, considering the disease patterns and compliance with the requirements of targeted studies. Blood was sampled into a test tube with an anticoagulant (10% Trilon B solution, heparin) for the hematological studies. Sections, prints, smears, and suspensions were prepared from the pathological material. Smears were sometimes made from blood and exudate, and print preparations were made from affected mucous membranes and organs. The smears were prepared on glass slides, dried in air or under a glass cover to avoid dust. For print preparations, a piece of the affected organ cut out with a sharp scalpel or a safety razor blade was grasped with tweezers and several thin prints were made on the glass with the free surface of the piece. Dried smears were fixed in methanol (methyl alcohol) for 15-20 min. The date of the smear preparation and the number of the bird were marked directly in the smear, closer to the edge of the slide.
Material for gastroenterological examination was taken from fresh (no later than 1 hour after death) corpses or from freshly euthanized birds (at least 5 heads). Samples were collected from those organs and tissues in which pathological changes were found. Small thin plates or

cubes no more than 1-2 cm thick were cut out of the affected organs. Together with the affected parts of the organs, the adjacent normal tissue was sampled.
The presence of infection was confirmed by the excrements of chickens infected with *C. jejuni*, plumage contamination, and characteristic intestinal distension (Fig. 1).

Figure 1: The manifestations of C. jejuni infection in chickens: a - litter of infected chickens; b – plumage contamination; c – hyperemia, intestinal distention.
Excrements were sampled with calcined tweezers (scalpel) and sent analyses in sterile test tubes, vials, glasses, which were tightly closed with parchment paper; samples from the bird corpses were sent in unopened intestines, tied at both ends. Excrement samples were collected on the 7th day, after changing the diets (prestarter to starter), on the 21st day (after vaccinations), and on the 35th day (slaughter).
Inoculation from organs were performed with Pasteur pipettes, tracheal swabs – with sterile cotton swabs, and excrements (0.5 g) were sampled with sterile glass rods (or a scalpel) into tubes with sterile saline (4.5 mL).
The studies were carried out by a certified laboratory, Federal State Budgetary Institution «Kemerovo Interregional Veterinary Laboratory» (certificate of accreditation RU.RA.21/PM52, issued on 19.08.2015.

Analysis of blood values
EDTA stabilized blood samples were placed in a thermocontainer with maintain-ing the temperature (2-8) °C immediately after collection, and within 1 day were deliv-ered and analyzed in the Shans-Bio laboratory (Moscow, Russia) for Hematocrit (Hct, PCV), leukocytes (WBC), hemoglobin (Hgb), erythrocytes (RBC), erythrocyte anisocy-tosis (RDW), mean erythrocyte hemoglobin concentration (MCHC), mean erythrocyte volume (MCV), mean erythrocyte hemoglobin (MCH), mature heterophils (pseu-do-eosinophils), eosinophils (Eos), monocytes (Mono), basophils (Bas), lymphocytes (Lym).
To obtain sera, samples of normal blood in a volume of at least 1 mL for each planned type of study, placed in Eppendorf tubes or plastic vacuum tubes with a vol-ume of 10 mL, were incubated for 30 min at 37 °C, then centrifuged for 3 min at 1200 rpm. Blood sera were stored at -30 °C for further analysis of the levels of antibodies to infectious chicken bronchitis

by enzyme immunoassay (ELISA) and Newcastle disease in the hemagglutination reaction (Hemagglutination Inhibition Test), as well as for analysis for biochemical parameters.

Gastroenterological examination

Samples of organs taken for gastroenterological examination during necropsy were fixed in a 10% solution of neutral formalin for at least 5 days. Tissues were pro-cessed in TLP-720 histocenter (Mt PointTM, Moscow, Russia), preparations were em-bedded using ESD-2800 station (Mt PointTM, Moscow, Russia), sections with a thick-ness of 5-8 microns were prepared on an RMD-3000 rotary semi-automatic microtome (Mt PointTM, Moscow, Russia), stained with hematoxylin and eosin using an ALS-96 linear automatic stainer (Mt PointTM, Moscow, Russia). The preparations were ex-amined using a Mikmed-6 microscope (LOMO LLC, Moscow, Russia), measurement and photo documentation were performed using an E31S PM video camera (MVTEAM Technology Co, Shenzhen, China) and ToupView software (MVTEAM Technology Co, Shenzhen, China) at a magnification of x100 and x400. The measuring scale of the video camera was calibrated using an object-micrometer of transmitted light (LOMO, Russia).

The thickness of the serous, muscular, mucous membranes of the glandular stomach, duodenal ulcer, rectum, and cecum, the diameter of the tubular gland of the glandular stomach were determined.

The nuclear-cytoplasmic ratio (N/C) in the liver was calculated according to the C. Tasca formula (1980) (Tasca, 1980):

$V = \pi/6 \times L \times B^2$;

where L – large diameter of cells and their nuclei, B – small diameter of cells and their nuclei.

The diameter of the renal corpuscle in the kidneys was determined. In the pan-creas, the diameters of the pancreatic acinus and the islet of Langerhans were measured. The area of the lobule was determined, and the ratio of the cortex and medulla was calculated in the thymus and bursa; the height of the epithelium was also meas-ured in the bursa.

