

Water treatment by contact non-equilibrium low-temperature plasma refers to innovative methods of processing food raw materials, which requires determining the level of safety of its use to meet the requirements of food safety for humans. The test animals were divided into four test groups with two different basic diets. The tested substances were drinking main water (control group) and plasma-chemically activated water (experimental group); wheat bread (control group) and wheat bread produced using plasma-chemically activated water (experimental group). It was found that there was no significant difference between the control and experimental groups of animals in body weight and its changes during 90 days of the introduction of the test substances into the diet. The amount of water and feed consumed was in direct proportion to the change in the weight of animals in the corresponding diets.

The hematological and biochemical analysis of the blood of the test rats did not confirm the toxic or allergenic effect of the studied feeding factors on their organism. An increase in the number of erythrocytes in the blood and activation of the phagocytic activity of experimental groups of animals were confirmed. This confirms the positive effect of plasma-chemically activated water and wheat bread made with its use on metabolic processes in the body of animals.

Macromorphological parameters of the body of animals and the results of histological studies of the stomach, liver, kidneys and femur as potential target organs demonstrated the absence of dystrophic-degenerative changes in these organs. A comprehensive study of the food safety of plasma-chemically activated water and wheat bread made with its use proves the possibility of using an innovative method of water treatment in food production

Keywords: plasma-chemically activated water, food safety, wheat bread, subchronic toxicity

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FOOD SAFETY OF PLASMA-CHEMICALLY ACTIVATED WATER AND BREAD MADE WITH ITS USE

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1. Introduction

Providing the population with safe food products that do not cause acute food poisoning, toxic infections or long-term negative consequences for human health is a global and urgent problem of our time [1–3]. A feature of making bread as a product of mass consumption is the use of water as the main raw material. The quality of bread depends significantly on the properties of water, which can be regulated by additional pre-treatment [4–7]. In particular, due to the treatment of water by physicochemical methods, it is possible to solve such urgent problems for bakery production as intensification of the production process [4, 5], lengthening the freshness of products [6] in an environmentally friendly way [7]. This allows to reduce the loss of raw materials and finished products along the food chain, achieving a resource-saving effect. Overestimated enthusiasm is caused by the introduction of various types of additional water treatment, which makes it possible to purposefully change its properties depending on technological needs [8, 9]. Water and its characteristics have a significant impact on the health of humans and living organisms. It is an activator of chemical and biological processes. In turn, with the help of controlled changes in the properties of water, it

is possible to correct its effect on living organisms. Water is a necessary component of human life and is in the body in direct form or in food. The modern food industry all over the world is faced with the problem of saving resources, increasing production efficiency against the background of food security volatility as a result of the gradual growth of the world's population [2, 10, 11].

Among the combined physicochemical methods of water treatment, attention is drawn to the use of contact non-equilibrium low-temperature plasma [4, 12–14]. The plasma-chemically activated water obtained by this method has high permeability due to the appearance of a small-cluster structure and pronounced antiseptic properties due to the presence of peroxide and superoxide compounds in its composition [4]. This method of water treatment is also classified as environmentally friendly [15–17]. Excluding the use of antimicrobial substances, treatment with non-equilibrium contact plasma leads to inactivation of opportunistic microorganisms in water due to the simultaneous action of electric discharges, ultraviolet radiation, and ozonation [15]. In this case, the antiseptic properties of the treated water are supplied with peroxide and super-peroxide compounds formed as a result of the action of plasma on water [16]. In particular, main water treated with

non-equilibrium contact plasma has an antimicrobial effect on opportunistic microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, which can cause food poisoning [16].

Taking into account the properties of plasma-chemically activated water, studies on the study of the possibility of its use as drinking water to meet human needs are relevant. In addition, there is a need to study the possibility of using in the food industry for the manufacture of various products. However, the possibilities of using plasma-chemically activated water are limited by the lack of comprehensive data on its safety.

2. Literature review and problem statement

The works [5, 6] present the results of using plasma-chemically activated water to intensify the accumulation of biomass of baker's yeast in the production of bakery products from wheat flour and dispersed grain mass of wheat grain. The efficiency of the introduction of treated water into the technological process has been proven, which helps to reduce the production process by 20–30 %, improve the quality of finished products by 5–21 %, and extend the shelf life of wheat bread by 1.5–2 times. But the assessment of the harmlessness of plasma-chemically activated water as the main raw material for the production of bakery products remained unresolved. The need to assess the safety of such water is due to the fact that the treatment with contact non-equilibrium plasma refers to unconventional methods of raw material preparation. An option for overcoming and solving the corresponding problem can be a cycle of scientific research involving laboratory rats.

In [18], the effect of plasma-chemically activated water on the functional state of *Riccia fluitans*, *Lémma minor L.*, *Paramecium caudatum*, *Artemia salina*, and *Cyprinus carpio* was studied. It was confirmed that the use of plasma-chemically activated water with a concentration of peroxide compounds in the amount of 500 mg/l contributed to an increase in the amount of viable zooplankton by 60–70 % compared to the use of main water without preliminary treatment [18]. In particular, the intensity of development of the root system of phytoplankton increased by 7–12 and 28–39 % compared with the control group in the case of using water exposed to plasma for 10 and 30 minutes, respectively [18]. The introduction of brine shrimp, duckweed, and speech into the diet of scaly carp, previously cultivated in plasma chemically activated water, led to an acceleration in the growth of these fish [19, 20]. The authors of [21] confirmed the effectiveness of the effect of plasma chemically treated water on individual bacteria in the planktonic state, which is correlated with the data of [18]. However, due to the fact that cold atmospheric pressure plasma was used, in addition to H₂O₂, active components were also present in the inactivation mechanism, and this does not allow this method of water treatment to be classified as environmentally friendly. The biological activity of plasma-chemically activated water was tested on bacteria in planktonic and biofilm forms [22]. In such water, active components were found – hydrogen peroxide and nitrogen compounds. Such compounds quickly suppressed gram-positive bacteria. However, it was shown that plasma-chemically activated was not effective against bacterial biofilms.

