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Assessment of the toxicity of modified dried egg white

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Abstract. The relevance of this study stems from the need to develop safe and functionally effective protein carriers for the immobilisation of biologically active substances and microorganisms, which show promise for use in animal husbandry, the food industry and biotechnology. The aim of the study was to assess the toxicological properties of modified dried egg white by determining its acute toxicity, its effect on the haematological, biochemical and antioxidant parameters of the blood of laboratory animals, as well as its irritant effect on the ocular mucosa. The study utilised toxicological, clinical, haematological, biochemical and statistical research methods. The acute toxicity of modified dried egg white was investigated following intragastric administration to white mice at doses ranging from 100 to 5,000 mg/kg body weight, and its irritant effect on the ocular mucosa of California rabbits was assessed. It was found that even at the maximum dose of 5,000 mg/kg, no fatalities were observed, and short-term manifestations of depression and diarrhoea were transient and resolved completely without further intervention. Haematological, biochemical and antioxidant blood parameters were analysed and found to remain within physiological norms: at a dose of 2,000 mg/kg, haemoglobin concentration was 132 g/L, erythrocyte count was $5.7 \times 10^{12}/\text{dm}^3$, total protein – 65.4 g/dm³, glucose concentration – 5.4 mmol/dm³, and total thiol groups – 16.3 µg/cm³; whereas at the maximum dose of 5,000 mg/kg, total protein reached 67.3 g/dm³, AST activity – 0.93 µmol/h/mL, ALT – 1.04 µmol/h/mL, and glucose concentration – 6.0 mmol/dm³, all

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within the physiological range. When assessing the irritant effect on the mucous membrane of the rabbits' eyes, only mild, transient clinical manifestations were observed, with no damage to the iris; whilst haematological, biochemical and antioxidant parameters also corresponded to physiological values, confirming the absence of haemotoxic, hepatotoxic, metabolic and pronounced oxidative effects of the modified dry egg white. The practical value of this work lies in the potential to use the results obtained to develop and implement safe protein carriers for biologically active substances and microorganisms in animal husbandry, the food industry, biotechnology and scientific research

Keywords: protein carrier; immobilisation; haematological parameters; biochemical parameters; laboratory animals; biotechnology

Introduction

The development of safe, biocompatible and functionally effective carriers for the immobilisation of biologically active substances and microorganisms is one of the priority areas in the development of modern food technology, biotechnology and animal husbandry. Such carriers ensure increased stability of active components, protection against adverse environmental factors, sustained release and preservation of biological activity, which significantly broadens the scope of their practical application. Of particular interest are materials of natural origin, which are characterised by high biocompatibility, biodegradability and safety, making them a promising basis for the creation of new functional delivery systems for biologically active compounds.

The work by T. Liu *et al.* (2024) notes that naturally derived protein matrices are among the most promising materials for the encapsulation of probiotics, enzymes, vitamins and other biologically active compounds. The authors found that the use of protein carriers provides a high level of protection for immobilised components during processing and storage, and also facilitates their controlled release in the required environment. Similar conclusions were reached by B. Ma *et al.* (2024), S. Razi *et al.* (2024) and Y. Tian *et al.* (2024), who demonstrated that delivery systems based on food proteins are characterised by high encapsulation efficiency, structural stability and

the ability to maintain the biological activity of functional ingredients. The researchers emphasise that, thanks to their emulsifying, gelling and stabilising properties, chicken egg proteins are among the most promising natural materials for the creation of next-generation carriers. J. Su *et al.* (2020) and L. Song *et al.* (2022) note that, alongside the study of the material's physicochemical characteristics, it is necessary to determine its acute toxicity, its effect on the body's functional state, and any possible local irritant effects, as it is precisely these indicators that determine the potential for the further practical application of new protein systems. Further development of immobilisation technologies involves the use of chicken egg proteins as natural carriers of biologically active substances (Rostamabadi *et al.*, 2023). Y. Zhang *et al.* (2023) note in their review that ovalbumin and other egg proteins, owing to their amphiphilic nature, self-assembly capacity, gel-forming ability and biodegradability, are promising materials for the creation of nanoparticles, emulsions, hydrogels and aerogels designed to deliver biologically active compounds and probiotic cultures. At the same time, the authors emphasise the need for further study of their biological safety and behaviour in biological systems.

Analysing current trends in the development of protein-based delivery systems, J. Zang *et al.* (2023) and E. Armanu *et al.* (2025)

have shown that modified gels based on chicken egg proteins can be effectively used as carriers for nutraceuticals due to their high mechanical strength, stability and ability to ensure the controlled release of active components. At the same time, J. Lu *et al.* (2020) and T. Mehrotra *et al.* (2021) emphasise that the development of such systems requires optimisation of the structure of protein matrices to enhance the bioavailability of immobilised substances and preserve their functional activity. The safety of protein carriers is also attracting considerable attention from researchers. Thus, an analysis of the current scientific literature indicates that most studies are devoted to the development of protein-based delivery systems, the improvement of their structural characteristics, and the evaluation of the effectiveness of encapsulating biologically active substances. At the same time, the toxicological safety of modified dried egg white as a potential carrier for the immobilisation of biologically active components and microorganisms remains insufficiently studied. In particular, there is a lack of comprehensive studies that simultaneously assess acute toxicity, local irritant effects, and changes in haematological, biochemical and antioxidant parameters in laboratory animals, which necessitated the present study. The lack of information regarding the toxicological properties of modified dried egg white limits its practical application as a carrier for biologically active substances and microorganisms in food and biotechnological systems. Conducting a comprehensive assessment of its biological safety is a necessary prerequisite for the further introduction of such materials into production, as it allows the identification of potential risks to the organism and justifies the feasibility of their practical application.