Microbiological analysis of excrements

Microbiological methods were used to detect bacteria of the *C. genus*. Primary enrichment was carried out in Bolton broth selective liquid medium (CM 0983, OXOID) in two stages. The first stage of incubation was carried out at a temperature of (37±2) °C for 4–6 h, and at the second stage, at a temperature of (41.5±2.0) °C for 44±4 h. After that, they were subcultured onto solid selective nutrient media (Preston agar and mCCD agar) and cultured at a temperature of (41.5±2.0) °C for 44±4 h in a modified gas atmosphere. Commercial gas generators were used to create a modified gas atmosphere. As a result of cultivation, isolated colonies were obtained and identified using commercial api test systems (Biomeriuex, France).

For PCR, the culture liquid obtained at the stage of primary enrichment was used to obtain biomass of microbial cells and subsequent isolation of DNA from it. A 1.0 cm^3 culture liquid was collected into sterile centrifuge tubes and centrifuged for 5 min at 6.5 thousand rpm (Eppendorf). The resulting supernatant was removed to obtain a biomass concentrate. Considering that the concentrate could contain the components of the nutrient medium, the obtained biomass samples were subjected to washing. A sterile saline solution was added to the decantant, suspended by shaking, and then recentrifuged under the same conditions. DNA was isolated from the concentrate obtained after draining the washing saline solution.

Also, for PCR, typical colonies were selected from agar selective media and a suspension of microorganisms with a given titer was prepared in sterile saline solution.

Total DNA from the samples was isolated using the Genomic DNA Purification Kit (Fermentas, Inc., Vilnius, Lithuania) according to the manufacturer's recommendations. The composition and structure of the bacterial community was determined by NGS sequencing. Amplification was performed using a Verity DNA amplifier (Life Technologies, Inc., New York, USA) with primers that amplify the 16S rRNA gene fragment: 343F 5-CTCCTACGGRRSGCAGCAG-3', 806R 5-GGACTACNVGGGTWTCTAAT-3'. Sequencing was performed on a MiSeq genomic sequencer (Illumina, Inc., San Diego, California, USA) using the MiSeq Reagent Kit v3 (Illumina, Inc., San Diego, California, USA). The processing of 2 x 300 nt reads and DNA sequences was performed using the CLC Bio GW7.0 bioinformatics platform (Qiagen, Amsterdam, the Netherlands). The total number of bacteria was determined by qPCR using the Reagent kit for RT-PCR in the presence of an intercalating dye EVA Green (Syntol, Moscow, Russia) and primers Eub338 5-ACTCCTACGGGAGGCAGCAG-3', Eub 5185' ATTACCGCGGCTGCTGG-3' on a DT Lite-4 detecting amplifier (DNK-Tekhnologiya, Protvino, Russia) under conditions: 95 °C - 3 min (1 cycle); 95 °C - 13 s, 57 °C - 13 s, 72 °C - 30 s (40 cycles).
Bacteria were inoculated on a selective medium – erythritol blood-carbon agar to isolate campylobacter.

Statistical data processing
Statistical processing of the research results consisted in finding significant differences between the mean values in the samples. The mean values for the groups, the errors of the mean in body mass, weight and relative weight of internal organs, mor-phometric indicators, the statistical significance of differences between the group of test systems receiving TS and the control group were determined, indicating, if there are differences, the values of the statistical criterion, the number of degrees of freedom (df) and the probability of forecast error (p). Outliers were detected according to the Grubbs' criterion (Lemeshko and Lemeshko, 2005; Sadeghi and Bijani, 2018).
The dynamics of body mass was assessed by the indicator of the relative increase in body mass according to Brody's equation (Sadeghi and Bijani, 2018):

$$K = \frac{(W_t - W_0)}{0.5 \times (W_t + W_0)} \times 100\ \%;$$

where K – relative growth (%) for a certain period; Wt – weight at a given age, g; W0 – initial weight, g.
The relative mass of organs (relative to body weight) was calculated according to the equation:
$M_T = m/M \times 100\%$;
where M_T – relative organ mass by body mass, %; m – organ mass, g; M – bodyweight of the bird, g

3. Results and discussion

30-days-old chickens were infected with the *C. jejuni* culture on January 22, 2021, after which the state of the test systems was monitored for 10 days, followed by euthanasia and necropsy. The body mass dynamics of chickens infected with *C. jejuni* are presented in Tables 2-3.

Table 2: Weight gain results in chickens infected with *C. jejuni* (stage 1)

Groups	Experiment design	Body weight[1], g		Average body weight, g		Change in body weight, g
		1	2	1	2	

