

8. Kozaeva RS, Klymenko MO, Kostenko VO. Lipopolysaccharide-induced systemic inflammatory response enhances the development of oxidative-nitrosative stress in salivary glands of rats under alcohol damage. *Fiziologichnyi Zhurnal*. 2021;67(6):60–67. doi: <https://doi.org/10.15407/fz67.06.060>.

9. McCarthy C. Common food additives and chemicals harmful to children. Harvard Health Publishing; 2021. Available from: <https://www.health.harvard.edu/blog/common-food-additives-and-chemicals-harmful-to-children-2018072414326>.

10. Yavtushenko IV, Nazarenko SM, Katrushov OV, Kostenko VO. Quercetin limits the progression of oxidative and nitrosative stress in the rats' tissues after experimental traumatic brain injury. 2020; 73(10):2127–2132. doi.org/10.36740/WLek202010104.

Стаття надійшла 15.08.2023 р.

DOI 10.267224/2079-8334-2024-3-89-203-207

UDC 611.344:613.29-036.14-071.3-092.9

S.M. Bilash, Ya.O. Oliinichenko, O.M. Pronina, M.M. Koptev, A.V. Pirog-Zakaznikova,
S.V. Donchenko, O.V. Mamai
Poltava State Medical University, Poltava

CHARACTERISTICS OF METRIC PARAMETERS OF THE ILEUM WALL IN RATS UNDER LONG-TERM COMPLEX INFLUENCE OF CHEMICAL FOOD ADDITIVES

e-mail: s.bilash@pdmu.edu.ua

It has been experimentally proven that chemical food additives can cause changes in the morphological structure of various internal organs, including the digestive system. To date, data on the complex effects of these substances on the ileum are rather limited. It justified the aim of the study, which was to investigate changes in the intestinal wall after long-term administration of a complex of chemical food additives, including monosodium glutamate, sodium nitrite and ponceau 4R. In this study, using histological and morphometric methods of research, we determined the dynamic changes in the overall average wall thickness and its separate layers. It was found that the ileum of rats of both the control group (receiving saline) and the experimental groups (receiving a complex of chemical food substances for 1, 4, 8, 12, 16, and 20 weeks) is represented by serosa, muscular layer, submucosa and mucosa. It has been established that changes occur for up to 8 weeks due to hyperhydration of connective tissue and pericellular oedema of the structural components of all layers. However, starting from week 12, there are signs of irreversible changes that progress to week 20 and manifest as dystrophic changes with subsequent atrophy of the ileal wall, confirmed by a decrease in all indicators. Thus, with their prolonged administration, chemical food additives disrupt the protective and adaptive mechanisms of the ileum.

Key words: ileum, intestinal wall, food additives, morphometric analysis, rats, mucosa, serosa, lymphocytes.

S.M. Білаш, Я.О. Олійніченко, О.М. Проніна, М.М. Коптев, А.В. Пирог-Заказникова,
С.В. Донченко, О.В. Мамай

ХАРАКТЕРИСТИКА МЕТРИЧНИХ ПОКАЗНИКІВ СТІНКИ КЛУБОВОЇ КИШКИ ЩУРІВ ПРИ ТРИВАЛОМУ КОМПЛЕКСНОМУ ВПЛИВІ ХІМІЧНИХ ХАРЧОВИХ ДОБАВОК

Експериментально доведено, що хімічні харчові добавки можуть викликати зміни морфологічної будови різних внутрішніх органів, зокрема травної системи. На сьогодні, дані щодо комплексного впливу цих речовин на клубову кишку є досить обмеженими. Це й обґрунтувало мету дослідження, яка полягала у вивченні змін кишкової стінки при тривалому введенні комплексу хімічних харчових добавок, зокрема глутамату натрію, нітриту натрію та понсо 4R. У роботі, за допомогою гістологічного та морфометричного методів дослідження, було проведено визначення динамічних змін показників загальної середньої товщини стінки та окремих її оболонок. Було встановлено, що клубова кишка щурів як контрольної групи (отримувала фізіологічний розчин), так і експериментальних груп (отримували комплекс хімічних харчових речовин протягом 1, 4, 8, 12, 16, 20 тижнів) представлена серозною, м'язовою, підслизистою та слизовою оболонками. Встановлено, що до 8 тижня виникають зміни обумовлені гіпергідратацією сполучної тканини та перичелюлярним набряком структурних компонентів усіх оболонок. Але починаючи з 12 тижня з'являються прояви необоротних змін, які прогресують до 20 тижня та проявляються у вигляді дистрофічних змін з подальшою атрофією стінки клубової кишки, що підтверджується зменшенням всіх показників. Таким чином, хімічні харчові добавки при їх тривалому введенні порушують захисні та адаптаційні механізми клубової кишки.