The aim of the study was to experimentally substantiate the toxicological safety of modified dried egg white as a promising protein carrier for the immobilisation of biologically active substances and microorganisms through

a comprehensive assessment of its acute toxicity, its local irritant effect and its impact on the haematological, biochemical and antioxidant parameters of laboratory animals.

Materials and Methods

The acute toxicity of modified dried egg white was investigated in male BALB/c mice weighing 21.5–22.0 g and aged 56–57 days. The mice were prepared for the experiment in accordance with the requirements of I. Kotsiumbas *et al.* (2006). The study was conducted in two stages. For each stage, four groups of mice were formed. Each group comprised six mice. The duration of the experiment was 14 days. During the first stage, the effect of low doses (100; 250; 500 and 1,000 mg/kg body weight) of modified dried egg white was investigated. During the second stage, the toxic effects of high doses of the carrier (2,000; 3,000; 4,000; 5,000 mg/kg body weight) were assessed. A solution of modified dried egg white was administered to the mice as a single intragastric dose using a metal probe with a welded lead tip to prevent damage to the oesophagus. The modified egg white was dissolved in a 1% starch solution. The animals were observed and housed in accordance with the recommendations set out by I. Kotsiumbas *et al.* (2006).

The irritant effect of modified dried egg white was investigated using California rabbits with a body weight of 2.7–2.8 kg. Three males were prepared for the experiment in accordance with the requirements of I. Kotsiumbas *et al.* (2006). A solution of modified dried egg white, at a dose of 2 drops, was instilled into the conjunctival sac of the left eye. The right eyes of the laboratory animals were used as controls. Observation of the rabbits over a 14-day period and determination of the carrier's toxicity level were carried out in accordance with the recommendations of W. Diener & E. Schleder (1999) and I. Kotsiumbas *et al.* (2006). The content of protein, low-molecular-weight and total sulphhydryl groups in the blood serum of rabbits

and mice was determined using the method of G. Ellman (1959); total protein was determined according to the method of O. Lowry *et al.* (1951), using the glucose oxidase-peroxidase method of P. Trinder (1969). Aminotransferase activity was investigated according to the method of S. Reitman & S. Frankel (1957). Haemoglobin content in the blood and erythrocyte count were determined according to the instructions of I. Tovstukha *et al.* (2025).

During the experiments, the air temperature in the laboratory animal quarantine rooms and procedure rooms was maintained at 17-18°C, relative humidity at 57-64%, and illuminance at the level of the laboratory animal cages was 305-320 lx. The animals had free access to standard pelleted feed and drinking water throughout the experimental period. All experimental studies were conducted in accordance with current methodological approaches and relevant requirements and standards, including ISO/IEC 17025:2017 (2017). Animal housing and all procedures were carried out in accordance

with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986) and Order No. 249 of the Ministry of Education and Science, Youth and Sport of Ukraine (2012). Statistical analysis of the research results was carried out using Microsoft Excel 2021 and Statistica 10.0 software. The arithmetic mean (M) and the standard error of the mean (m) were determined. The significance of differences between the indicators was assessed using the Student's t-test. A difference was considered statistically significant at $p < 0.05$.

Results and Discussion

The results of the acute low-dose toxicity study of modified dried egg white showed that a single intragastric administration to mice at doses ranging from 100 to 1,000 mg/kg body weight did not result in any fatalities in any of the test groups (Table 1). This indicates the absence of any marked acute toxic effects of the test substance when administered at low doses.

Table 1. Results of acute low-dose toxicity testing of modified dried egg white in mice

Group	Number of animals	Dose, mg/kg body weight	Number of dead animals	Mortality rate, %	Time of death	Adverse clinical signs
1 st	6	100	0	0	–	Not specified
2 nd	6	250	0	0	–	Not specified
3 rd	6	500	0	0	–	Not specified
4 th	6	1,000	0	0	–	Not specified

Source: compiled by the authors

During clinical observation of the mice, no adverse changes in the animals' general physiological condition were observed. All mice remained active, responded appropriately to external stimuli, and maintained normal mobility and coordination of movement. Feed and water intake were within physiological norms; no signs of central nervous system depression, convulsions, paresis, respiratory distress or gastrointestinal dysfunction were observed. When mice were administered modified dried egg white at doses of 100 and 250 mg/kg body

weight, their clinical condition did not differ from the physiological norm. A similar pattern was observed when the dose was increased to 500 and 1,000 mg/kg body weight. The absence of adverse clinical signs, even at the maximum dose tested, indicates that the carrier is well tolerated by laboratory animals.

The results obtained provide grounds for concluding that modified dried egg white is characterised by a low level of acute toxicity within the range of doses tested. The results of the acute toxicity study of high doses of

modified dried egg white showed that a single administration of the preparation to mice at doses ranging from 2,000 to 5,000 mg/kg body weight did not result in the death of any animals in any of the test groups. All mice

survived throughout the entire observation period, and the mortality rate was 0%. This indicates the absence of any pronounced acute toxic effects of the test preparation, even at high doses (Table 2).