2	Control	190.95	208.98	191.92±12.39	210.85±16.48	18.93
		186.13	194.92			
		199.24	238.09			
		211.72	207.38			
		186.54	232.94			
		198.82	212.03			
		172.80	dead[2]			
		184.71	199.23			
		191.63	194.78			
		196.69	209.33			
3	$BD+Inf_{100}$	185.38	195.39	195.61±12.13	199.28±18.10	3.67
		189.93	214.22			
		199.34	220.16			
		205.16	171.84			
		201.11	185.29			
		184.23	203.60			
		208.82	n/sp[3]			
		180.92	n/sp			
		197.64	195.88			
		203.59	207.82			
4	$BD+Inf_{500}$	196.83	231.10	200.47±12.06	207.43±46.15	6.96
		224.04	206.81			
		201.25	188.86			
		199.22	135.08			
		201.24	231.02			
		186.03	282.50			
		201.83	182.59			
		182.24	249.54			
		214.15	159.33			
		197.82	n/sp			
5	$BD+Inf_{1000}$	209.29	212.28	197.87±7.73	207.23±32.43	9.36
		198.23	185.01			
		179.99	250.76			
		201.13	236.08			
		204.04	193.93			
		192.29	217.71			
		200.27	218.07			
		201.65	144.01			
		205.51	237.41			
		197.74	177.08			
6	$BD+Inf_{5000}$	201.49	215.56	195.57±13.15	183.55±24.89	-12.01
		184.57	166.77			
		167.08	175.61			
		200.23	200.34			
		204.04	198.86			
		195.32	140.89			
		184.16	186.83			
		204.51	dead[4]			

		210.11	dead			
		204.15	dead			
7	BD+Inf$_{10000}$	201.27	193.82	207.95±34.50	212.17±45.45	4.22
		254.94	263.44			
		172.29	178.01			
		201.11	dead			
		210.12	213.41			

1 – before infection (21.01.2023); 2 – 10 days after infection (04.02.2023); 1data is not pairwise related; 2slaughtered for comparative histology; 3 n/sp – nonspecific death; 4dead – presumably death due to experimental infection

Table 3: Weight gain results in chickens infected with C. jejuni (stage 2)

Groups	**Experiment design**	**Body weight[1], g**		**Average body weight, g**		**Change in body weight, g**
		1	**2**	**1**	**2**	
2	Control	189.81	236.68	195.97±12.40	215.74±16.50	19.77
		188.19	193.75			
		200.32	218.25			
		211.16	209.16			
		189.54	230.72			
		199.94	217.39			
		174.84	slaughtered [2]			
		198.73	215.48			
		201.59	208.74			
		205.61	211.53			
8	BD+Inf$_{100}$+SPro	187.21	197.36	198.51±12.40	200.88±16.50	2.37
		192.38	215.68			
		199.96	222.13			
		207.78	173.39			
		204.41	188.49			
		187.3	205.41			

		9				
		209.12	200.16			
		193.33	196.38			
		202.59	199.70			
		200.92	210.13			
9	BD+Inf$_{500}$+SPro	210.11	218.33	201.37±12.40	208.45±16.50	7.08
		216.81	210.71			
		210.35	198.68			
		200.70	220.01			
		195.37	219.49			
		200.62	237.18			
		190.82	200.10			
		199.84	208.31			
		190.12	173.22			
		198.91	198.49			
10	BD+Inf$_{1000}$+SPro	207.17	209.71	197.78±12.40	209.35±16.50	11.6
		191.44	181.33			
		209.73	247.38			
		179.16	231.49			
		201.53	190.77			
		190.02	227.16			
		197.98	212.11			
		200.15	180.73			
		202.03	209.08			
		198.59	203.74			

11	BD+Inf_{5000}+SPro	203.15	198.94	194.20±12.40	187.17±16.50	-7.03
		187.71	188.31			
		178.16	176.94			
		202.35	176.33			
		207.81	201.96			
		199.41	dead[4]			
		186.67	183.73			
		187.93	179.42			
		201.85	179.09			
		186.95	199.78			
12	BD+Inf_{10000}+SPro	198.31	196.90	194.13±12.40	200.23±16.50	6.10
		206.17	205.42			
		165.40	189.84			
		199.16	dead			
		201.62	208.74			
13	BD+Inf_{100}+Mus	201.09	207.99	202.75±12.40	215.28±16.50	12.53
		190.32	171.90			
		218.59	201.38			
		206.48	228.13			
		201.20	231.02			
		204.31	249.54			
		198.03	207.38			
		203.42	213.27			
		204.11	229.04			
		199.9	213.16			

		5				
14	BD+Inf$_{500}$+Mus	212.18	220.41	203.28±12.40	211.45±16.50	8.17
		220.01	213.28			
		213.42	200.08			
		202.41	221.73			
		199.26	220.16			
		202.14	240.92			
		192.91	202.17			
		200.16	210.58			
		190.28	173.74			
		200.04	n/sp[3]			
15	BD+Inf$_{1000}$+Mus	209.02	211.93	199.93±12.40	215.97±16.50	16.04
		193.75	184.74			
		211.18	249.19			
		185.41	234.32			
		206.03	193.71			
		192.47	229.57			
		199.13	215.75			
		204.75	183.79			
		204.37	209.03			
		193.16	n/sp			
16	BD+Inf$_{5000}$+Mus	205.18	211.75	195.16±12.40	202.48±16.50	6.32
		189.33	199.74			
		179.46	182.41			
		204.17	206.10			

		209.53	210.39			
		200.48	207.48			
		188.64	198.89			
		191.36	209.41			
		199.59	200.74			
		193.83	197.93			
17	BD+Inf10000+Mus	200.18	198.74	195.86±12.40	203.13±16.50	7.27
		208.61	dead			
		167.57	182.73			
		201.24	217.29			
		201.68	213.76			

1 – before infection (25.01.2023); 2 – 10 days after infection (04.02.2023); [1]data is not pairwise related; [2]slaughtered for comparative histology; [3] n/sp – nonspecific death; [4]dead – presumably death due to experimental infection

The observation data on chickens infected with *C. jejuni* are presented in Tables 4-5.

