EFEITO DO ESTRESSE INDUZIDO POR HORMÔNIOS NO COAGULOGRAMA DE CARPA (*CYPRINUS CARPIO*)**EFFECT OF HORMONE-INDUCED STRESS ON CARP (*CYPRINUS CARPIO*) COAGULOGRAM****ВЛИЯНИЕ ГОРМОНИНДУЦИРОВАННОГО СТРЕССА НА КОАГУЛОГРАММУ КАРПА (*CYPRINUS CARPIO*)**BEREZINA, Daria I.^{1*}; FOMINA, Lyubov L.²¹ Vologda State Dairy Farming Academy named after N.V. Vereshchagin, "Aquabiocenter", Department of Veterinary Medicine and Biotechnology. Russia.² Vologda State Dairy Farming Academy named after N.V. Vereshchagin, Department of Veterinary Medicine and Biotechnology. Russia.

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RESUMO

A carpa (*Cyprinidae*) é uma das espécies de peixes dominantes e mais valiosas em termos de piscicultura. Em condições de cultivo de alta intensidade, os peixes são sistematicamente expostos a fatores extremos que causam reações de estresse que são acompanhadas por mudanças no estado funcional dos sistemas de defesa do organismo e exercem impacto principalmente nos parâmetros hematológicos. O sistema hemostático é um desses sistemas de defesa, que neutraliza o sangramento por meio de um mecanismo de coagulação. A hemocoagulação segue o mesmo padrão em todos os vertebrados, de peixes sem mandíbula a mamíferos, e representa uma adaptação ancestral dos animais a condições estressantes, frequentemente associada à perda de sangue na natureza. O objetivo desta pesquisa foi estudar o efeito do estresse induzido por hormônios na hemostasia plasmática (secundária) em peixes. Dada a fragmentação dos dados e diferenças na metodologia e condições, a falta de padronização no estudo da hemostasia em peixes, especialmente em condições críticas, esse problema permanece não totalmente divulgado na ciência global. O artigo apresenta os resultados do estudo dos parâmetros do coagulograma da carpa (*Cyprinus carpio*) sob a influência de respostas de estresse agudo e crônico simulados por injeções de análogos de cortisol sintético (dexametasona para estresse de curto prazo e betametasona para estresse crônico) durante 21 dias. A dinâmica desses indicadores foi analisada em comparação com peixes intactos. Foi estabelecido que, ao acelerar o tempo de tromboplastina parcial ativada, tempo de protrombina e ao aumentar a quantidade de fibrinogênio no sangue de peixes, os processos de coagulação do sangue foram claramente acelerados em todos os grupos de animais testados até o último dia do experimento. A dinâmica de outros parâmetros, como o conteúdo dos complexos monoméricos de fibrina solúveis ou o conteúdo da antitrombina III, indicou o desenvolvimento simultâneo de processos de hipercoagulação em alguns grupos. Suposições foram feitas para explicar o padrão de mudanças observadas não apenas em peixes tratados, mas também em animais de controle.

Palavras-chave: coagulação, hemostasia, cortisol.**ABSTRACT**

Carp (*Cyprinidae*) is one of the dominating and most valuable fish species in fish farming. Under conditions of high-intensity cultivation, fish are systematically exposed to extreme factors that cause stress reactions, accompanied by changes in the functional state of the defense of the body systems and exert impact, primarily, on hematological parameters. The hemostatic system is one such defense systems, which counteracts bleeding through a coagulation mechanism. Hemocoagulation follows the same pattern in all vertebrates, from jawless fish to mammals, and represents an ancient adaptation of animals to stressful conditions, often associated with blood loss in nature. This research aimed to study the effect of hormone-induced stress on plasma (secondary) hemostasis in fish. Given the data fragmentation and differences in methodology and conditions, the lack of standardization in studying hemostasis in fish, especially in critical conditions, this problem remains not fully disclosed in global science. The article presents the results of studying carp (*Cyprinus carpio*) coagulogram parameters under the influence of acute and chronic stress responses, simulated by injections of synthetic cortisol

