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Assessment of the safety and toxicity of sulphur-enriched spirulina in white mice

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Abstract. The study of *Spirulina platensis* biomass enriched with sulphur is of particular relevance due to its potential as a valuable feed supplement for livestock. This study aimed to assess the safety and acute toxicity of sulphur-enriched spirulina biomass. Linear mice were used for the experiment. To evaluate the safety, the mice were administered 0.35 cm^3 of physiological saline (control) and 25% and 50% suspensions of sulphur-enriched spirulina biomass (experimental groups I and II) via intragastric administration. The animals were observed for 12 days, and protein metabolism indicators were examined. For acute toxicity assessment, laboratory animals received intragastric doses of spirulina biomass ranging from 5 to 6,000 mg/kg body weight. The observation period lasted 14 days. It was established that during the 12-day observation period following the administration of spirulina biomass suspensions, no lethal cases were recorded in the mice. No pathological or anatomical changes in the internal organs were observed in the experimental animals. No statistically significant increase or decrease in total protein levels or aminotransferase activity was detected in mice from experimental groups I and II compared with the control group. Assessment of acute toxicity indicated that spirulina biomass belongs to toxicity class 4, categorising it as a low-toxicity substance. No significant behavioural changes or notable effects on metabolic processes were observed, suggesting the potential safety of this biomass for use in animal husbandry. The findings of this study may be applied in livestock production to introduce innovative approaches to feed enrichment with beneficial elements that enhance animal productivity and health

Keywords: *Spirulina platensis*; liver; laboratory animals; feed supplement; clinical condition of animals

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Introduction

Spirulina platensis, a multicellular blue-green microalga, is widely used as a valuable feed additive and bioactive ingredient in the food industry. Assessing safety parameters is essential for determining whether sulphur-enriched spirulina is safe for consumption at various doses and by different consumer groups, including children, pregnant women, individuals with allergies, and those with chronic conditions. Additionally, the toxicity of supplements, particularly those containing sulphur, must be thoroughly investigated, as excessive sulphur consumption can lead to harmful effects, such as poisoning or impaired organ function. Scientific research in this area is crucial for understanding how sulphur enrichment alters the properties of spirulina and what potential risks may arise from its consumption, thereby contributing to public health protection. Furthermore, it will enable the development of safe dosage recommendations and guidelines for the use of this supplement, as well as establish clear regulations for the production and sale of such products on the market, considering the specific characteristics of sulphur-enriched spirulina.

According to O.M. Tytariova & O.A. Kuzmenko (2021), *Spirulina platensis* contains a significant amount of essential amino acids, polyunsaturated fatty acids, B vitamins, iron, magnesium, and other micronutrients, making it a promising feed for animals and poultry. It contains between 55% and 70% protein by dry weight, significantly exceeding similar indicators of traditional protein products such as soybeans. The biomass of this microalga can also be used as a dietary and therapeutic supplement.

Significant attention is paid to the adaptive capabilities of *Spirulina platensis* in response to changing cultivation conditions. One of the important features of this microalga is its ability to accumulate certain mineral elements in its biomass, allowing for the adjustment of its chemical composition depending on the needs of the end consumer. With an increased content of a particular mineral element in the cultivation medium, its active accumulation in the biomass of the microalga occurs (Abbas *et al.*, 2024). Of particular interest is the ability of spirulina to accumulate sulphur – a vital element involved in the synthesis of proteins, enzymes, antioxidants (glutathione), as well as in the metabolism of vitamins (biotin, thiamine) and coenzymes. This is confirmed by the research of E. Henao *et al.* (2020), who found that sulphur-enriched *Spirulina platensis* biomass can acquire unique properties, including influencing the bioavailability of other micronutrients and potentially enhancing the immune status of animals. It was established that they can modulate the Nrf2 factor or other regulatory pathways that determine the body's resistance to oxidative stress.

Research by T. Clifford *et al.* (2021) has shown that sulphur is involved in the synthesis of Sadenosylmethionine (SAM), γ-glutamylcysteine glycine, and a range of hormones, enzymes, and vitamins. Sulphur-containing compounds are key components of many antioxidants that influence physiological processes in the body. This opens up possibilities for the use of sulphur-enriched spirulina biomass as a means of improving immune status and antioxidant protection in animals.