Table 4: Observations on chickens infected with *C. jejuni* (stage 1)

Days after infection	**Infectious dose, CFU/mL**			**Results**
	1000	**5000**	**10000**	
1	-	-	+	1 chicken from group 5 died
2	+	+	+	distress in experimental groups 3-5
3	+	+	+	distress in experimental groups 3-5
4	+	+	+	distress in experimental groups 3-5
5	+	+	+	the condition persisted, no dead chickens were found when examining
6	-	+	-	the condition persisted, no dead chickens were found when examining
7	-	+	-	1 chicken from group 4 died
8	-	+	-	the condition persisted, no dead chickens were found when examining
9	-	+	-	the condition persisted
10	-	+	-	2 chickens from group 1 and 1 chicken from group 2 died, all 3 had signs of pecking

"-" – the absence of infection dose; "+" – the presence of infection dose

Table 5: Observations on chickens infected with *Campylobacter jejuni* (stage 2)

Days after infection	Diet						Results
	II			III			
	Infectious dose, CFU/mL						
	1000	5000	10000	1000	5000	10000	
1	-	-	+	-	-	-	the condition persisted, no dead chickens were found when examining
2	-	-	+	-	-	+	the condition persisted, no dead chickens were found when examining
3	-	-	+	-	-	-	the condition persisted, no dead chickens were found when examining
4	-	-	+	-	-	+	the condition persisted, no dead chickens were found when examining
5	-	-	+	-	-	+	the condition persisted, no dead chickens were found when examining
6	-	-	+	-	-	+	the condition persisted, no dead chickens were found when examining
7	-	+	+	-	-	+	the condition persisted, no dead chickens were found when examining
8	-	-	+	-	-	+	1 chicken from group 5 died
9	-	+	+	-	+	+	the condition persisted, no dead chickens were found when examining
10	-	+	+	-	+	+	1 chicken from group 2 and 1 chicken from group 3 died

"-" – absence of infection dose; "+" – presence of infection dose

The situation presented in Figure 1 is true for all birds with no shungite-based mineral supplements in their diets. Severe intestinal inflammation, disseminated necrohemorrhagic enteritis, and hepatic necrotizing cholangiohepatitis were observed. No enteritis, plumage contamination, intestinal distention, or hepatic necrotizing cholangiohepatitis were observed in groups of birds that had shungite-based mineral supplements with water or feed.

Data weight indices of chickens of the control and experi-mental groups after the first stage of the research are presented in Tables 6-7.

Table 6: Data on livability and body weight indices of chickens of the control and experimental groups (stage 1)

Groups	Experiment design	Chickens, heads	Fatality, heads	Days after infection	Average body weight, g		Change in body weight, g
					1	2	

2	Control	10	1[1]	4	192.31±12.39	215.72±16.48	23.41
3	BD+Inf$_{100}$	10	1[2]	10	194.27±12.13	198.42±18.10	4.15
4	BD+Inf$_{500}$	10	2[3]	10	200.47±12.06	207.43±46.15	6.96
5	BD+Inf$_{1000}$	10	-	-	197.87±7.73	207.23±32.43	9.36
6	BD+Inf$_{5000}$	10	1, 1, 1	4, 7, 9	195.57±13.15	183.55±24.89	-12.01
7	BD+Inf$_{10000}$	5	1	1	207.40±34.50	211.76[1]±45.45	4.35

1 – before infection; 2 – 10 days after infection; [1] slaughtered for comparative histological material; [2] n/sp – nonspecific death; [3] individual body weights of chickens in this group were 193.82 g, 178.01, and 263.44; thus 2 out of 3 chickens were underweight

Table 7: Data on livability and body weight indices of chickens of the control and experimental groups (stage 2)

Groups	**Experiment design**	**Chickens, heads**	**Fatality, heads**	**Days after infection**	**Average body weight, g**		**Change in body weight, g**
					1	**2**	
2	Control	10	1[1]	4	213.52±12.39	225.94±16.48	12.42
8	BD+Inf$_{100}$+SPro	10	-	-	214.39±12.13	228.75±18.10	14.36
9	BD+Inf$_{500}$+SPro	10	-	-	210.19±12.06	218.13±46.15	7.94
10	BD+Inf$_{1000}$+SPro	10	-	-	207.49±7.73	217.47±32.43	9.98
11	BD+Inf$_{5000}$+SPro	10	1[2]	7	205.16±13.15	198.63±24.89	-6.53
12	BD+Inf$_{10000}$+SPro	5	1	1	216.31±34.50	223.16[1]±45.45	6.85
13	BD+Inf$_{100}$+Mus	10	1	1	209.73±12.13	221.84±12.13	12.11
14	BD+Inf$_{500}$+Mus	10	-	1	208.95±12.06	224.03±12.06	15.08
15	BD+Inf$_{1000}$+Mus	10	-	-	210.74±7.73	210.95±7.73	0.21
16	BD+Inf$_{5000}$+Mus	10	-	-	210.57±13.15	213.16±13.15	2.59
17	BD+Inf$_{10000}$+Mus	5	-	-	205.79±34.50	207.99±34.50	2.2

1 – before infection; 2 – 10 days after infection; 1 slaughtered for comparative histological material; 2 n/sp – nonspecific death