analogs (dexamethasone for short-term stress, and betamethasone for chronic stress) during 21 days. The dynamics of these indicators were analyzed in comparison to intact fish. It has been established that by accelerating the activated partial thromboplastin time, prothrombin time, and increasing the amount of fibrinogen in the blood of fish, blood coagulation processes were clearly accelerated in all groups of animals tested by the last day of the experiment. The dynamics of other parameters, such as the content of soluble fibrin monomer complexes or antithrombin III content, indicated the simultaneous development of hypercoagulation processes in some groups. Assumptions have been made to explain the pattern of changes observed not only in treated fish but also in control animals.

Keywords: *coagulation, hemostasis, cortisol.*

АННОТАЦИЯ

Карп (*Cyprinidae*) является одним из доминирующих и наиболее ценных видов рыб с точки зрения рыбоводства. В условиях высокоинтенсивного выращивания рыба систематически подвергается воздействию экстремальных факторов, вызывающих стрессовые реакции, которые сопровождаются изменением функционального состояния систем защиты организма и оказывают влияние, прежде всего, на гематологические параметры. Одной из таких систем защиты является гемостатическая система, противодействующая кровотечениям через механизм коагуляции. Гемокоагуляция проходит по одной и той же схеме у всех позвоночных животных, от безчелюстных рыб до млекопитающих, и представляет собой древнюю адаптацию животных к стрессовым условиям, часто связанным с кровопотерей в природе. Целью данного исследования было изучение влияния гормонального стресса на плазменный (вторичный) гемостаз у рыб. Учитывая фрагментацию данных и различия в методологии и условиях, отсутствие стандартизации при изучении гемостаза у рыб, особенно в критических условиях, эта проблема остается не до конца раскрытой в мировой науке. В статье представлены результаты исследования параметров коагулограммы карпа (*Cyprinus carpio*) под влиянием острой и хронической стрессовых реакций, симитированные синтетическими аналогами кортизола в течение 21 дня. Проанализирована динамика этих показателей в сравнении с интактными рыбами. Установлено, что за счет ускорения активированного частичного тромбопластинового времени, протромбинового времени и увеличения количества фибриногена в крови рыб к последнему дню эксперимента процессы свертывания крови явно ускоряются у всех групп животных, участвующих в опыте. Динамика других параметров, таких, как содержание растворимых фибрин-мономерных комплексов или содержание антитромбина III, говорят об одновременном развитии гипокоагуляционных процессов у некоторых групп. Были высказаны предположения, призванные объяснить картину изменений, наблюдаемую не только у обработанных рыб, но и у контрольных животных.

Ключевые слова: *коагуляции, гемостаз, кортизол.*

1. INTRODUCTION:

As one of the dominant *Cyprinidae* species, *C. carpio* (common carp) is cultivated in more than 100 countries around the world and accounts for up to 10% of the world's annual freshwater aquaculture production (Xu *et al.*, 2014). *C. carpio* is also an important species of ornamental fish; they are an object of contemporary veterinary medicine, which has recently entered a new stage in the development of surgical care for these animals. These fish are readily available and suitable for carrying out hemostasiological studies, in contrast to species of more value or smaller size, since such studies involve sampling large volumes of biomaterial. Routine hematological examinations in fish require 0.5-1 ml of blood with preservation of the fish life (Blaxhall and Daisley, 1973), while for the coagulogram studies (without conducting morphological and biochemical blood tests), 5-9

ml are required according to medical techniques (Momot, 2006). Carp contains 5.08 ml circulating blood per 100 g of body weight (Itazawa, Takeda, Yamamoto, and Azuma, 1983). In contrast, this amount varies in some other cultured fish: 2.8-3.5 ml/100 g in trout, 1.8 ml/100 g in catfish (Conte, Wagner, and Harris, 1963).