Studies on the effects of spirulina on farm animals demonstrate its positive impact. For instance, O. Tytariova *et al.* (2022) found that adding spirulina to rabbit feed reduced the accumulation of heavy metals in meat. This confirms its ability to influence mineral metabolism and improve the quality of the final product. Meanwhile, G. Kolluri *et al.* (2022) investigated the effects of spirulina added to the drinking water of broilers during cyclic chronic heat stress and found improvements in productivity, physiological status, and immunobiochemical parameters of the poultry.

The study of M. Marino *et al.* (2021) confirmed that sulphur-containing compounds influence biochemical processes in the body and can have a positive effect in the context of animal feed. Specifically, they participate in the synthesis of amino acids (methionine, cysteine), coenzymes, and vitamins, which are essential for the normal growth and development of animals. Additionally, these compounds can positively influence the absorption of other micronutrients such as iron, magnesium, and calcium (Francioso *et al*., 2020). This highlights the importance of controlling the level of sulphur-containing compounds in feed and their potential impact on the metabolism of other beneficial substances.

Despite numerous studies dedicated to the nutritional value and biological activity of spirulina, the question of its toxicological safety, when enriched with sulphur, remains insufficiently studied. In particular, there is a lack of comprehensive toxicological studies that would assess the impact of such biomass on the mammalian organism when used as a feed additive. This creates a need for an expanded study of the safety and acute toxicity of sulphur-enriched spirulina. Thus, the aim of this study is to experimentally assess the safety and toxicity of sulphurenriched *Spirulina platensis* biomass using a white mouse model.

Materials and Methods

Building on the known ability of *Spirulina platensis* to accumulate mineral elements, the biomass of the blue-green alga with increased sulphur content was obtained in 2024 at the Institute of Animal Husbandry and Food Technologies of the Bila Tserkva National Agrarian University. To evaluate the safety of sulphur-enriched spirulina biomass, three groups of linear mice (four individuals in each) of the same age (55 days), sex, size, and body weight (19.5-20.5 g) were formed using a random assignment. The saline solution and the tested spirulina suspensions were administered intragastrically to the animals using a metal probe. The distribution of animals into groups is presented in Table 1. This approach allowed for a standardised study with a clear consideration of the influencing factors being studied.

Table 2. *Basic physical and chemical parameters of raw materials (n = 4)*

Group	Number of animals	Investigated factor
Control	4	Physiological saline at a dose of 0.35 cm^3 per animal
Experimental I	4	25% spirulina biomass suspension at a dose of 0.35 cm^3 per animal
Experimental II	4	50% spirulina biomass suspension at a dose of 0.35 cm^3 per animal

Source: developed by the authors

After intragastric administration of the experimental samples to the mice, they were observed for 12 days. At the end of the experiment, the mice were anaesthetized and euthanised for pathological and anatomical studies of the internal organs and sampling for biochemical analysis. A homogenate was prepared from the collected liver, in which certain indicators of protein metabolism were determined. The total protein content was determined using the method of O.H. Lowry *et al*. (1951), and the activity of aminotransferases was determined using the method of S. Reitman & S. Frankel (1957).

In addition to assessing safety parameters such as total protein levels in the liver, aminotransferase activity (aspartate aminotransferase and alanine aminotransferase), and the pathological condition of internal organs, the acute toxicity of sulfur-enriched spirulina biomass in white mice was determined. The experiments consisted of two stages: preliminary and expanded. The suspension was administered to the experimental animals once and twice (depending on the dose) intragastrically. For 6 hours before the administration of the spirulina suspension prepared in a 1% starch solution, the mice were kept on a fasting diet with free access to water. Each group of mice consisted of five individuals.

In the preliminary study, doses of 5, 500, and 5,000 mg of spirulina per kg of body weight were administered intragastrically to mice. In the expanded study, doses of 3,000, 4,000, 5,000, and

6,000 mg/kg body weight were investigated. Observations of the mice were carried out for 14 days. Doses of 4,000-6,000 mg/kg body weight were divided in half and administered twice. All experimental studies were conducted following modern methodological approaches and in compliance with relevant requirements and standards, in particular, meeting the requirements of DSTU ISO/IEC 17025:2005 (2006). Animal maintenance and all manipulations were carried out by the provisions of the Procedure for Conducting Scientific Research and Experiments on Animals by Research Institutions (Law of Ukraine No. 249, 2012) and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986).

