ФИЗИОЛОГИЯ И БИОХИМИЯ ГИДРОБИОНТОВ

PHYSIOLOGY AND BIOCHEMISTRY OF HYDROCOLE

Original article
UDK 664-4,575.224.2
https://doi.org/10.24143/2073-5529-2022-3-48-59
EDN BSCKRL

Evaluating possible genotoxicity of three feed additives recommended for aquaculture by using micronucleus test on *Danio rerio* erythrocytes

N. I. Kochetkov[1], S. V. Smorodinskaya[2], D. L. Nikiforov-Nikishin[3], V. A. Klimov[4], N. A. Golovacheva[5], A. L. Nikiforov-Nikishin[6], Yu. N. Grozescu[7]✉

[1–6]K. G. Razumovsky Moscow State University of technologies and management (the First Cossack University), Moscow, Russia

[7]Astrakhan State Technical University, Astrakhan, Russia, grozesku@yandex.ru ✉

Abstract. Based on the internal study results there has been examined the effect of three feed additives of different functional status (butyric acid, organomineral chelate compounds, lycopene) on occurrence of micronuclei (MN) and other nuclear anomalies (NA) in *Danio rerio* erythrocytes. Evaluation of the genotoxicity of butyric acid didn't show any genotoxic effect throughout the experiment. The highest frequency of MN occurrence was recorded on the 5th day of the experiment at a concentration of 1 mg/l and amounted to 0.28%. When using organomineral chelate compounds in feed composition, it was also not found that the threshold values for the occurrence of MN (5/1 000 cells) were exceeded. Lycopene showed the pronounced antigenotoxic properties expressed in a decrease in the occurrence of MN and NA up to the control values, which is significantly lower than in testing other feed additives. The data obtained helped to find out that when using all the studied feed additives on the 5th day of the experiment the frequency of occurrence of NA significantly increased and then decreased to the control values. This effect may be explained by three factors: adaptation of fish to a new diet; increased erythropoiesis and greater number of erythroblasts in the peripheral blood; high affinity of the chelating agent with trace element ions. The biosafety tests of three feed additives on the *Danio rerio* model object showed the absence of a genotoxic effect in the entire range of concentrations studied. Consequently, these functional additives can be recommended for including into food products.

Keywords: *Danio rerio,* genotoxicity, feed additives, micronucleus test

For citation: Kochetkov N. I., Smorodinskaya S. V., Nikiforov-Nikishin D. L., Klimov V. A., Golovacheva N. A., Nikiforov-Nikishin A. L., Grozesku Yu. N**.** Evaluating possible genotoxicity of three feed additives recommended for aquaculture by using micronucleus test on *Danio rerio* erythrocytes. *Vestnik of Astrakhan State Technical University. Series: Fishing Industry*. 2022;3:48-59. (In Russ.). https://doi.org/10.24143/2073-5529-2022-3-48-59. EDN BSCKRL.

Научная статья

Оценка возможной генотоксичности трех кормовых добавок, рекомендуемых для аквакультуры, методом микроядерного теста на эритроцитах *Danio rerio*

Н. И. Кочетков[1], С. В. Смородинская[2], Д. Л. Никифоров-Никишин[3], В. А. Климов[4], Н. А. Головачева[5], А. Л. Никифоров-Никишин[6], Ю. Н. Грозеску[7]✉

[1–6]Московский государственный университет технологий и управления имени К. Г. Разумовского (Первый казачий университет), Москва, Россия

[7]Астраханский государственный технический университет, Астрахань, Россия, grozesku@yandex.ru✉

Аннотация. По результатам собственного исследования изучено влияние трех кормовых добавок различного функционального статуса (масляная кислота, органоминеральные хелатные соединения, ликопин) на возникновение микроядер (МЯ) и других ядерных аномалий (ЯА) в эритроцитах крови *Danio rerio*. Оценка генотоксичности масляной кислоты показала отсутствие генотоксического эффекта на протяжении всего эксперимента. Наибольшая частота встречаемости МЯ была зафиксирована на 5 день опыта в концентрации 1 мг/л и составила 0,28 %. При применении в составе кормов органоминеральных хелатных соединений также не было установлено превышения пороговых значений встречаемости МЯ (5/1 000 клеток). Ликопин проявил выраженные антигенотоксические свойства, которые выражались в снижении частоты встречаемости МЯ и ЯА до контрольных значений, что значительно ниже, чем при испытании других кормовых добавок. Полученные данные позволили установить, что при использовании всех исследуемых кормовых добавок на 5 сутки опыта значительно увеличивалась частота встречаемости ЯА, которая затем снижалась до значений контроля. Данный эффект может быть следствием трех факторов: адаптацией рыб к новому кормовому рациону; увеличением скорости эритропоэза и количества эритробластов в периферической крови; высоким сродством хелатообразующего агента с ионами микроэлементов. Проведенные испытания биобезопасности трех кормовых добавок на модельном объекте *Danio rerio* показали отсутствие генотоксического эффекта во всем исследуемом спектре концентраций. Таким образом, данные функциональные добавки могут быть рекомендованы к включению в продукты питания.

Ключевые слова: *Danio rerio*, генотоксичность, кормовые добавки, микроядерный тест

Для цитирования: *Кочетков Н. И., Смородинская С. В., Никифоров-Никишин Д. Л., Климов В. А., Головачева Н. А., Никифоров-Никишин А. Л., Грозеску Ю. Н.* Оценка возможной генотоксичности трех кормовых добавок, рекомендуемых для аквакультуры, методом микроядерного теста на эритроцитах *Danio rerio* // Вестник Астраханского государственного технического университета. Серия: Рыбное хозяйство. 2022. № 3. С. 48–59. https://doi.org/10.24143/2073-5529-2022-3-48-59. EDN BSCKRL.

Introduction

Functional additives used in aquaculture usually are not tested for biosafety because it is believed that their properties can be determined from their chemical composition or by comparison with similar substances [1]. This formal evaluation cannot provide a guaranteed safety, and therefore additional testing of possible toxic properties is required for feed additives.

The Ames test is a standard technique for evaluating genotoxicity, when genotoxic properties are determined based on the reverse mutation of *Salmonella typhimurium*. The disadvantage of this method is an early low sensitivity and a high percentage of false positive results [2]. An alternative way to assess genotoxicity is the comet assay, which requires sophisticated analytical equipment [3].

In such conditions it is possible to use a micronucleus test based on the estimation of the frequency of nuclear anomalies. The micronucleus test can be performed *in vivo*, on living objects, and *in vitro*, on cell cultures. For example, the OECD chemical testing guidelines have methods for the micronucleus test on mammalian red blood cells [4] and *in vitro* on mammalian cells [5], in which the recommended organism is a laboratory mouse. Other model systems are used for rapid toxicity and ecotoxicological studies, in particular bony fish species [6].

Danio rerio is a common model system for toxicological and ecotoxicological studies. This fish species has several advantages, in particular: small size, ease of maintenance, growth and maturation rate, established transgenetic methods, as well as the presence of orthologous genes with humans [7, 8]. Thus, the use of *Danio rerio* to evaluate the genotoxicity of feed additives will allow the interpretation of the results on humans.

At the moment, there is a need for the inclusion of additional components in food and feed for animals that contribute to improving their functional properties [9]. Among the groups of such additives of the greatest interest are: antioxidants [10], pre/probiotic preparations [11] and micronutrient and vitamin complexes. In this paper, the possible genotoxicity of the following

additives was considered: (i) butyric acid, which is an alternative to probiotics, has positive effects on the intestinal microflora and digestive health of fish [12]; (ii) organomineral chelated micronutrient compounds, which are a source of micronutrients with greater bioavailability than inorganic salts [13, 14]; (iii) lycopene, which is a promising antioxidant with proven multiple properties [15].