Table 2. Results of acute toxicity testing of high doses of modified dried egg white in mice

Group	Number of animals	Dose, mg/kg body weight	Number of dead animals	Mortality rate, %	Time of death	Adverse clinical signs
1 st	6	2,000	0	0	–	Not established
2 nd	6	3,000	0	0	–	Not established
3 rd	6	4,000	0	0	–	Temporary suppression
4 th	6	5,000	0	0	–	Suppression, diarrhoea during the first 24 hours

Source: compiled by the authors

During clinical observation of animals administered 2,000 and 3,000 mg/kg body weight of modified dried egg white, no adverse clinical signs were observed. The mice remained active, responded appropriately to external stimuli, and consumed food and water in physiologically normal quantities. No signs of impaired coordination, convulsions, central nervous system depression or gastrointestinal disturbances were observed. When the dose was increased to 4,000 mg/kg body weight, temporary depression was noted in the animals. The mice exhibited reduced mobility and decreased activity during the first 24 hours following administration of the drug. However, these changes were transient and resolved without further intervention, indicating an adaptive response of the organism to a high toxicological load. In the group of animals administered the maximum dose of 5,000 mg/kg body weight, more pronounced clinical changes were observed in the form of depression and diarrhoea during the first day of the experiment. Despite this, no fatalities were recorded, and the clinical condition of the mice gradually returned to normal. A return to normal physiological activity and feed intake was observed as early as the second day of the study.

The results obtained indicate that the modified dried egg white is characterised by a low level of acute toxicity, even when administered at high doses. The absence of fatalities suggests that the median lethal dose (LD_{50}) exceeds 5,000 mg/kg body weight, which, in accordance with toxicological classifications, provides grounds for classifying the carrier under investigation as a low-toxicity or virtually non-toxic substance. These data confirm the findings of X. Lv *et al.* (2024), who note that the use of natural protein carriers in food and biotechnological systems requires a comprehensive assessment of their biological safety, including the determination of acute toxicity, effects on the body's functional state and possible local irritant effects, as it is precisely these indicators that determine the potential for the further practical application of new protein systems. The results of the analysis of haematological parameters in mice following the administration of high doses of modified dried egg white indicate that the test preparation had no significant adverse effect on the haematopoietic system. It was found that the haemoglobin content and erythrocyte count in the blood of animals in all experimental groups were within the physiological range for laboratory mice (Table 3).

Table 3. *Haematological parameters of the blood of mice following the administration of high doses of modified dried egg white*

Administered dose, mg/kg	Haemoglobin concentration, g/L	Erythrocyte count, $10^{12}/\text{dm}^3$
2,000	132 ± 3.9	5.7 ± 0.32
3,000	128 ± 5.6	5.4 ± 0.28
4,000	135 ± 4.8	5.9 ± 0.33
5,000	137 ± 3.7	6.0 ± 0.31

Source: compiled by the authors

Following the administration of modified dried egg white to mice at a dose of 2,000 mg/kg body weight, the haemoglobin concentration was 132 g/L and the erythrocyte count was $5.7 \times 10^{12}/\text{dm}^3$. These results indicate a normal functional state of the haematopoietic system and an adequate supply of oxygen to the tissues. When the dose was increased to 3,000 mg/kg body weight, a slight decrease in haemoglobin concentration of 3.0% and in erythrocyte count of 5.2% was observed compared with the group administered 2,000 mg/kg of the preparation. These changes were minor and remained within physiological limits, indicating that no anaemia or suppression of erythropoiesis developed. When a dose of 4,000 mg/kg body weight was used, a tendency towards an increase in haematological parameters was observed. Haemoglobin levels were 2.2% higher, and erythrocyte counts were 3.5% higher, compared with mice administered the vehicle at a dose of 2,000 mg/kg

body weight. A similar pattern was observed at the maximum dose tested – 5,000 mg/kg body weight.

The moderate increase in haematological parameters observed with the use of high doses of modified dried egg white is likely to be of an adaptive nature and is not associated with any toxic effects of the preparation. The absence of marked changes in haemoglobin levels and erythrocyte count indicates that the test preparation does not exert a pronounced haematotoxic effect on laboratory animals, even at high doses. The results of the study of serum protein metabolism parameters in mice following the administration of high doses of modified dried egg white indicate that the test preparation has no pronounced adverse effect on liver function or the body's protein metabolism. It was found that total protein content and aminotransferase activity in all experimental groups remained within the physiological range for laboratory mice (Table 4).

Table 4. *Protein metabolism parameters in the blood serum of mice following the administration of high doses of modified dried egg white*

Administered dose, mg/kg	Protein concentration, g/dm^3	AST activity, $\mu\text{mol}/\text{h}/\text{mL}$	ALT activity, $\mu\text{mol}/\text{h}/\text{mL}$
2,000	65.4 ± 1.16	0.81 ± 0.052	0.92 ± 0.031
3,000	66.3 ± 2.06	0.79 ± 0.015	0.94 ± 0.023
4,000	66.8 ± 2.33	0.84 ± 0.023	0.98 ± 0.011
5,000	67.3 ± 1.78	0.93 ± 0.049	1.04 ± 0.031