The hemostatic system that coagulates the blood and regulates its aggregation state in the bloodstream is of considerable interest from the veterinary, medical, and evolutionary viewpoints. The hemostatic system is designed to ensure the integrity of the internal environment of the body and stop bleeding in case of damage to the vascular wall, its permeability and resistance, and to maintain the liquid state of the blood in the vascular bed. Studies conducted on Teleostei indicate that the coagulation process is fundamentally similar to other vertebrates, particularly mammals (Kudryashov, 1975; Jagadeeswaran, Gregory, Day, Cykowski, and

Thattaliyath, 2005), the only difference is in its being adapted to lower temperatures. And the enzymes involved in coagulation can work on a broader temperature range than in hematothermal species (Botyazhova, 2000).

The dynamics and influence of endogenous cortisol in stress responses resulting from hypoxia (Prychepa, 2015), intoxication (Gluth and Hanke, 1984, 1985; Romanenko, Potrokhov, and Zinkovsky, 2010), temperature disturbances (Chen, Sun, Tsai, Song, and Chang, 2002; Strange, Schreck, and Golden, 1977), catching (Pottinger, 1998; Ruane, Huisman, and Komen, 2001), increased stocking density (Ruane, Carballo, and Komen, 2002) and even transportation (Möck and Peters, 1990) have been well studied in fish. As is known, an enhanced content of this hormone causes destabilization in the state of cellular and humoral factors of immunity, and depletion of the immune system (Mikryakov, 2004; Tort, 2011). Besides, it was noted that hemostatic mechanisms were triggered in fish exposed to endogenous cortisol impact induced by acute and chronic hypoxia (Berezina, 2020). This effect was previously confirmed by other authors who studied the coagulation time, platelet count, fibrinogen level (Bouck and Ball, 1966; Hattingh and Van Pletzen, 1974) and activated partial thromboplastin time (APTT) (Fujikata and Ikeda, 1985) in stressed fish. Some of them noted that the clotting time is advantageous as an indicator of stress (Tavares-Dias and Oliveira, 2009; Casillas and Smith, 1977; Barton and Iwama, 1991), although they concluded that hypercoagulation is likely to be associated with increased platelet count in the blood (Casillas and Smith, 1977; Wedemeyer *et al.*, 1976; Hunn *et al.*, 1992) and activation of the vascular- thrombocytic link of hemostasis caused by the production of catecholamines and corticosteroids during stress.

It is assumed that interspecies differences in blood coagulation in captured fish might well be the result of differences in the stress resistance of these fish (Ivanov, 2011). An experience was obtained in simulating stress responses with dexamethasone phosphate in carps (Balabanova, Mikryakov, and Mikryakov, 2009), as well as other synthetic corticosteroids (Houghton and Matthews, 1986; Roth, 1972) in freshwater fish, which resulted in marked processes of immune depression comparable to the endogenous cortisol effect. Information on the influence of synthetic corticosteroid analogs on fish blood coagulation is currently not available in the literature. Therefore, it seems relevant to compare

the exogenous and endogenous cortisol effects on the plasma-coagulation hemostasis unit.

Therefore, this study aimed to assess changes in the functional state of secondary hemostasis in fish under the impact of hormone-induced stress.

2. MATERIALS AND METHODS:

2.1. Ethics

The research was conducted in strict accordance with ethical principles established by the European Convention on the protection of the Vertebrata used for experimental and other scientific purposes (adopted in Strasbourg on March 18, 1986, and confirmed in Strasbourg in June 15, 2006) and approved by the local Ethics Committee of the Vologda State Dairy Farming Academy named after N.V. Vereshchagin (Record No. 12 dated December 3, 2015).

2.2. Animals and groups of study

The research was carried out in the Aquabiocenter, aquaculture development center, the Vologda State Dairy Farming Academy. The experiment involved 24 *Cyprinus carpio* L., grown in the Diana fishery (Kaduy village, the Vologda Region), which were previously divided into three groups (n=8 to each group): the first experimental group that underwent hormonal induction of acute stress; the second experimental group with the induced chronic stress, and the control group, which remained intact (Table 1).