Ключові слова: клубова кишка, кишкова стінка, харчові добавки, морфометричний аналіз, щури, слизова оболонка, серозна оболонка, лімфоцити.

The work is a fragment of the research project "Pathogenetic mechanisms of post-stress disorders under conditions of exogenous influences and wartime factors and the search for methods of its correction", state registration No. 0124U003313.

Dysfunctions of internal organs resulting from their morphological changes often occur due to the negative impact of endogenous and exogenous factors. Chemical food additives, widely used in the modern food industry, play an important role among the latter. They not only give food products a pleasant taste, smell, and attractive appearance but also prevent early spoilage, thereby extending the shelf life of food products. The results of studies show that these chemicals can cause changes in various internal organs [4, 5, 10]. However, given its direct contact with them, they have the most negative impact on the digestive

system. The small intestine, in particular its terminal section, the ileum, plays an important role in digestion. It secretes intestinal juice, conducts hydrolysis of substances through cavity and membrane digestion, and is the main site of water and electrolyte reabsorption in the small intestine. The complex morphological structure of the organ (leaf-shaped villi, accumulation of lymphoid follicles in the form of Peyer's patches) explains the possibility of various morphological changes under the influence of various exogenous agents [3], including chemical food additives.

Given the large number of substances classified as chemical food additives, a literature search was conducted to determine the frequency of their use. The results showed that monosodium glutamate, sodium nitrite and ponceau 4R are the most common. Monosodium glutamate (MSG, E621) is a glutamic acid salt that stimulates orosensory receptors, acting as a chemical flavour enhancer, improving the taste of the product [6] and is widely used in the manufacture of fast food, meat and sausage products, canned food, sauces, and convenience food. Sodium nitrite (E250) is used in the production of sausages and other meat products. It gives them a distinctive pink colour and aroma through the formation of nitrosyl myoglobin, and also inhibits the growth of *Clostridium botulinum* and other foodborne pathogens, which delays oxidative processes and extends the shelf life of products [9]. Ponceau 4R (E124) is a bright red food colouring agent that belongs to aromatic heterocyclic compounds. It finds extensive use in the confectionery industry, where it is employed to give cakes and pastries an attractive appearance, and also in the production of sweet carbonated drinks.

In view of the above, the study of changes occurring in the ileum with the complex use of these chemical compounds is of considerable interest to scientists and requires detailed study.

The purpose of the study was to determine the changes in the morphometric parameters of the ileal wall with prolonged administration of monosodium glutamate, sodium nitrite and ponceau 4R in combination.

Materials and methods. The study was carried out on 70 white mature rats, which were kept in standard conditions of the vivarium of Poltava State Medical University. The experimental animals were divided into control and 6 experimental groups of 10 rats each. Animals in the control group received saline. Animals of the experimental groups received a complex of chemical food additives (monosodium glutamate, sodium nitrite, Ponceau 4R), once at the rate of 20 mg/kg of monosodium glutamate, 5 mg/kg of Ponceau 4R and 0.6 mg/kg of sodium nitrite in 0.5 ml of distilled water orally for 1, 4, 8, 12, 16 and 20 weeks. The doses used were two times lower than the maximum permissible doses for food. The rats had free access to water and standard vivarium food for the rest of the time. The experimental animals were euthanised by overdose with ether anaesthesia. The extracted experimental material (ileal biopsies) was embedded in paraffin according to conventional methods [1], followed by histological and morphological examination. Sections made from paraffin blocks were stained with haematoxylin and eosin, according to Hart, according to Van Gieson. Further study of histological sections was performed using a light microscope with a digital microphotocamera Olympus C 3040-ADU with programs adapted for these types of studies (Olympus DP-Soft, licence No. VJ285302, VT310403, 1AV4U13B26802) and Vorex 3 (serial number 5604). The following morphometric parameters of the ileal wall were determined: total average wall thickness, average serosa thickness, average muscle thickness, average submucosa thickness, and average mucosa thickness.