The work [14] presents the results of using plasma-chemically activated water for various biological and medical objects. This water was tested as an antitumor, antimetastatic, antimicrobial, regenerative agent, including for blood coagulation and even as a dental treatment. However, the need to search

for prospects for solutions in applied biomedical applications of plasma-chemically activated water was noted. Promising for further research is not only the establishment of the mechanisms and stability of the properties of such water after treatment, but also its safety as a food ingredient [14, 23].

In work [12] it was proved that the use of cold atmospheric plasma to activate water is an alternative to the traditional methods of its disinfection. In particular, it was shown in [24] that plasma-chemically activated water can act as an alternative disinfectant for inactivation of the S-protein and protection against the SARS-CoV-2 virus, and the possible mechanism of action on the virus was substantiated. The results of studies [25] indicate a high efficiency of the decomposition of pesticides and allergens due to the treatment of water with plasma, and in [26], plasma-chemically activated water was used to enhance plant resistance to diseases. Due to its microbiocidal properties, plasma-chemically activated water is attracting more and more attention for use in the food, agricultural and biomedical industries. But the use of such water should overcome the obstacles associated with the lack of determining the level of its safety in relation to complex living organisms to assess the impact on the human body. All this suggests that it is advisable to conduct a study to determine the subchronic toxicity of plasma-chemically activated water and food products made with its use.

3. The aim and objectives of research

The aim of research is to assess the safety of plasma-chemically activated water and wheat bread made with such water by determining cytotoxicity and general toxicity after prolonged administration to laboratory rats. This will allow the widespread use of plasma-chemically activated water for the production of food products while saving resources, increasing the efficiency and environmental friendliness of production processes.

To achieve the set aim, the following objectives are set:

- to determine the effect of plasma-chemically activated water and bread made with its use on the consumption of water and feed, changes in the weight of animals during the study;
- to analyze the hematological and biochemical parameters of the blood of the test animals;
- to establish the macromorphological parameters of the test animals under the influence of the studied factors and the histological changes in the target organs of rats after 90 days of consumption of the corresponding rations.

4. Materials and methods of research

4.1. Characteristics of water exposed to contact non-equilibrium plasma and bread made with its use

Drinking water of the city water supply network in the city of Dnipro, pretreated with contact non-equilibrium low-temperature plasma (NLP), was used as the test substance. Plasma-exposed water acquires new properties (Table 1). During plasma-chemical activation, water is exposed to ultraviolet radiation, electrolysis, water hammer, pulsed discharge, and the action of atomic oxygen and hydrogen occurring during one operation. The peculiarities of plasma-activated water include the appearance of stable hydrogen peroxide and super-peroxide compounds in it, which has been confirmed by many scientific works [15, 27–29]. Also, plasma-chemical treatment of water

leads to partial or almost complete destruction of clusters with the formation of an additional amount of free, unconnected water molecules. This leads to an increase in the reaction and permeable water [30–32]. The studies used drinking water exposed to contact non-equilibrium plasma for 30 minutes to the content of peroxide compounds in the amount of 500 mg/l, stored for no more than 24 hours after processing.

Table 1

Characteristics of water used in research

Water characteristic	Duration of water treatment, min	Water pH	Concentration of peroxide compounds, mg/l	Redox potential, mV	Surface tension 10^{-3} N/m
Mainline	–	7.3–7.6	–	210–260	68.2
Plasma chemically activated water	30	9.9–10.4	500	80–124	59.1

Plasma-chemically activated water (PAW), obtained with other parameters of the activation process, has less pronounced antiseptic and permeable properties. It should be noted the stability of the properties acquired during the plasma-chemical activation, which retain in the case of preparation of drinking water for 7 days. The water used in the studies underwent mandatory control of the main indicators reflecting the degree of activation (pH, concentration of peroxide compounds) before its introduction into the diet of animals. Plasma-chemically activated water (PAW) was used instead of main water without additional treatment in the diet of rats ad libitum separately without mixing with other components, which ensured the uniformity of its administration.

To obtain wheat bread, let's use drinking water without additional treatment and water previously exposed to the action of contact non-equilibrium plasma, according to the recommendations given in [6]. For the production of bread was used: wheat flour of the highest grade, pressed yeast; table salt; water exposed to NLP for 30 minutes; main water without additional treatment for the preparation of control samples. The dough was prepared in a safe way. After kneading, fermentation, kneading, separating, shaping and finally standing the dough pieces, baking was carried out in a laboratory oven with a humidified baking chamber at a temperature of 220–230 °C. The duration of baking pan bread was 30 minutes, hearth bread – 28 minutes. To ensure the optimal structural and mechanical properties of the combined feed mixtures for animals, wheat bread was dried after 12–24 hours at a temperature of 40–60 °C and used in a form dried to a moisture content of 12–14 %. The uniformity of distribution of wheat bread in the diet of rats was monitored daily.