According to Mead's resource equation (Van Zutphen, Baumans, and Beynen, 2001), and the local "Guidelines for the development of water quality standards for water bodies of fishery importance" (Registered in the Ministry of Justice of Russia on 03.09.2009 under N 14702), this number of animals (n=8) was considered appropriate for the research (6-10 individuals) and statistically relevant.

2.3. Procedures

Dexamethasone phosphate (4 mg/ml) was used as a hormonal drug simulating acute stress (Balabanova *et al.*, 2009) and metabolizing within four hours. This synthetic hormone is an analog of natural cortisone. Betamethasone suspension (2.63 mg of betamethasone sodium phosphate + 6.43 mg of betamethasone dipropionate/ml) was used as a hormonal drug simulating chronic stress; its run-out period took more than ten days.

Fish of the first experimental group were treated with dexamethasone phosphate (Ellara, Russia) by parenteral injection at a dose of 0.2 ml or 0.8 mg of the dexamethasone phosphate active substance per individual. Fish of the second experimental group were injected with Diprospan (Schering-Plow Labo N.V., Belgium) at a dose of 0.5 ml per individual, which corresponded to 3.5 mg of the active substance.

The fish were kept in an experimental setup providing continuous water circulation between the aquariums and forced aeration at a water temperature of 18–20°C; feeding schedule implied feeding fish once a day with granulated feed. Before blood collection, the fish were anesthetized by adding clove oil to the water at a dose of 0.033 ml/l (Hamackova, Kouril, Kozak, and Stupka, 2006), followed by holding for 15 minutes. Blood was collected with a syringe from the caudal artery into glass tubes containing 3.8% sodium citrate solution, and into glass tubes with a coagulation activator. Blood sampling in animals participating in the experiment was performed immediately after acclimatization, and then 7, 14 and 21 days after injection of drugs (Balabanova *et al.*, 2009).

The plasma coagulation hemostasis parameters were determined on a Thrombostat coagulometer manufactured by Behnk Elektronik (Germany). To assess the state of plasma coagulation hemostasis, the following parameters were determined: APTT, prothrombin time (PT), and TT (thrombin time) using human thrombin, and quantitative analysis of fibrinogen (Fomina, Kulakova, and Berezina, 2017). The anticoagulant properties of blood were evaluated by the content of Antithrombin III in plasma. Plasma fibrinolytic activity was measured by detecting soluble fibrin monomer complexes (SFMC) in O-phenanthroline test (plate version).

2.4. Statistics

The obtained results are presented in the form of the mean value and the standard error of the mean ($M \pm m$). The statistical significance of carp coagulogram indicators for multiple independent samples was determined using the Kruskal-Wallis test, and the Wilcoxon test was used for paired dependent samples. The research results with a value of the alpha error probability being equal to or less than 5% ($p < 0.05$) were considered statistically significant. The difference between the two indicators was considered significant if it was equal to or exceeded its average error of difference by two or more times.

Also, the statistical criteria (the Kruskal-Wallis H test, which is a generalization of the Mann-Whitney U-test, and the Wilcoxon test) used in this research allowed the authors to conduct statistical analysis on small ($n \geq 3$) samples.

3. RESULTS AND DISCUSSION:

The coagulogram data presented in Tables 2-4. APTT characterizes the first phase of blood clotting (prothrombinase formation) and is responsible for the internal path of hemocoagulation. The research results show that the APTT changed significantly in all groups: synchronously and unidirectionally toward the decrease compared to baseline (Figures 1-3). No differences between the control and experimental groups were marked at the beginning of the experiment. Thus, the degree of influence of coagulation factors on thrombus formation increased with the experiment days, which indicates the acceleration of blood coagulation (hypercoagulation).