The data was analysed using a PC and the InStat software package used for statistical processing of data from biomedical and epidemiological studies. The difference was considered significant at $p < 0.05$.

All manipulations were performed in accordance with the "Rules for the Use of Laboratory Experimental Animals" (2006, Appendix 4) and the Helsinki Declaration for the Humane Treatment of Animals, the Law of Ukraine "On the Protection of Animals from Cruelty" (No. 3447-IV of 21.02.2006), in compliance with the requirements of the Bioethics Committee of Poltava State Medical University (Protocol No. 208 of 22.09.2022), in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

Results of the study and their discussion. The ileal wall of rats of the control (Fig. 1A) and experimental groups is represented by serosa, muscular layer, submucosa and mucosa, but their morphometric parameters change after the first week of the experimental study (Fig. 1B).

When analysing the results of the morphometric study of the average total intestinal wall thickness, it was found that after the first week of administration of the chemical food additives complex, the indicator was statistically significantly reduced by 51.78 % (at $p < 0.05$) compared to the control group, after the fourth week this index was 21.56 % higher compared to the control and 19.75 % lower compared to the previous observation period, at $p < 0.05$. After 8 weeks of the study, the index was 40.98 % higher than the

control group and 15.98 % lower than the previous period. After week 12, the total intestinal wall thickness increased by 16.6 % compared to the control group but was 17.29 % lower than in the previous study period, at $p < 0.05$. As for the 16th week of the experimental study, it was determined that this index was 12.76 % higher than in the control group and 3.29 % lower than in the previous observation period. After 20 weeks, the total intestinal wall thickness was lower compared to the control group and the previous experiment period, namely by 7.45 % and 17.93 %, respectively (Fig. 2).

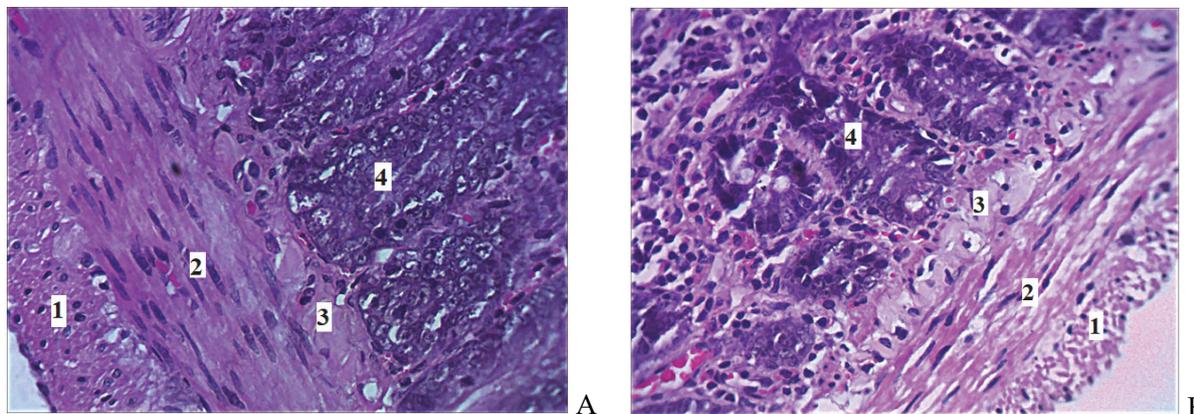


Fig. 1. Ileal wall of the control group rats (A) and changes in the ileal wall of rats after 1 week of administration of a complex of chemical food additives (B). Paraffin section. Hematoxylin and eosin staining. Magnification: okh. 10; obh. 40. 1. Serosa; 2. Muscular layer; 3. Submucosa; 4. Mucosa.

Evaluating the dynamic changes in the metric indices of the mean thickness of the ileal mucosa at different observation periods, it was found that after the first week of the experiment, this index increased by 40.11 % compared to the control group. After the 4th week, it was 26.07 % higher than in the control group but 23.45 % lower than in the previous period. At the next stage of the experimental study (after 8

