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INFLUENCE OF NANOPARTICLES ON THE YIELD OF EMBRYOS OF THE ROE COMMON CARP (*CYPRINUS CARPIO* LINNAEUS, 1758) IN THE FERMENTATION PROCESS

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Abstract. The main purpose of the present study is to study the effect of Fe_3O_4(20-30 nm) nanoparticles on common carp (*Cyprinus carpio* Linnaeus, 1758) aquaculture process before fertilization to germ cells (sperm, egg cell) and embryonic developmental stages after fertilization.

The research material was implemented in between June-July 2021 at the Samukh-fish farm of the Republic of Azerbaijan. These nanoparticles were added to the germ cells (sperm, egg cell) of common carp (*Cyprinus carpio* Linnaeus, 1758) before fertilization in different amounts (0.0001 g, 0.001 g, 0.005 g and 0.05 g), as well as to the fertilized egg and their embryonic.

Studies have indicated that when Fe_3O_4 (20-30 nm) nanoparticles are added to 0.0001 g and 0.001 g of common carp yeast (sperm) before fertilization, the fertilization rate of the eggs and the subsequent spawning of free embryos is higher than the control (59.12%) and was 61.65% and 71.45%, respectively. Fe_3O_4 (20-30 nm) nanoparticles have a stimulating effect in the amount of 0.05 g. It can be assumed that these nanoparticles in the specified doses have a catalytic effect on the acrosomes of sperm, accelerate energy activity and, consequently, increase the activity of sperm, which leads to an increase in the fertilization rate of eggs.

Correspondingly, the results that obtained can be used to reduce weights, especially in the process of carp aquaculture, to reduce losses during embryonic development and increase overall productivity.

Keywords: *common carp, nanoparticles, sexual cells, fertilization of roe, fermentation process.*

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1. Introduction

A carp aquaculture is one of the most progressive and effective areas of artificial fish farming. Common carp (*Cyprinus carpio* Linnaeus, 1758) is now widely grown for commercial purposes in majority countries due to its high adaptability to environmental conditions, in addition for short incubation period, rapid growth rate and a some additional specific purposes (Moiseev *et al*., 1985; Guliyev, 2006; Emre & Kurum, 2008; Grigoriev & Sedova, 2008; Mamedov *et al*., 2016).

The use of nanomaterials and nanoparticles in consumer goods has significantly increased their likelihood of exposure to water, soil and air. Metal oxides of nanoparticles interact with the aquatic environment at different stages of the life cycle, and these interactions may lead to an urgent need for species for adapting to the changing environments regarding the disruption of the body's biochemical balance (Ju-Nam & Lead 2008; Gaiser *et al*., 2012; Xia *et al*., 2013). Nanoparticles, unlike their main analogues (larger particles with the same chemical composition), have the ability

to be more absorbed and keep organisms under their influence. However, data on its involvement in metabolism and its excretion from the body are limited, although hepatic excretion is the most likely pathway (Handy *et al*., 2008; Dahle, & Arai, 2015).

The fate of nanoparticles in aquatic ecosystems, their interactions with biotic and abiotic components, and their potential for adverse events have not yet been fully explored, and these uncertainties raise concerns about risks both for human and environmental health (Handy & Shaw, 2007; Ju-Nam & Lead, 2008). Thus, the study of the ecotoxicity of nanomaterials in the aquatic environment is one of the major areas in the research studies.

Due to the rapid development of nanotechnology and its application to various fields, there is insufficient information in the scientific literature in the effect during the fertilization process and embryonic developmental stages, which are more sensitive to hydrobionts, especially environmental factors. Therefore, the main purpose of the present study is to study the effect of Fe_3O_4(20-30 nm) nanoparticles on common carp (*Cyprinus carpio* Linnaeus, 1758) aquaculture process before fertilization to germ cells (sperm, egg cell) and embryonic developmental stages after fertilization.