The intensity of the external coagulation pathway, which is predominant for fish (Berezina *et al.*, 2017, Doolittle and Surgenor, 1962), is indicated by the PT that characterizes the first and second phases of coagulation. After analyzing the data obtained, we should note a gradual reduction of this time by the last day of exposure to hormone-induced stress in all fish groups. While the nature of changes in each group was different: in the control (significantly) and the second experimental groups, the changes had a stepped shape (Figures 1 and 3) in fish with simulated acute stress, the changes were smoother (Figure 2). The PT shortening also indicates the activation of the external link of hemostasis and hypercoagulation, which may be conditioned by corticosteroid effects. For example, it was shown in the experiments on the effects of stress on hemocoagulation involving mice (Polidanov, Skorokhod, and Babichenko, 2020).

In the same publication, the authors noted a stress-induced increase in plasma fibrinogen levels, this also proved to be true for fish as we showed earlier (Berezina and Fomina, 2018). Quantitative characteristics of fibrinogen, together with TT, characterize the third phase of blood coagulation (fibrin formation). The amount of this protein in the blood plasma increased in animals of all groups during the experiment, as stated above. The curve of changes in the first and second experimental groups of animals (Figures 2 and 3) treated with hormones has a similar shape, in contrast to the control group (Figure 1).

Fibrinogen is an important functional indicator of the plasma hemostasis system, providing the formation of a clot, and its increase indicates the activation of coagulation.

The TT is an equally significant indicator that reflects the rate of fibrinogen conversion to fibrin. Its analysis in dynamics indicates that in the control group (Figure 1) and the first experimental group (Figure 2) the TT underwent a peak increase in its duration on the 14th day of the research, and by the 21st day it showed a similar sharp decrease. In the 2nd experimental group of fish exposed to long-acting hormones, on the contrary, the TT sharply decreased by the 14th day and then remained at a level below the initial one (Figure 3). A sharp increase in the TT means a high risk of developing coagulopathies toward hypocoagulation, while its reduction indicates a tendency toward hypercoagulation and thrombosis. Both processes undoubtedly lead to disturbances in the body homeostasis.

Antithrombin III exerts a major depressant (anticoagulant) effect on blood clotting processes. The level of this anticoagulant factor changed significantly: more sharply in the fish of the control group (Figure 1), more smoothly toward the decrease in the 1st experimental group (Figure 2), which may be associated with blood sampling or with increased demand in compensatory processes of hemostasis because of hypercoagulation. The content of AT III in fish of the second experimental group also changed significantly over time, but eventually exceeded the initial level (Figure 3). Excess of antithrombin III in blood plasma indicates hypocoagulation processes in the plasma coagulation unit.

The amount of SFMCs, which are thrombinemia markers in intravascular coagulation in humans, still remains quite high in fish compared to most mammals, which is consistent with previous studies (Berezina *et al.*, 2017). In the experiment, the same significant fluctuations of fibrin-monomer complexes with a tendency to decrease could be observed in the control and experimental groups. The SFMC concentration decreased more smoothly in the second experimental group (Figure 3). The mechanism for increasing the number of fibrin monomer complexes implied creating a large amount of SFMC during the activation of coagulation processes and increased thrombin content. And if the coagulation processes accelerated by the end of the experiment, it can be assumed that this indicator should increase. However, the quantitative characterization of SFMCs in the blood of fish and their role in the

physiology of aquatic organisms has not yet been described in the literature. Therefore, any conclusions in this regard should be made with caution.

4. CONCLUSIONS:

It should be noted that changes in the coagulogram, such as APTT reduction, increased fibrinogen level, shortened PT and TT, indicate a change in the tendency for blood to clot toward its acceleration during the experiment, not only in treated fish, but also in the control group. At the same time, a reduced concentration of SFMC may theoretically indicate hypocoagulable state. Other parameters did not change similarly in all groups, for example, AT III increased in 2nd experimental group. However, it should be highlighted that the form of the above changes between groups often differed greatly from each other.