Source: compiled by the authors

Following the administration of modified dried egg white to mice at a dose of 2,000 mg/kg body weight, the total protein content

in blood serum was $65.4 \text{ g}/\text{dm}^3$. AST activity was $0.81 \mu\text{mol}/\text{h}/\text{mL}$, and ALT activity was $0.92 \mu\text{mol}/\text{h}/\text{mL}$. These results indicate normal

protein metabolism and the absence of any impairment in hepatocyte function. When the dose of the drug was increased to 3,000 mg/kg body weight, a slight increase in total protein content of 1.3% was observed. AST activity remained virtually unchanged, whilst ALT activity showed a tendency towards a slight increase of 2.1% compared with the values in animals administered a vehicle dose of 2,000 mg/kg body weight. These changes were minor and showed no signs of a pathological nature. In the group of mice administered 4,000 mg/kg body weight of modified dried egg white, protein metabolism parameters increased within the margin of error compared with mice administered the lowest dose of the carrier. At the maximum dose studied – 5,000 mg/kg body weight – the highest protein metabolism indices were observed: total protein content was 67.3 g/dm³, AST activity was 0.93 µmol/h/mL, and ALAT activity was 1.04 µmol/h/mL.

The observed moderate increase in total protein content and transaminase activity was

dose-dependent; however, all parameters remained within the physiological range. The absence of a sharp increase in AST and ALT activity indicates that the modified dried egg white did not cause pronounced toxic liver damage or impairment of hepatocyte cell membrane permeability. The results obtained provide grounds for concluding that the carrier under investigation is well tolerated by laboratory mice, even at high doses. The observed changes in protein metabolism parameters are of an adaptive nature and do not indicate the development of marked hepatotoxic effects. The results of the study of thiol group content in the blood serum of mice following the administration of high doses of modified dried egg white indicate the absence of marked disturbances in the antioxidant status of the laboratory animals. It was found that the levels of protein-bound, total and low-molecular-weight (free) thiol groups in all experimental groups remained stable and fell within the range of physiological variations (Table 5).

Table 5. Thiol group content in the blood serum of mice following the administration of high doses of modified dried egg white, µg/cm³

Administered dose, mg/kg	Protein-bound	Total	Low-molecular-weight (free)
2,000	14.6 ± 0.32	16.3 ± 0.22	1.7 ± 0.09
3,000	14.9 ± 0.26	16.6 ± 0.18	1.7 ± 0.07
4,000	15.2 ± 0.29	17.0 ± 0.35	1.8 ± 0.05
5,000	15.2 ± 0.30	17.1 ± 0.42	1.9 ± 0.11

Source: compiled by the authors

Following the administration of modified dried egg white to mice at a dose of 2,000 mg/kg body weight, the concentration of protein thiol groups in blood serum was 14.6 µg/cm³, that of total thiol groups was 16.3 µg/cm³, and that of low-molecular-weight (free) thiol groups was 1.7 µg/cm³. These results indicate that the body's antioxidant system continued to function normally and that there were no signs of marked oxidative stress. When the dose of the preparation was increased to 3,000 mg/kg

body weight, a slight increase in the content of protein-bound and total thiol groups was observed, by 2.0% and 1.8% respectively, compared with the values in mice administered the vehicle at a dose of 2,000 mg/kg. The content of low-molecular-weight thiol groups remained unchanged. These changes were not statistically significant and likely reflected the body's physiological adaptation to the administration of high doses of the preparation. In the group of mice administered 4,000 mg/kg body weight

of modified dried egg white, a further trend towards an increase in the content of thiol groups was observed. A similar pattern was observed at the maximum dose of 5,000 mg/kg body weight.

The observed moderate increase in thiol group content may indicate activation of the body's antioxidant system in response to the administration of high doses of modified dried egg white. At the same time, the absence of sharp changes in the indicators confirms that the test substance does not cause pronounced oxidative stress or disruption of the cellular redox balance. Thus, the results of the analysis of thiol groups in the blood serum of

mice indicate the stability of the body's antioxidant status when high doses of modified dried egg white are administered and confirm the absence of any pronounced toxic effect on cellular metabolic processes. The results of the study of glucose levels in the blood serum of mice following the administration of high doses of modified dried egg white indicate that the test preparation has no significant adverse effect on carbohydrate metabolism in laboratory animals. It was found that glucose concentrations in all experimental groups remained within the physiological normal range for mice (Fig. 1).

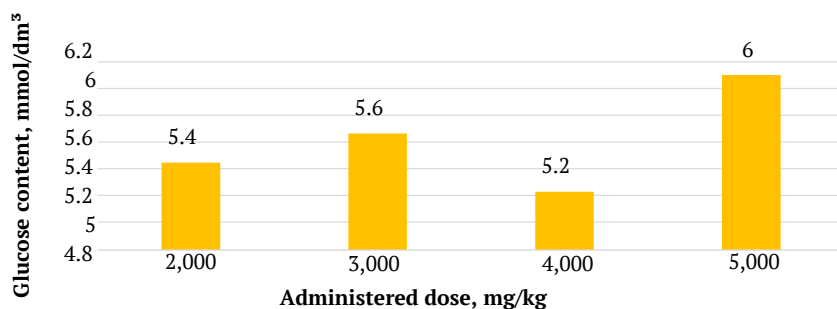


Figure 1. Serum glucose levels in mice following the administration of high doses of modified dried egg white, mmol/dm³