Thus, the *aim of this work* is to evaluate the possible genotoxic properties of three promising functional feed additives (butyric acid, organomineral chelated compounds of microelements and lycopene) by micronucleus test on *Danio rerio*.

Materials and Methods

Object of Research. Aged 3 months *Danio rerio* were kept in 20 L aquariums at 20-24°C and pH 7.2-7.4 with natural light conditions (l/n 12 h), 25 individuals in each aquarium, according to the standard housing protocols [16]. The fish were fed with Coppens vital (0.5-0.8 mm) commercial pelleted feed.

Individuals without visible injuries 2.03 ± 0.17 cm in size and weighing 0.26 ± 0.03 g were selected for the experiment. Each experimental group included 25 individuals in three replicates ($n = 25 \cdot 3$). Identical conditions were maintained in the experimental tanks as well as in the holding tanks.

Preparing experimental diets. Coppens vital 0.5-0.8 mm (Coppens, Netherlands) was used as a base feed. Dosages of the feed were determined on the basis of previous studies.

Butyric acid (BA) in concentrations (mg/kg): 0.5, 1.0, and 2.0 [17, 18] was introduced into the base feed by microencapsulation with sodium alginate. For this purpose, sodium alginate was dissolved in distilled water (0.5 mg/100 ml) and sprayed onto the feed pellets. Then, the described dosages of BA were applied to the sprayed feed.

Organomineral chelated micronutrients (Ch) (Jupiter LLC, Russia) with the following composition (g/l): Fe – 10; Mn – 15; Zn – 35; Se – 0.3; I – 1.1; Cu – 3. This composition was formed based on a number of publications describing the micronutrient requirements of vertebrate animals [19-21]. The chelating agent for all the elements was ethyl diamindianthic acid (EDDS). The chelated compounds were applied to the feed using the same method as the butyric acid at the following dosages (mg/kg): 0.5, 1.0, and 2.0.

Lycopene extract (Lic), as a fat-soluble compound, was dissolved in 20 ml of corn oil. The following concentrations of lycopene (g/kg) were used in the experiment: 25, 50, 75 [22, 23]. The resulting solution was evenly incorporated into the feed.

The experimental feed was dried at 40°C to the original moisture content and stored at 4°C. The composition of the experimental feed is shown in Table 1.

Table 1

Composition of experimental feeds

Ingredients	Con	Ch05	Ch1	Ch2	BA0.5	BA1	BA2	Lic25	Lic50	Lic75
Crude protein, %	42	42	42	42	42	42	42	42	42	42
Cride Fat, %	13	13	13	13	13	13	13	13	13	13
Crude Fibre, %	2.39	2.39	2.39	2.39	2.39	2.39	2.39	2.39	2.39	2.39
Crude Ash, %	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3
Phosphorus, %	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85
Calcium, %	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
Sodium, %	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin A, IU/kg	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000
Vitamin D3, IU/kg	2 274	2 274	2 274	2 274	2 274	2 274	2 274	2 274	2 274	2 274
Propulgallate, mg/kg	53	53	53	53	53	53	53	53	53	53
Butylated hydroxyanisole, mg/kg	53	53	53	53	53	53	53	53	53	53
Chelate trace elements, mg/kg	–	0.5	1.0	2.0	–	–	–	–	–	–
Butyric acid, g/kg	–	–	–	–	0.5	1.0	2.0	–	–	–
Lycopene, mg/kg	–	–	–	–	–	–	–	25	50	75

Micronucleus test. Before blood sampling, fish were sedated in MS-222 solution (1 mg/L), after which blood was drawn from the caudal vein. The micronucleus test was performed on days 5, 10, and 15 of the experiment according to the approved technique. On these dates, 3 individuals ($n = 3 \cdot 10$) of the same size and without visible lesions were randomly selected from each study group.

Blood smears were prepared according to the standard technique [24]. The blood sample was air-dried for 2-3 min, then fixed in Nikiforov mixture (1 : 1, methyl alcohol: diethyl ether) for 20 min and dried. Next, azure-eosin staining with Romanowsky-Giemsa was performed. For this purpose, 9 ml of dye was added to 190 ml of buffer solution (pH 7.0), after which the preparations were stained for 10 minutes. The obtained preparations were washed three times in containers with distilled water and dried for 30 minutes.

The frequency of micronuclei and nuclear anomalies in *Danio rerio* erythrocytes was determined by the methods [25, 26]. At least 2 500 blood cells were counted on each prepared blood slide and nuclear abnormalities were counted. ImageJ software (National Institutes of Health, USA) with a specially written script was used for automatic cell counting. The process of automatic cell counting is shown in Fig. 1.

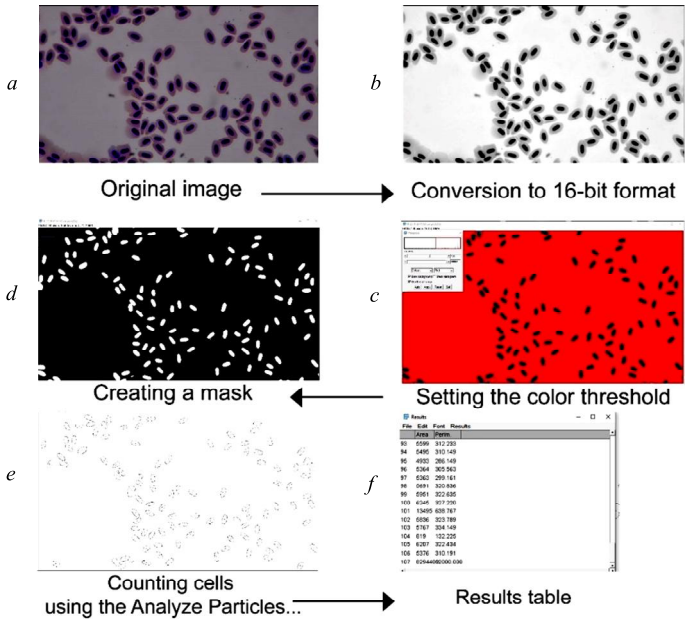

Fig. 1. Automatic blood cell count using the ImageJ program: *a* – initial photograph of erythrocytes at 400x magnification; *b* – image conversion to 16-bit format to highlight cell boundaries; *c* – setting color threshold with Image > Adjust > Threshold...; *d* – creating a two-color mask; *e* – cell counting with Analyze > Analyze Particles... (parameters: size 200-infinity; rounding 0.00-2.00); *f* – counting the final cell counts

After making, the smears were viewed under an Olympus BX53 light microscope (Olympus Corporation, Japan) with an ocular attachment Carl Zeiss ERc 5s (Zeiss, Germany) and ZEN lite software (Zeiss, Germany).

In addition to micronuclei (MN), the following nuclear anomalies were considered: notched nuclei (NN), lobbed nuclei (LN), and blebed nuclei (BN). Controlling for multiple nuclear anomalies increases the validity of testing. A number of distinguishing characteristics were used to reliably locate MN: same morphology of the nucleus and MN, small distance from the nucleus, size of 1/16 to 1/3 of the nucleus, minimal difference in color from the nucleus [27]. Examples of nuclear anomalies are shown in Fig. 2.

