Original article
UDK 639.3
https://doi.org/10.24143/2073-5529-2025-3-51-58
EDN JXQQLU

# The effect of the beet molasses on the growth rate and resistance of carp fingerlings (*C. carpio* L.) exposed to a single sublethal exposure to copper sulfate

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**Abstract.** The effect of the addition of beet molasses to fish feed on lipid peroxidation, fish-breeding, biological and morphological parameters of carp fingerlings (*C. carpio* L.) under control conditions and subjected to a single sublethal exposure to copper sulfate was studied. The fish were fed daily, at the rate of 2% of the body weight feed. Copper sulfate treatment was carried out once at the rate of 3 ml of 10% solution per 20 liters of water, for 24 hours. The duration of the experiment was 30 days. Growth parameters and survival were determined in all fish with an interval of 2 weeks. The intensity of lipid peroxidation was determined in the gills by the content of malonic dialdehyde. The liver was taken for histological analysis. The best fish-breeding and biological indicators were found in control individuals and experimental feed variants with the addition of beet molasses, including under the influence of copper sulfate. All feed options led to an increase in the intensity of lipid peroxidation processes in the gills. Beet molasses showed an antioxidant effect only under conditions of increased oxidative processes caused by copper sulfate. There was a decrease in the content of malonic dialdehyde in the gills by 12%, but the intensity of lipid peroxidation still remained 34% higher compared with the control. During the studied period, no morphological changes were detected in the liver.

**Keywords:** carp fingerlings, copper sulfate, fish feed, beet molasses, production, biochemical and morphophysiological parameters of carp

**Acknowledgment:** the publication was prepared as part of the implementation of the state assignment of the Southern Scientific Center of the Russian Academy of Sciences for 2025, state registration number 125011200145-8 on the topic “Features of the processes of formation of the ichthyofauna of the pools of the southern seas of Russia under the influence of anthropogenic factors, climate change, and the development of scientific foundations for improving technologies for restoring populations of valuable fish species for the sustainable functioning of aquatic ecosystems”.

**For citation:** Shirina Yu. M., Kotelnikov A. V., Kotelnikova S. V., Kuzov A. A. The effect of the beet molasses on the growth rate and resistance of carp fingerlings (*C. carpio* L.) exposed to a single sublethal exposure to copper sulfate. *Vestnik of Astrakhan State Technical University. Series: Fishing industry*. 2025;3:51-58. (In Russ.). https://doi.org/10.24143/2073-5529-2025-3-51-58. EDN JXQQLU.

Научная статья

# Влияние свекловичной патоки на интенсивность роста и резистентность сеголеток карпа (*C. carpio* L.), подвергшихся однократному сублетальному воздействию сульфата меди

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**Аннотация.** Исследовано влияние добавления в рыбный корм свекловичной патоки на перекисное окисление липидов, рыбоводно-биологические и морфологические показатели сеголеток карпа (*C. carpio* L.) в контрольных условиях и подвергшихся однократному сублетальному воздействию сульфата меди. Рыб кормили ежедневно, из расчета 2 % корма от массы тела. Обработку сульфатом меди проводили однократно из расчета 3 мл 10 % раствора на 20 л воды, в течение 24 ч. Продолжительность эксперимента составила 30 дней. С интервалом в 2 недели у всех рыб определяли параметры роста и выживаемость. Интенсивность перекисного окисления липидов определяли в жабрах по содержанию малонового диальдегида. Для гистологического анализа брали печень. Наилучшие рыбоводно-биологические показатели были у контрольных особей и экспериментальных вариантов корма с добавлением свекловичной патоки, в том числе в условиях воздействия сульфата меди. Все варианты кормов привели к увеличению интенсивности процессов перекисного окисления липидов в жабрах. Свекловичная патока проявляла антиоксидантный эффект только в условиях усиления окислительных процессов, вызванных сульфатом меди. Было отмечено уменьшение содержания малонового диальдегида в жабрах на 12 %, но при этом интенсивность перекисного окисления липидов все равно оставалась выше на 34 % по сравнению с контролем. За исследованный срок морфологических изменений в печени выявлено не было.

**Ключевые слова:** сеголетки карпа, сульфат меди, корм для рыб, свекловичная патока (меласса), продукционные, биохимические и морфофизиологические показатели карпа

**Благодарности:** публикация подготовлена в рамках реализации ГЗ ЮНЦ РАН на 2025 г., № ГР 125011200145-8, по теме «Особенности процессов формирования ихтиофауны бассейнов южных морей России в условиях влияния антропогенных факторов, изменений климата и разработка научных основ совершенствования технологий восстановления популяций ценных видов рыб для устойчивого функционирования водных экосистем».