Source: compiled by the authors

Following the administration of modified dried egg white to mice at a dose of 2,000 mg/kg body weight, the serum glucose concentration was 5.4 mmol/dm³. This result indicates normal energy metabolism and an adequate supply of glucose to the body. When the dose of the preparation was increased to 3,000 mg/kg body weight, a slight increase in glucose concentration was observed. These changes were minor and showed no signs of being pathological. In the group of mice administered 4,000 mg/kg body weight of modified dried egg white, the glucose content was 3.7% lower compared with the group of mice administered the lowest dose of the vehicle. At the maximum dose studied – 5,000 mg/kg body weight – the highest serum

glucose level was recorded, amounting to 6.0 mmol/dm³. Despite a slight increase in this parameter, it remained within the physiological range and did not indicate the development of marked disturbances in carbohydrate metabolism or toxic damage to the body. Thus, the results of the biochemical studies confirm the good tolerability of modified dried egg white and the absence of significant disturbances in carbohydrate metabolism, even when high doses of the preparation were used. It was found that, following administration of the feed additive into the conjunctival sac, the test animals exhibited only mild and transient clinical signs of irritation, which resolved within the first day of observation (Table 6).

Table 6. Irritant effects of the feed additive on the ocular mucosa of rabbits (n = 3)

Experimental animal	Observation time	Ocular discharge (0-3 points)	Conjunctival hyperaemia (0-3 points)	Conjunctival oedema (0-4 points)	Condition of the iris (0-2 points)
No. 1	1 hour	1	0	0	0
	24 hours	1	0	0	0
	2 days	0	0	0	0
	3 days	0	0	0	0
	14 days	0	0	0	0
No. 2	1 hour	2	0	0	0
	24 hours	1	1	1	0
	2 days	0	0	1	0
	3 days	0	0	0	0
	14 days	0	0	0	0
No. 3	1 hour	1	0	0	0
	24 hours	1	0	0	0
	2 days	0	0	0	0
	3 days	0	0	0	0
	14 days	0	0	0	0

Source: compiled by the authors

In rabbit No. 1, one hour after administration of the product, slight discharge from the eye was observed, which was assessed as 1 point. Similar changes were also observed 24 hours after administration of the feed additive. No signs of conjunctival hyperaemia, oedema or pathological changes in the iris were observed. By the second day of the experiment, the clinical signs of irritation had completely disappeared, and no pathological changes to the ocular mucous membrane were detected during the subsequent observation period. In rabbit No. 2, the reaction of the mucous membrane was slightly more pronounced. One hour after administration of the feed additive, moderate discharge from the eye was observed, rated at 2 points. After 24 hours, slight discharge, mild hyperaemia and oedema of the conjunctiva were recorded, corresponding to 1 point. On the second day of the study, discharge and hyperaemia were absent, although slight conjunctival oedema persisted. By the third day, all clinical signs of irritation had completely disappeared. The condition of the iris remained unchanged throughout the study period. In rabbit No. 3, only mild discharge from the eye was observed 1 and 24 hours after administration of the preparation, which was assessed

as 1 point. No signs of hyperaemia, conjunctival oedema or damage to the iris were detected. From the second day of observation onwards, the ocular mucosa was in a physiological state.

The results obtained indicate that the feed additive under investigation did not cause any pronounced toxic or irritant effects on the mucous membrane of the rabbits' eyes. The clinical changes observed were mild and transient in nature, were not accompanied by damage to the iris or persistent pathological changes, and resolved spontaneously without further intervention. Thus, the results of the studies confirm the good local tolerability of the feed additive and indicate its low level of irritation to the mucous membrane of the rabbits' eyes. Given its low toxicity, the absence of any adverse effects on haematological and biochemical blood parameters, and its good tolerability by laboratory animals, the carrier under investigation may be a promising candidate for the immobilisation of biologically active substances, probiotic cultures and other functional components. The results obtained are consistent with the findings of C. Ferraro *et al.* (2024), who note that the introduction of new protein-based materials into food and biotechnology processes should

be accompanied by a comprehensive toxicological assessment, including the determination of acute toxicity, analysis of haematological and biochemical parameters, as well as studies of biocompatibility and possible local irritant effects. The results of the analysis of haematological parameters in the rabbits' blood indicate

that the feed additive under investigation had no adverse effect on the haematopoietic system or the animals' general physiological condition. It was established that the haemoglobin content and erythrocyte count in the blood of all test rabbits were within the physiological range typical of clinically healthy animals (Table 7).

Table 7. Haematological parameters of the blood of rabbits (n=3)

Experimental animal	Haemoglobin concentration, g/L	Erythrocyte count, $10^{12}/\text{dm}^3$
No. 1	140 ± 2.98	6.4 ± 0.23
No. 2	137 ± 3.22	6.5 ± 0.23
No. 3	144 ± 4.22	7.0 ± 0.28

Source: compiled by the authors

In rabbit No. 1, the haemoglobin concentration was 140 ± 2.98 g/L, and the erythrocyte count was $6.4 \times 10^{12}/\text{dm}^3$. These results indicate that erythropoiesis is proceeding normally and that the body's tissues are receiving an adequate supply of oxygen. No signs of anaemia or suppression of haematopoietic function were detected. In rabbit No. 2, the haemoglobin concentration was 2.14% lower than that of the first rabbit. Despite a slight decrease in haemoglobin concentration compared with the other animals, the values remained within the range of physiological variation and did not indicate the development of pathological processes or toxic damage to the body. The highest haematological values were recorded in rabbit No. 3. The haemoglobin concentration in its blood was 2.8% higher, and the erythrocyte count was 9.3% higher than those of the first rabbit. The increase in these parameters compared with the other animals did not exceed the physiological norm and was likely due to the individual characteristics of the animal's organism.

