Assessment of acute toxicity of the organomineral chelate complex for Danio rerio embryos

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Abstract. The work evaluates the acute toxicity of the organomineral chelate complex for *Danio rerio* embryos. To date, very few studies have been presented on the mineral component of diets in aquaculture. Currently, a number of preparations (supplements) containing microelements are used to correct mineral feeding in livestock farming. A number of studies have shown that the use of organomineral chelate complexes as a feed additive provides better assimilation of the metal than when it is introduced into the diet in inorganic or any other organic form. The domestic market does not have scientifically based organomineral chelate complexes for industrial aquaculture. The search for effective and non-toxic chelate complexes for aquaculture in the Russian Federation is an urgent task. Purpose of the work: to determine the median lethal concentration of the organomineral chelate complex on *Danio rerio* embryos. The acute toxicity of an organomineral chelate complex (JUPITER LLC, Russia) was determined. The active basis of the dietary supplement (hereinafter referred to as dietary supplement): a complex of ethylenediaminedisuccinic acid and lysine with microelements (Fe, Mn, Cu, Zn, Co, Se, I). The studies were carried out in accordance with OECD recommendations No. 236 on the basis of the Department of Biochemistry and Physiology of the St. Petersburg State University of Veterinary Medicine. Based on the study, the following conclusions can be drawn: the maximum concentration that does not cause death during testing is 0.25 g/l and the minimum concentration that causes 100% death during testing is 16 g/l. Based on the results of the survival of *Danio rerio* embryos, the median lethal concentration of the organomineral chelate complex was calculated. The LC50 of the study drug is 4.67 g/l.

1 Introduction

The active development of aquaculture in the Russian Federation is hampered by a number of reasons, the most important of which is the lack of competitive domestic feed [1]. The

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lack of balanced feeding leads to a decrease in the efficiency and profitability of fisheries enterprises [2]. To date, very few studies have been presented on the mineral component of diets in aquaculture [3]. It is also noteworthy that diets for feeding fish in individual biogeochemical provinces have not yet been developed. An excess or deficiency of elements in a particular province contributes to the development of a number of pathological conditions (initiates free radical oxidation, reduces productive qualities, and leads to the death of livestock) [4,5].

Currently, a number of preparations (supplements) containing microelements are used to correct mineral feeding in livestock farming. Among organic preparations, three main groups can be distinguished: salts of organic acids, organomineral chelate complexes and metal complexes with analogues of endogenous substances [6]. A number of studies have shown that the use of organomineral chelate complexes as a feed additive provides better assimilation of the metal than when it is introduced into the diet in inorganic or any other organic form [7-9]. This, in turn, helps to increase animal productivity and reduce feed consumption per unit of production [10].

Chelated complex compounds have the following advantages over inorganic salts: they do not cake during long-term storage; do not disturb the pH of the gastrointestinal tract; have lower competition between metals during absorption; have a higher efficiency of transport of metals through the walls of the gastrointestinal tract (hydroxypolymerization is prevented, neutral complexes pass through the charged mucosal layer more successfully, equally charged ions (for example, copper and zinc) do not compete for binding sites in the intestinal mucin); do not react with other feed elements, including phytates and polyphenols [11-12]. However, despite the considered positive aspects of the use of chelate complexes, today the domestic market does not have scientifically based organomineral chelate complexes for industrial aquaculture [13]. The search for effective and non-toxic chelate complexes for aquaculture in the Russian Federation is an urgent task.

Purpose of the work: to determine the median lethal concentration of the organomineral chelate complex on *Danio rerio* embryos

2 Materials and methods

2.1 Experimental animals

Danio rerio embryos (0-96 hours) were used in the experiment. Parental pairs (wild type) were obtained from the vivarium of aquatic organisms of the federal state budgetary educational institution “St. Petersburg State University of Veterinary Medicine”.

The animals were kept in aquariums with a light intensity of 300–330 lux; Photoperiod: 14 hours light/10 hours dark. The water temperature was maintained at 28 ± 0.5 °C. Adults were fed 3 times a day (dry Tetra Min Granules daily; shrimp (*Artemia sp.*) once every three days. The qualitative characteristics of the water used to keep the fish are presented in Table 1.

Table 1. Water quality parameters*.

Parameter	Characteristic
pH	7.1±0.3
Hardness, mg/l	79±4
Conductivity, μs	923±31
Dissolved oxygen concentration, mg/l	5.8±0.2

Note: * - data is given for the month (August 2023)

Parental pairs were placed in spawning tanks (Tecniplast, Italy) and left until eggs were obtained. After spawning, the eggs were sorted (unfertilized eggs were discarded) and washed with E3 medium and transplanted into 24-well plates (one egg in each well).