4. 2. Features of the injection and actual doses of the tested substances in the diet of animals

Water exposed to contact non-equilibrium plasma, or bread made with its use, was administered orally. The tested food components were included in the diet of animals instead of drinking water and wheat bread made according to traditional technology, respectively (Table 2). The tested food ingredient (plasma chemically activated water) or food product (bread made using plasma chemically activated water) was introduced into the diet of animals daily for 90 days.

Due to the complexity of the experiment, two control groups and two experimental ones were formed. The first group of an-

imals – control group 1 “Water” – consumed a full diet of food and consumed main water without any treatment. The second group of animals – experimental group 1 “Plasma-chemically activated water” – consumed a full-fledged diet of food and consumed main water, exposed to the action of non-equilibrium contact plasma to a concentration of peroxide compounds of 500 mg/l. The third group of animals – control group 2 “Bread” – consumed a diet in which carbohydrate-containing feed – rye bread, oats and wheat groats – were replaced with wheat bread and consumed main water without treatment. The fourth group of animals – experimental group 2 “Bread made using plasma-chemically activated water” – consumed a diet similar to the previous group, but it included wheat bread made using plasma-chemically activated water.

Table 2

Animal rations during the research

Component	Content in the recipe, % for a group of animals			
	«Water»	«PAW»	«Bread»	«Bread made using PAW»
Wheat bread	–	–	65.2	–
Wheat bread based on PAW	–	–	–	65.2
Oat grain	23.0	23.0	–	–
Rye bread	21.2	21.2	–	–
Wheat grain	21.0	21.0	–	–
Juicy feed (beets, carrots)	18.0	18.0	18.0	18.0
Powdered milk	4.4	4.4	4.4	4.4
Meat and bone meal	11.0	11.0	11.0	11.0
Fodder yeast	0.7	0.7	0.7	0.7
Stone salt	0.7	0.7	0.7	0.7

When testing plasma chemically activated water for 90 days of the study, the actual dose of the substance was 152–278 g/kg of animal weight per day, the equivalent of which is the dose of water consumption by humans 11–20 g/kg of body weight per day. For wheat bread made using plasma-chemically activated water, the actual dose was 85–152 g/kg of rat weight per day. The equivalent of such a dose for humans corresponds to 6–11 g/kg of body weight per day. The calculated actual doses exceed the human consumption of water and wheat bread required to maintain normal body function and an adequate quality of life.

4. 3. Test animals: characteristics and features of the content

To determine toxicity according to the requirements of the OECD Test guideline 408, nonlinear rats were used, kept under standard conditions by the vivarium of the veterinary clinic of the Dnieper State Agrarian and Economic University. Before the start of the study, the animals were kept under experimental conditions for 2 weeks to monitor their health. The study was carried out in accordance with the rules of humane treatment of animals and the requirements of the “European Convention for the Protection of Vertebrate Animals used for Scientific and Other Purposes” (Strasbourg, 1985).

The experiment involved 4 test groups, including 80 animals: 20 animals in each of the two experimental and two control groups. Moreover, each group of animals included 10 males and 10 females. The test animals were 1–1.5 months old. The weight of the rats was 91.5 ± 10.3 g, which did not ex-

ceed 20 % of the average weight of animals of each sex. Groups of animals were formed by randomizing rats according to their weight, each of which was individually identified.

The test rats were kept at a temperature of 22 ± 2 °C and a relative air humidity in the room of 55 ± 10 %; these parameters were monitored daily. Any deviations from these indicators during the study were insignificant and did not affect the overall results of the experiment. The lighting in the room was artificial, and the daylight hours were 12 hours. For feeding the animals, glass containers were used, the design of which prevented loss of feed during feeding. Food products and water supplied in drinkers were available to animals without restriction during the stage of animal acclimatization and the actual experiment. The rats were kept in groups of 5 rats of the same sex in one cage.

The recording of clinical changes in the test animals and their behavior was carried out twice a day during the entire duration of the experiment.

4.4. Analysis of food safety of tested substances based on clinical studies

During the study, visual changes in the state of the external integument, mucous membranes of animals, stool, changes in gait and behavior were recorded daily.

The determination of body weight and the amount of food and water consumed for the test animals was carried out every week for 90 days of the study. In this case, the weight of males and females was set separately.

Removal of animals from the experiment was carried out by decapitation under ether anesthesia in accordance with ethical provisions. Blood samples for morphological studies were taken in EDTA-K₃ tubes; blood serum was obtained by centrifugation at 3500 rpm for 10 minutes. The resulting serum was immediately frozen at a temperature of -20 °C and stored in this state until the moment of biochemical studies.

Immediately after collection, blood samples were analyzed for the number of leukocytes, erythrocytes, platelets, erythrocytes and hematocrit, as well as the hemoglobin content on an automatic hematological analyzer PCE 90-Vet (High Technology, USA). Various leukocyte forms were counted in blood smears stained according to Wright-Giemsa using a light microscope (Olympus CH 20, Japan). In the

blood serum, the content of total protein, albumin, globulins, glucose, the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase was determined. The analysis was carried out on an automatic biochemical analyzer BioChem FC-200 (High Technology, USA) using High Technology reagents.