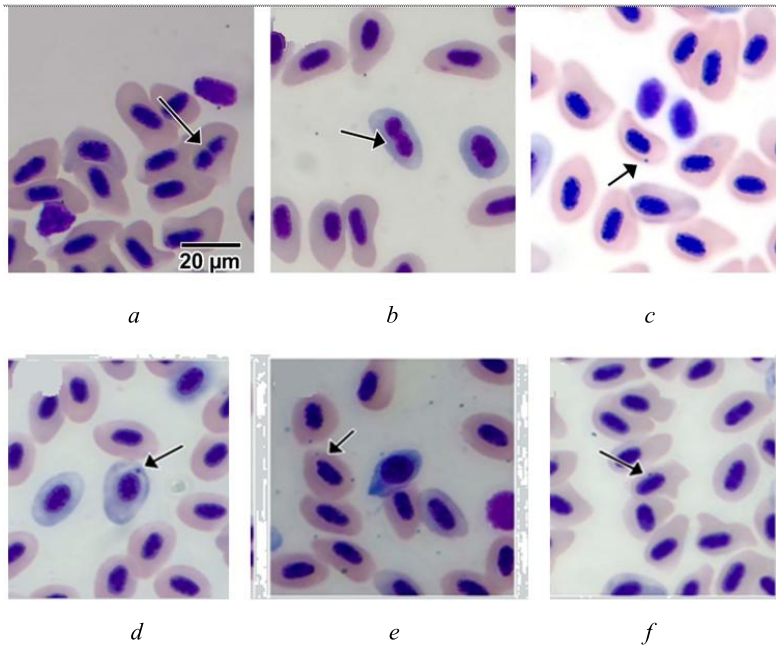

Fig. 2. Nuclear anomalies detected in different experiments. The arrows indicate cells with nuclear anomalies: *a*, *e* – lobbed nuclei (LN); *b*, *d* – micronuclei (MN); *c* – notched nuclei (NN); *f* – blebed nuclei (BN)

Positive control. Cr (VI) was obtained by dissolving potassium dichromate (chemical pure 99.7%; CAS 7778-50-9) in pure distilled water for each experimental group. Concentrations of 2.5 and 4 mg/L, traditionally used as a positive control in the micronucleus test [28, 29], were used in the experiment. The conditions of the experiment with potassium bichromate were repeated and conducted in parallel with the feed additive test.

In our work, the threshold value of MN occurrence, the excess of which is interpreted as a genotoxic effect for *Danio rerio*, was 0.5% per 1 000 blood cells. For other species of nuclear anomalies, no generally accepted threshold of genotoxicity was established, and their evaluation was performed by direct compared with control.

Statistical processing. Numerical data distribution normality was determined using the Shapiro-Wilk test. Numerical data were compared between different groups using a two-way ANOVA with Tukey's post hoc test. The level of significance was chosen as $P \leq 0.05$ and results are presented as mean ± SD (standard deviation). Data were statistically processed using GraphPad Prism version 9.0 software (GraphPad, San Diego, CA, USA).

Results

Positive control (potassium bichromate). When potassium bichromate (Dic) was used as a positive control, a genotoxic effect was observed on the fifth day of the experiment (Fig. 3, *a*).

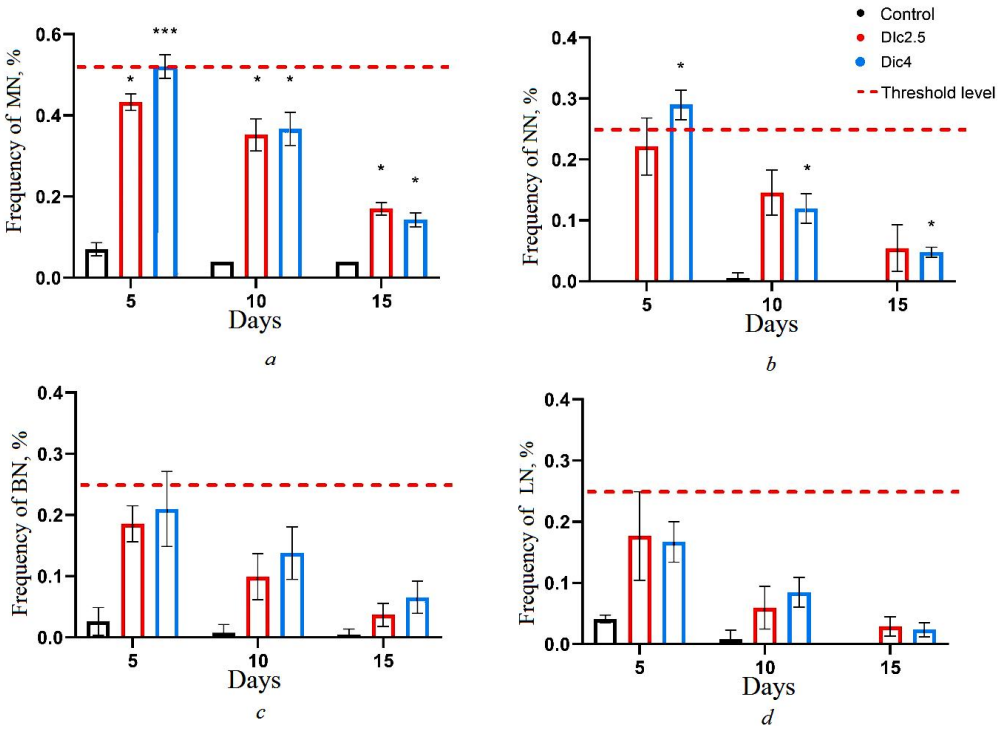

Fig. 3. Frequency of MN and various nuclear anomalies when using potassium bichromate in different concentrations: *a* – percentage of MN (red line – genotoxicity threshold); *b* – percentage of NN; *c* – percentage of BN; *d* – percentage of LN

There was also a reliable difference in nuclear anomalies of the "notched nucleus" type, on days 5, 10, and 15 of the experiment (Table 2).

Table 2

Numerical values of the occurrence of MN and various nuclear anomalies using potassium bichromate at different concentrations*

Days	Variants	MN	NN	LN	BN
5	Control	0.07 ± 0.013	0	0.025 ± 0.018	0
	Dic2.5	**0.432 ± 0.016****	0.217 ± 0.038	0.167 ± 0.059	0.184 ± 0.024
	Dic4	**0.519 ± 0.023*****	**0.288 ± 0.019****	0.164 ± 0.027	0.203 ± 0.05
10	Control	0.064 ± 0.031	0.015 ± 0.018	0.027 ± 0.14	0
	Dic2.5	**0.35 ± 0.032****	0.142 ± 0.03	0.051 ± 0.028	0.093 ± 0.03
	Dic4	**0.365 ± 0.033****	**0.117 ± 0.019****	0.082 ± 0.019	0.133 ± 0.035
15	Control	0.032 ± 0.023	0	0.014 ± 0.01	0.021 ± 0.002
	Dic2.5	**0.169 ± 0.012****	0.046 ± 0.031	0.024 ± 0.013	0.034 ± 0.015
	Dic4	**0.142 ± 0.014****	**0.047 ± 0.006****	0.021 ± 0.009	0.062 ± 0.021

*Values are presented as mean ± standard deviation. Significance values from controls were obtained using two-direction ANOVA test with Tukey's post hoc test: **$p < 0.01$; ***$p < 0.05$.

When evaluating other nuclear anomalies, no reliable differences were found.

Butyric acid. Record of MN and other nuclear anomalies showed that on day 5 of the experiment, the incidence of MN in all groups studied was no more than 0.23% (Fig. 4). The highest value was recorded in the group with butyric acid concentration of 1 mg/L and made 0.28%. This is the maximum recorded value throughout the experiment, nevertheless, it is well below the threshold of detectable genotoxicity.