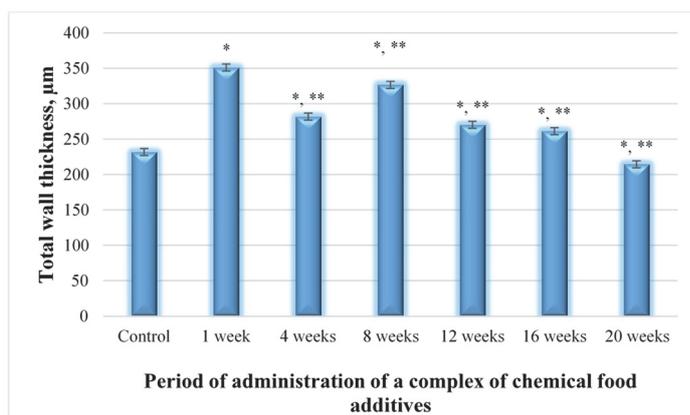


Fig. 2. Dynamics of changes in the average values of the total wall thickness of the ileum of rats at different periods of administration of chemical food additives.

Note: * – statistically significant at $p < 0.05$ compared to the control group, ** – statistically significant at $p < 0.05$ compared to the previous observation period.

The following changes were found when assessing the mean values of the intestinal wall submucosa. After 1 week, the index was 10.05 % lower than in the control group. After 4 weeks, compared with the control and the previous period of the experimental study, the indicator increased by 21.53 % and 33.74 %, respectively ($p < 0.05$). After 8 weeks, the reverse dynamics was observed, i.e. the index was 6.46 % lower than in the control group and 23.03 % lower than in the experimental group of animals administered a complex of chemical food additives for 4 weeks. After 12 weeks, the average submucosa thickness was 6.46 % higher than in the control group and 13.81 % higher than in the previous observation period. After 16 weeks, the index was characterised by negative dynamics, as it was 6.65 % lower than in the control group and 12.32 % lower than at the previous time point. The worst metric was found after week 20 – lower by 17.74 % compared to the control group and 11.88 % compared to the previous period (Fig. 3B).

Further, we analysed the metric indices of the mean thickness of the ileal muscle layer. Thus, after 1 week of administration of the complex of chemical food additives, the index increased by 36.76 %

compared to the control. After 4 weeks, this index increased by 18.66 % compared to the control group but was 24.96 % lower than the previous observation period ($p < 0.05$). Analysing the changes after week 8, it was found that the mean thickness of the muscle layer of the intestinal wall increased both in comparison with the control group (by 61.26 %) and with the previous period (by 35.9 %). Positive changes in the index were also characteristic after week 12, in particular, an increase of 81.22 % compared to the control and 12.37 % compared to the previous experimental period. After week 16, this index was 18.17 % higher than in the control group, although it decreased by 34.79 % compared to the previous study period, at $p < 0.05$. The most significant changes were detected after week 20, characterised by a decrease of 18.6 % and 31.12 % compared to the control group and the previous observation period, respectively (Fig. 4A).

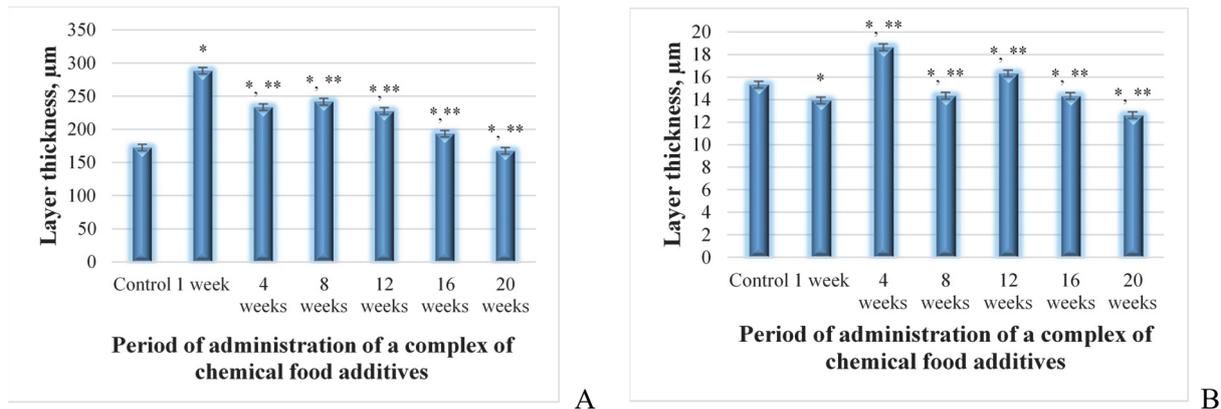


Fig. 3. Dynamics of changes in the average indicators of the mucosa (A) and submucosa (B) of the ileal wall in rats at different periods of administration of chemical food additives.