The overall fertilization rate was ≥91.3% in each batch tested, meeting the OECD validation criteria (≥70%).

2.2 Study drug

The acute toxicity of an organomineral chelate complex (Jupiter LLC, Russia) was determined. The active basis of the dietary supplement (hereinafter referred to as dietary supplement): a complex of ethylenediaminedisuccinic acid and lysine with microelements (Fe, Mn, Cu, Zn, Co, Se, I). The trace element composition of the chelate complex is presented in Table 2.

Table 2. Microelement composition of the studied organomineral chelate complex.

Microelement	Content g/l
Iron	33.1
Manganese	6.7
Copper	3.4
Zinc	18.6
Cobalt	0.76
Selenium	0.43
Iodine	1.01
Total number (sum) of elements	64

The chelate complex (multicomponent substance) was a dark brown solution, odorless, highly soluble in water and insoluble in organic solvents, pH of the dietary supplement solution was 6.3.

Quality control of the drug was carried out using the ion exchange chromatography method. Compliance of the drug with the declared parameters was 98%. Permissible deviations in the content of microelements, according to the manufacturer, should be no more than 5%.

The standard aqueous medium for *Danio rerio* E3 embryos (the medium is used when raising fry) was used as a solvent for the studied dietary supplement. The composition of the aqueous medium for embryos is presented in Table 3.

Table 4. Composition of the aqueous environment for embryos.

Substance	Content grams per liter
NaCl	0.292
KCl	0.013
CaCl	0.044
$MgSO_4$	0.081

15 concentrations of the studied dietary supplement were prepared, differing by a factor of 2.0 (64...0.0039 g/l). Working concentrations were prepared by diluting dietary supplements with E3 medium.

2.3 Experimental system

The studies were carried out according to OECD recommendations No. 236 [14]. Embryos were placed in standard transparent flat-bottomed 24-well polystyrene plates. The plates

were covered with transparent lids during testing to prevent evaporation. The studies were carried out under static conditions.

The eggs were distributed into the wells of the plates in the following quantities:

- 20 eggs per plate for each tested concentration;
- 20 eggs as a control for E3 medium per plate;
- 20 eggs as a positive control per plate (3,4-DCA4 mg/l);
- 4 eggs in E3 medium as an internal control in a plate for each concentration.

Plates with embryos were incubated in a thermostatic chamber (The Thermo Scientific, USA), in which a constant temperature (28 ± 0.1 °C) and photoperiod (14 hours light:10 hours dark, at 300 lux) were maintained.

According to the standards of OECD Test No. 236, after 96 hours, the hatching rate of the negative control should be ≥ 80%, the minimum mortality of the positive control should be 30%, and the survival and hatching rate of the solvent control should not be different from the negative control.

2.4 Testing

The studies were carried out at the Department of Biochemistry and Physiology of the St. Petersburg State University of Veterinary Medicine.

After spawning, the eggs were sorted (unfertilized eggs were discarded), washed (with E3 medium) and transplanted into the wells of 24-well plates (filled with 2.0 ml of the test drug at a given concentration). The studies were carried out in a static system. Every 24 hours (24...96 hours after fertilization) observations were made under a stereomicroscope (ZEISS SteREO Discovery.V12, Germany). The following pathologies were recorded: coagulation of fertilized eggs, lack of somite formation, non-separation of the tail, and lack of heartbeat. Observational data is used to determine death: any positive result in one of the observational data means that the embryo has died.

In accordance with OECD recommendations No. 236, the following toxicity indicators were determined during the experiments: the maximum concentration that does not cause death during the test; minimum concentration causing 100% mortality during the test; Cumulative mortality for each concentration during the recommended observation period; LC50 values at 96 hours with 95% confidence interval.