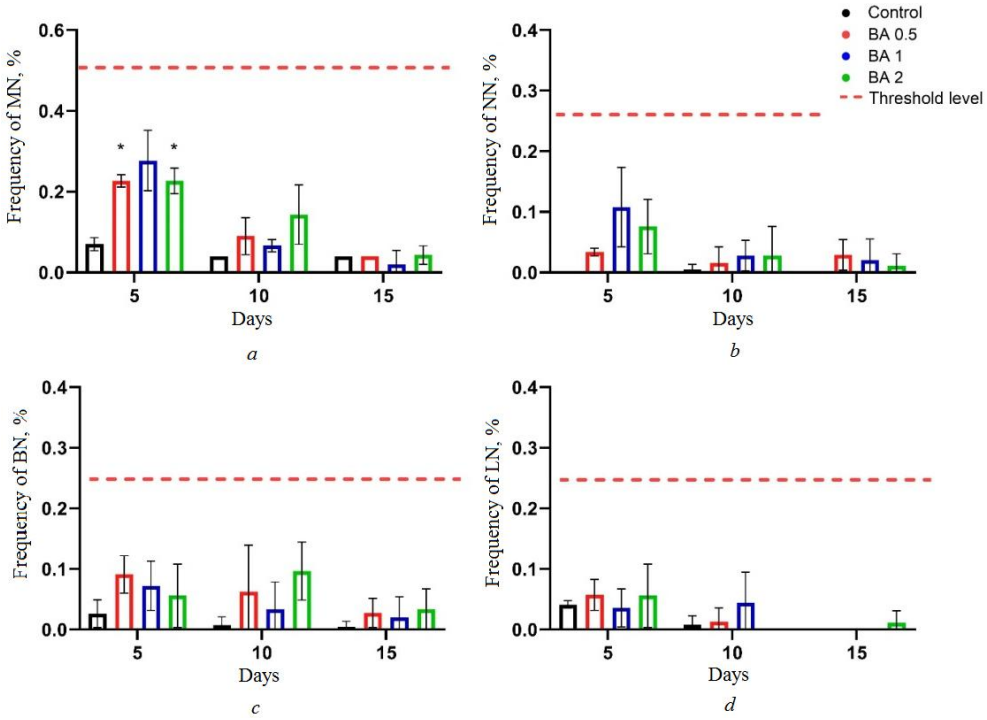

Fig. 4. Frequency of MN and various nuclear anomalies when using butyric acid in different concentrations:
a – percentage of MN (red line – genotoxicity threshold);
b – percentage of NN; *c* – percentage of BN; *d* – percentage of LN

On day 10 of the experiment, the number of MN at concentrations of 0.5 and 1 mg/l significantly decreased to values of 0.08 and 0.06%, respectively (Table 3).

Table 3

Numerical values of the occurrence of MN and various nuclear anomalies when using butyric acid in different concentration*

Days	Variants	MN	NN	LN	BN
5	Control	0.07 ± 0.013	0	0.025 ± 0.018	0
	BA0.5	0.232 ± 0.012	0.033 ± 0.005	0.057 ± 0.02	0.091 ± 0.091
	BA1	**0.28 ± 0.063****	**0.107 ± 0.053****	0.035 ± 0.025	0.072 ± 0.033
	BA2	**0.23 ± 0.028****	0.075 ± 0.036	0.055 ± 0.042	0.055 ± 0.42
10	Control	0.064 ± 0.031	0.015 ± 0.018	0.027 ± 0.14	0
	BA0.5	0.088 ± 0.037	0.015 ± 0.021	0.013 ± 0.018	0.062 ± 0.062
	BA1	0.067 ± 0.014	0.027 ± 0.02	0.044 ± 0.04	0.033 ± 0.036
	BA2	0.14 ± 0.059	0.027 ± 0.039	0	0.096 ± 0.039
15	Control	0.032 ± 0.023	0	0.014 ± 0.01	0.021 ± 0.002
	BA0.5	0.042 ± 0.002	0.029 ± 0.02	0	0.027 ± 0.019
	BA1	0.02 ± 0.028	0.02 ± 0.028	0	0.019 ± 0.028
	BA2	0.015 ± 0.045	0.011 ± 0.016	0.011 ± 0.016	0.033 ± 0.027

*Values are presented as mean ± standard deviation. Significance values from controls were obtained using two-direction ANOVA test with Tukey's post hoc test: ** $p < 0.05$.

At the same time, in the group of fish fed with butyric acid added at a concentration of 2 mg/l, the number of MN was 0.14%.

On the last day of the experiment, there was also a negative dynamics in the number of MN; in all studied groups their values did not exceed 0.4%. The obtained data on the number of MN did not differ signifi-

cantly from the reference values of the control, which suggests the absence of genotoxicity of the studied substance in the given concentrations.

Over the entire period of the experiment, the number of other nuclear anomalies did not exceed the control values (Fig. 4, *b*, *c*, *d*)). Just like the number of MN, the percentage of nuclear anomalies decreased with the course of the experiment. Thus, on the last day of the experiments, their number did not exceed 0.02%.

Organomineral chelate compounds of trace elements. Record of MN and nuclear anomalies showed the following results (Fig. 5, Table 4).

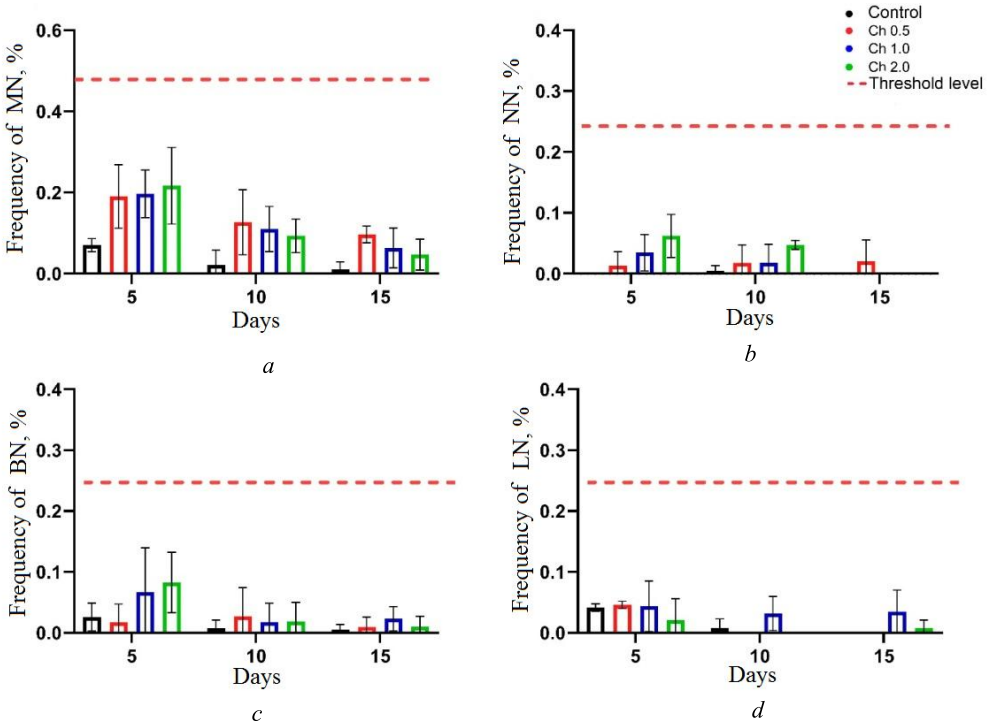

Fig. 5. Frequency of MNlei and various nuclear anomalies when using an organomineral chelated trace element compound at different concentrations: *a* – percentage of MN (red line – genotoxicity threshold); *b* – percentage of NN; *c* – percentage of BN; *d* – percentage of LN

Table 4

Numerical values of the occurrence of MN and various nuclear anomalies when using organomineral chelate compounds of trace elements in different concentrations*

Days	Variants	MN	NN	LN	BN
5	Control	0.07 ± 0.013	0	0.025 ± 0.018	0
	CH0.5	0.133 ± 0.023	0.013 ± 0.018	0.045 ± 0.0004	0.017 ± 0.017
	CH1	0.177 ± 0.047	0.034 ± 0.024	0.043 ± 0.033	0.066 ± 0.059
	CH2	0.195 ± 0.07	0.062 ± 0.028	0.02 ± 0.028	0.082 ± 0.04
10	Control	0.064 ± 0.031	0.015 ± 0.018	0.027 ± 0.14	0
	CH0.5	0.127 ± 0.064	0.017 ± 0.024	0	0.027 ± 0.038
	CH1	0.111 ± 0.043	0.017 ± 0.025	0.031 ± 0.022	0.017 ± 0.025
	CH2	0.091 ± 0.036	**0.047 ± 0.006****	0	0.018 ± 0.025
15	Control	0.032 ± 0.023	0	0.014 ± 0.01	0.021 ± 0.002
	CH0.5	**0.096 ± 0.017****	0.02 ± 0.028	0	0.009 ± 0.013
	CH1	0.061 ± 0.037	0	0.034 ± 0.028	0.023 ± 0.016
	CH2	0.048 ± 0.029	0	0.007 ± 0.01	0.009 ± 0.014

*Values are presented as mean ± standard deviation. Significance values from controls were obtained using two-direction ANOVA test with Tukey's post hoc test; **$p < 0.05$.