The absence of sharp fluctuations in haemoglobin concentration and erythrocyte count indicates that the feed additive under investigation does not exert a pronounced haemotoxic effect and does not adversely affect the functional state of the haematopoietic system. The results also confirm the absence of disturbances in oxygen transport and the stability of the erythrocyte component of the blood in the test animals. Thus, the results of the haematological analyses of the rabbits' blood confirm the good tolerability of the feed additive under investigation and indicate that it is safe with regard to its effect on the haematopoietic system. The results of the study of protein metabolism parameters in rabbit serum indicate the absence of any marked adverse effect of the feed additive under investigation on liver function and protein metabolism in the animals. It was established that the total protein content and aminotransferase activity in all test rabbits were within the physiological normal range (Table 8).

Table 8. Protein metabolism parameters in the blood serum of rabbits (n=3)

Experimental animal	Protein concentration, g/dm ³	AST activity, $\mu\text{mol}/\text{h}/\text{mL}$	ALT activity, $\mu\text{mol}/\text{h}/\text{mL}$
No. 1	70.1 ± 2.15	1.22 ± 0.45	0.85 ± 0.22
No. 2	65.4 ± 1.67	1.17 ± 0.23	0.91 ± 0.17
No. 3	68.3 ± 3.56	1.20 ± 0.16	0.94 ± 0.21

Source: compiled by the authors

In rabbit No. 1, the total protein concentration in the blood serum was 70.1 g/dm^3 , which was the highest value among the laboratory animals. AST activity was $1.22 \text{ } \mu\text{mol/h/mL}$, while ALT activity was $0.85 \text{ } \mu\text{mol/h/mL}$. These results indicate normal protein metabolism and the absence of signs of toxic liver damage. In rabbit No. 2, a lower total protein concentration was established. AST activity was $1.17 \text{ } \mu\text{mol/h/mL}$, while ALT activity was $0.91 \text{ } \mu\text{mol/h/mL}$. Despite the slight decrease in total protein concentration compared with the other animals, the values obtained remained within the physiological range and did not indicate impaired protein-synthetic function of the organism. In rabbit No. 3, the total protein concentration and aminotransferase activity corresponded to physiological norms, indicating a stable functional

state of hepatocytes and normal metabolic processes. The absence of a pronounced increase in AST and ALT activity in the blood serum of the rabbits indicates that the feed additive under study did not cause disruption of hepatic cell membrane permeability or the development of pronounced hepatotoxic effects. The slight variation in the parameters between the animals was probably due to individual physiological characteristics. The results of the study of thiol group concentrations in the blood serum of rabbits indicate a stable antioxidant status in the laboratory animals and the absence of pronounced oxidative stress following use of the carrier. It was established that the concentrations of protein-bound, total and low-molecular-weight (free) thiol groups in all rabbits remained within the physiological range (Table 9).

Table 9. Thiol group content in the blood serum of rabbits, $\mu\text{g/cm}^3$, ($n = 3$)

Experimental animal	Protein-bound	Total	Low-molecular-weight (free)
No. 1	11.3	13.5	2.2
No. 2	10.7	13.2	2.5
No. 3	10.5	12.9	2.4

Source: compiled by the authors

In rabbit No. 1, the concentration of protein-bound thiol groups was $11.3 \text{ } \mu\text{g/cm}^3$, total thiol groups $13.5 \text{ } \mu\text{g/cm}^3$ and low-molecular-weight (free) thiol groups $2.2 \text{ } \mu\text{g/cm}^3$. These values indicate the preservation of normal functioning of the body's antioxidant system and maintenance of cellular redox balance. In rabbit No. 2, the concentrations of protein-bound and total thiol groups were slightly lower than those in the first rabbit. The concentration of low-molecular-weight thiol compounds was the highest among the laboratory animals, at $2.5 \text{ } \mu\text{g/cm}^3$. These changes showed no signs of a pathological nature and were probably associated with individual characteristics of metabolism. Despite a slight decrease in some parameters in rabbit No. 3 compared with the other animals, the values remained stable and corresponded to physiological levels. The minor

variations in thiol group parameters between the experimental rabbits did not indicate the development of pronounced oxidative stress or toxic effects of the feed additive under study on the animals. Thus, the results of the study of thiol groups in the blood serum of rabbits confirm the absence of an adverse effect of the feed additive under study on the antioxidant status of the organism and indicate its good tolerability by laboratory animals. The results of the study of glucose concentration in the blood serum of rabbits following exposure to modified dried egg white indicate the absence of an adverse effect of the preparation under study on carbohydrate metabolism in the animals. It was established that glucose concentrations in all experimental rabbits remained within the physiological range, indicating a stable state of energy metabolism in the organism (Fig. 2).

