

STUDIES ON THE TOXICITY OF C-PHYCOCYANIN IN GUPPY FISH AND INFUSORIA *PARAMECIUM CAUDATUM*

*I. P. Patereha, candidate of veterinary sciences,
V. I. Kushnir, candidate of veterinary sciences,
Yu. M. Kosenko, doctor of biological sciences,
I. M. Kushnir, doctor of veterinary sciences*

State Scientific-Research Control Institute of Veterinary Medicinal Products and Feed Additives
11, Donetska str., Lviv, 79019, Ukraine
ipater2@gmail.com

One of the important and urgent tasks, whose successful solution depends on the supply of food to the population, is to increase the production of livestock products. Ensuring a high level of production of livestock products largely depends on the quality of feed, which is determined, first of all, by toxicological control.

C-phycoyanin is a protein complex that belongs to phycobiliproteins and can be used as one of the potential natural components to improve the feeding of commercial fish.

In order to establish the safety of the use of active feed components for feeding and ready-made feed into which these components are introduced, first of all, it is necessary to study their effect on the organisms of protozoa and fish, which are used for laboratory research.

The high sensitivity of ciliates to the appearance of toxic substances in their environment and changes in their concentration led to the use of these organisms for testing the most diverse chemical elements and compounds.

*The article presents the results of studying the effect (harmlessness) of C-phycoyanin on the bodies of protozoa (*Paramecium caudatum*) and guppy fish.*

*When studying the effect of C-phycoyanin on the body of protozoa (*Paramecium caudatum*), it was established that its use for 48 hours in doses of 5, 10, 25, 50, and 100 mg/l did not cause the death of ciliates. When studying the effect of C-phycoyanin on the body of guppy fish, it was established that its use in doses of 5, 10, 25, 50, and 100 mg/l did not cause the death of fish.*

*So, on the basis of the conducted research, it was established that C-phycoyanin belongs to class 4, and is a non-toxic substance for *Paramecium caudatum* ciliates. In addition, it was established that its use in the above doses did not cause the death of fish, and its LC₅₀ is higher than 100 mg/l.*

Keywords: TOXICITY, ACUTE TOXICITY LC₅₀, *PARAMECIUM CAUDATUM*, C-PHYCOCYANIN, GUPPY.

ДОСЛІДЖЕННЯ ТОКСИЧНОСТІ С-ФІКОЦІАНІНУ У РИБ ГУППІ ТА ІНФУЗОРИЇ *PARAMECIUM CAUDATUM*

І. П. Патерега, В. І. Кушнір, Ю. М. Косенко, І. М. Кушнір

Державний науково-дослідний контрольний інститут ветеринарних препаратів
та кормових добавок
вул. Донецька, 11, м. Львів, 79019, Україна
ipater2@gmail.com

Одним з важливих і невідкладних завдань, від успішного вирішення яких залежить

постачання населенню продуктів харчування, є збільшення виробництва продуктів тваринництва. Забезпечення високого рівня виробництва продукції тваринництва значною мірою залежить від якості кормів, яка визначається, перш за все, токсикологічним контролем.

C-фікоціанін є протеїновим комплексом, який належить до фікобіліпротеїнів і може застосовуватись як один із потенційних природних компонентів для покращення годівлі промислових риб.

З метою встановлення безпечності використання для годівлі діючих компонентів корму і готового корму, до складу якого вводяться ці компоненти, в першу чергу, необхідно вивчити їх вплив на організм найпростіших і риб, які використовуються для лабораторних досліджень.

Висока чутливість інфузорій до появи в оточуючому її середовищі токсичних речовин та зміна їхньої концентрації зумовила використання цих організмів для тестування найрізноманітніших, за хімічною природою, елементів та сполук.

У статті наведені результати вивчення впливу (нешкідливість) C-фікоціаніну на організм найпростіших (*Paramecium caudatum*) і риб гупі.

При вивченні впливу C-фікоціаніну на організм найпростіших (*Paramecium caudatum*) було встановлено, що його застосування упродовж 48 год у дозах 5, 10, 25, 50 та 100 мг/л не викликало загибелі інфузорій.

При вивченні впливу C-фікоціаніну на організм риб гупі було встановлено, що його застосування у дозах 5, 10, 25, 50 та 100 мг/л не викликало їх загибелі.