3 Results and Discussion

Studies were conducted on the dependence of embryo death (coagulation of eggs, absence of somites, heartbeat and tail separation) on the concentration of the drug under study. The results of the experiments (Table 4) showed that when embryos are exposed to solutions with concentrations of the organomineral chelate complex of 32-64 mg/l, coagulation of all fertilized eggs is observed (Figure 1). It should be noted that in some embryos, deformation of the chorion was observed in the first two hours after placing the embryo in the solution. When using 16 g/l, coagulation of 18 embryos was observed and the absence of somite formation was noted in 2 embryos (48 hours). At a concentration of 8 g/l, there was no formation of somites in 6 embryos, no heartbeat in 5 embryos (72 hours) and no separation of the tail bud in 1 embryo (Figure 2). Application of concentrations of 4 g/l, 2 g/l, 1 g/l and 0.5 g/l resulted in the death of 45%, 40%, 20% and 15% of embryos, respectively. When embryos were incubated in a medium with a chelate complex at a concentration of 0.25...0.0039 g/l, the survival rate was 100% (Figure 4).

Table 4. Survival of *Danio rerio* embryos during incubation with various concentrations of organomineral chelate complex.

Concentration, g/l	Coagulation of fertilized eggs, pieces	Lack of formation of somites, pieces	Absence of tail bud separation, pieces	Absence of heartbeat, pieces	Number of dead embryos, %
64	20	-	-	-	100
32	20		-	-	100
16	18	2	-	-	100
8	-	6	1	5	60
4	2	1	2	4	45
2	-	1	5	2	40
1	1	2	-	1	20
0.5	3	-	-	-	15
0.25	-	-	-	-	-
0.125	-	-	-	-	-
0.625	-	-	-	-	-
0.0312	-	-	-	-	-
0.0156	-	-	-	-	-
0.0078	-	-	-	-	-
0.0039	-	-	-	-	-

The maximum concentration that does not cause death during testing is determined to be 0.25 g/l and the minimum concentration that causes 100% death during testing is 16 g/l.

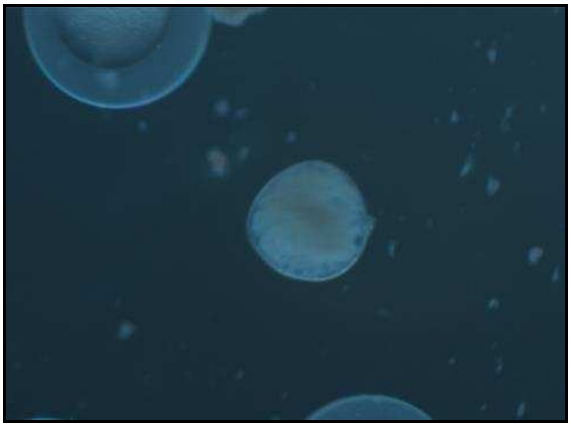

Fig.1. Coagulation of fertilized eggs.

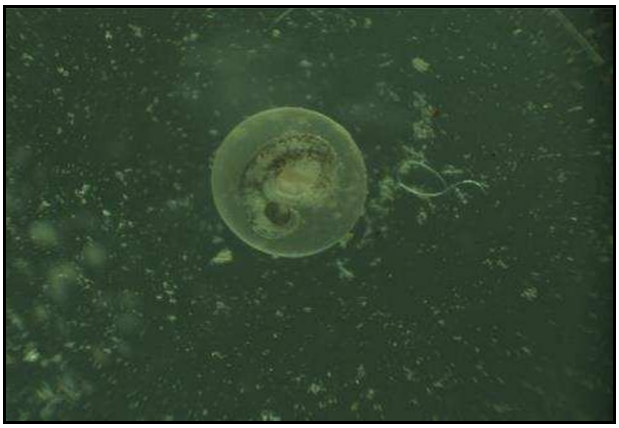

Fig. 2. No tail bud separation (48 hours).

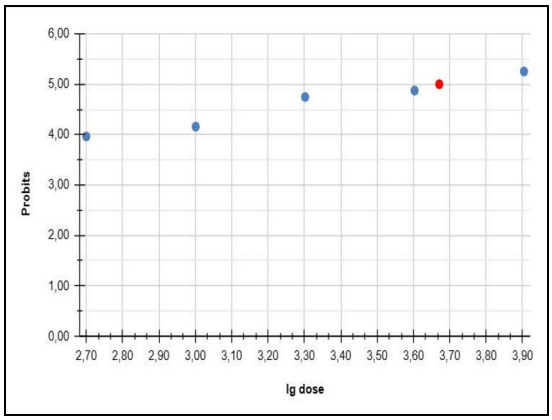

Fig. 3. LC_{50} by probit analysis.