**Для цитирования:** *Ширина Ю. М., Котельников А. В., Котельникова С. В., Кузов А. А.* Влияние свекловичной патоки на интенсивность роста и резистентность сеголеток карпа (*C. carpio* L.), подвергшихся однократному сублетальному воздействию сульфата меди // Вестник Астраханского государственного технического университета. Серия: Рыбное хозяйство. 2025. № 3. С. 51–58. https://doi.org/10.24143/2073-5529-2025-3-51-58. EDN JXQQLU.

## Introduction

Copper is an important trace element for the healthy growth and reproduction of all plants and animals. This element plays an important role in cell physiology, nutrition, and metabolism in vertebrates [1]. Although the essential role of copper in some enzymatic processes has been proven [2], this heavy metal can have an adverse toxicological effect when its content is high. Copper is absorbed through the gills and digestive tract of fish and mainly accumulates in various tissues such as gills, liver, kidneys and internal organs [3]. As a rule, as the concentration of metals in the environment increases, fish accumulate more of them in their tissues [4]). When consumption is not balanced with elimination processes and detoxification mechanisms, metals can have toxic effects [5]. The gills, given that they are in direct contact with the aquatic environment, are the main entrance for dissolved substances [6]. These substances can subsequently enter the liver, which is the main organ for removing metals from the body, through the circulatory system. When the liver's capacity is exceeded, they can accumulate in other tissues, such as muscles. The accumulation of metals in muscles is usually insignificant, since it is not a metabolically active tissue, but it is important for the transfer of metals along the food chain [7].

With prolonged exposure to copper in fish in sublethal doses, a number of physiological and metabolic changes or adaptations occur that allow fish to survive for a long time. These adaptations are usually reversible, and fish recover after exposure stops. Research on chronic toxicity is limited, and the mechanisms of chronic copper toxicity for fish are less well understood than the mechanisms of acute toxicity. Feeding speed is one of the most important factors affecting fish productivity and growth, and toxic substances can affect fish growth both directly and indirectly, affecting feeding or increasing maintenance costs [8].

Recently, much attention has been paid to the interaction between food and copper toxicity. Most of these studies focus on the toxicity of copper ingested from food and compare its importance to the effects of copper ingested from water [9]. Some studies have focused on the effect of feed composition and availability on fish viability when exposed to copper supplied with water [10] or a combination of both aspects in the study [11].

There are many compounds (nucleotides, plant extracts, etc.) that enhance immunity and the effectiveness of fish nutrition, which can be used as food additives in aquaculture [12]. One of these additives may be beet molasses (molasses).

The beet molasses is a thick concentrated liquid syrup, a by-product of the processing of sugar beet into sugar. In general, beet molasses contains approximately 50% sucrose, 1% raffinose and 0.25% glucose and fructose in terms of dry weight. In addition to sugar, molasses contains many important trace elements, such as minerals and vitamins [13]. In beet molasses, potassium, calcium, sodium, magnesium and iron are present in noticeable amounts. It is important to note that the minerals in molasses are dissolved and, therefore, easily absorbed. Molasses also contains B vitamins and is free of fat, fiber, or cholesterol. The secondary components of beet molasses include proteins, betaine, glutamic acid, purine and pyrimidine bases, organic acids, pectin and melanoidins. Apart from the fact that molasses is a rich source of many important nutrients, there is some evidence that molasses has significant antioxidant potential. It has recently been discovered that molasses extracts have significant antioxidant activity combined with interesting physiological functions that include anti-inflammatory properties, action as a vaccine adjuvant and resistance to infections, as well as a protective effect against oxidative DNA damage [14]. The rate of introduction into fish feed is 3-5% [15]. However, there is no data in the literature on the use of molasses to neutralize the toxic effects of copper sulfate on the immune system of fish. Therefore, the purpose of this study was to evaluate the effect of beet molasses on the physiological status of carp fingerlings exposed to a single sublethal exposure to copper sulfate.

**Materials and methods**

The research was carried out in the Laboratory of Aquaculture and Aquatic Bioresources of the Southern Scientific Center of the Russian Academy of Sciences. The object of the study is fingerlings of carp *C. carpio* D. in the amount of 120 pieces. The average weight and length of the fish were 15.3 g and 10 cm, respectively. Before the start of the experiment, the fish were kept in aerated dechlorinated tap water at a temperature of 23 ± 1 °C and fed daily with commercial feed for juvenile carp (52% crude protein), produced by “Fish Feeds JSC”, at the rate of 2% of body weight.