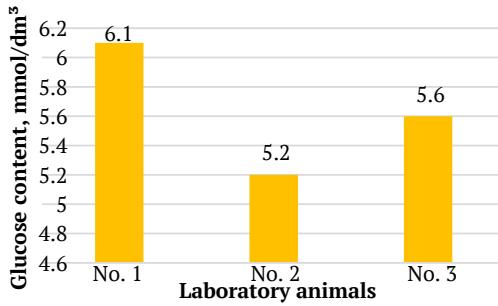


Figure 2. Serum glucose levels in rabbits following administration of modified dried egg white, mmol/dm³

Source: compiled by the authors

No statistically significant differences were found between the animals in terms of their blood serum glucose levels. The results obtained indicate the stability of carbohydrate metabolism in rabbits when a modified dry egg white is used. The absence of sharp changes in glucose concentration indicates that the feed additive under investigation does not have a pronounced toxic effect on the energy metabolism of laboratory animals. Thus, the results of the biochemical studies confirm the good tolerability of modified dried egg white by rabbits and indicate that the product has no adverse effect on carbohydrate metabolism parameters.

The research findings indicate a low level of acute toxicity of the modified dried egg white and its good tolerability by laboratory animals. The absence of fatalities in mice, even when high doses of the preparation of up to 5,000 mg/kg body weight were administered, indicates that the carrier under investigation can be classified as a low-toxicity or virtually non-toxic substance. These results are consistent with data from other researchers, who note that protein carriers of natural origin are characterised by high biocompatibility and low toxicity due to their natural origin and biodegradability (Ferraro *et al.*, 2024; Li *et al.*, 2024).

The absence of pronounced clinical signs of intoxication in animals when low doses of the

preparation were used indicates that the modified dried egg white is well tolerated by mice. Even when high doses were administered, the clinical manifestations observed were short-lived and mild. The temporary suppression of activity and diarrhoea in animals at the maximum dose were likely associated with the body's adaptive response to a significant load of protein components, rather than with the development of toxic damage to organs and systems. Similar data on the safety of protein-based delivery systems are presented in the works of M. Fathi *et al.* (2018) and A. Martínez-López *et al.* (2020), which indicate that protein nanostructures and carriers do not induce pronounced toxic reactions in laboratory animals. The absence of significant changes in haematological parameters confirms that modified dried egg white has no adverse effect on the haematopoietic system. Haemoglobin levels and erythrocyte counts in the mice's blood remained within physiological ranges even when high doses of the preparation were administered. This indicates the absence of haematotoxic effects and disturbances in erythropoiesis. Such results are characteristic of natural protein carriers, which, according to E. Abeyrathne *et al.* (2013), are characterised by high biological compatibility and do not cause significant disturbances to the body's physiological state.

The results of biochemical studies also confirm the absence of any marked toxic effect of modified dried egg white on liver function. Total protein levels and aminotransferase activity in the blood serum of mice remained within the physiological range. A slight dose-dependent increase in AST and ALT activity at the maximum dose was not accompanied by clinical signs of toxicity and was likely of an adaptive nature. It is known that slight fluctuations in transaminase activity may occur in response to an increased metabolic load without the development of structural damage to hepatocytes. Similar conclusions regarding the safety of protein carriers are presented in

the works of H. Cheng *et al.* (2023) and S. Wu *et al.* (2024), which emphasise the stability of the functional state of cells when using protein delivery systems.

An important indicator of the safety of the carrier under investigation is the stability of the antioxidant status in laboratory animals. It was found that the levels of protein-bound, total and low-molecular-weight thiol groups in the blood serum of mice remained stable and did not exceed physiological ranges. The results obtained indicate the absence of pronounced oxidative stress and disturbances in cellular redox balance. A slight increase in the concentration of thiol compounds when high doses of the preparation were used is likely associated with the activation of the body's compensatory antioxidant mechanisms. Similar patterns have been described in the work of J. Žur *et al.* (2016), which notes that immobilised protein systems are capable of maintaining a stable functional state of cells without the development of oxidative damage. The absence of marked changes in serum glucose concentration in mice indicates the stability of carbohydrate metabolism and the body's energy supply when using modified dried egg white. Even when the maximum dose was administered, glucose levels remained within the physiological range, confirming the absence of any adverse effect of the preparation on metabolic processes. This is an important criterion for toxicological safety, as disturbances in carbohydrate metabolism often accompany the development of intoxication and stress responses in the body.

The results of the assessment of the local irritant effect of modified dried egg white on the ocular mucous membrane of rabbits also deserve particular attention. It was established that only mild, transient signs of irritation were observed after administration of the preparation and that these resolved spontaneously during the first days of the study. The absence of damage to the iris, persistent hyperaemia or pronounced conjunctival oedema indicates a

low level of local irritation caused by the preparation. The results obtained confirm the good local tolerability of the carrier under study and are consistent with data on the safety of natural protein-based systems reported by B. Ruan *et al.* (2018) and M. Maggonage *et al.* (2024). The results of the studies confirm the potential for using modified dried egg white as a protein carrier in biotechnology and the food industry. Given its low toxicity, the absence of any adverse effects on haematological and biochemical blood parameters, and its good tolerability by laboratory animals, the carrier under investigation may be promising for the immobilisation of biologically active substances, probiotic cultures and other functional components. The results obtained lay the groundwork for further research into the efficacy and functional properties of modified dried egg white within biotechnological systems.