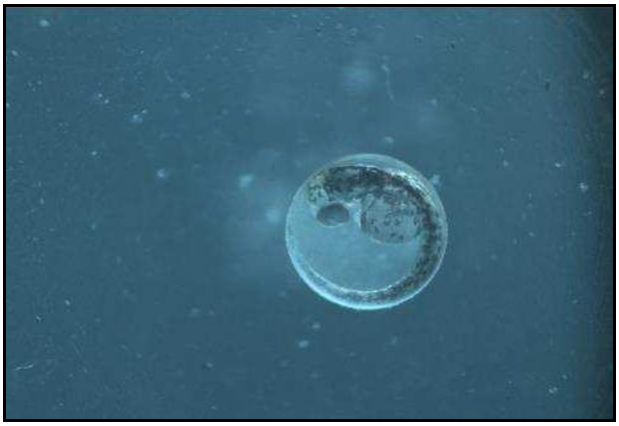

Fig. 4. Normal embryo development (48 hours).

Based on the results of the survival of Danio rerio embryos, the median lethal concentration of the organomineral chelate complex was calculated. The LC_{50} of the study drug is 4.67 g/l (Fig. 3).

4 Conclusion

Thus, studies conducted to assess the toxicity of the organomineral chelate complex for *Danio rerio* embryos have established that the maximum concentration that does not cause death during testing is 0.25 g/l and the minimum concentration that causes 100% death during testing is 16 g/l. Based on the results of the survival of *Danio rerio* embryos, the median lethal concentration of the organomineral chelate complex was calculated. The LC_{50} of the study drug is 4.67 g/l.

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References

1. V.P. Korotkiy, E.A. Maksim, S.S. Marisov, V.A. Ryzhov, A.S. Zenkin, Development of a feed phytonutrient to increase the efficiency of growing juvenile sturgeon fish in fish farms, Animal husbandry and feed production, **4**, **140** (2011)
2. T.G. Litvinova, D.V. Kondratyev, Increasing the economic efficiency of fish farming based on optimizing the feed ration (using the example of the State Unitary Enterprise "Fish Farm "Pikhtovka" of the Udmurt Republic), Russian Journal of Entrepreneurship, **18**, **20**, 3083-3096 (2017)
3. I. N. Ostroumova, Mineral nutrition of fish in conditions of technogenic pollution of water bodies, Fish farming and fishing, **2**, 28-29 (2001)
4. Mike Davies, Clinical Signs in Humans and Animals Associated with Minerals, Trace Elements and Rare Earth Elements (2021)
5. R.N. Selimov, The influence of the mineral feed additive "Helavit" on the mineral metabolism of horses: dissertation... Candidate of Veterinary Sciences: 02/06/05 /, Place of protection: St. Petersburg. state acad. vet. medicine, St. Petersburg, 142 (2011)
6. K.S. Koroleva, D.V. Khodos, Problems and challenges of the modern fishing industry, EV, **3**, **26** (2021)
7. P.A. Krasochko, T.I. Lebedeva, Irina Aleksandrovna, A.E. Stankut, Analysis of preparations based on nanoparticles of microelements used in animal husbandry and veterinary medicine, Collection of scientific papers of SKNIIZh, 1 (2021)
8. A.E. Arinzhanov, E.P. Miroshnikova, Yu.V. Kilyakova, Prospects for the use of nanoparticles in animal husbandry (review), Animal husbandry and feed production, **2**, **85** (2014)
9. H.M. Lin, S.G. Deng, S.B. Huang, Y.J. Li, R. Song, The effect of ferrous-chelating hairtail peptides on iron deficiency and intestinal flora in rats, J Sci Food Agric, Jun, **96**, **8**, 2839-44 (2016) doi: 10.1002/jsfa.7452
10. V.G. Skopichev, L.Yu. Karpenko, I.O. Bogolyubova, Physiology of fish. Book 2. Nutrition and digestion (Quadro, St. Petersburg, 2017)
11. K. Ajisaka, Y. Oyanagi, T. Miyazaki, Y. Suzuki, Effect of the chelation of metal cation on the antioxidant activity of chondroitin sulfates, Biosci Biotechnol Biochem, Jun, **80**, **6**, 1179-85 (2016) doi: 10.1080/09168451.2016.1141036
12. D.S. Ponko, A.N. Prikhodko, O.L. Yankina, N.A. Kim, Prospects for the use of chelates in feeding farm animals, News of OGAU, 12-1 (2018)

13. V.M. Kosolapov, V.A. Chuikov, Kh.K. Khudyakova, V.G. Kosolapova, Mineral elements in feed and methods of their analysis: monograph, Moscow, 272 (2019)
14. Fish Embryo Acute Toxicity (FET), Test No. **236**