The objects of histological studies were the stomach, liver, kidneys, and femur. Selected whole organs were fixed in 5 % chilled formalin solution ($+4$ °C) in a refrigerator for 48 hours. The subsequent fixation was carried out at room temperature in a 10 % formalin solution for 10–14 days. Paraffin sections with a thickness of 2–3 μ m were stained with hematoxylin and eosin. In total, more than 60 histosections were made and analyzed. Microphotography was performed using a Leica DM1000 microscope.

4.5. Statistical processing

The data obtained were evaluated by the least squares method for each group. Objective disagreement scores with control groups were determined statistically by comparing the least squares of each test group with the corresponding least squares of the corresponding control group of animals. The obtained numerical results of the studies were processed using the Student's t-test to determine the degree of probability of the difference (p) between the rats in the control and experimental groups. The results of the mean values were considered statistically probable at $p \leq 0.05$ and $p \leq 0.01$ in comparison with the data of the control group.

5. Results of studies of food safety of plasma-chemically activated water and wheat bread made with its use

5.1. Consumption of water and feed, changes in the weight of the test animals during the study

As seen from Fig. 1, at the initial stage of research, the weight of animals of different sex for both the experimental and control groups differed within the margin of error and was insignificant. Starting from the fourth week of the study, there was a gradual increase in the difference between the growth of the mass of the male and female animals, which at the end of the 14th week reached 13–15 %.

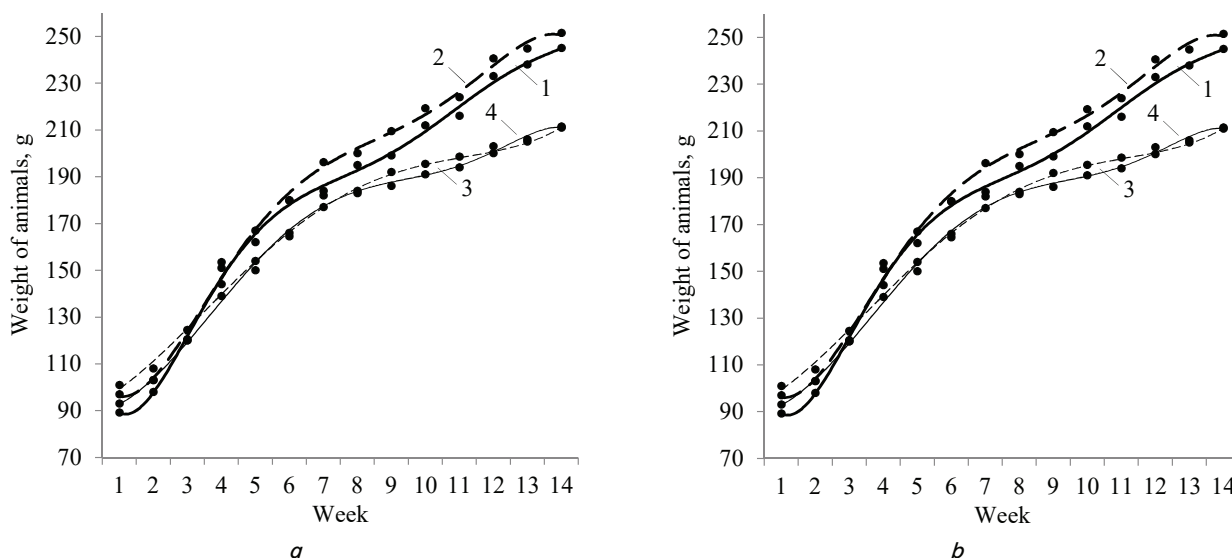


Fig. 1. Change in the weight of rats in test groups: *a* – water; *b* – wheat bread; 1 – male, control; 2 – male, experiment; 3 – female; control; 4 – female, experiment

Consumption of water exposed to the action of non-equilibrium contact plasma instead of main water without additional preparation did not significantly affect the weight of animals, regardless of their sex. However, the test animals of the groups with replacement of carbohydrate components with wheat bread (experiment and control) had a slightly reduced weight. This is due to the lower balance of nutrients in such diets (Table 2). However, the weight of both male and female rats who consumed bread made using plasma-chemically activated water did not actually differ from animals that were fed bread made using traditional technology during the study.

Rats of all experimental and control groups were characterized by a gradual increase in the amount of food consumed, which slightly increased at 8–9 weeks of the experiment. It should be noted the difference between the level of food consumption by animals of the Water and Plasma-chemically activated water groups and the Bread and Bread, made using plasma-chemically activated water during the entire period of the experiment. In the first two groups, the rats consumed more food. This was due to the greater balance of the diet of these animals compared to the groups whose diet was less balanced as a result of replacing oats, rye bread and wheat with wheat bread. The indicated research results are corrected with a change in the weight of animals of the corresponding groups by a direct proportional relationship.

The water consumption of the test animals gradually increased with the increase in the lifespan of the rats. Comparison of the experimental and control groups shows that there is no influence of the corresponding configurations of the animals' diets on the amount of water consumed. Separately, it should be noted that when rats were soldered in water previously exposed to the action of contact non-equilibrium plasma, no differences were observed in the use of such water along with water without additional preparation. The use of plasma chemically activated water did not lead to the development of thirst or refusal to consume it in rats. In turn, this indicates the absence of vivid manifestations of its non-perception by the body of animals and sufficient satisfaction of vital functions with such water.