Conclusions

Toxicological studies conducted on modified dried egg white indicate its low toxicity and good tolerability in laboratory animals. It was found that a single intragastric administration of the preparation to white mice at doses ranging from 100 to 5,000 mg/kg body weight did not result in any fatalities. Even at the maximum dose of 5,000 mg/kg, only transient depression and diarrhoea were observed, which resolved spontaneously without further intervention, indicating that the body's response was of an adaptive nature. The results of haematological studies showed that modified dry egg white has no adverse effect on the haematopoietic system of laboratory animals. Following administration of the preparation at a dose of 2,000 mg/kg, the haemoglobin concentration in mice was 132 g/L, and the erythrocyte count was $5.7 \times 10^{12}/\text{dm}^3$. In rabbits, the haemoglobin concentration ranged from 137 to 144 g/L, and the erythrocyte count was $6.4\text{--}7.0 \times 10^{12}/\text{dm}^3$, which corresponded to physiological norms and confirmed the absence of haemotoxic effects of the preparation.

Biochemical studies revealed that indicators of protein metabolism and liver function remained stable. At a dose of 2,000 mg/kg, the total protein content in the mice's serum was 65.4 g/dm³, with AST activity at 0.81 μ mol/h/mL and ALT activity at 0.92 μ mol/h/mL, whereas at the maximum dose of 5,000 mg/kg, these values were 67.3 g/dm³, 0.93 and 1.04 μ mol/h/mL, respectively. The values obtained did not exceed physiological norms and did not indicate toxic damage to hepatocytes. An assessment of antioxidant status showed that the use of modified dried egg white did not induce the development of marked oxidative stress. Following administration of the preparation at a dose of 2,000 mg/kg, the content of protein thiol groups was 14.6 μ g/cm³, total thiol groups at 16.3 μ g/cm³, and low-molecular-weight thiol groups at 1.7 μ g/cm³, indicating that cellular redox balance was maintained and the body's antioxidant system was functioning stably. No adverse effect of the modified dried egg white on carbohydrate metabolism parameters was observed. The serum glucose concentration in the mice was 5.4 mmol/dm³ at a dose of 2,000 mg/kg and 6.0 mmol/dm³ at the maximum dose of 5,000 mg/kg, remaining within the physiological range and showing no evidence of impaired energy metabolism.

The results of the local irritation assessment confirmed that the preparation was well

tolerated by the ocular mucosa of rabbits. The clinical signs of irritation observed were mild, short-lived and resolved completely within the first day of observation, without damage to the iris or the development of pathological tissue changes. The results obtained confirm the safety of modified dried egg white and justify its potential use as a protein carrier for the immobilisation of biologically active substances and microorganisms in animal husbandry, the food industry and biotechnology. Prospects for further research include investigating the chronic toxicity, allergenic and immunobiological safety of the modified dried egg white; studying the effectiveness of immobilising various biologically active compounds and probiotic microorganisms using it as a carrier; and assessing the stability, bioavailability and functional efficacy of the developed systems under conditions of practical application in animal husbandry, the food industry and biotechnology.

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Conflict of Interest

None.

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Встановлення токсичності модифікованого сухого яєчного білка

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Анотація. Актуальність роботи зумовлена необхідністю створення безпечних та функціонально ефективних білкових носіїв для іммобілізації біологічно активних речовин і мікроорганізмів, перспективних для використання у тваринництві, харчовій промисловості та біотехнології. Метою дослідження було оцінити токсикологічні властивості модифікованого сухого яєчного білка шляхом визначення його гострої токсичності, впливу на гематологічні, біохімічні та антиоксидантні показники крові лабораторних тварин, а також подразнювальної дії на слизову оболонку ока. У роботі використано токсикологічні, клінічні, гематологічні, біохімічні та статистичні методи дослідження. Було досліджено гостру токсичність модифікованого сухого яєчного білка за внутрішньошлункового введення білим мишам у дозах від 100 до 5000 мг/кг маси тіла та оцінено його подразнювальну дію на слизову оболонку ока кролів каліфорнійської породи. Встановлено, що навіть за максимальної дози 5000 мг/кг летальних випадків не спостерігали, а короточасні прояви пригнічення та діареї були транзиторними й повністю зникали без додаткового втручання. Проаналізовано гематологічні, біохімічні та антиоксидантні показники крові, які залишалися в межах фізіологічної норми: за дози 2000 мг/кг вміст гемоглобіну становив 132 г/л, кількість еритроцитів – $5,7 \times 10^{12}/\text{дм}^3$, загального білка – 65,4 г/дм³, концентрація глюкози – 5,4 ммоль/дм³, загальних тілових груп – 16,3 мкг/см³, тоді як за максимальної дози 5000 мг/кг вміст загального білка досягав 67,3 г/дм³, активність АсАт – 0,93 мкмоль/год/мл, АлАт – 1,04 мкмоль/год/мл, а концентрація глюкози – 6,0 ммоль/дм³, не виходячи за межі фізіологічної норми. Під час оцінки подразнювальної дії на слизову оболонку ока кролів було виявлено лише слабо виражені транзиторні клінічні прояви без ушкодження райдужної оболонки, при цьому гематологічні, біохімічні та антиоксидантні показники також відповідали фізіологічним значенням, що підтверджувало відсутність гемотоксичної, гепатотоксичної, метаболічної та вираженої оксидативної дії модифікованого сухого яєчного білка. Практична цінність роботи полягає у можливості використання отриманих результатів для розроблення та впровадження безпечних білкових носіїв біологічно активних речовин і мікроорганізмів у тваринництві, харчовій промисловості, біотехнології та наукових дослідженнях

Ключові слова: білковий носій; іммобілізація; гематологічні показники; біохімічні показники; лабораторні тварини; біотехнологія