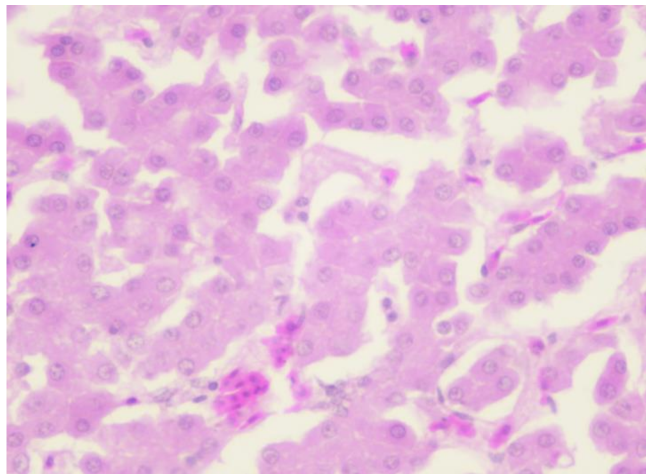
The idea of a smart city differs from a people's perspective in terms of technology[48]. This is clear as the countries embark on smart cities projects because they provide a wide range of insights into the problem. While intelligent cities worldwide are becoming increasingly common, their significance is still a myster. "The smart cities market continues in the 'I know it when I see it' process without a generally recognized definition." The notion of an intelligent city is not widely known, and identifying one has proved difficult. On the other hand, several definitions stress standard features, characteristics, and elements that can help define intelligent city perspectives. The use of hardware, applications, and networks for information technology, data on different urban areas and services, for instance, would enhance the quality of life for a particular population group.

The study of the antimicrobial activity of the shungite mineral against the background of infection of chickens with *C. jejuni* resulted in establishing this activity by two shungite-based supplements (Sirtila Pro and Mustala) by the absence of severe intestinal inflammation, disseminated necrohemorrhagic enteritis and hepatic necrotizing cholangiohepatitis in chickens receiving these supplements with water or feed.

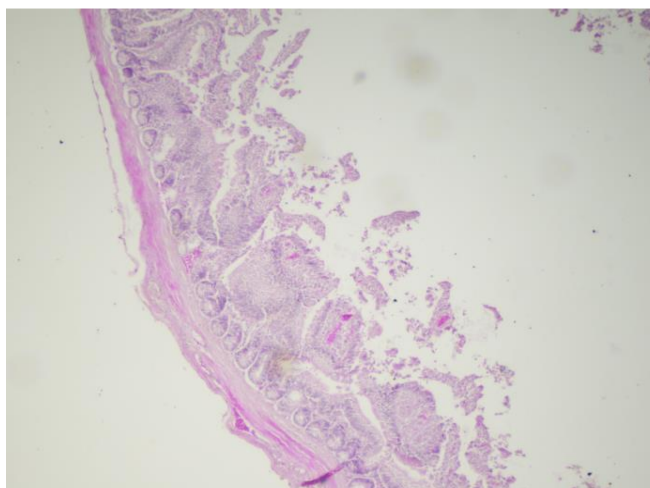
The antimicrobial activity of the shungite-based supplements is confirmed by the data presented in Table 6. It follows from the table data that the greatest weight was observed in the chickens of the experimental groups infected with 500 CFU/0.1 mL with the introduction of Sirtila Pro; 1 g/L in drinking water and 500 CFU/0.1 mL with the introduction of Mustala; 1g/kg in feed. The average weight in the groups was 201.37 g and 203.28 g, respectively, in group 2, 208.45 g and 211.45 g, respectively, in group 4. Thus, despite the infection, the chickens continued to grow and develop within the normal range due to the suppression of *C. jejuni* infection with shungite-based supplements.

Histological examination of the pathological material of a chicken that died from a hyperacute infection during the first day after infection with *C. jejuni* at the maximum dose (10,000 CFU/mL) revealed granular liver dystrophy, edema of the submucosa in the duodenum, necrosis of the apical part of the villi, in the absence of pathology in other studied organs (glandular and gizzard, heart, spleen, large intestine, bursa), which indicates the development of acute catarrhal enteritis.

Pathomorphological manifestations of hyperacute *C. jejuni* infection in chickens are shown in Figure 2.



A



B

Figure 2: Pathomorphological manifestations of a hyperacute C. jejuni infection in a chicken: *A – liver (×400 magnification); B – wall of the duodenum (magnification ×40).*

The fact of infection of chickens with non-pathogenic for poultry Campylobacter was proved as a result observing the bird and pathological cutting of carcasses. Infection manifested in the form of severe intestinal inflammation, disseminated necro-hemorrhagic enteritis, and hepatic necrotizing cholangiohepatitis. Figure 2 shows that as a result of infection with *C. jejuni*, chickens experience an enlargement and uneven staining of the liver, the presence of numerous punctate hemorrhages, and the formation of lymphoid follicles, as well as miliary necrosis. There is a catarrh-al-hemorrhagic inflammation in the duodenum. The duodenum of infected chickens, in comparison with normal ones, is enlarged, its walls are thinned, it is filled with liquid contents. Numerous plaques and hemorrhages are observed.

The are presented in the Table 8 isolation rate of campylobacter from poultry excrements.