5. 2. Hematological and biochemical parameters of the blood of test animals

Blood is an integrating system. During anabolic and catabolic processes in the body of animals, some changes can affect the characteristics of the hemogram and blood leukogram. As it is possible to see from the Table 3, when assessing the physiological state of the organism of rats by the number of erythrocytes, hematocrit, hemoglobin

concentration, no significant changes were found between the experimental and control groups in relation to physiological norms.

Analysis of the leukogram in experimental studies against the background of the use of the feed factor is informative, since one of the leading functions of leukocytes is to protect the body from microorganisms alien to it. The results of the analysis of the leukocyte blood profile of the test and control animals are presented in Table 4.

There was no significant difference in each group of the experiment, except for the ratio of segmented leukocytes and monocytes in the blood of experimental rats in relation to the control values. There was no pronounced toxic and allergenic effect of the food factor on the rat organism. Segmented neutrophils and a small number of stab neutrophils were present in the blood. When analyzing the ratio of such blood cells in the control group Bread in comparison with other experimental animals, a significant difference in values was recorded. At the same time, in the experimental groups, there was a tendency to an increase in lymphocytes in the blood of rats (Table 4).

Table 3

Results of hematological analysis of animal blood

Indicator	«Water»	«Plasma-chemically activated water»	«Bread»	«Bread, made using plasma-chemically activated water»
Erythrocytes, 10 ¹² /l	5.99±0.43	6.500.20±	5.22±0.27	5.67±0.21
Hematocrit, %	34.6±2.40	37.8±1.05	31.0±1.28	33.1±1.17
Hemoglobin, g/l	114.2±7.78	123.8±3.42	101.8±4.43	106.8±3.26
MCV, 10 ⁻¹⁵ /l	57.7±0.20	58.2±0.57	59.6±0.95	58.5±0.50
MCH, pg	19.1±0.37	19.1±0.7=17	19.6±0.30	18.9±0.31
MCHC, %	33.1±0.56	32.8±0.07	32.9±0.16	32.3±0.28
Color index, units	0.96	0.95	0.98	0.95

Note: MCV – mean corpuscular volume, MCH – mean corpuscular hemoglobin and MCHC – mean corpuscular hemoglobin concentration

Table 4

Analysis of the leukocyte blood count of test animals

Indicator	«Water»	«Plasma-chemically activated water»	«Bread»	«Bread, made using plasma-chemically activated water»
Leukocytes, G/L	7.65±1.35	9.82±1.03	10.37±1.64	9.43±0.61
Basophils, %	absent	absent	absent	absent
Eosinophils, %	0.50±0.32	0.50±0.32	0.83±0.57	0.33±0.37
Rod neutrophils, %	2.17±0.31	2.00±0.37	3.00±0.77	1.83±0.52
Segmented neutrophils, %	26.2±0.70	28.2±1.25	22.3±1.26*	24.5±1.26
Lymphocytes, %	68.2±0.60	67.5±1.18	71.3±1.96	70.5±1.18
Monocytes, %	3.00±0.37	1.83±0.31▲	2.50±0.68	2.83±0.31
Leukogram				
Eosinophils, G/L	0.04±0.03	0.05±0.04	0.06±0.05	0.03±0.03
Rod neutrophils, G/L	0.16±0.03	0.19±0.04	0.31±0.09	0.19±0.06
Segmented neutrophils, G/L	2.00±0.37	2.71±0.21	2.32±0.35	2.29±0.14
Lymphocytes, G/L	5.25±0.94	6.67±0.79	7.42±1.28	6.67±0.50
Monocytes, G/L	0.21±0.03	0.19±0.04	0.25±0.07	0.26±0.02

Note: * – the difference is significant between parallel experimental groups in comparison with another control group “Bread” or “Water” at the level of p<0.05; ▲ – the difference is significant compared with the control group “Bread” or “Water” at the level of p<0.05

According to the results of a biochemical blood test (Table 5), the total protein in serum between the tested groups of animals had insignificant differences (within 5–11 %). Between the control and experimental groups of rats, this indicator can be considered the same, which indicates that there is no effect of the studied food component/product on the total protein content. Rats fed a balanced diet and drinking plasma-chemically activated water had the same level of albumin protein fraction. Similarly, the level of albumin was the same in rats, the basis of the diet of which was both bread made using traditional technology and using water exposed to contact non-equilibrium plasma. That is, the fractional composition of the protein in the blood serum of animals did not undergo significant changes under the influence of the investigated substances. The results of the study also indicate a constant glucose level in the animals of the control and experimental groups.

AST activity in animals of all test groups was at the same level. At the same time, the activity of ALT localized in the cytoplasm of liver cells was 23–45 % lower for rats kept on diets with wheat bread, at the level of $p < 0.01$. The activity of alkaline phosphatase, when water exposed to contact non-equilibrium plasma was included in the diet of rats, or wheat bread made with its use, did not undergo significant changes.