Results

In assessing the safety of spirulina, it was established that no fatalities occurred among the experimental or control groups of white mice over the twelve-day experiment. It was found that within the first 2.5-3.5 hours after the administration of 0.35 cm^3 of a 50% suspension of sulphurenriched spirulina biomass, animals from the second experimental group exhibited lethargy. Over time, the mice regained mobility and responded to created noise, turning lights on and off, and touching. In 50% of mice, gastrointestinal disturbances were observed during the first day. Consumption of food and water resumed after 16-24 hours. Animals periodically approached feeders and drinkers.

According to observations of mice that were administered 0.35 cm^3 of a 25% suspension of spirulina biomass, no behavioural disturbances were detected during the twelve-day experiment. Upon dissection of the mouse carcasses and conducting pathological and anatomical studies, it was found that the condition of the internal organs of animals from the first and second experimental groups did not differ from the condition of the internal organs of control mice. When studying certain indicators of protein metabolism in the liver of mice during the establishment of the safety of sulphur-enriched spirulina biomass, it was established that on the 12th day after administration, the activity of aspartate aminotransferase in animals from the first experimental group was not significantly lower compared to the control. The difference was within the margin of error (Table 2).

***Table 2.** Protein content and aminotransferase activity in the liver of mice exposed to Spirulina, M ± m, n = 4*

Group	Activity of AST, μmol/h/g	Activity of ALT, μmol/h/g	Content of total protein, g/kg
Control	10.3 ± 0.36	13.2 ± 0.68	44.7 ± 2.32
Experimental I	9.9 ± 0.47	12.9 ± 0.82	43.8 ± 3.56
Experimental II	10.5 ± 0.43	13.6 ± 0.76	45.5 ± 1.97

Note: AST – aspartate aminotransferase; ALT – alanine aminotransferase
Source: developed by the authors

Analysing the activity of aspartate aminotransferase in the liver of mice from the second experimental group, it was found that the increase in the indicator was also not statistically significant. Similarly, it was established that the activity of alanine aminotransferase in the liver of animals from the experimental groups did not statistically differ from the indicator in animals from the control group. The content of total protein in the liver of mice from the control group was at the level of 44.7 g/kg. Deviations from the control in the first and second experimental groups were 2% and 1.7%, respectively, which did not exceed the limits of statistical error. Thus, it was established that with a single administration of 0.35 cm^3 of 25% and 50% suspensions of spirulina biomass, no disturbances in protein metabolism were observed in the liver of mice.

Along with determining safety, the acute toxicity of sulphur-enriched spirulina biomass was investigated. According to the results of a preliminary study on the administration of *Spirulina platensis* biomass, enriched with sulphur, at doses from 5 to 5,000 mg/kg of body weight, it was established that over 14 days, no changes in the behaviour of animals were observed. Mice responded to stimuli, moved freely, and periodically consumed food and water. Also, no lethal cases were recorded (Table 3).

Table 3. *Results of observation of mice during the determination of the toxicity of sulphur-enriched Spirulina platensis biomass (preliminary study)*

Number of mice in the group	Dose of spirulina, mg/kg	Number of animals that died	Behavioural changes	Changes in body weight (g)	Gastrointestinal disorders
5	5	0	None	None	None
5	500	0	None	None	None
5	5,000	0	Temporary refusal of food and water for 1.5-2 hours after administration; normal behaviour restored within 4-10 hours	Slight weight loss (up to 5%)	None

Source: developed by the authors

For a dose of 5,000 mg/kg, mice refused food and water for 1.5-2 hours after the administration of Spirulina. After 4-10 hours, the behaviour of mice in this group was within physiological norms. Under the conditions of the expanded study, no deviations in the behaviour of mice were noted. After the administration of the second part of the dose of 5,000 mg/kg of body weight to 4 animals, temporary lethargy was observed (2-3 hours), and the mice did not eat or drink. By the 6th hour of observation, the ethological indicators of mice in this group were normal (Table 4)

Table 4. *Results of observation of mice during the determination of the toxicity of sulphur-enriched Spirulina platensis biomass (expanded study)*