While considering the results of the data analysis from control animals, not subjected to artificial hormonal exposure, the hypercoagulation effect may be associated with the strong short-term stress caused by handling, even in anesthetized fish. In any case, at this stage the coagulogram response to the acute and chronic action of exogenous cortisol during the days of the experiment was similar in its vector to that of intact fish. As a result, it can be concluded about a weak effect of synthetic hormones, and either insignificant or missing plasma coagulation factors to hormone-induced stress.

However, these findings require refinement and further research to describe the impact of corticosteroid treatment on plasma endogenous cortisol levels and related effects and investigate the response force of synthetic cortisol analogs on the hemostasis system compared to natural stress responses.

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Table 1. Distribution of the experimental groups.

Group	Description	Number of animals
Control group	Intact fish	8
1 st experimental group	Hormonal induction of acute stress	8
2 nd experimental group	Induced chronic stress	8
Total		24

Table 2. Coagulogram dynamics in fish after the 7th day. Source: the authors

Indicator	Prior to treatment			7 th day		
	Control group (n=8)	1 st experimental group (n=8)	2 nd experimental group (n=8)	Control group (n=8)	1 st experimental group (n=8)	2 nd experimental group (n=8)
1	2	3	4	5	6	7
TT, sec	128,43±10,38*	157,85±24,17 ^a	221,29±33,37 ^{bc}	144,9±20,4 ^c	128,75±19,98 ^b	184,58±39,30 ^b
PT, sec	296,88±101,59 ^{abc}	211,93±60,56	249,5±116,93	160,75±16,45 ^c	157,18±18,59*	257,10±69,11 ^{bc}
APTT, sec	27,05±1,06 ^{abc}	20,85±2,63 ^{abc}	25,35±4,93 ^{abc}	8,90±0,23 ^{bc}	8,13±0,38* ^b	10,30±0,53 ^{bc}
Fibrinogen, g/l	0,86±0,03 ^{ac}	0,69±0,20 ^{abc}	0,60±0,17 ^{abc}	0,77±0,05* ^{bc}	0,97±0,01	1,06±0,01 ^{bc}
SFMC, mg/100 ml	29,00±0,41 ^{bc}	28,00±0,71* ^{bc}	30,00±0,71 ^{abc}	28,50±0,29 ^{bc}	29,00±0,58 ^{bc}	27,75±1,31
AT III, %	95,54±1,99 ^{abc}	101,38±4,63 ^{abc}	90,57±13,52	65,17±3,91 ^{#b}	78,21±2,91 ^{bc}	76,69±9,08 ^c

The difference from the indicator of the first experimental group on the same day of the experiment is significant (p≤0.05)

*The difference from the indicator of the second experimental group on the same day of the experiment is significant (p≤0.05)

^a The difference from a similar indicator of a similar group on the 7th day of the experiment is significant (p≤0.05)

^b The difference from a similar indicator of a similar group on the 14th day of the experiment is significant (p≤0.05)

^c The difference from a similar indicator of a similar group on the 21st day of the experiment is significant (p≤0.05)

Notes: TT, sec – thrombin time; PT, sec - prothrombin time; APTT, sec - activated partial thromboplastin time; Fibrinogen, g/l – fibrinogen level, SFMC, mg/100 ml – soluble fibrin-monomeric complexes level; AT III, % - antithrombin III level