Note: * – statistically significant at $p < 0.05$ compared to the control group, ** – statistically significant at $p < 0.05$ compared to the previous observation period.

Studying the dynamics of metric changes in the average thickness of the serosa of the ileum, it was found that after 1 week of administration of the complex of chemical food additives, the index increased by 30.41 % compared to the control group. After 4 weeks, a positive trend was observed, characterised by an increase of 57.77 % compared to the control and 9.8 % compared to the previous observation period. After week 8, the index increased by 9.61 % compared to the control group but was 30.52 % lower than at the previous time point ($p < 0.05$). After 12 weeks of the experimental study, the mean thickness of the intestinal wall serosa increased in comparison with the control group (by 40.1 %) and the previous observation period (27.81 %). The increase in the index was also characteristic after week 16 of the experiment, in particular, by 46.31 % and 4.44 % compared to the control group and the previous observation period, respectively ($p < 0.05$). After week 20 of the study, the index increased by 10.78 % compared to the control group, although it decreased by 24.29 % compared to the previous period (Fig. 4B).

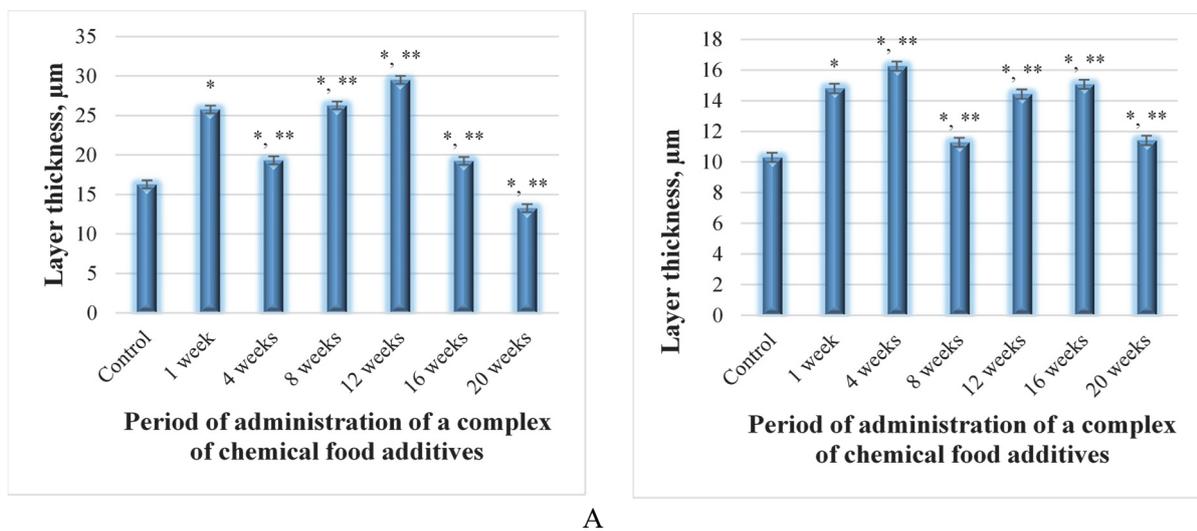


Fig. 4. Dynamics of changes in the average indicators of the muscle layer (A) and serosa (B) of the ileal wall in rats at different periods of administration of chemical food additives. Note: * – statistically significant at $p < 0.05$ compared to the control group, ** – statistically significant at $p < 0.05$ compared to the previous observation period.

Thus, we have found that the administration of a complex of chemical food additives (monosodium glutamate, sodium nitrite, ponceau 4R) for 20 weeks causes changes in the metric parameters of both the total thickness of the ileal wall and its separate layers. The results of our previous studies show that in the early stages of combined administration of the selected food additives, significant metric changes in the ileal wall occur due to hyperhydration of connective tissue, impaired blood circulation, significant lymphocytic infiltration, and pericellular oedema of the structural components of all layers [2]. In the dynamics of the experimental study, it was found that these changes are characteristic even after 8 weeks of observation, which can be explained by the activation of compensatory and adaptive mechanisms to the effects of exogenous harmful factors. After 12 weeks of administration of monosodium glutamate, sodium nitrite and ponceau 4R, irreversible changes in the intestinal wall structure appear, which progress to 20 weeks. It manifests in the form of dystrophic changes with subsequent atrophy of the ileal wall, which is confirmed by a decrease in not only the total thickness of the intestinal wall but also each of its layers. It can most likely be explained by the prolonged exposure to aggressive exogenous factors, which include chemical food additives. In our opinion, these substances deplete the cellular and tissue components of the ileum, disrupting its protective and adaptive mechanisms.