Number of mice in the group	Dose of feed additive, mg/kg	Number of animals that died	Ethological indicators	Ethological changes	Body weight changes (g)	Gastrointestinal disturbances
5	3,000	0	Within normal limits	None	Slight weight loss (up to 3%)	None
5	4,000	0	Within normal limits	None	Slight weight loss (up to 3%)	None
5	5,000	0	Within normal limits	None	Slight weight loss (up to 5%)	Disturbances observed for 16-18 hours after administration; recovery of functions within 48 hours
5	6,000	0	Within normal limits	None	Slight weight loss (up to 5%)	Disturbances observed for 16-18 hours after administration; recovery of functions within 48 hours

Source: developed by the authors

When studying a dose of sulphur-enriched *Spirulina platensis* of 6,000 mg/kg in mice, gastrointestinal disorders were detected. The intensity of the movements of animals from this group was reduced. 16-18 hours after the administration of spirulina biomass, the consumption of food and water by mice resumed. By the 48^{th} hour of the study, the functioning of the gastrointestinal tract of animals was normal. Ethological changes in mice that were administered Spirulina in amounts of 5,000 and 6,000 mg/kg are associated with the administration of a large amount of feed additive to the animals. It was proved that in an expanded study when administering sulphurenriched spirulina biomass to white mice at a dose of 3,000 to 6,000 mg/kg of body weight for a 14 day observation, no animal deaths were detected.

Discussion

The results of this study on the safety and acute toxicity of sulphur-enriched *Spirulina platensis* biomass are of significant importance for the further development of feed additives and the enrichment of animal diets with microelements. In particular, it was established that even at high doses (3,000-6,000 mg/kg body weight), spirulina biomass does not cause lethal cases in white mice. This is consistent with the findings of H.V. Merzlova (2015), according to which, when 5,000 mg/kg body weight of zinc-enriched spirulina biomass was administered to laboratory animals, no lethal cases were observed during the experimental period. It is important to note that, unlike studies with zinc biomass, the current study shows that sulphur-containing spirulina may be safer for intragastric administration.

The absence of changes in protein metabolism indicators in the liver of mice that were administered a single dose of 0.35 cm^3 per head of a 50% suspension of sulphur-enriched spirulina biomass (safety assessment) is confirmed by studies by authors G. Merzlova & O. Melnichenko (2012), which indicate that when laboratory animals were administered spirulina biomass enriched with Cobalt, no statistically significant increase or decrease in aminotransferase activity and total protein was observed. Therefore, it has been experimentally proven that *Spirulina platensis* biomass enriched with sulphur belongs to low-toxicity substances – class 4. The lethal dose (LD_{50}) of spirulina biomass for intragastric administration to laboratory animals (white mice) is greater than 5,000 mg/kg (Khan *et al.,* 2005).

Researchers M.B. Colovic *et al.* (2018) highlighted the crucial role of sulphur-containing amino acids in protecting cells from oxidative stress and the toxic effects of heavy metals. These amino acids have been shown to reduce damage to cellular structures by neutralising free radicals, playing a critical role in maintaining the antioxidant balance in the body. *Spirulina platensis* biomass, enriched with sulphur, contains a significant amount of these amino acids, which can contribute to increasing the body's resistance to the effects of exogenous toxicants. Studies of the toxicological profile of this biomass have not revealed a significant increase in the level of aminotransferases in animals receiving high doses of sulphur-enriched spirulina, confirming the absence of a negative impact on the liver and potential antioxidant effects. Given this, the application of such biomass may be promising in veterinary medicine and animal husbandry as an effective means of preventing oxidative stress, which can occur as a result of intensive animal farming, exposure to environmental pollutants, and metabolic stress.

C.A. Houghton (2019) highlighted sulforaphane as a promising nutraceutical with potent antioxidant properties. Although the mechanism of action of individual compounds was not analysed in this study, the results demonstrate a similar positive effect of sulphur-containing spirulina biomass on the physiological state of animals. Unlike sulforaphane, which activates specific antioxidant pathways, the sulphur contained in spirulina biomass is involved in general metabolic processes and provides a more prolonged effect on cellular metabolism. This could make it an effective component in animal feed, as sulphur-enriched biomass can contribute to a stable increase in the antioxidant status of the organism and improve its overall resistance to negative external factors.