Отже, на основі проведених досліджень було встановлено, що C-фікоціанін належить до 4 класу, тобто є не токсичною речовиною для інфузорій *Paramecium caudatum*. Крім того, було встановлено, що його застосування у вище вказаних дозах не викликало загибелі риб, а його ЛК₅₀ є вищою, ніж 100 мг/л.

Ключові слова: ТОКСИЧНІСТЬ, ГОСТРА ТОКСИЧНІСТЬ ЛК₅₀, ІНФУЗОРИЇ *PARAMECIUM CAUDATUM*, C-ФІКОЦІАНІН, ГУПІ.

One of the important and urgent tasks, whose successful solution depends on the supply of food to the population, is to increase the production of livestock products.

Ensuring a high level of livestock production largely depends on the quality of feed, which is determined, first of all, by toxicological control.

C-phycocyanin is a protein complex that belongs to phycobiliproteins and can be used as one of the potential natural components to improve the feeding of commercial fish.

In order to establish the safety of the use of active feed components for feeding and ready-made feed into which these components are introduced, first of all, it is necessary to study their effect on the organisms of the simplest and fish, which are used for laboratory research.

The high sensitivity of infusorium to the appearance of toxic substances in their environment and the change in their concentration led to the use of these organisms for testing the most diverse chemical elements and compounds.

As for biotesting, infusorium has a number of advantages. Compared to other test systems.

In the morphological aspect, it is a cell, and in the physiological aspect, it is a whole organism that has a large specific surface area of contact with the surrounding environment. Due to the rapidity of life and high reproductive capacity, these test objects make it possible to trace the effects of toxicants on many generations in a relatively short period of time. As a result of the low ability to regulate the internal concentration in the target's body, a large number of compounds accumulate. As a result, the process of disordering the normal functioning of body systems is accelerated, which often leads to their deaths. Thus, these advantages characterize infusoriums as a test object that is highly sensitive to the influence of external compounds.

Tetrahymena pyriformis and *Paramecium caudatum* are the most popular in biological testing. this is due to the wide distribution of these infusoriums in nature and the ease of their cultivation.

In most cases, the toxic effect on free-living infusoriums is recorded by the following test

reactions:

- change in body shape;
- death of infusoriums in an acute experiment;
- inhibition and stimulation of the rate of division in acute and chronic experiments;
- pathological violations of the division process;
- increase and decrease in the number of conjugative pairs;
- suppression and strengthening of the phagocytosis process;
- paralysis of the contractile vacuole;
- loss of orientation in space and change in movement.

Most scientists studied the survival of infusoriums under the influence of external factors in an acute experiment for 10 minutes, 1, 5, 24, 48, 72, 96 and 120 hours, and a chronic experiment for 7, 10, 14, 15, 25, 28 and 30 days.

Guppy is a small fish from the *Pecilia* family. The average size of a male's body is 3 cm, and a female's is 6 cm. The scales have the appearance of a rhombic grid, which is why the fish received the species epithet from the Latin "reticulum" - net. Fish are characterized by pronounced sexual dimorphism.

Females are much larger and thicker than males, do not have strongly developed veil fins, and are much more modestly colored: mainly the body and tail are gray, sometimes the caudal fin is colored or spotted. The most important distinguishing feature of males is the presence of a modified anal fin, which is called a gonopodium. Thanks to it, males are able to place sexual products directly into the female's abdominal cavity, where fertilization takes place.

The purpose of the work is to study the effect (harmlessness) of C-phycoyanin on the bodies of the simplest (infusoria *Paramecium caudatum*) and guppy fish.

C-phycoyanin obtained from the biomass of *Cladophora sp.* was used in the experiments.

Test objects were used to establish the toxicity class of veterinary drugs:

- infusoria *Paramecium caudatum*;
- guppy fish.

Materials and methods.

Study of C-phycoyanin toxicity on infusoria *Paramecium caudatum*

Experiments were conducted to study the toxicity of C-phycoyanin on infusorias according to the methodology (Kosenko et al., 1997; Kotsiumbas et al., 1999, Kotsiumbas et al., 2006).