The maximum number of MN was observed with chelated trace element compounds on day 5 of the experiment, at a concentration of 2 mg/kg and reached 0.19% (1 000 cells). At a concentration of 1 mg/kg and 0.5 mg/kg, the number of MN was 0.17% and 0.13%, respectively. Significant differences were observed in the number of MN at day 15 at a concentration of 0.5 mg/kg; attached nuclei at day 10 at a concentration of 2.0 mg/kg ($p < 0.05$). These values are lower than the genotoxicity threshold determined as 0.5% (or 5 MN per 1,000 cells).

All types of nuclear anomalies occurred in all experimental groups. Their frequency did not exceed 0.06%, which is also a sign of the absence of genotoxicity. Numerical values of anomalies and MN are shown in Fig. 5 (*b*, *c*, *d*).

On day 10 of the experiment, the number of MN decreased significantly compared to the beginning of the experiment. Thus, in all groups under study, the number of MN did not rise above 0.12%. The distribution of nuclear anomalies had a random character and was not observed in all replications, remaining at a low level, not significantly different from the control.

On day 15 of the experiment, there was a further negative dynamic in the number of MN. At the concentration of 0.5 mg/kg the number of MN was 0.09%, which was the highest value among all concentrations for this period of time. The dynamics of the number of MN and nuclear anomalies in the CH study is shown in Fig. 5 and Table 4.

The studies suggest that the investigated concentrations of organomineral chelate compounds of trace elements are not genotoxic.

Lycopene. The study of the genotoxic properties of lycopene showed that this substance in the investigated concentrations presumably possesses I antigenotoxic effect. If chelated compounds and fatty acids on day 5 of the experiment showed an increased level of MN compared to the control, when lycopene was used, its level did not exceed 0.14%. On days 10 and 15 of the experiment, the number of MN in *Danio rerio* blood erythrocytes decreased to values not different from those of the control in all concentrations studied (Fig. 6 and Table 5).

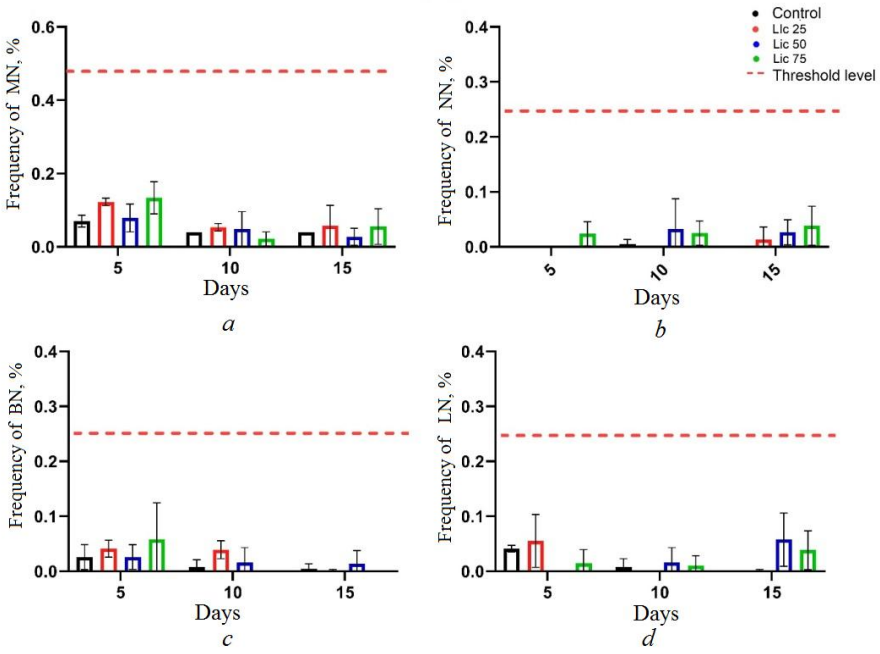

Fig. 6. Frequency of MN and various nuclear anomalies when using lycopene at different concentrations:
a – percentage of MN (red line – genotoxicity threshold); *b* – percentage of NN;
c – percentage of BN; *d* – percentage of LN

Table 5

Numerical values of the occurrence of MN and various nuclear anomalies using potassium bichromate at different concentrations*

Days	Variants	MN	NN	LN	BN
25	Control	0.07 ± 0.013	0	0.025 ± 0.018	0
	LIC25	**0.122 ± 0.008****	0	0.055 ± 0.039	0.041 ± 0.012
	LIC50	0.078 ± 0.031	0	**0****	0.026 ± 0.018
	LIC75	0.133 ± 0.035	0.023 ± 0.018	0.014 ± 0.02	0.05 ± 0.054
50	Control	0.064 ± 0.031	0.015 ± 0.018	0.027 ± 0.14	0
	LIC25	0.053 ± 0.008	0	0	0.039 ± 0.013
	LIC50	0.048 ± 0.039	0.032 ± 0.045	0.015 ± 0.022	0.015 ± 0.022
	LIC75	0.021 ± 0.015	0.024 ± 0.018	0.01 ± 0.014	0
75	Control	0.032 ± 0.023	0	0.014 ± 0.01	0.021 ± 0.002
	LIC25	0.057 ± 0.045	0.013 ± 0.018	0.001 ± 0.001	0.001 ± 0.001
	LIC50	0.0270 ± 0.019	0.026 ± 0.018	0.057 ± 0.039	0.013 ± 0.019
	LIC75	0.055 ± 0.039	0.038 ± 0.029	0.038 ± 0.029	0

*Values are presented as mean ± standard deviation. Significance values from controls were obtained using two-direction ANOVA test with Tukey's post hoc test: **$p < 0.05$.

The measured number of nuclear anomalies was also lower than the values obtained with the other feed additives studied. Despite this, nuclear anomalies were present throughout the experiment and their number and distribution by type was random. Anomalies of the BN type were noted most frequently on blood preparations, the number of which on average reached 0.057% at the concentration of 50 mg/kg on day 5 of the experiment, which is also a very low value.

Discussion

The studies showed the absence of genotoxic effect of the applied feed additives on *Danio rerio* blood erythrocytes in the whole concentration range. As a result of long-term use of the supplements under consideration, a slight increase in the number of MN and nuclei anomalies on day 5 of the experiment and their further decrease on days 10 and 15 were recorded. The mechanism of MN reduction is probably based on the following reactions: natural cycles of erythropoiesis in *Danio rerio*; adaptation to the new composition of the feed mixture, which can take up to 10 days.

In the course of work to establish the genotoxicity of butyric acid there was shown the absence of a genotoxic effect on all the studied indicators, including MN and nuclear anomalies. The negative dynamics of the number of MN, also recorded in the study of organomineral chelate compounds of trace elements, is probably a consequence of the adaptation of the organism to the new composition of feed. It is worth noting that butyric acid is an energy substrate and can be included in energy metabolism without additional catabolic reactions. Earlier studies [30] on the inclusion of butyric acid in the diet of animals, including fish, showed a significant change in blood biochemical parameters, as well as increased glycogen deposition in the liver. This suggests that butyric acid can cause some acceleration of erythropoiesis processes by direct incorporation into metabolic pathways. However, this hypothesis requires additional verification by molecular biology methods.