5.3. Macromorphological parameters and histological changes in the internal organs of the test animals

As it is possible to see from the Table 6, the established weight of rats before their autopsy was slightly higher in the “Water” and “Plasma-chemically activated water” groups. However, the established difference was within the measurement error for experimental group 2 and exceeded the error at the level of $p < 0.05$ for rats included in control group 2. Such results are associated with the characteristics

Biochemical analysis of the blood of test animals

Indicator	«Water»	«Plasma-chemically activated water»	«Bread»	«Bread, made using plasma-chemically activated water»
Total protein, g/l	74.0±3.06	72.3±4.70	68.4±0.43	65.7±2.19
Albumin, g/l	44.0±2.08	46.0±3.21	44.0±1.00	42.0±0.58
Globulins, g/l	30.0±2.52	26.3±1.76	24.3±0.88	23.7±1.76
Protein coefficient, units	1.37±0.24	1.73±0.09	1.77±0.09	1.80±0.12
AST, U/l	132.7±5.46	143.3±15.92	141.3±14.77	144.3±11.89
ALT, U/l	43.0±2.52	45.0±0.58**	23.7±1.67**	30.3±2.03*
De Ritis index, units	3.07±0.09	3.17±0.41	6.07±0.99	4.83±0.70
Alkaline phosphatase, U/l	419.3±30.48	447.3±23.70	341.7±26.35	358.7±37.68
Glucose, mmol/l	5.78±0.19	5.86±0.44	5.26±0.54	5.13±0.07

Note: ** – the difference is significant between parallel experimental groups in comparison with another control group “Bread” or “Water” at the level of $p < 0.01$; * – the difference is significant between parallel experimental groups in comparison with another control group “Bread” or “Water” at the level of $p < 0.05$

Table 5

of the diets of the experimental animals. At the same time, no differences were recorded in the weight of animals in the experimental and control groups of related diets.

During autopsy, the mass of such vital organs of animals as lungs, heart, liver, kidneys, spleen and stomach was determined, as well as their relation to body weight. As it is possible to see from the Table 6, significant at the level of $p < 0.1$, the difference between the control and experimental groups of rats in weight and the ratio of weight to body weight of the lungs, which is 25–30 % compared to the control. In other cases, the difference between test animals kept on related diets with replacement of water or bread for the corresponding component, the preparation of which included the use of plasma chemically treated water, was insignificant. However, the heart, liver and spleen of the rats in the control group “Bread” had a weight 14–23 % less than in the control group “Water”, which is due to the difference in the composition of the diets of the animals.

Table 6

Macromorphological parameters of test animals after autopsy

Indicator	«Water»	«Plasma-chemically activated water»	«Bread»	«Bread, made using plasma-chemically activated water»
Body weight before autopsy	233.9±9.1	243.9±13.5	193.9±10.8*	193.8±15.8
Lungs, g	1.57±0.08	1.63±0.06	1.55±0.16	1.16±0.10▲
Lungs/Body weight, %	0.620.03±	0.640.02±	0.610.06±	0.460.04±▲
Heart, g	1.040.05±	0.970.04±	0.820.05±*	0.700.05±
Heart/body weight, %	0.41±0.02	0.38±0.02	0.32±0.02*	0.27±0.02
Liver, g	6.65±0.26	6.66±0.50	5.73±0.29*	5.94±0.28
Liver/Body weight, %	2.62±0.10	2.63±0.20	2.26±0.11*	2.34±0.11
Right kidney, g	0.90±0.02	0.97±0.07	0.83±0.05	0.71±0.07
Right kidney/Body weight, %	0.35±0.01	0.38±0.03	0.33±0.02	0.28±0.03
Left kidney, g	0.89±0.02	0.97±0.07	0.83±0.05	0.71±0.07
Left kidney/Body weight, %	0.35±0.01	0.38±0.03	0.33±0.02	0.28±0.03
Spleen	0.75±0.05	0.68±0.05	0.58±0.02*	0.55±0.04
Spleen/Body weight, %	0.300.02±	0.270.02±	0.230.01±	0.220.02±
Stomach	1.85±0.22	1.89±0.07	1.90±0.25	1.64±0.10
Stomach/Body weight, %	0.73±0.09	0.74±0.03	0.75±0.10	0.65±0.04

Note: * – the difference is significant between parallel experimental groups in comparison with another control group “Bread” or “Water” at the level of $p < 0.05$; ▲ – the difference is significant compared with the control group “Bread” or “Water” at the level of $p < 0.1$

Histological examination revealed that in the group of rats that received water exposed to contact non-equilibrium plasma, there were no signs of an inflammatory reaction on the part of the lamina propria of the gastric mucosa. In the mucous membrane of the fundus of the stomach, both in the control and in the experimental groups of animals, no signs of dysregenerative changes associated with a violation of the ratio of the main and parient cells were found. The absence of signs of pathological processes and a significant effect of plasma-chemically activated water and bread made with its use on the histological structure of the rat stomach was established. Dystrophic degenerative changes in the liver parenchyma were absent for the experimental and control groups of rats, and the size of hepatocytes was equal. The study of the morphological structure of the kidneys of the experimental group of animals that received plasma-chemically activated water, and animals that were fed bread prepared with its use, showed the preserved microscopic structure of the organ without dystrophic degenerative changes in the kidneys. The revealed structural characteristics of bones showed a high functional activity of the bone marrow and the absence of signs of degradation of bone tissue and other components of the hematopoietic microenvironment. Both in the case of the direct use of plasma chemically activated water by rats, and in the case of its use as an ingredient in food products, the absence of its toxic effect on the bone tissue of animals was established.