Equipment, materials, reagents

- ✓ Binocular stereoscopic microscope, MBC;
- ✓ pH meter EB-74;
- ✓ electric thermostat TC-80M-2;
- ✓ KVS drying cabinet-100/250;
- ✓ general purpose laboratory scales of the 4th accuracy class;
- ✓ laboratory grinder MPR-2;
- ✓ Bogorov cameras;
- ✓ laboratory glasses;
- ✓ glass tubes with a capacity of 25 ml;
- ✓ pipettes with a capacity of 10 ml; Pasteur pipettes;
- ✓ microscope slides;
- ✓ slides with a recess;
- ✓ coverslips;
- ✓ Petri dishes;
- ✓ Antibiotic bottles;
- ✓ filter paper;
- ✓ fresh pressed yeast according to TU U 00383320.001-97;
- ✓ tripod for test tubes;

- ✓ acetone according to TU 6-09-3513-86;
- ✓ dechlorinated tap water;
- ✓ test cultures of the infusoria *Paramecium caudatum*.

Study of the toxicity of C-phycoyanin on guppy fish

According to the OECD № 203 "Fish. Acute toxicity testing" (OECD, 2019) feed research was conducted using guppy fish. The essence of the method is the poisoning and death of guppy fish with toxins that enter through the gills directly into the blood, bypassing the protective mechanisms of the digestive system.

The principle of the test is that the fish are exposed to the test substance for 96 hours under any static, semi-static, or flow-through conditions. At the same time, at the time of death of fish, visible deviations related to appearance and behavior are recorded. If possible, determine the concentration that causes the death of 50 % of fish (LC₅₀).

Equipment

The following laboratory equipment was used for the analysis:

1. pH meter;
2. appropriate temperature control devices (thermometers);
3. reagents for water quality control Tetra Pond Test Set;
4. equipment for maintaining water temperature and oxygen content (heaters, compressors).

Holding of fish

All fish should be held in the laboratory for at least 9 days before being used in the study. The first 48 hours were the settling-in period. Then, fish should be acclimatized for at least 7 days (48 hours of housing + 7 days of acclimatization = 9 days) in water similar to the test water immediately before the start of the test.

During the acclimatization period, deaths were recorded, and the following criteria were applied:

- the death of less than 5% of the population in seven days: the batch was accepted.

Fish were kept under the following conditions:

- ✓ photoperiod: day/night - 16/8 hours;
- ✓ temperature: 23-25 °C
- ✓ oxygen concentration: at least 60 % of the air saturation value.
- ✓ feeding: daily up to 24 hours before the start of the application of the researched

product. Feed was given until saturation. Excess food and feces were removed to avoid waste accumulation.

Dechlorinated tap water was used in the experiments, in which guppy fish were mobile, consumed food well, and actively responded to external stimuli.

Tested solutions were prepared by simply mixing the substance under study with water. The drug was studied in doses of 100 mg/l, 50 mg/l, 25 mg/l, 10 mg/l and 5 mg/l. These doses were selected according to OECD № 203 (OECD, 2019).

10 fish were used in each experimental and control group. The fish were distributed randomly. The study was conducted in 2 repetitions.

Result and discussion.

Study of C-phycoyanin toxicity on infusoria *Paramecium caudatum*

To obtain the tested concentrations of the drug:

- 1) mixed 9 ml of infusoria culture and 1 ml of C-phycoyanin solution, the concentration of which was 100 mg in 1 l;
- 2) mixed 9 ml of infusoria culture and 1 ml of C-phycoyanin solution, the concentration of which was 50 mg in 1 l;
- 3) mixed 9 ml of infusoria culture and 1 ml of C-phycoyanin solution, the concentration of which was 25 mg in 1 l;

4) mixed 9 ml of infusoria culture and 1 ml of C-phycoyanin solution, the concentration of which was 10 mg in 1 l;

5) mixed 9 ml of infusoria culture and 1 ml of C-phycoyanin solution, the concentration of which was 5 mg in 1 l.

The complete cessation of movement (locomotor function) and deformation of the body served as indicators of the loss of vital activity in the paramecium. Each experiment was carried out in 5-fold repetition, the total number of infusorias - at least 100–150 specimens.

To observe the development of acute intoxication, 0.1–0.4 ml of the prepared mixture of culture and solution was introduced into the recess of the glass slide, covered with a cover glass, and changes in movement, body shape, destruction of the shell, dispersion of cytoplasm, and death of infusorias were monitored under a microscope. All manipulations were carried out using a micropipette.

To conduct acute experiments, cultures in the stationary phase of growth were used, in which individuals with large body sizes prevailed.

With the help of a microscope (magnification 6x4), the total number of infusoria before the start and the number alive after 48 hours of the experiment were recorded.

Toxicity was assessed according to the criteria given in the table 1.