Researchers Y. Ingenbleek & H. Kimura (2013) indicated that a sulphur deficiency can significantly disrupt protein metabolism and lead to a decrease in the efficiency of energy metabolism processes. In this regard, the introduction of sulphur-containing additives to the diet is of great importance for maintaining the normal physiological state of animals. According to the obtained results, the level of total protein in the liver of animals that received *Spirulina platensis* biomass, enriched with sulphur, remained within the physiological norm. This indicates the absence of a negative impact on protein metabolism and confirms the possibility of using such biomass to support metabolic homeostasis.

K. Jankowski *et al.* (2014) studied the impact of sulphur fertilisers on the concentration of microelements in rapeseed and found that increasing the sulphur content in the soil contributes to an increase in the concentration of zinc and manganese in plant tissues. Similarly, the process of enriching *Spirulina platensis* biomass with sulphur can influence the improvement of the bioavailability of microelements for animals, contributing to a more efficient absorption of nutrients. In addition, there may be a synergistic interaction between sulphur-containing compounds and other microelements, which may have a positive impact on the overall level of mineral metabolism. Further research can help clarify how sulphur-containing biomass affects the metabolism of microelements and what concentrations of sulphur are optimal for enriching feed.

In the research of A. Maruyama-Nakashita (2017), it was established that plants can adapt their metabolism to sulphur deficiency through the activation of enzymatic pathways. The obtained results confirm that *Spirulina platensis* has a high ability to accumulate sulphur, which opens up possibilities for its use as a source of this microelement in animal feed. The variability of the chemical composition of spirulina biomass depending on cultivation methods can have a significant impact on its biological activity. I.D. Nwachukwu *et al.* (2012) emphasised the significance of sulphur-containing natural compounds in the formation of plant defence mechanisms against pathogens. Since the role of sulphur-enriched *Spirulina platensis* biomass in modulating the immune response of animals remains insufficiently studied, this aspect is a promising direction for further research. In particular, it is necessary to assess the possible impact of such biomass on the state of the animal immune system, its ability to regulate inflammatory processes and increase resistance to infectious agents.

Y. Yagishita *et al.* (2019) emphasised the importance of the form in which sulphur-containing compounds are consumed to achieve maximum efficiency. The introduction of sulphur-containing spirulina biomass in the form of a suspension ensures a uniform distribution of microelements in the animal's body, which may be an advantage compared to other sources of sulphur. This confirms the need for further research on optimal dosages and administration methods to achieve maximum efficiency.

Thus, the results of the study confirm the significant role of sulphur in supporting metabolic processes, particularly in protein and energy metabolism, as well as in regulating the antioxidant status of the organism. The absence of lethal cases in white mice when administered *Spirulina platensis* biomass enriched with sulphur at high doses (up to 6,000 mg/kg body weight) indicates its low toxicity and safety for intragastric administration. Analysis of biochemical indicators showed that the level of total protein in the liver of mice from the experimental groups did not differ from the control values, confirming the absence of a negative impact on metabolic homeostasis. The results also demonstrated the stability of aminotransferase activity, indicating the absence of a toxic effect of sulphur-enriched spirulina on liver function. Temporary behavioural changes and minor disturbances of gastrointestinal tract functions, recorded in groups with the highest doses, were short-term and completely disappeared within 48 hours, confirming the adaptive capabilities of the organism to such dosages.

The results obtained demonstrate not only the safety of sulphur-enriched *Spirulina platensis* biomass but also its potential usefulness for feed enrichment. Spirulina's ability to accumulate sulphur can contribute to improving the bioavailability of microelements in animal diets, which is consistent with previous research on the role of sulphur in the absorption of zinc, manganese, and other important elements. Additionally, the antioxidant properties of sulphur-containing compounds can help protect cells from oxidative stress, which is particularly important in the context of intensive animal farming.

The practical significance of the obtained results includes the possibility of using sulphurenriched spirulina biomass in veterinary dietetics to increase animal productivity and as a prophylactic agent against stress factors. However, further research should be aimed at determining the optimal dosages for long-term use, assessing its impact on the performance indicators of different animal species, and the mechanisms of interaction between sulphur-containing compounds and other biologically active components of the feed ration.