6. Discussion of the results of assessing the food safety of plasma-chemically activated water and wheat bread made with its use

Analyzing the obtained results of using plasma-chemically activated water in its natural form and for the production of bakery products, it was found that the following aspects are decisive. The change in the weight of rats upon consumption of activated water and wheat bread with its use practically did not differ from the weight of rats during their development as compared with the control (Fig. 1). This indicates the absence of any effect on the vital processes of animals, such as a change in appetite, an increase or decrease in the degree of assimilation of feed. Tables 3–5 are demonstrating the absence of significant changes in the hematological and biochemical parameters of the blood of experimental animals. The reason for the absence of any changes, obviously, is the fact that plasma-chemically activated water does not provoke or stimulate such changes, but, on the contrary, makes them stable in the case of hematopoiesis. Its use as a feed factor even contributes to the formation of components in a cow capable of responding to infectious threats (Table 4).

The use of plasma chemically activated water per os in laboratory animals did not cause significant disturbances in the physiological processes of vital activity in the experimental animals (Fig. 1). This is consistent with the results of studies [14, 16, 33], according to which it was found that there is no negative effect of plasma-chemically activated water on the indicators of biochemical blood analysis and histological analysis of such internal organs as the heart, liver, spleen, lungs, and kidneys. Moreover, histological analysis of the skin tissues of the rats showed a significant decrease in inflammation in the test animals, whose wounds were treated with water exposed to contact non-equilibrium

plasma. In general, as noted earlier, the use of cold non-equilibrium plasma has been widely used in biomedicine over the past two decades. It is known that direct plasma treatment promotes wound healing, but there are difficulties in the convenience of its implementation [16]. The use of plasma chemically activated water has the expected effect of inactivating several types of bacteria, usually infecting wounds [33]. The results of biochemical blood tests and histological analysis of the main internal organs of the mice showed that plasma-chemically activated water does not show obvious side effects. Taken together, these results indicate that plasma-chemically activated water may be a new and effective method for accelerating wound healing [33].

Due to the complex clinical, morphometric (Table 5) and histological examination of the organs of the test animals, it can be argued that plasma-chemically activated water and bread made with its use are safe components of nutrition. In this sense, the results of studies of the biochemical parameters of the blood of the tested animals are of particular interest (Table 3). An increase in the number of erythrocytes and the content of hemoglobin was noted in the blood of animals from the experimental group of animals that consumed plasma-chemically activated water, with an increased level of hematocrit. Since the value did not have a probable difference, only an upward trend can be noted, which may characterize a more active course of erythropoiesis in the blood of research animals [34]. Thus, the total number of erythrocytes in the “Plasma-chemically activated water” group (Table 4) exceeded the value of the corresponding control group by an average of 8.5%. At the same time, in the experimental group, which was fed bread made with plasma-chemically activated water, this difference was significantly less.

Despite the tendency to an increase in the number of red blood cells, an incredible decrease in the concentration of hemoglobin in the red blood cell was observed in the experimental groups. The color index of blood confirms this fact (0.95 units). In experimental group 2, the difference in the average concentration of hemoglobin in the erythrocyte was 1.82% compared to the control. It should be noted that Wintrob's erythrocyte indices characterize the morphology of erythrocytes and are important in the study of diagnostic signs of various anemias and properties of erythrocytes. There was no significant difference in the average erythrocyte volume, the average hemoglobin content, and the average hemoglobin concentration in the erythrocyte of the experimental and control groups.

The hemoglobin content in the blood of the experimental rats was higher than the parameters in the control group. This can be explained by the activation of synthetic processes in their bodies and an increase in the number of erythrocytes [34]. Since the established changes did not exceed physiological values, this indicates a positive activating effect of plasma chemically activated water on the course of metabolic processes in the body of test animals, which is consistent with works [14, 16, 33]. It is obvious that the intake of a substance with active properties contributes to the body's neuro-humoral response to such a factor.

The totality of all leukocytes creates a specific system in which each type of them is independent and characterizes the body's resistance to the action of a certain factor. In the experimental groups of test animals, a tendency towards an increase in lymphocytes in the blood of rats was noted (Table 4), which may be a confirmation of the activation of this

function [35]. Since one of the functions of neutrophils is to protect the body from foreign agents, it can be assumed that phagocytic activity is activated in the body of rats. It is known that monocytes are formed in the bone marrow and belong to the system of phagocytic mononuclear cells. Considering this, a decrease in their particles in the blood of animals drinking plasma-chemically activated water can be explained by an increase in the number of neutrophils.

The index of total protein in the blood serum was lower for the control and experimental groups of animals that consumed the substituted part of the feed for bread, which is obviously due to the different levels of balance in the diet of the corresponding groups of animals (Table 5). The lower ALT activity in rats kept on diets with wheat bread can be explained by a lower functional load on the liver. Considering that the enzyme is cytosolic, let's assume a greater stability of hepatocyte membranes on diets containing bread, the probability of getting into which ballast substances is less. The absence of changes in the activity of alkaline phosphatase in rats of the experimental groups confirms the absence of the toxic effect of the studied substances on the state of the hepatobiliary system of animals.

