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Reproductive functions of males of a carp at addition of vitamin E and selenium into feed during prespawning season

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The purpose. To study effect of different concentrations of vitamin E in combination to selenium upon reproductive function of males of a carp. **Methods.** Methodological basis of scientific researches is the microscopic study of cells of sperm with the use of computerized system CASA (Computer Assisted Semen Analysis). **Results.** Assessment of quality of sexual products of males of a carp is carried out by fixation of effect of addition into their ration of vitamin-mineral additive during prespawning season. Parameters of mobility and fertilizing ability of sperm are probed. It is determined that it is expedient to feed vitamin E (at the rate of 75 mg/kg of feedstuff) in a complex with selenium (at the rate of 0,3 mg/kg of feedstuff), considering raise of productivity of sperm. Increase in quantity of sperm with rectilinear-forward motion and speed of movement of sexual cells in particular is revealed. **Conclusions.** Addition in feedstuff of vitamin E and selenium in prespawning season positively influences quantity and quality of sexual products of males of a carp. In particular, total activity of sexual cells grows and the quantity of motionless ones decreases.

Key words: *males of a carp, sexual products, spermatozoon, computer diagnosis, mobility, rectilinearly-forward motion, manege movement.*

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Formulation of the problem. The primary function of the reproductive system in animals is the production of reproductive gametes. The most important in this aspect is the transfer of genetic potential to the next generation. One of the most important factors responsible for successful reproduction is the quality of the sperm cells. In assessing the quality of sperm, in addition to visual signs (volume, color, smell, consistency), the following are considered first of all: mobility, concentration, content of pathological forms and survival of sperm. The use of native semen of inadequate quality is ineffective in order to obtain an optimal genetically determined result [1,2,3].

The technological aspect of reproduction of carp is worked out, therefore the main negative factor during spawning campaign is inadequate quality of reproductive material, in particular the quality of male sperm products [4,5].

Analysis of recent research and publications. There are a large number of factors, both exogenous and endogenous, that negatively affects the reproductive function of not only females but also fish males [6,7]. In particular, from a literature source it is known about a number of pathological states of the reproductive system of males observed in vitamin E deficiency, namely: testicular degeneration, sperm quality decline, mobility and sperm motility [8].

The action of vitamin E in the body of animals is closely related to the action of the micronutrient Selenium, which is a biologically active element necessary for the normal functioning of the reproductive system of animals. Experimental studies have established the link between selenium

content in the body and the problems associated with infertility of females and males, their overall development and resistance to disease [9,10].

Studies of physiological and biochemical evaluation of sperm production of pedigree males in the conditions of their introduction of vitamin E and Selenium into their diet indicate that feeding animals to organic selenium in combination with vitamin E contributes to the increase of the activity of enzymes - succinate dehydrogenase (SDG) and cytochrome oxidase (CCHO), which are localized in mitochondria of sperm.

During the 90th day of feeding the males of the experimental group of vitamin and mineral supplements, the activity of the SDG increased by 21.7% ($p < 0.05$), and the CCHO by 9.3% ($p < 0.05$), as compared with the control about the positive effect of this compound on the metabolic processes in the sperm, their viability and fertilizing ability [11].

The evaluation of the dynamic movement of sperm has become widespread in reproduction technology, since this method allows to determine the quality of the received germ cells, to identify abnormalities and to prevent the ineffectiveness of fertilization. A detailed analysis of mobility and the evaluation of the dynamics of sperm movement from pedigree fish males allows the formation of sperm by categories of mobility, distinguishing samples with fast and straightforward movement; sample with slow rectilinear motion; non-linear motion tests and completely immobile sperm tests [2,12].

The purpose of research. To evaluate the reproductive functions of carp males for the introduction into the main diet of various concentrations of vitamin E in combination with selenium throughout the spawning period.

Materials and methods of research. The research was conducted in the conditions of the Lviv research station of the Institute of Fisheries of the National Academy of Sciences of Ukraine of Lviv Region. The object of the study was three-year-old male carp, of which three groups were formed - two experimental and control. The males were kept within a month prior to spawning in closed water supply systems under conditions of gradual increase of water temperature and adherence to generally accepted standards in fish farming. Twice a day fish fed balanced nutrients with forage, the total protein content of which was 45%. The feeding was normalized according to the growth of the carp and the environment. For males of the first experimental group, vitamin E was added additionally to the main diet in the form of an oil solution of the preparation of alpha-tocopherol acetate at a concentration of 25 mg / kg and the Selenium trace element Sel-Plex (based on selenium 0.3 mg / kg). For males of the second experimental group - on the basis of vitamin E 75 mg / kg and Selenium 0.3 mg / kg. The control group of fish received feed without vitamins and elements added.

The spawning was carried out by the factory method, stimulating males with pituitary injections of crucian carps at a rate of 1 mg / kg. Following the completion of pre-nourishment, samples from the control and experimental groups were selected for specimens to determine the quality of sperms and their fertility. Sperm quality studies were carried out on the equipment of the German firm Minitub (a microscope connected to a computer camcorder equipped with the SpermVision program). To ensure focus and activation of the motion, the samples of semen were diluted with pond water.