Comparing the results obtained on the number of MN when adding butter acid and organomineral chelated micronutrient compounds to the feed, we can conclude that butter acid has a greater effect on the formation of nuclear abnormalities. The use of these feed additives, based on the results of the studies obtained, can help to reduce oxidative stress, which may have a positive effect on the incidence of genetic abnormalities.

Since low concentrations of chelate compounds were used in the study, the absence of their genotoxic effect during the entire experiment was shown. The maximum values of the number of MN and nuclear abnormalities recorded on day 5 of the experiment are probably the result of adaptation of fish to the conditions of maintenance and food ration. It is also likely that as a result of micronutrient deficiency, more red blood cells are released into the bloodstream because the chelate supplement includes iron compounds in a convenient form for assimilation. After adaptation and habituation to the feed, on the 10th day of the experiment, this effect decreases, and the negative dynamics of the number of MN continues until the end of the experiment. It should be noted that the number of MN as well as other nuclear anomalies did not approach the values recorded in individuals when genotoxic compounds were used.

The antigenotoxic effect demonstrated by lycopene in all concentrations studied is probably related to its antioxidant properties. Since lycopene has the property of accumulating in tissues and being released into the blood [15], the negative dynamics of MN throughout the chronic experiment is evident. The presence of other abnormalities of the erythrocyte nucleus shape may be related to other DNA repair mechanisms unrelated to oxidative stress. For these reasons, the decrease in the number of NA may not occur as intensively in comparison with MN.

In general, the results of this experiment demonstrate significant prospects for the use of lycopene in aquaculture feeds, since it not only does not show genotoxic properties, but, on the contrary, has an antigenotoxic effect.

Conclusions

Testing the genotoxic properties of feed additives is an important step in biosafety evaluation, because without such measures their use carries significant risks. *Danio rerio* is a suitable model for the evaluation of genotoxic effects. Butyric acid has no genotoxicity in the entire range of concentrations studied, even under the conditions of a long experiment (15 days). The maximum recorded value of micronuclei (0.28%) was well below the genotoxicity thresholds. Organomineral chelated metal compounds based on EDDS have no genotoxic effect at concentrations from 0.5 to 2 mg/kg. Lycopene showed pronounced antigenotoxic properties, which makes it the most promising component of aquaculture. Its properties are probably based on its high antioxidant activity preventing the formation of reactive oxygen species.

References

1. European Food Safety Authority (EFSA). Technical Guidance for assessing the safety of feed additives for the environment. *EFSA Journal*, 2008, no. 6 (10), p. 842.

2. Hui Yang, Xiaopeng Zhang, Haibo Liu, Wenming Cui, Qiannan Zhang, Yongning Li, Zhou Yu, Xudong Jia. Lanthanum nitrate genotoxicity evaluation: Ames test, mouse micronucleus assay, and chromosome aberration test. *Muta-*

tion Research. Genetic Toxicology and Environmental Mutagenesis, 2016, vol. 810, pp. 1-5.

3. Sasaki Y. F., Kawaguchi S., Kamaya A., Ohshita M., Kabasawa K., Iwama K., Taniguchi K., Tsuda Sh. The comet assay with 8 mouse organs: results with 39 currently used food additives. *Mutation Research. Genetic Toxicology and Environmental Mutagenesis*, 2002, vol. 519, no. 1-2, pp. 103-119.

4. *OECD, 2016a. OECD Guidelines for the Testing of Chemicals. Test No. 474: Mammalian Erythrocyte Micronucleus Test. Organization for Economic Cooperation and Development, Paris, France*. Available at: https://www.oecd.org/env/test-no-474-mammalian-erythrocyte-micronucleus-test-9789264264762-en.htm (accessed: 06.06.2022).

5. *OECD, 2016b. OECD Guidelines for the Testing of Chemicals. Test No. 487. In: Vitro Mammalian Cell Micronucleus Test. Organization for Economic Cooperation and Development, Paris, France*. Available at: https://www.oecd.org/env/ehs/testing/test-no-487-in-vitro-mammalian-cell-micronucleus-test-9789264264861-en.htm (accessed: 06.06.2022).

6. Canedo A., de Jesus L. W. O., Cardoso Bailão E. F. L., Rocha Th. L. Micronucleus test and nuclear abnormality assay in zebrafish (*Danio rerio*): Past, present, and future trends. *Environmental Pollution*, 2021, vol. 290, p. 118019.

7. Canedo A., Rocha Th. L. Zebrafish (*Danio rerio*) using as model for genotoxicity and DNA repair assessments: Historical review, current status and trends. *Science of The Total Environment*, 2021, vol. 762, p. 144084.

8. Zub A. V., Zagrebin V. L., Dvoriashina I. A., Terent'ev A. V. Vozmozhnost' ispol'zovaniia biologicheskoi modeli presnovodnoi ryby *Danio rerio* v doklinicheskikh issledovaniiakh [Possibility of using biological model of freshwater fish *Danio rerio* in preclinical studies]. *Vestnik Volgogradskogo gosudarstvennogo meditsinskogo universiteta*, 2020, no. 1 (73), pp. 10-13.

9. Day L., Seymour R., Pitts K., Konczak I., Lundin L. Incorporation of functional ingredients into foods. *Trends in Food Science & Technology*, 2009, vol. 20, no. 9, pp. 388-395.

10. Simakov Iu. G., Nikitin I. A., Ivanov S. A., Shterman V. S., Shterman S. V., Sidorenko M. Iu., Sidorenko Iu. I. Izuchenie toksikologicheskikh kharakteristik rastitel'nykh ekstraktov dlia ispol'zovaniia v produktakh sportivnogo pitaniia [Studying toxicological characteristics of plant extracts for using in sports nutrition products]. *Pishchevaia promyshlennost'*, 2021, no. 11, pp. 74-79. DOI: 10.52653/PPI.2021.11.11.009.

11. Loiko N. G., Karetkin B. A., Simon N. A., Shanenko E. F., Pichugiga T. V., Grinevich A. I., Ganina V. I. Probioticheskie produkty. Novye priemy povysheniia kachestva [Probiotic products. New techniques for improving quality]. *Molochnaia promyshlennost'*, 2013, no. 10, pp. 57-59.

12. Mingbao Zhang, Yanan Wang, Xianqi Zhao, Chang Liu, Baozhen Wang, Jun Zhou. Mechanistic basis and preliminary practice of butyric acid and butyrate sodium to mitigate gut inflammatory diseases: a comprehensive review. *Nutrition Research*, 2021, vol. 95, pp. 1-18.

13. Gharibzahedi S. M. T., Jafari S. M. The importance of minerals in human nutrition: Bioavailability, food fortification, processing effects and nanoencapsulation. *Trends in Food Science & Technology*, 2017, vol. 62, pp. 119-132.

14. Walters M. E., Esfandi R., Tsopmo A. Potential of food hydrolyzed proteins and peptides to chelate iron or calcium and enhance their absorption. *Foods*, 2018, vol. 7, no. 10, p. 172.

15. Arballo J., Amengual J., Erdman J. W. Lycopene: A critical review of digestion, absorption, metabolism, and excretion. *Antioxidants*, 2021, vol. 10, no. 3, p. 342.

16. Aleström P., D'Angelo L., Midtlyng P. J., Schorderet D. F., Schulte-Merker S., Sohm F., Warner S. Zebrafish: Housing and husbandry recommendations. *Laboratory animals*, 2020, vol. 54, no. 3, pp. 213-224.