After the acclimatization period, the fish were divided into 4 groups: 1 – Control (without exposure to copper sulfate and a diet without molasses); 2 – Feed option 1 (with exposure to copper sulfate and a diet without molasses); 3 – Feed option 2 (with exposure to copper sulfate and a diet containing 3% beet molasses); 4 – Feed option 3 (without exposure to fish with copper sulfate and a diet containing 3% beet molasses).

Copper sulfate treatment was performed once: 10% solution at the rate of 3 ml per 20 liters of water for 24 hours. After that, 90% of the aquarium water was replaced with clean water [12].

During the experiment, fish conditions were monitored daily. The temperature was maintained in the range of 22-21 °C, pH – 7.41, dissolved oxygen – 6.4 mg/l. Continuous ventilation with compressed air was carried out in each aquarium. To maintain clean and healthy water throughout the experiment period, three quarters of the aquarium water was pumped daily to remove feces and feed residues and replaced with clean, aerated water from the storage tank.

The fish were fed with beet molasses 2 times a day at the rate of 2% of the total fish biomass in each aquarium, adjusting the daily amount of feed every two weeks depending on the fish biomass. The nutritional composition of the feed is shown in Table 1.

*Table 1*

**Nutritional composition of the feed forcarp fingerlings with the addition of beet molasses**

| Indicator | Content |
|---|---|
| Crude protein, % | 54.3 |
| Raw fat, % | 12.3 |
| Raw ash, % | 6.86 |
| Raw fiber, % | 1.86 |
| Humidity, % | 6.01 |
| Gross energy, MJ/kg | 22.93 |

The duration of the experiment was 30 days. With an interval of 2 weeks, growth parameters and survival were determined in all fish according to the following formulas [16, 17]:

$$\text{Absolute weight gain} = W_f - W_s;$$

$$\text{Average daily weight gain} = \frac{W_f - W_s}{t};$$

$$\text{Average daily growth rate} = \left[\left(\frac{W_f}{W_s}\right)^{1/t} - 1\right]100;$$

$$\text{Specific growth rate} = \frac{\log(W_f) - \log(W_s)}{t}100;$$

$$\text{Mass accumulation coefficient} = \; x = \frac{\left(\left(W_f^{\frac{1}{3}} - W_s^{\frac{1}{3}}\right)3\right)}{t},$$

where $W_s$ and $W_f$ are the mass of fish at the beginning and at the end of the experiment, g; $t$ is the duration of the experiment, day.

The intensity of lipid peroxidation was determined in the gills, since they are in direct contact with the medium where the feed and copper sulfate were supplied. The indicator was assessed by the content of malonic dialdehyde (MDA) using the thiobarbiturine method [18].

For histological analysis, the liver was taken as the organ that reacts most quickly to the effects and mobilely reflects the functional state of the body and takes an active part in the digestive process. A liver sample was fixed in a Buena mixture, embedded in paraffin wax, and sections with a thickness of 7 microns were made [19]. The resulting sections were stained with hematoxylin-eosin and photographed using an Olympus BX 53 microscope.

The Student's criterion was used for statistical processing of the analysis of the obtained results [20].

**Results and discussions**

A lot of natural and artificial chemicals are dissolved in fresh water. Aquatic organisms have adapted to the effects of natural compounds present in water and can be exposed to various pollutants such as heavy metal salts (for example, $CuSO_4$). Copper can accumulate in the organs of aquatic animals [21], which can lead to redox reactions with the formation of free radicals and, consequently, cause morphological changes and disrupt some physiological processes. Copper also has a toxic effect, leading to a decrease in feed intake, increased oxidative stress, a decrease in body weight, as well as biochemical and hematological changes [22].

After adding a sublethal dose of copper sulfate to the water (variants No. 1 and 2), during the first day the fish behaved sluggishly and sedately, rose to the surface of the water, periodically fell on its side, it had rapid movement of the gill cover, tremor of the fins, and rapid ingestion of air. The waste during this period was the maximum for the entire duration of the experiment and reached 20%.

The control individuals and experimental variants No. 2 and 3 had the best fish-breeding and biological indicators (Table 2).