2. Materials and methods

The research material was implemented in between June-July 2021 at the Samukh-fish farm of the Republic of Azerbaijan. The object of the experiment was the use of sexual products (eggs, sperm) of the common carp (*Cyprinus carpio* Linnaeus, 1758). During the experiments, mature male and female individuals were pre-marked and grouped, and their length, weight, and fullness coefficient (Fulton) were determined. Mature spawns were obtained from sexually mature spawners by the "milking method" and placed on smooth surface porcelain dishes according to the number of variants. In order to specify the number of caviar in the options and to calculate the total productivity, 1 gram of caviar was taken from each caviar used and fixed in 4% formalin solution after determining the number of caviar grains.

Fertilization of caviar was carried out by the "dry" method according to the accepted methodology (Moiseev *et al*., 1985; Guliyev, 2006; Grigoriev & Sedova, 2008; Mamedov *et al*., 2016). The quality of sperm taken from male was initially assessed visually, and the sperm activity in the samples was determined on a 5-point scale, magnified 200 times with the assistance of the Karl Zeiss Axio Imager M2 binoculars in accordance with the obtained methodology (Persov, 1953). Skyspring Nanomaterials in the course of experiments. Inc,USA, Houston TX. Fe_3O_4 (20-30 nm) nanoparticles from the company were used.

These nanoparticles were added to the germ cells (sperm, egg cell) of common carp (*Cyprinus carpio* Linnaeus, 1758) before fertilization in different amounts (0,0001 g, 0,001 g, 0,005 g and 0,05 g), as well as to the fertilized egg and their embryonic. The effect on developmental stages was studied with the help of Carl Zeiss Axio Imager M2 binoculars. Every day in the incubation process, dead embryos are separated from the device in each version, noted in special notebook, and the free embryos are the counted.

3. Results and discussion

In accordance with the issues raised, the experiments were carried out on different options. The results of the experiments were compared to the control. Studies have

indicated that when Fe_3O_4 (20-30 nm) nanoparticles are added to 0,0001 g and 0,001 g of common carp yeast (sperm) before fertilization, the fertilization rate of the eggs and the subsequent spawning of free embryos is higher than the control (59,12%) and was 61,65% and 71,45%, respectively (Table 1; Figure 1).

Table 1. Addition of Fe_3O_4(20-30nm) nanoparticles to common carp yeast (sperm)

Amount of Fe_3O_4 (20-30nm), g	Length of the fish, L, cm	Mass of fish P, g	Amount of roe, g	Number in 1 g of roe, pcs	Total number of roe, pcs	Release		Number of dead	
						%	pcs	%	pcs
0,001	50	1981	30	182	5460	71,45	3901	28,55	1559
0,0001	50	1981	30	182	5460	61,65	3366	38,35	2094
Control	50	1981	30	182	5460	59,12	3228	40,88	2232

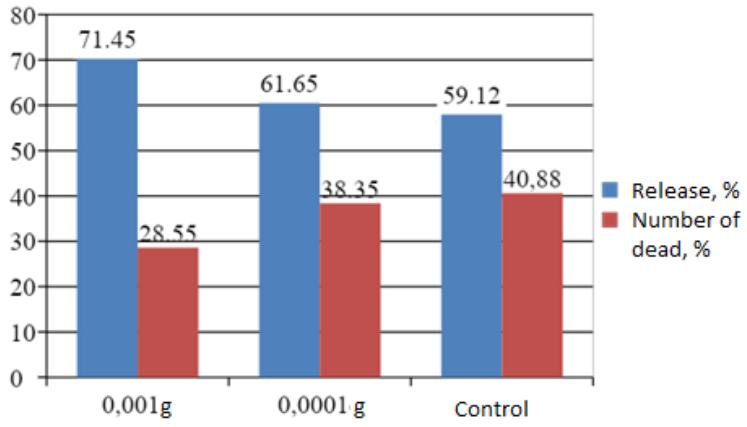

Fig 1. Addition of Fe_3O_4(20-30nm) nanoparticles to common carp yeast (sperm)

Table 2. Addition of Fe_3O_4(20-30nm) nanoparticles to common carp caviar before fertilization