Other studies have shown that the combined use of monosodium glutamate, sodium nitrite and ponceau 4R leads to a disruption in the morphological structure of organs of the digestive system [7, 8] and others [11]. Thus, it can be assumed that chemical food additives have a negative impact not only on the ileum, but also on other parts of the gastrointestinal tract.

Conclusion

Long-term use of chemical food additives (monosodium glutamate, sodium nitrite, and ponceau 4R) leads to pronounced morphometric changes in the ileal wall, which is characterised by atrophic changes due to disruption of adaptation processes caused by a negative, damaging exogenous factor.

References

- Horalskyy LP, Khomych VT, Kononskyy OI. Osnovy histolohichnoyi tekhniki i morfofunktsionalni metody doslidzhen u normi ta pry patolohiyi. Zhytomyr: "Polissya"; 2015. 286 s. [in Ukrainian].
- Oliynichenko YaO. Metrychni zminy stinky klubovoyi kyshky shchuriv pry diyi kompleksu kharchovykh khimichnykh dobavok na rannikh terminakh eksperymentu. Aktualni problemy suchasnoyi medytsyny. 2024;24(2(86)):131–136. doi: <https://doi.org/10.31718/2077-1096.24.2.131>. [in Ukrainian].
- Shepitzko KV. Kharakterystyka morfometrychnykh parametriv stinky klubovoyi kyshky pry odnorazovomu vvedenni kriokonservovanoyi platsenty ta aseptychnomu zapalenni. Svit medytsyny ta biolohiyi. 2014;4(46):174–178. [in Ukrainian].
- Akimov OY, Kostenko VO. Functioning of nitric oxide cycle in gastric mucosa of rats under excessive combined intake of sodium nitrate and fluoride. Ukrainian Biochemical Journal. 2016;88(6):70–5. doi: 10.15407/ubj88.06.070.
- Banerjee A, Mukherjee S, Maji BK. Monosodium glutamate causes hepato-cardiac derangement in male rats. Hum Exp Toxicol. 2021;40:359–369. doi: 10.1177/09603271211049550.
- Banerjee A, Mukherjee S, Maji BK. Worldwide flavor enhancer monosodium glutamate combined with high lipid diet provokes metabolic alterations and systemic anomalies: an overview. Toxicology Reports. 2021; 8:938–961. doi: 10.1016/j.toxrep.2021.04.009.
- Grigorenko A, Yeroshenko G, Shevchenko K, Lisachenko O, Perederii N. Remodeling of the rat duodenal wall under the effect of complex food additives of monosodium glutamate, sodium nitrite and Ponceau 4R. Georgian Medical News. 2021;5(314):145–150.
- Koka VM, Starchenko II, Mustafina GM, Roiko NV, Filenko BM, Proskurnia SA. Features of the structure of the epithelium of the mucous membrane of the tongue under the effect of complex of food additives in the experiment. World of medicine and biology. 2022;1(79):200–204. doi: 10.26724/2079-8334-2022-1-79-200-204.
- Lavado G, Higuero N, León-Camacho M, Cava R. Formation of Lipid and Protein Oxidation Products during In Vitro Gastrointestinal Digestion of Dry-Cured Loins with Different Contents of Nitrate/Nitrite Added. Foods. 2021; 10:1748. doi: <https://doi.org/10.3390/foods10081748>.
- Song Z, Song R, Liu Y, Wu Z, Zhang X. Effects of ultra-produced foods on the microbiota-gut-brain axis: The bread-and-butter issue. Food Res Int. 2023;167:112730. doi: 10.1016/j.foodres.2023.112730.
- Yeroshenko GA, Donets IM, Shevchenko KV, Vatsenko AV, Ulanovska-Tsyba NA, Riabushko OB, et al. Morphometric and morphological features of rat bronchus-associated lymphoid tissue under the impact of the complex of food additives. World of medicine and biology. 2023;3(85):209–215. doi: 10.26724/2079-8334-2023-3-85-209-215.

Стаття надійшла 3.08.2023 р.