*Table 2*

**Fish-breeding and biological indicators of carp fingerlings**

| **Indicators** | **Experimental options** | | | |
|---|---|---|---|---|
| | **Control** | **Feed option 1** | **Feed option 2** | **Feed option 3** |
| Initial mass, g | 16.95 ± 3.61 | 13.04 ± 5.69 | 15.5 ± 7.28 | 15.82 ± 4.86 |
| Final mass, g | 26.01 ± 1.58* | 17.33 ± 7.49** | 24.41 ± 5.85* | 25.12 ± 2.25* |
| Absolute initial length, mm | 10.36 ± 0.89 | 9.4 ± 1.69 | 9.9 ± 1.86 | 10.04 ± 1.19 |
| Absolute final length, mm | 10.82 ± 0.83 | 10.04 ± 1.67 | 10.06 ± 1.97 | 10.43 ± 1.15 |
| Absolute increase, g | 9.06 | 4.29 | 8.91 | 9.3 |
| Average daily increase, g | 0.302 | 0.143 | 0.297 | 0.31 |
| Average daily growth rate, % | 1.423 | 0.943 | 1.51 | 1.537 |
| Specific growth rate, % | 0.62 | 0.412 | 0.657 | 0.667 |
| Mass accumulation coefficient, units | 0.039 | 0.023 | 0.04 | 0.041 |
| Survival rate, % | 100 | 80 | | 100 |
| Duration of the experiment, day | 30 | | | |

* Statistically significant differences are sufficient $p \le 0.05$; ** $p \le 0.01$.

The absolute increase here was 9.06, 8.91 and 9.3 g, respectively. The low growth rate in option 1 (the absolute increase was 4.29 g) can be caused by two factors: detoxification increased energy consumption, and the high copper content reduced feed intake, which ultimately led to slower growth. The fish from feeding option No. 2 was also subjected to a single treatment with copper sulfate, but here beet molasses was added to the feed, acting as an antioxidant, which helped reduce the toxic effects of copper on the carp's body. The results indicate that beet molasses has a significant potential effect on carp growth by protecting the body from the immunotoxic effects of $CuSO_4$.

All feed variants led to an increase in the intensity of lipid peroxidation processes in the gills (Table 3).

*Table 3*

**The content of malonic dialdehyde in the gills**

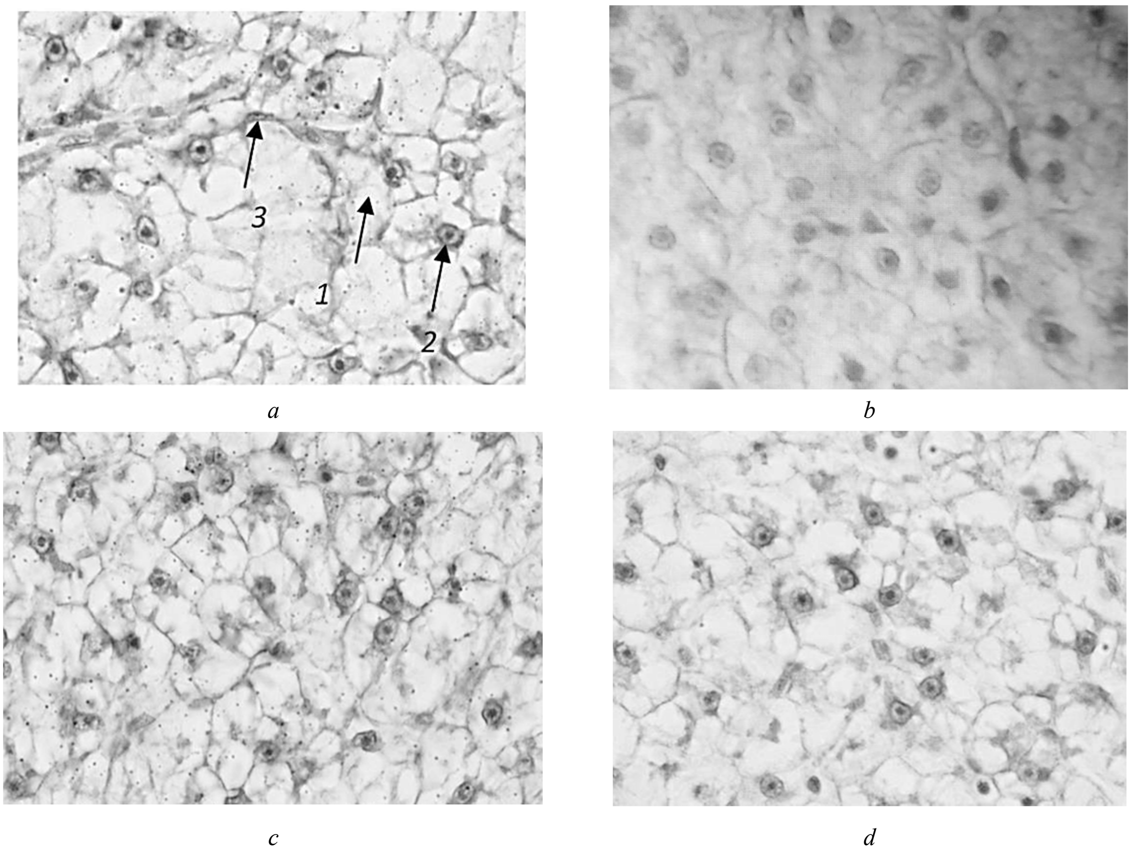
| Experimental options | MDA content, nm/g |
|---|---|
| Control | 23.21 ± 0.992 |
| Feed option 1 | 34.82 ± 1.453* |
| Feed option 2 | 31.20 ± 0.211*^ |
| Feed option 3 | 29.12 ± 0.510* |