Table 8: The isolation rate of campylobacter from poultry excrements, LG genomes/G (EQUIVALENT CFU/G

Age of birds, gays	**Control**		**C. jejuni**	
	1	**2**	**1**	**2**
7	7.56±0.26	6.9±0.36	8.3±0.24	7.11±0.39
21	8.01±0.35	7.56±0.15	9.04±0.40	7.94±0.39
35	7.5±0.56	6.45±0.59	9.45±0.45	8.49±0.59

1 – isolation substrate (blind appendages); 2 – isolation substrate (excrement samples).

The contamination degree of chickens (on the 6th day) with *C. jejuni* in samples by quantitative PCR are presented in Table 9.

Table 9: Evaluation of contamination degree of chickens (on the 6th day) with *c. Jejuni* in samples by quantitative PCR, LG GENOMES/G (EQUIVALENT CFU/G

Isolation substrate	***C. jejuni* infection background**	
	Control	***C. jejuni***
Excrement samples	7.2±0.58	3.16±0.28
Washings from carcasses	6.13±0.34	4.42±0.45

The respiratory tract of infected chickens was not tested as *C. jejuni* usually affects the digestive system of the bird.

The first dead chicken from group 7 (infection dose 10,000 CFU/mL) was found at the end of the first day after infection lying on its side. Necropsy: the area of the cloaca was not contaminated. Feathers were tousled, partly plucked on the chest. The subcutaneous tissue was dry, with severe dehydration. The crop was full. No abnormality was found in the heart, lungs, liver, spleen, lower intestine, cecum, muscular and glandular stomach. The small intestine was hyperemic on the outside and on the cut.

Three days after infection, the presence of pathological litter was observed, and a specific fetid odor appeared in the room. Four days after infection, the presence of un-formed excrement was observed.

One chicken from the experimental group 6 (5,000 CFU) died. No contamination of the cloaca was noted during necroscopy. When pressed, a white smearing mass was released from the cloaca. Feathers are tousled, partly plucked on the chest. The subcutaneous tissue was dry, with severe dehydration. The crop and intestines were empty. No abnormality was found in the heart, lungs, liver, spleen, glandular stomach, cecum. The muscle layer of the gizzard was exhausted, the mucous membrane had a sulfur-yellow color. Exhaustion was determined during the dissection of the organs, when catarrhal inflammation and hemorrhages were found in the gizzard and severe changes in the cuticle of the gizzard were found in the glandular stomach. The normal color of the gizzard lining is yellow, during the disease it acquired a dark brown color. The small intestine was hyperemic on the outside, and in the section, the intestinal wall was thin. Pathological signs characteristic of an acute inflammatory process in the small intestine were observed.

Fragments of the small intestine, gastric mucosa, and spleen were collected from the dead chicken and a chicken from the control group were collected for histological examination. One chick from the control group was euthanized to provide control material. When examining the chickens after 5-6 days after infection, the condition of the chickens was the same; no chicken died. At the end of the 7th day after infection 1 chicken from group 6 (5.000 CFU/mL) died. External examination of the corpse revealed exhaustion and dehydration. The plumage was disheveled, the plumage in the area of the cloaca was contaminated with excrement. Signs of autolysis were noted, so no autopsy was performed. Pathological signs characteristic of acute dyspeptic disorder were established. When examining the chickens after 8 days after infection, the condition of the chickens was the same; no chicken died.

When examining the chickens after 9 days after infection, the condition of the chickens was the same. One chicken from group 6 died. External examination of the corpse revealed exhaustion and dehydration. The plumage was disheveled, the plum-age in the area of the cloaca was contaminated with excrement, and toes were pecked. The respiratory organs, liver, kidneys were unchanged. In the atrial area, the sur-rounding area of discolored tissue was found (necrosis or purulent process). On the luminal surface of the gizzard, an outgrowth of pink-red tissue was found. The out-lines were indistinct; the edges were smoothed. Organs had no visible changes. The mucous membrane of the duodenum was moderately hyperemic, the rest of the intestine was unchanged. Material for histological examination was collected. Pathological signs indicating a moderate inflammatory process in the small intestine were observed. Possible nonspecific death due to pecking. Ten days after the infection, 2 chickens from group 1 and 1 chicken from group 4 died; all had signs of pecking. The necropsy revealed exhaustion and no signs of indigestion and pathology of internal organs. It was found that the death of chickens is nonspecific.

There was no significant statistical difference between the chickens of the experimental and control groups; however, there was a tendency of average weight decrease in the chickens exposed to infection, as well as the low body weight among them, which may be a sign of dyspeptic disorder. The presence of low body weight chickens in the experimental groups

receiving lower doses of *C. jejuni* (100 CFU and 500 CFU) might be associated with asymptomatic chronic infection.

The individual body weights of chickens within experiment groups 2-5 showed a significant variation, which may indicate that the individual susceptibility of chickens to experimental infection differs. After necropsy examination of groups 5 (1.000 CFU/mL) and 6 (5.000 CFU/mL) revealed distress and tousled feathers. Examination of two chickens from group 6 showed exhaustion, dehydration, tousled feathers, pale skin, and mucous membranes. Feathers were clean. No abnormality was found in the heart, lungs, liver, spleen, lower intestine, cecum, muscular and glandular stomach. The goiter, stomachs, intestines are filled with normal contents. Hyperemia of the small intestine, with signs of gas formation, isolated cases of uneven staining of the liver with areas of a lighter color, or mosaic coloration with signs of hyperemia were revealed. Examination of chickens from group 7 (10.000 CFU) revealed that 2 out of 3 chickens were distressed, had unkempt plumage and exhaustion, necropsy demonstrated signs of an inflammatory process in the small intestine.