The following parameters were used to analyze the parameters of the sperm movement: VCL is the curvilinear velocity (μm / sec), VCL is the velocity of rectilinear movement of the sperm head along the straight line between the initial and the end points of the trajectory (μm / sec), VAP is the velocity of sperm head propagation along the average trajectory motion (μm / sec), WOB - oscillation - value describing the variation of the real trajectory relative to the average (VAP / VCL,%); LIN - linearity of the real trajectory (VSL / VCL,%), STR - degree of straightforwardness of the sperm

movement (VSL / VAP%); ALH is the amplitude of lateral displacement of the head of sperm from the average trajectory of its movement, or the mean deviation of the head (μm) [2,7].

The obtained digital results were statistically analyzed using the Microsoft Excel software, determining the mean arithmetic values (M), the mean squared error (m), and the probability of the differences (P) between the mean arithmetic values under investigation [1].

Research results. Comparing the effectiveness of the use of the investigated mineral-vitamin supplement in feeding male carp, significant differences in the activity of sperm (Table 1).

1. Quality of the sperm of the male fish depending on the composition of the fed forage, % (M ± m, n = 9)

Parameters of sperm quality	Groups of carp males		
	Control	Experimental 1	Experimental2 2 2 2
Average sperm motility	76,6±2,34	87,5±2,17	94,0±1,97
Sperms with straight-forward movement	56,3±2,06	58,3±2,83	69,9±2,7
Sperms with manege movement	20,3±0,36	24,2±0,17	29,2±0,54
Immobile sperms	23,4±0,21	12,5±0,27	5,98±0,18

After activating sperm in water, the total activity of the germ cells of the experimental groups was 10.9% and 17.4% higher, respectively, relative to the control group. In the second experimental group, the number of sperm with rectilinear translational motion was 11.6% more control. The inverse dependence was determined by the number of immovable sperm, which was less in the experimental samples, compared with the control, respectively, at 10.9% and 17.42% (Table 1).

Consequently, the addition of Selenium sow and different concentrations of vitamin E provides increased vitality of carp sperm.

The results of approbation of the method for assessing the mobility of fish sperm in the experimental conditions using modern computer diagnostic methods are presented in Table 2.

2. Dynamic characteristics of motion of sperm fish using automated software CASA% (M ± m, n = 9)

Parameters of sperm quality	Groups of carp males		
	Control	Experimental 1	Experimental 2
VCL, m / sec.	56,98	71,01	114,6
VAP, m / sec.	41,22	40,71	60,18
VSL, m / sec.	31,19	26,46	39,03
WOB, %	0,72	0,57	0,67
LIN, %	0,55	0,41,	0,48
STR, %	0,76	0,72	0,71
ALH	3,97	5,64	4,89

Describing a dynamic variable such as the speed of rectilinear movement of the sperm head along the straight line segment between the initial and final points of the trajectory (VSL), it should be noted that this indicator was the highest in the second experimental group (Table 2). The least

variation of the sign was observed in the group of males who showed high sexual activity. The average rate of sperm movement in this group was 39.03 m / sec.

The minimum average value of this index was observed in the first experimental group of males with low sexual activity - 26.46 m / sec. The dynamics of the sperm of the control group males by this indicator was 31.19 m / sec.

According to the straightforwardness of the movement (STR), the male semen of the fish did not have significant differences and was in the range of 0.71-0.76%.

In the study of such an indicator as the amplitude of the lateral displacement of the head of sperm from the average trajectory of its movement (mean deviation of the head) and the frequency of oscillatory sperm movements - no significant significant difference was found between the indices.

As a result of the research, it was found that the mean curvature rate of sperm (VCL), which is the average time sperm velocity along its real trajectory, in the second experimental group increased to 114.6 m / sec and was significantly higher in control. At the same time, the average velocity along the trajectory (VAP) in the same group was 60.18 m / sec.

Conclusions

Thus, the introduction of vitamin E and Selenium during the spawning period has a positive effect on the quantity and quality of male reproductive organs. Effective is the additional feeding of vitamin E (at a rate of 75 mg / kg) and trace element Selenium (at a rate of 0.3 mg / kg) in view of the increase in sperm productivity, which is expressed in their higher activity, as well as twice the higher mean curvilinear velocity Sperm (VCL) relative to the indicators of the control group of fish.

The analysis of mobility and estimation of dynamics of motion of sperm obtained from pedigree carp males allows to classify sperm samples according to categories of mobility: with fast and straight motion, with slow rectilinear movement, with non-linear motion and with completely immobile sperms. In the course of the research, it was found that evaluation of sperm motility by computer analysis of CASA sperm is effective, since it provides for obtaining accurate and detailed information on the qualitative characteristics of male reproductive products.

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