Table 1

Assessment of the degree of toxicity of the studied drug using infusorias based on LC₁₀₀ after 48 hours of the experiment

LC ₁₀₀	Toxicity class
concentration of 0.001 % and higher	1
concentration of 0.1 % and higher	2
concentration of 1 % and above	3
there is no complete death at any concentration	4

Note: LC₁₀₀ - is a lethal concentration of 100 %.

The results of the conducted research are shown in table 2.

Table 2

Assessment of the degree of toxicity of C-phycoyanin for 48 hours of the experiment

№	Number of infusorias	Drug dose, mg/l	Survived infusoria, %	Toxicity class
1	100-150	5	98,7±3,6	4
2	100-150	10	97,6±6,1	4
3	100-150	25	95,7±4,8	4
4	100-150	50	92,4±5,6	4
5	100-150	100	91,1±4,3	4

In the conducted studies, no complete death of infusorias was established in any of the studied concentrations 48 hours after the start of the experiment.

It was established that C-phycoyanin belongs to class 4, i.e. it is a non-toxic substance for infusoria *Paramecium caudatum Ehrenberg*.

Study of the toxicity of C-phycoyanin on guppy fish

Guppies are best kept in small groups of 6 or more. They swim at the entire depth of the aquarium. Fish must be selected so that there are 2-3 females per male - in this case, females will be less susceptible to attacks by males ready to spawn. For a fish of 6–10 pieces, you will need an aquarium with a volume of 50 liters or more. It is advisable to use bright lighting: with it, the guppies look better, acquire a richer color. It is better to cover the aquarium with a lid, because the fish can quite easily jump out of the water and get on the floor.

The guppy feels good in clean, oxygenated water. The main thing is that the stream from the filter is not very strong: it is difficult for small fish to resist a strong stream. The optimal temperature

for keeping this tropical fish is 24–26 °C, although guppies can exist in the range of 18–30 °C. Its life depends greatly on the temperature. The higher this indicator is, the faster the metabolism, the more frequent reproduction, and the shorter the life. If the fish is at its lower limit of temperature, then its metabolism slows down, reproductive function stops.

Water with a hardness of 10–25 °dGH and an acidity of 7–8.5 is suitable for keeping guppies. However, water parameters are not an axiom. If the changes were not too sharp, guppies could adapt to almost any condition in a short time without any problems. To maintain good health, weekly water changes in the aquarium (25–30 %) are necessary.

Preparation for the test

During the first 24 hours of the study, fish were observed every 3 hours. On days 2–4 of the test, all containers of live fish were inspected twice daily (early morning and late afternoon to best cover a 24-hour period). Visible abnormalities in balance (loss of balance, raising or lowering of head, floating or sinking), appearance (weak or dark pigmentation, exophthalmia), ventilatory behavior (hyper, hypo, or irregular ventilation, coughing), and swimming behavior (increased or hypo-activity, immobility, convulsions, near the surface or bottom).

The results of the research conducted are shown in table 3.

Table 3

Assessment of the degree of toxicity of C-phycoyanin for 96 hours of the experiment

№	The number of guppy fish, individuals	Drug dose, mg/l	Survived guppy fish, individuals	Died guppy fish, individuals	Died guppy fish, %
1	10	5	10	0	0
2	10	10	10	0	0
3	10	25	10	0	0
4	10	50	10	0	0
5	10	100	10	0	0
6	10	Control	10	0	0

In the conducted studies, no deaths of guppy fish were found in any of the studied concentrations 96 hours after the start of the experiment. Guppy fish were mobile, consumed food well, and actively responded to external stimuli. Their behavior did not differ from the behavior of fish of the control group.

So, on the basis of the conducted research, it was established that C-phycoyanin belongs to class 4, and is a non-toxic substance for *Paramecium caudatum* ciliates. In addition, it was established that its use in the above doses did not cause the death of fish, and its LC₅₀ is higher than 100 mg/l.

CONCLUSIONS

1. As a result of the conducted research, it was established that C-phycoyanin belongs to class 4, and is a non-toxic substance for *Paramecium caudatum* ciliates.
2. C-phycoyanin is a non-toxic substance for guppy fish. The LC₅₀ of C-phycoyanin is higher than 100 mg/l.

Prospects for research. Studies of composition of *Cladophora* sp. biomass toxicity in Guppy fish and infusoria *Paramecium caudatum*.

Gratitude

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