Thus, pathoanatomical signs of *C. jejuni* infection include exhaustion, dehydration, pallor of the skin and mucous membranes, hyperemia, swelling of the small intestine, and uneven liver color.

Clinical and pathological manifestations of the disease are not always pronounced. In some cases, based on the exhaustion of birds in the absence of visible pathology at the autopsy, an asymptomatic course of the infection can be suspected. When studying the organs of chickens from the experimental groups that died during the observation period and surviving birds with external manifestations of the disease, slaughtered at the end of the experiment, granular dystrophy, and isolated punctate hemorrhages in the liver, heterochromatic staining of hepatocyte nuclei were noted. The results of histological examinations indicate that the studied chickens died as a result of the acute course of the infection caused by *C. jejuni*.

The increase in mortality in the negative control group can be explained by the fact that the chickens of this group did not receive additional biologically active agents that had a corrective effect on metabolic processes and activation of protective forces, productivity correctors; anti-stress and immunostimulating agents. The same factors contributed to the fact that mortality was inconsistent, highest in the groups with lower amounts of *C. jejuni*. Infection was not the cause of death.

Recently, scientists have been looking for the prevention and control of *C. jejuni* infection in chickens and, subsequently, in humans. There are some studies aimed at combating this disease.

The study (Taha-Abdelaziz *et al*., 2018) was developed to evaluate the solubility of unmethylated CpG motifs (E-CpG ODN) and PLGA-encapsulated oligodeoxynucleotides (ODN) and *C. jejuni* lysate when colonizing *C. jejuni* for the protective effect of multi-antigen vaccines. The results showed that oral administration of low (5 μg) or high (50 μg) doses of CpG significantly reduced the rate of *C. jejuni* colonization in laying hens by 1.23% and 1.32%, respectively (P <0.05). On 22nd day after infection (slaughter age), E-CpG significantly reduced *C. jejuni* levels by 1.89% and 1.46% in laying hens and broilers, respectively. A similar pattern was observed for *C. jejuni* lysate; oral administration of *C. jejuni* lysate reduced the intestinal load of *C. jejuni* in layers and broilers by 2.24% and 2.14%, respectively, 22 days after infection. In addition, the combination of E-CpG and *C. jejuni* lysate reduced the bacterial count in the caecal contents by 2.42% 22 days after infection of broilers. Small intestinal IgG (Ab) antibody titers in broiler chicks receiving low or high dose E-CpG or low dose *C. jejuni* lysate were significantly higher than in broiler chickens receiving placebo. In addition, these groups showed a positive correlation between the titer of serum IgG antibodies and the amount of *C. jejuni* in the cecum. These data indicate that PLGA-

encapsulated CpG or *C. jejuni* lysate may be a promising strategy for controlling *C. jejuni* in chickens.

The study (Sahin *et al*., 2003) used a laboratory challenge experiment to test whether specific maternal antibodies (MAB) against Campylobacter play a protective role in young chickens that do not normally contain Campylobacter in their bodies. The kinetics of *C. jejuni* colonization were compared for infected 3 day old broilers that were naturally positive for Campylobacter specific MAB and 21 day old broilers that were negative for Campylobacter specific MAB. Colonization occurs faster in birds infected at 21 days of age than in birds infected at 3 days of age, suggesting that certain MABs may be involved in delayed colonization. To further explore this possibility, in the presence or absence of Campylobacter infection, laboratory-reared laying hens free of specific pathogens and their 3-day-old offsprings with (MAB+) or without (MAB-) Campylobacter-specific MAB were orally infected with *C. jejuni*. Compared to the MAB- group, during the first week there was a significant decrease in the percentage of colonized chickens in the MAB+ group. These results indicate that Campylobacter-specific MAB play a role in protecting young chickens from colonization by *C. jejuni*. Obviously, the presence of MAB in young chickens does not affect the development of the systemic immune response after infection with *C. jejuni*. However, compared to birds infected on day 3, an active immune response to Campylobacter appeared earlier and stronger in birds infected on day 21. Clearance of Campylobacter infection was also observed in chicks infected at 21 days of age. These results (I) show that anti-Campylobacter MAB contributes to the absence of Campylobacter infection in wild young chickens, and (II) provide additional evidence to support the development of an immune approach to control Campylobacter infection in poultry.