Amount of Fe_3O_4 (20-30nm), g	Length of the fish, L, cm	Mass of fish P, g	Amount of roe, g	Number in 1 g of roe, pcs	Total number of roe, pcs	Release		Number of dead	
						%	pcs	%	pcs
0,05	52	2050	30	190	5700	46,64	2658	53,36	3041
0,005	52	2050	30	190	5700	44,1	2514	55,9	3186
Control	52	2050	30	190	5700	35,37	2016	64,63	3684

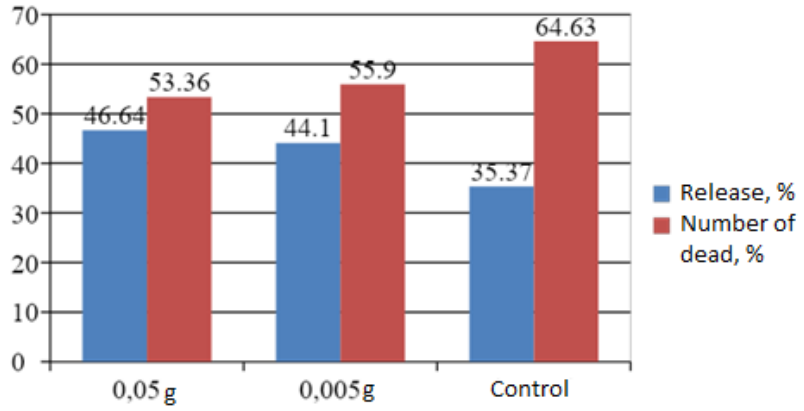

Fig 2. Addition of Fe_3O_4(20-30nm) nanoparticles to common carp caviar before fertilization

Although 0,005 g and 0,05 g of Fe_3O_4 (20-30 nm) nanoparticles were added to the common carp caviar before fertilization, the spawning rate of free embryos was higher than the control (35,37%), however the overall mortality rate was higher, 53,36% and 55,9%, respectively (Table 2; Figure 2).

Similar results were obtained when 0,005 g and 0,05 g of Fe_3O_4(20-30 nm) nanoparticles were added to fertilized eggs of normal weight, and the yield of free embryos varied between 65,35-75,35% in different variants (Table 3; Figure 3).

Table 3. Addition of Fe_3O_4(20-30nm) nanoparticles to common carp fermented caviar

Amount of Fe_3O_4 (20-30nm), g	Length of the fish, L, cm	Mass of fish P, g	Amount of roe, g	Number in 1 g of roe, pcs	Total number of roe, pcs	Release		Number of dead	
						%	pcs	%	pcs
0,05	52	2050	30	190	5700	75,35	4295	24,65	1405
0,005	52	2050	30	190	5700	27,01	1540	72,99	4160
Control	52	2050	30	190	5700	35,37	2016	64,63	3684

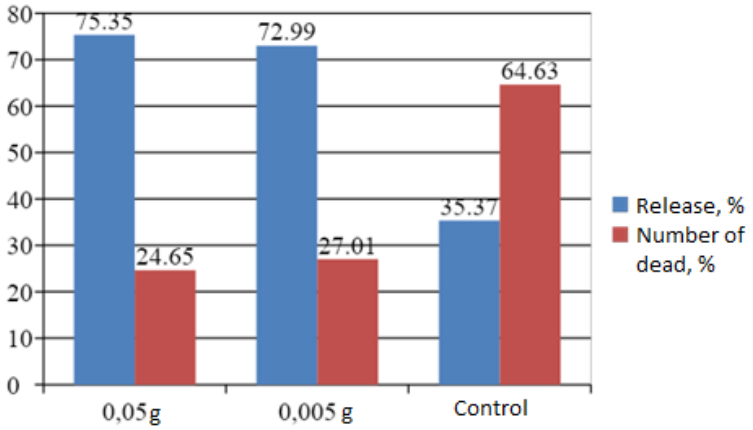

Fig 3. Addition of Fe_3O_4 (20-30nm) nanoparticles to common carp fermented caviar

In the study, the amount of Fe_3O_4 (20-30 nm) nanoparticles was tested in a wide range (0,0001 g, 0,001 g, 0,005 g and 0,05 g). As the final impact indicators were determined, the number of nanoparticles was tested in a relatively small range (0,005 g and 0,05 g) and the number of variants was reduced (Table 4, 5; Figure 4, 5) as the study continued.