Conclusions

The conducted study assessed the safety and acute toxicity of sulphur-enriched *Spirulina platensis* biomass using a white mouse model. The experimental results confirmed that spirulina biomass belongs to low-toxicity substances (safety class 4) and can be used in veterinary practice and animal husbandry as a feed additive. Under conditions of a single intragastric administration of 0.35 cm^3 of a 50% suspension of spirulina biomass, no lethal cases were recorded among white mice. In the first hours after administration, animals exhibited short-term signs of lethargy and gastrointestinal disturbances, expressed in reduced activity and temporary refusal of food. However, within 24 hours, the condition of the animals normalised, indicating the absence of a long-term toxic effect.

The study of biochemical parameters revealed that the administration of 0.35 cm^3 of 25% and 50% suspensions of spirulina biomass did not cause significant changes in protein metabolism indicators in the liver of mice. The activity of AST and ALT remained within the physiological norm and did not differ from the indicators of the control group. The content of total protein also did not undergo significant changes, confirming the absence of a negative impact of sulphurenriched spirulina biomass on the functional state of the liver of laboratory animals. Studies of the acute toxicity of sulphur-enriched spirulina biomass showed that it is characterised by a high safety margin. The administration of doses ranging from 5 to 5,000 mg/kg body weight to mice did not result in lethal outcomes, and the observed behavioural changes were temporary. Even when the maximum dose of 6,000 mg/kg body weight was administered, no serious pathological changes were observed in the animals. A slight decrease in mouse activity, short-term gastrointestinal disturbances, and a minor loss of body weight were reversible and completely disappeared within 48 hours after administration. It was determined that the LD_{50} of spirulina biomass exceeds 5,000 mg/kg, confirming its safety for use.

A promising direction for further research is the evaluation of the effectiveness of using sulphur-enriched spirulina biomass in the feeding of young dogs. An important task is to determine the optimal dosages, long-term effects on the animal's body, and possible mechanisms of the positive effect on physiological indicators. Further experimental studies can provide new data on the bioavailability of sulphur-containing compounds, their impact on metabolic processes, and the overall physiological state of animals, which will allow for a more effective application of spirulina biomass in veterinary practice and animal husbandry.

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Conflict of Interest

None.

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Перевірка показників нешкідливості та токсичності спіруліни збагаченої сульфуром на білих мишах

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Анотація. Вивчення біомаси *Spirulina platensis*, збагаченої сульфуром, є актуальним через її потенціал як цінної кормової добавки для сільськогосподарських тварин. Метою роботи було вивчення нешкідливості та гострої токсичності біомаси спіруліни збагаченої сульфуром. Для дослідження використовували лінійних мишей. Вивчаючи нешкідливість біомаси спіруліни мишам внутрішньошлунково вводили по 0,35 $см^3$ фізіологічного розчину (контроль) та 25 % і 50 % розчину суспензії біомаси спіруліни збагаченої сульфуром (I і II дослідні групи). Спостереження за тваринами проводили продовж 12 діб. У організмі мишей вивчали показники білкового обміну. За вивчення гострої токсичності лабораторним тваринам водили внутрішньошлунково біомасу спіруліни у кількості від 5 до 6000 мг/кг маси тіла. Спостереження за мишами тривало 14 діб. Встановлено, що, досліджуючи нешкідливість, протягом 12-добового спостереження після введення суспензій біомаси спіруліни летальних випадків у мишей не було виявлено. У дослідних тварин не виявлено патолого-анатомічних змін внутрішніх органів. Статистично значущого збільшення або зменшення вмісту загального білка, активності амінотрансфераз у мишей із I і II дослідних груп у порівнянні із тваринами контрольної групи не було встановлено. Визначаючи гостру токсичність, виявлено, що біомаса спіруліни відноситься до малотоксичних речовин – 4 клас. Значних поведінкових змін та суттєвого впливу на метаболічні процеси у тварин не зафіксовано, що свідчить про потенційну безпечність цієї біомаси для використання у тваринництві. Результати дослідження можна використати у тваринництві для впровадження інноваційних підходів до збагачення кормів корисними елементами, що покращують продуктивність і здоров’я тварин

Ключові слова: *Spirulina platensis*; печінка; лабораторні тварини; кормова добавка; клінічний стан тварин