The study (Wang *et al*., 2018) showed that *C. jejuni* is a major cause of human Campylobacter disease worldwide. Chicken is the main host of *C. jejuni*. The cecum is the main colonization site for *C. jejuni*. The CLOCK gene was shown to play an important role in regulating the response to *C. jejuni* vaccination and is associated with immune genes. The correlation network between the CLOCK gene and microRNA (miRNA) plays a crucial role in the development of colorectal tumors. MiRNA plays a key role in bacterial invasion by regulating several target genes. To study the regulatory correlation between miRNAs and target genes, five miRNAs that regulate the expression of CLOCK genes (miRNAs interact with CLOCKs) and five immune-related target genes predicted using Targetscan and miRDB were selected for expression patterns analysis. Expression of patterns was determined in the cecum of chickens at 4, 8, 12, 16, 20 and 24 h after inoculation with *C. jejuni* using quantitative PCR. The results showed that the expression of gga-miR-148a, gga-miR-1416-5p, gga-miR-30b and gga-miR-30c was significantly higher than the expression of 8 genes of the island of pathogenicity (hpi) and gga-miR. -30a-5p, Gga-miR-30b, and gga-miR-30c were significantly inhibited at 24 hpi, while gga-miR-1416-5p were significantly inhibited at 20 hpi ($P<0.05$). Gene expression levels BCL9 (B-cell CLL / lymphoma 9), STX16 (syntaxin16), IL4R (interleukin 4 receptor), and IRF4 (interferon regulation factor 4) increased significantly after 8 hpi, while SOCS3 (cytokine signaling 3) were significantly suppressed. In addition, the expression pattern of gga-miR-30b corresponds to gga-miR-30c. miRNA and mRNA showed fluctuating expression patterns in both inoculated and non-inoculated groups. The direction of SOCS3 regulation by 8 hpi contradicts the direction of regulation of gga-miR-148a, gga-miR-1416-5p, gga-miR-30b, and gga-miR-30c. Therefore, the MiR-30 and miR-148 / miR-152 families time-dependently regulate the response to inoculation of *C. jejuni* into the cecum of chickens. BCL9, STX16, IRF4 and IL4R play an important role in the response to *C. jejuni* inoculation. MiRNA, which interacts with the CLOCK gene and immune-related target genes, responds to *C. jejuni* inoculation. The SOCS3 gene interacts with gga-miR-30b, gga-miR-30c, gga-miR-148a, and gga-miR-1416-5p in response to inoculation of 8 hpi *C. jejuni*. The

correlation between miRNAs that interact with CLOCK and immune-related target genes plays a critical role in the response to *C. jejuni* inoculation. These results form the basis for further studies of the regulation mechanism of chicken microRNA and its target genes in response to inoculation with *C. jejuni*.
Enterobactin (Ent) with sideoform plays a crucial role in the colonization of *C. jejuni* in the intestine. The innovative Ent conjugate vaccine was recently released. The vaccine has been shown to induce high levels of Ent-specific antibodies in rabbits; Ent-specific antibodies show strong binding to Ent and inhibit Ent-dependent growth of *C. jejuni*. In this study using Specific Pathogen Free (SPF) chickens, three trials were performed to evaluate the immunogenicity of the Ent conjugate vaccine and its efficacy against intestinal colonization of *C. jejuni*. Purified Ent was bound to carrier lymphohemocyanin (KLH). Up to 3 intramuscular immunizations of chickens with Ent-KLH were unsuccessful. Immunization does not affect weight gain, the development of key immune organs, or the gut microbiota (Cui *et al*., 2020).
In the first two trials, compared with the control group, immunization of chickens according to different protocols (2 or 3 vaccinations) always elicited a strong Ent-specific immune response. Consistent with high levels of systemic anti-EntIgG, caecal colonization of *C. jejuni* was significantly reduced in two independent vaccination trials. A third test showed that a single Ent-KLH vaccination is sufficient to detect high levels of systemic antibodies specific to Ent, which can persist in chicks for up to eight weeks. The Ent-KLH combination vaccine can induce high levels of Ent-specific antibodies in chickens and protect them from *C. jejuni* colonization. This vaccine represents a novel strategy for the control of Campylobacter in poultry and humans (Masoudi *et al*., 2020).

4. Conclutions

The experimental infection caused illness with signs of digestive tract damage in chickens. The first clinical signs of dyspeptic disorder, expressed in distress, rumple-ness, and contamination of the plumage, unformed fetid greenish excrements, appeared in birds infected with doses of the pathogen from 1.000 CFU and above after 2 days, becoming more pronounced 3 days after infection.
After infection with a high dose of the pathogen (10.000 CFU), the development of hyperacute intestinal infection and death of the chicken was observed within the first day after infection.
The experiment demonstrated that when the chickens were fed shungite-based supplement Sirtila Pro, the livability of the chickens was up to 100%, and the greatest increase in the bodyweight of the chickens was noted (9.36 g). The amount of *C. jejuni* in chicken excrements decreased to 7.11±0.39 lg genomes/g (equivalent CFU/g) when using a shungite-based feed supplement compared to the control (7.56±0.26 lg genomes/g (equivalent CFU/g). Thus, the antimicrobial activity of the roasted shungite-based mineral supplement against *C. jejuni* infection was proven.

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Conflict of Interest declaration
The authors declare that they have NO affiliations with or involvement in any organization or entity with any financial interest in the subject matter or materials discussed in this manuscript.

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Author Contributions
Anna B. Balykina - wrote the paper; **Kirill A. Zaitsev -** analyzed and interpreted the data; **Viktor P. Murygin -** analyzed and interpreted the data; **Ilya N. Nikonov** – Conceived, designed the research, wrote the paper; **Anastasiya A. Kabanova -** contributed reagents, materials, analysis tools or data; **Olga O. Babich** - Conceived and designed the research. All authors accepted the final draft.

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