The results of the research were in line with the results obtained during the resumption of research. Thus, when Fe_3O_4 (20-30 nm) nanoparticles were added to common carp yeast (sperm) in the amount of 0,05 g before fertilization, the fertilization rate of spawns and subsequent release of free embryos from spawning was higher than the control (82%) and was 92% (Table 4; Figure 4).

Table 4. Addition of Fe_3O_4 (20-30 nm) nanoparticles to common carp yeast (sperm)

Amount of Fe_3O_4 (20-30nm), g	Length of the fish, L, cm	Mass of fish P, g	Amount of roe, g	Number in 1 g of roe, pcs	Total number of roe, pcs	Release		Number of dead	
						%	pcs	%	pcs
0,05	52	2050	30	190	5700	92,0	5244	8,0	456
Control	52	2050	30	190	5700	82,0	4674	18,0	1026

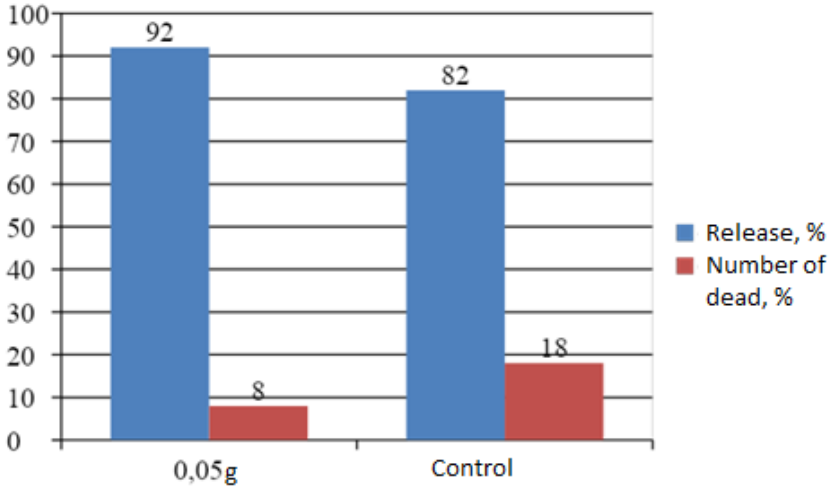

Fig 4. Addition of Fe_3O_4(20-30nm) nanoparticles to common carp yeast (sperm)

Table 5. Addition of Fe_3O_4(20-30nm) nanoparticles to common carp fermented caviar

Amount of Fe_3O_4 (20-30nm), g	Length of the fish, L, cm	Mass of fish P, g	Amount of roe, g	Number in 1 g of roe, pcs	Total number of roe, pcs	Release		Number of dead	
						%	pcs	%	pcs
0,05	52	2050	30	190	5700	87,4	4982	12,6	718
Control	52	2050	30	190	5700	82,0	4674	18,0	1026

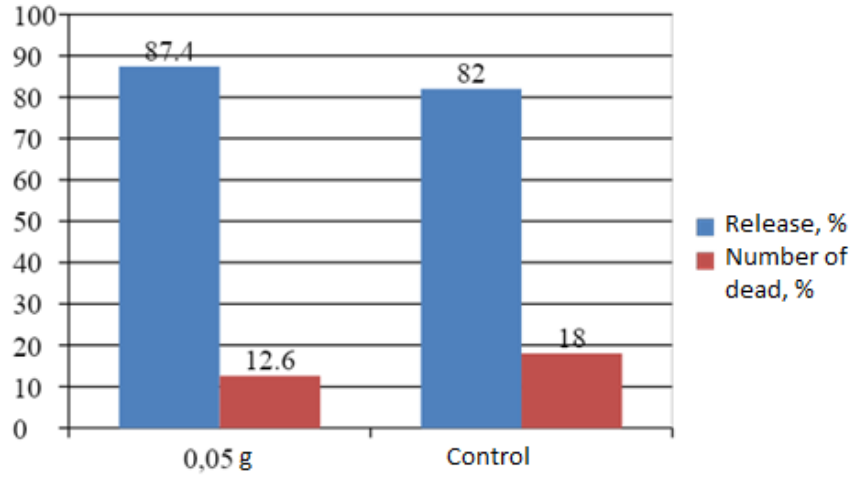

Fig 5. Addition of Fe_3O_4(20-30nm) nanoparticles to common carp fermented caviar