17. Abdel-Latif H. M. R., Abdel Tawwab M., Dawood M., Menanteau-Ledouble S., El-Matbouli M. Benefits of dietary butyric acid, sodium butyrate, and their protected forms in aquafeeds: a review. *Reviews in Fisheries Science & Aquaculture*, 2020, vol. 28, no. 4, pp. 421-448.

18. Aalamifar H., Soltanian S., Vazirzadeh A., Akhlaghi M., Morshedi V., Gholamhosseini A., Mozanzadeh M. T. Dietary butyric acid improved growth, digestive enzyme activities and humoral immune parameters in Barramundi (*Lates calcarifer*). *Aquaculture Nutrition*, 2020, vol. 26, no. 1, pp. 156-164.

19. Gramm S. Y., Beketov S. V., Kochetkov N. I., Klimov V. A., Nikifirov-Nikishin A. L., Nikifirov-Nikishin D. L. Histological changes in the liver, intestines and kidneys of *Clarias gariepinus* when using feed with chelated compounds. *International Journal of Pharmaceutical Research*, 2020, vol. 12, no. 3, pp. 2380-2391.

20. Nikiforov-Nikishin D. L., Kochetkov N. I., Smorodinskaya S. V., Tatarenko P. Yu., Matveeva D. M. Toxicity of metal chelates mixture in aquatic environment at *Danio rerio*. *IOP Conference Series: Earth and Environmental Science*, 2021, vol. 839, no. 5, p. 052010.

21. Mézes M. et al. Deposition of organic trace metal complexes as feed additives in farm animals. *Eur Chem Bull.*, 2012, vol. 1, no. 10, p. 410.

22. Dawood M. A. O., Abdel-Tawwab M., Abdel-Latif H. M. R. Lycopene reduces the impacts of aquatic environmental pollutants and physical stressors in fish. *Reviews in Aquaculture*, 2020, vol. 12, no. 4, pp. 2511-2526.

23. Langi P., Kiokias S., Varzakas Th., Proestos Ch. Carotenoids: From plants to food and feed industries. *Microbial carotenoids*, 2018, pp. 57-71.

24. Bolognesi C., Hayashi M. Micronucleus assay in aquatic animals. *Mutagenesis*, 2011, vol. 26, no. 1, pp. 205-213.

25. Brand M., Granato M., Nüsslein-Volhard C. Keeping and raising zebrafish. *Zebrafish: a practical approach*, 2002, pp. 7-37.

26. Bagdonas E., Vosylienė M. Z. A study of toxicity and genotoxicity of copper, zinc and their mixture to rainbow trout (*Oncorhynchus mykiss*). *Biologija*, 2006, vol. 52, no. 1, pp. 8-13.

27. Fenech M. Mechanisms by which genotoxins cause micronuclei and other nuclear anomalies. *The micronucleus assay in toxicology*, 2019, pp. 8-23.

28. de Lemos C. T., Rödel P. M., Terra N. R., Erdtmann B. Evaluation of basal micronucleus frequency and hexavalent chromium effects in fish erythrocytes. *Environmental Toxicology and Chemistry: An International Journal*, 2001, vol. 20, no. 6, pp. 1320-1324.

29. Normann C. A. B. M., Moreira J. C. F., Cardoso V. V. Micronuclei in red blood cells of armored catfish *Hypostomus plecotomus* exposed to potassium dichromate. *African Journal of Biotechnology*, 2008, vol. 7, no. 7, pp. 001-004.

30. Yang Liu, Zhichu Chen, Jihong Dai, Pei Yang, Weiqi Xu, Qinghui Ai, Wenbing Zhang, Yongan Zhang, Yanjiao Zhang, Kangsen Mai. Sodium butyrate supplementation in high-

soybean meal diets for turbot (*Scophthalmus maximus* L.): effects on inflammatory status, mucosal barriers and microbiota in the intestine. *Fish & shellfish immunology*, 2019, vol. 88, pp. 65-75.

Список источников

1. *European* Food Safety Authority (EFSA). Technical Guidance for assessing the safety of feed additives for the environment // EFSA Journal. 2008. N. 6 (10). P. 842.

2. *Hui Yang, Xiaopeng Zhang, Haibo Liu, Wenming Cui, Qiannan Zhang, Yongning Li, Zhou Yu, Xudong Jia*. Lanthanum nitrate genotoxicity evaluation: Ames test, mouse micronucleus assay, and chromosome aberration test // Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2016. V. 810. P. 1–5.

3. *Sasaki Y. F., Kawaguchi S., Kamaya A., Ohshita M., Kabasawa K., Iwama K., Taniguchi K., Tsuda Sh.* The comet assay with 8 mouse organs: results with 39 currently used food additives // Mutation Research. Genetic Toxicology and Environmental Mutagenesis. 2002. V. 519. N. 1-2. C. 103–119.

4. *OECD, 2016a.* OECD. OECD Guidelines for the Testing of Chemicals. Test No. 474: Mammalian Erythrocyte Micronucleus Test. Organization for Economic Cooperation and Development, Paris, France. URL: https://www.oecd.org/env/test-no-474-mammalian-erythrocyte-micronucleus-test-9789264264762-en.htm (дата обращения: 06.06.2022).

5. *OECD, 2016b*. OECD Guidelines for the Testing of Chemicals. Test No. 487. In: Vitro Mammalian Cell Micronucleus Test. Organization for Economic Cooperation and Development, Paris, France. URL: https://www.oecd.org/env/ehs/testing/test-no-487-in-vitro-mammalian-cell-micro nucleus-test-9789264264861-en.htm (дата обращения: 06.06.2022).

6. *Canedo A., de Jesus L. W. O., Cardoso Bailão E. F. L., Rocha Th. L.* Micronucleus test and nuclear abnormality assay in zebrafish (*Danio rerio*): Past, present, and future trends // Environmental Pollution. 2021. V. 290. P. 118019.

7. *Canedo A., Rocha Th. L.* Zebrafish (*Danio rerio*) using as model for genotoxicity and DNA repair assessments: Historical review, current status and trends // Science of The Total Environment. 2021. V. 762. P. 144084.

8. *Зуб А. В., Загребин В. Л., Дворяшина И. А., Терентьев А. В.* Возможность использования биологической модели пресноводной рыбы Данио рерио в доклинических исследованиях // Вестн. Волгоград. гос. мед. ун-та. 2020. № 1 (73). С. 10–13.

9. *Day L., Seymour R., Pitts K., Konczak I., Lundin L.* Incorporation of functional ingredients into foods // Trends in Food Science & Technology. 2009. V. 20. N. 9. P. 388–395.

10. *Симаков Ю. Г., Никитин И. А., Иванов С. А., Штерман В. С., Штерман С. В., Сидоренко М. Ю., Сидоренко Ю. И.* Изучение токсикологических характеристик растительных экстрактов для использования в продуктах спортивного питания // Пищевая промышленность. 2021. № 11. С. 74–79. DOI: 10.52653/PPI.2021.11.11.009.

11. *Лойко Н. Г., Кареткин Б. А., Симон Н. А., Шаненко Е. Ф., Пичугина Т. В., Гриневич А. И., Ганина В. И.* Пробиотические продукты. Новые приемы повышения качества // Молочная промышленность. 2013. № 10. С. 57–59.

12. *Mingbao Zhang, Yanan Wang, Xianqi Zhao, Chang Liu, Baozhen Wang, Jun Zhou*. Mechanistic basis and preliminary practice of butyric acid and butyrate sodium to mitigate gut inflammatory diseases: a comprehensive review // Nutrition Research. 2021. V. 95. P. 1–18.

13. *Gharibzahedi S. M. T., Jafari S. M.* The importance of minerals in human nutrition: Bioavailability, food fortification, processing effects and nanoencapsulation // Trends in Food Science & Technology. 2017. V. 62. P. 119–132.