The safety of food components definitely requires monitoring the state of target organs when they are introduced into the diet of test animals [14]. Plasma-chemically activated water contains hydrogen peroxide and super-peroxide compounds. Therefore, it is obvious that the primary control should be carried out according to the state of the organs of the alimentary canal, since they are the first to come into contact with such a product. Considering that the regulation of the water-electrolyte balance in the body is under the influence of the kidneys as an organ of the urinary system, changes in their structure may indicate the negative effect of plasma-chemically activated water. In addition, the structure of the femur is able to reflect the effect of the components of plasma-chemically activated water on the structure of the bone tissue. The results of histological studies give grounds to assert the absence of dystrophic degenerative changes in these organs, which is consistent with the work [33].

The reproducibility of the results obtained is determined by adherence to the procedure of the international regulation OECD Test guideline 408 for the assessment of subchronic toxicity of food. But the results obtained in identifying food safety are limited to testing only wheat bread made using plasma-chemically activated water. Such a product is characterized by a rather prolonged high-temperature treatment, which can lead to the complete decomposition of peroxide compounds formed as a result of the plasma-chemical treatment of water. Despite the comprehensive testing, it would be advisable to additionally reveal the effect of plasma-chemically activated water and food products based on it on individual cells of target organs *in vitro*. Also, in view of the peculiarities of the influence of the studied food ingredients on the leukocyte blood count, provocative testing of experimental animals, for example, those affected by infectious diseases, should be carried out. The absence of side effects due to exposure to plasma-chemically activated water is a phenomenon.

The presence of a certain stimulating effect on the erythropoiesis system, as well as on the redistribution of leukocyte cells in the blood of rats, gives grounds for further research. In particular, they should be aimed at deeper studies of the effect of plasma-chemically activated water on living organisms of a complex organization. In particular, a detailed study of the effect of plasma-chemically activated water on

the proliferative activity of the bone marrow and leukopoiesis in laboratory animals looks relevant.

The use of plasma chemically activated water in laboratory rats does not cause a cellular reaction on the part of the mucous membrane of the digestive canal organs and does not lead to changes in the histological structure of internal organs. In addition, there are no changes indicating functional disorders of the liver and kidneys, and no signs of inhibition of the hematopoietic system were revealed. In view of this, it can be argued about a high level of safety of plasma-chemically activated water as a separate raw material for the production of a wide range of food products in the future. That is, the food safety of plasma-chemically activated water and bread with plasma-chemically activated water complements the theoretical foundations for the implementation of innovative methods of processing food raw materials and food products. This opens up opportunities for finding ways to use such water as an alternative and environmentally friendly method of water treatment in the food industry. In particular, it can be used to create environmentally friendly and functional food products. At the same time, certain limitations of the widespread use of the results obtained are imposed by the lack of testing with the involvement of people, which requires further research.

The processes of water activation by contact non-equilibrium plasma are gaining widespread implementation in world scientific and industrial practice. Their peculiarity lies in the direct (contact) effect of a plasma discharge on an aqueous medium. In laboratory conditions, various methods are used for the formation of non-equilibrium plasma: corona, barrier, or glow discharges, each of which is successful in achieving the final goal. But the technical implementation of such processing methods also has fundamental differences. Corona and barrier discharges, for example, require ultra-high voltages and currents to generate plasma. In contrast to the glow discharge used to obtain plasma-chemically activated water, the food safety of which has been studied, corona and barrier discharges require sophisticated electrical and technological equipment. Contact non-equilibrium plasma as an activation method makes it possible to achieve effective water treatment with acceptable technological and technical and economic indicators. The practical implementation of the process of obtaining plasma-chemically activated water is equipped with complex equipment that is effective both from a technological and energy point of view. Taking into account the established food safety of such water as a food ingredient, the widespread introduction of this method of water treatment in the food industry is promising.

7. Conclusions

1. Replacing the main water with plasma-chemically activated water showed the absence of thirst development in laboratory rats and did not lead to the animals refusing to consume water treated with contact non-equilibrium plasma during the 90-day experiment. The feed consumption in the test groups of animals with the replacement of oats, wheat and rye bread for wheat bread was lower, which is associated with a less balanced diet in terms of nutrients. It was found that the introduction of wheat bread made using plasma-chemically activated water into the diet showed a similar level of feed consumption. The change in the weight of the animals during the experiment correlated with the amount of food consumed, but the test substances intro-

duced into the diet did not lead to a decrease in the weight gain of both female and male animals.

2. Plasma-chemically activated water and bread made with its use, according to the number of erythrocytes, hematocrit, hemoglobin concentration in the blood, showed the absence of toxic or allergenic effects on the animal organism. A constant level of glucose was established in animals of the control and experimental groups. The activity of ALT localized in the cytoplasm of liver cells was 23–45 % lower for rats kept on diets with wheat bread, at $p < 0.01$. But wheat bread prepared with plasma-chemically activated water did not lead to a likely difference in ALT activity compared to the control group of animals. In the experimental groups of test animals, a tendency for an increase in the number of lymphocytes in the blood was established, which can contribute to an increase in the level of the body's defense against infectious diseases.

3. Test water and bread in the amount of 152–278 and 85–152 g/kg of body weight per day, respectively, for 90 days did not affect the change in the mass of animal organs. The heart, liver and spleen of laboratory rats were 14–23 % less in weight in diets on wheat bread due to their lower nutritional value. Histological studies of the

stomach, liver, kidneys and femoral cysts of experimental animals with the introduction of plasma-chemically activated water or wheat bread made with its use showed the absence of dystrophic degenerative changes in organs. This testifies to the appropriate level of food safety of plasma-chemically activated water and wheat bread made with its use.

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