When 0,05 g of Fe_3O_4 (20-30 nm) nanoparticles were added to the fertilized caviar of common carp, the yield of free embryos was 87,4% (Table 5; Figure 5).

Thus, the general results of the research suggest that Fe_3O_4 (20-30 nm) nanoparticles have a stimulating function when added to yeast and fertilized caviar of common carp.

Bioaccumulation, distribution and localization of nanoparticles in organs, removal from tissues and the mechanism of their action are intensively studied (Kovalenko & Folmanis, 2006; Schrand *et al*., 2010; Arinzhanov *et al*., 2012; Gajewicz *et al*., 2012; Xia *et al*., 2013; Ivask *et al*., 2014). The toxic effects of nanoparticles are studied comparatively using model organisms from both marine and freshwater fish, and the main purpose of these studies is to determine the risk of nanomaterials (Krysanov & Demidova, 2012; Abramenko, 2017).

References to the scientific literature (Kovalenko, 2006, Gaiser *et al*., 2009; Lee *et al*., 2009; Van Hoecke *et al*., 2009, Krysanov & Demidova, 2012; Arnold *et al*., 2013; Felix *et al*., 2013, Xia *et al*., 2013;), a number of studies evaluated the effect of various nanoparticles on hydrobionts, found that nanoparticles of some metals have high biological activity, accelerate metabolism and increase the body's resistance to natural factors, growth rate, as well as external signs. It was noted that metals in the form of nanoparticles, in addition to having high bactericidal properties, are also less toxic and do not accumulate in the body as they are assimilated as a trace element in the metabolic process (Bikmukhametov, 2009).

It should be noted that there is no information in the scientific literature on the direct effect of Fe_3O_4 (20-30 nm) nanoparticles on common carp (*Cyprinus carpio* Linnaeus, 1758) on germ cells (sperm, egg cells), as well as on fertilized eggs. In the preparation of research the effect of nanoparticles on the process of embryonic development, the species *Danio rerio* (*Danio rerio* Hamilton, 1822) was used as a model object. During the experiments, *Danio rerio* embryos were exposed to CeO_2 and silver (Ag) nanoparticles (LC50) for 96 hours, their morphological changes in embryonic developmental stages, release of free embryos depending on the number of nanoparticles and mortality rate were studied (Krysanov, Demidova, 2012; Abramenko, 2017). It was noted that in its pure form, CeO_2 nanoparticles do not cause complications

in the development of *Danio rerio* embryos, but the increase in the amount of silver (Ag) nanoparticles has led to an increase in embryonic mortality and the number of defective embryos.

Studies have shown that when Fe_3O_4 (20-30 nm) nanoparticles were added to the yeast of common carp (*Cyprinus carpio* Linnaeus, 1758) before fertilization, during the process the fertilization rate of the eggs was higher and the yield of free embryos was higher than in other variants. Fe_3O_4 (20-30 nm) nanoparticles have a stimulating effect in the amount of 0,05 g. It can be assumed that these nanoparticles in the specified doses have a catalytic effect on the acrosomes of sperm, accelerate energy activity and, consequently, increase the activity of sperm, which leads to an increase in the fertilization rate of eggs.

Common carp has been used in aquaculture for many years at fish farms in Azerbaijan, and in most cases, plant-formed offspring are used effectively. In such circumstances the high level of kinship between individuals leads to inbreeding. For this reason, the infant mortality rate is high, additionally in most cases the quality of the offspring's sexual products is low, and the new generation formed from them is intolerant to various environmental influences.

Correspondingly, the results that obtained can be used to reduce weights, especially in the process of carp aquaculture, to reduce losses during embryonic development and increase overall productivity.

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