* Differences compared to the control ($p \leq 0.001$); ^ differences between feed options 1 and 2 ($p \leq 0.05$).

At the same time, the greatest increase was noted in the feed variant with the addition of only copper sulfate (Feed option 1), where the content of MDA in the gills increased 1.5 times. The beet molasses feed (Feed option 3) also contributed to an increase in the content of MDA in the gills, but in this case the increase was 25% compared with the control. The addition of molasses to the feed variant with copper (Feed option 2) had an antioxidant effect, reducing the content of MDA in the gills by 12%, but the intensity of peroxidation processes still remained 34% higher compared with the control.

Thus, beet molasses showed an antioxidant effect only under conditions of increased pro-oxidant processes, in this case caused by copper sulfate, and an excess of antioxidants in the feed can lead to the opposite effect. At the same time, an increase in the concentration of TBA-reactants in the gills of fish treated with beet molasses may be a consequence not so much of the pro-oxidant effect of the feed as an indirect result of the intensification of metabolism, and, consequently, the excretion of metabolic products from the body through the gills in aquarium conditions. The fish treated with molasses were characterized by higher fish-breeding and biological parameters. At the same time, the gills are quite sensitive to the increased ammonia content and react to its increase by increasing lipid peroxidation [23].

Micro-preparations of the liver of carp fingerlings receiving experimental feed options (Fig.) practically did not differ from the control group.




Liver of juvenile carp (magnification 400): *1* – liver cells; *2* – nucleus with a nucleolus; *3* – blood vessels; *a* – control; *b* – feed option 1; *c* – feed option 2; *d* – feed option 3

The liver of juvenile carp is a parenchymal tissue without a pronounced girder structure. Hepatocytes have a multifaceted shape and fit snugly to each other. The cytoplasm of cells is usually light, but does not contain vacuoles.

On histological preparations, the nuclei of hepatocytes are clearly visible, having a rounded shape with one clearly distinguishable nucleolus in the middle. The destruction of nuclear structures is not observed.

The liver tissue is abundantly supplied with blood vessels. At the same time, infiltration accumulations of leukocyte cells, which could indicate the presence of pronounced inflammatory processes, were not detected. Thus, the morphological picture of the liver indicates its satisfactory condition.

**Conclusion**

The studies performed make it possible to evaluate the effectiveness and safety of adding beet molasses to fish feed when they are grown under artificial conditions and treated with copper sulfate.

When treating fish with copper sulfate, a significant inhibition of the increase in size and body weight of carp fingerlings was observed. The death rate of individuals reached 20%. An increase in the intensity of peroxidation processes in the gills was noted under these conditions. Beet molasses is able to exhibit an antioxidant effect, but only under conditions of increased lipid peroxidation, in this case caused by copper sulfate. There was a decrease in the content of malondialdehyde in the gills of fish exposed to copper sulfate and fed a molasses diet, but the intensity of lipid peroxidation still remained higher compared with the control.

The analysis of the morphological picture of the liver during the studied period did not reveal the presence of pronounced inflammatory and necrotic processes when using all feed options. Obviously, the liver has a fairly large margin of safety and is able to withstand negative effects on the body for quite a long time.

Thus, the addition of beet molasses can have a beneficial effect on the fish-breeding and biological parameters of fish, reduce the negative effects of copper sulfate on the body, but it does not completely eliminate them. In addition, there were no significant differences from the control group. Additionally, it is worth considering the possibility of intensification of lipid peroxidation in fish gills when beet molasses is added to the feed under conditions of their artificial cultivation.

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The article was submitted 26.05.2025; approved after reviewing 16.06.2025; accepted for publication 11.09.2025
Статья поступила в редакцию 26.05.2025; одобрена после рецензирования 16.06.2025; принята к публикации 11.09.2025

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