14. *Walters M. E., Esfandi R., Tsopmo A*. Potential of food hydrolyzed proteins and peptides to chelate iron or calcium and enhance their absorption // Foods. 2018. V. 7. N. 10. P. 172.

15. *Arballo J., Amengual J., Erdman J. W.* Lycopene: A critical review of digestion, absorption, metabolism, and excretion // Antioxidants. 2021. V. 10. N. 3. P. 342.

16. *Alesträm P., D'Angelo L., Midtlyng P. J., Schorderet D. F., Schulte-Merker S., Sohm F., Warner S.* Zebrafish: Housing and husbandry recommendations // Laboratory animals. 2020. V. 54. N. 3. P. 213–224.

17. *Abdel-Latif H. M. R., Abdel Tawwab M., Dawood M., Menanteau-Ledouble S., El-Matbouli M.* Benefits of dietary butyric acid, sodium butyrate, and their protected forms in aquafeeds: a review // Reviews in Fisheries Science & Aquaculture. 2020. V. 28. N. 4. P. 421–448.

18. *Aalamifar H., Soltanian S., Vazirzadeh A., Akhlaghi M., Morshedi V., Gholamhosseini A., Mozanzadeh M. T*. Dietary butyric acid improved growth, digestive enzyme activities and humoral immune parameters in Barramundi (*Lates calcarifer*) // Aquaculture Nutrition. 2020. V. 26. N. 1. P. 156–164.

19. *Gramm S. Y., Beketov S. V., Kochetkov N. I., Klimov V. A., Nikifirov-Nikishin A. L., Nikifirov-Nikishin D. L.* Histological changes in the liver, intestines and kidneys of *Clarias gariepinus* when using feed with chelated compounds // International Journal of Pharmaceutical Research. 2020. V. 12. N. 3. P. 2380–2391.

20. *Nikiforov-Nikishin D. L., Kochetkov N. I., Smorodinskaya S. V., Tatarenko P. Yu., Matveeva D. M.* Toxicity of metal chelates mixture in aquatic environment at *Danio rerio* // IOP Conference Series: Earth and Environmental Science. 2021. V. 839. N. 5. P. 052010.

21. *Mézes M. et al.* Deposition of organic trace metal complexes as feed additives in farm animals // Eur Chem Bull. 2012. V. 1. N. 10. P. 410.

22. *Dawood M. A. O., Abdel-Tawwab M., Abdel-Latif H. M. R.* Lycopene reduces the impacts of aquatic environmental pollutants and physical stressors in fish // Reviews in Aquaculture. 2020. V. 12. N. 4. P. 2511–2526.

23. *Langi P., Kiokias S., Varzakas Th., Proestos Ch.* Carotenoids: From plants to food and feed industries // Microbial carotenoids. 2018. P. 57–71.

24. *Bolognesi C., Hayashi M.* Micronucleus assay in aquatic animals // Mutagenesis. 2011. V. 26. N. 1. P. 205–213.

25. *Brand M., Granato M., Nüsslein-Volhard C.* Keeping and raising zebrafish // Zebrafish: a practical approach. 2002. C. 7–37.

26. *Bagdonas E., Vosylienė M. Z.* A study of toxicity and genotoxicity of copper, zinc and their mixture to rainbow trout (*Oncorhynchus mykiss*) // Biologija. 2006. V. 52. N. 1. P. 8–13.

27. *Fenech M.* Mechanisms by which genotoxins cause micronuclei and other nuclear anomalies // The micronucleus assay in toxicology. 2019. P. 8–23.

28. *de Lemos C. T., Rödel P. M., Terra N. R., Erdtmann B.* Evaluation of basal micronucleus frequency and hexavalent chromium effects in fish erythrocytes // Environmental Toxicology and Chemistry: An International Journal. 2001. V. 20. N. 6. P. 1320–1324.

29. *Normann C. A. B. M., Moreira J. C. F., Cardoso V. V.* Micronuclei in red blood cells of armored catfish Hypostomus

plecotomus exposed to potassium dichromate // African Journal of Biotechnology. 2008. V. 7. N. 7. P. 001–004.

30. *Yang Liu, Zhichu Chen, Jihong Dai, Pei Yang, Weiqi Xu, Qinghui Ai, Wenbing Zhang, Yongan Zhang, Yanjiao Zhang, Kangsen Mai*. Sodium butyrate supplementation in high-soybean meal diets for turbot (*Scophthalmus maximus* L.): effects on inflammatory status, mucosal barriers and microbiota in the intestine // Fish & shellfish immunology. 2019. V. 88. P. 65–75.

The article is submitted 24.06.2022; approved after reviewing 26.07.2022; accepted for publication 20.09.2022
Статья поступила в редакцию 24.06.2022; одобрена после рецензирования 26.07.2022; принята к публикации 20.09.2022

Information about the authors / Информация об авторах

Nikita I. Kochetkov – Junior Researcher of the Center "Aquaculture"; K. G. Razumovsky Moscow State University of technologies and management (the First Cossack University); samatrixs@gmail.com

Svetlana V. Smorodinskaya – Laboratory Manager of the Center "Aquaculture"; K. G. Razumovsky Moscow State University of technologies and management (the First Cossack University); s.smorodinskaya@mgutm.ru

Dmitry L. Nikiforov-Nikishin – Candidate of Biology; Leading Researcher of the Center "Aquaculture"; K. G. Razumovsky Moscow State University of technologies and management (the First Cossack University); akvabiotex@rambler.ru

Victor A. Klimov – Researcher of the Center "Aquaculture"; K. G. Razumovsky Moscow State University of technologies and management (the First Cossack University); mirvar@rambler.ru

Natalia A. Golovacheva – Candidate of Veterinary Sciences; Assistant Professor of the Department of Biology and Ichthyology; K. G. Razumovsky Moscow State University of technologies and management (the First Cossack University); molekula00@inbox.ru

Alexei L. Nikiforov-Nikishin – Doctor of Biology, Professor; Decan Faculty of Biotechnology and Fisheries; K. G. Razumovsky Moscow State University of technologies and management (the First Cossack University); 9150699@mail.ru

Yulia N. Grozesku – Doctor of Agricultural Sciences, Assistant Professor; Professor of the Department of Aquaculture and Fisheries; Astrakhan State Technical University; grozesku@yandex.ru

Никита Ильич Кочетков – младший научный сотрудник центра «Аквакультура»; Московский государственный университет технологий и управления имени К. Г. Разумовского (Первый казачий университет); samatrixs@gmail.com

Светлана Валерьевна Смородинская – заведующий лабораторией центра «Аквакультура»; Московский государственный университет технологий и управления имени К. Г. Разумовского (Первый казачий университет); s.smorodinskaya@mgutm.ru

Дмитрий Львович Никифоров-Никишин – кандидат биологических наук; ведущий научный сотрудник центра «Аквакультура»; Московский государственный университет технологий и управления им. К. Г. Разумовского (Первый казачий университет); akvabiotex@rambler.ru

Виктор Александрович Климов – научный сотрудник центра «Аквакультура»; Московский государственный университет технологий и управления имени К. Г. Разумовского (Первый казачий университет); mirvar@rambler.ru

Наталья Алексеевна Головачева – кандидат ветеринарных наук; доцент кафедры биологии и ихтиологии; Московский государственный университет технологий и управления имени К. Г. Разумовского (Первый казачий университет); molekula00@inbox.ru

Алексей Львович Никифоров-Никишин – доктор биологических наук, профессор; декан факультета биотехнологий и рыбного хозяйства; Московский государственный университет технологий и управления имени К. Г. Разумовского (Первый казачий университет); 9150699@mail.ru

Юлия Николаевна Грозеску – доктор сельскохозяйственных наук, доцент; профессор кафедры аквакультуры и рыболовства; Астраханский государственный технический университет; grozesku@yandex.ru