Table 3. Coagulogram dynamics in fish after the 14th day. Source: the authors

Indicator	Prior to treatment			14 th day		
	Control group (n=8)	1 st experimental group (n=8)	2 nd experimental group (n=8)	Control group (n=8)	1 st experimental group (n=8)	2 nd experimental group (n=8)
1	2	3	4	5	6	7
TT, sec	128,43±10,38*	157,85±24,17 ^a	221,29±33,37 ^{bc}	177,20±27,99 ^c	217,65± 35,54* ^c	107,63± 16,92
PT, sec	296,88±101,59 ^{abc}	211,93±60,56	249,5±116,93	175,10±19,76 ^c	139,03± 13,39	129,60± 7,39
APTT, sec	27,05±1,06 ^{abc}	20,85±2,63 ^{abc}	25,35±4,93 ^{abc}	7,28± 0,24 ^c	6,95± 0,30	7,33± 0,13 ^c
Fibrinogen, g/l	0,86±0,03 ^{ac}	0,69±0,20 ^{abc}	0,60±0,17 ^{abc}	0,85± 0,05* ^c	0,98± 0,02*	1,17± 0,03
SFMC, mg/100 ml	29,00±0,41 ^{bc}	28,00±0,71* ^{bc}	30,00±0,71 ^{abc}	24,25± 2,25	23,25± 1,49*	28,00± 0,00
AT III, %	95,54±1,99 ^{abc}	101,38±4,63 ^{abc}	90,57±13,52	47,66±12,55* ^{bc}	87,80± 2,75	85,01± 4,99 ^c

The difference from the indicator of the first experimental group on the same day of the experiment is significant (p≤0.05)

*The difference from the indicator of the second experimental group on the same day of the experiment is significant (p≤0.05)

^a The difference from a similar indicator of a similar group on the 7th day of the experiment is significant (p≤0.05)

^b The difference from a similar indicator of a similar group on the 14th day of the experiment is significant (p≤0.05)

^c The difference from a similar indicator of a similar group on the 21st day of the experiment is significant (p≤0.05)

Notes: TT, sec – thrombin time; PT, sec - prothrombin time; APTT, sec - activated partial thromboplastin time; Fibrinogen, g/l – fibrinogen level, SFMC, mg/100 ml – soluble fibrin-monomeric complexes level; AT III, % - antithrombin III level

Table 4. Coagulogram dynamics in fish after the 21st day. Source: the authors

Indicator	Prior to treatment			21 st day		
	Control group (n=8)	1 st experimental group (n=8)	2 nd experimental group (n=8)	Control group (n=8)	1 st experimental group (n=8)	2 nd experimental group (n=8)
1	2	3	4	5	6	7
TT, sec	128,43±10,38*	157,85±24,17 ^a	221,29±33,37 ^{bc}	119,70± 25,50	132,23± 30,92	130,33± 14,39
PT, sec	296,88±101,59 ^{abc}	211,93±60,56	249,5±116,93	110,90± 14,81	137,90± 26,45	134,88± 10,24
APTT, sec	27,05±1,06 ^{abc}	20,85±2,63 ^{abc}	25,35±4,93 ^{abc}	7,98± 0,35	7,58± 0,33	8,28± 0,52
Fibrinogen, g/l	0,86±0,03 ^{ac}	0,69±0,20 ^{abc}	0,60±0,17 ^{abc}	0,97± 0,05*	0,99± 0,02*	1,22± 0,03
SFMC, mg/100 ml	29,00±0,41 ^{bc}	28,00±0,71* ^{bc}	30,00±0,71 ^{abc}	25,50± 1,26*	24,25± 1,18*	28,00± 0,00
AT III, %	95,54±1,99 ^{abc}	101,38±4,63 ^{abc}	90,57±13,52	75,64± 10,41*	95,15± 2,83	107,04± 3,67

The difference from the indicator of the first experimental group on the same day of the experiment is significant (p≤0.05)

*The difference from the indicator of the second experimental group on the same day of the experiment is significant (p≤0.05)

^a The difference from a similar indicator of a similar group on the 7th day of the experiment is significant (p≤0.05)

^b The difference from a similar indicator of a similar group on the 14th day of the experiment is significant (p≤0.05)

^c The difference from a similar indicator of a similar group on the 21st day of the experiment is significant (p≤0.05)

Notes: TT, sec – thrombin time; PT, sec - prothrombin time; APTT, sec - activated partial thromboplastin time; Fibrinogen, g/l – fibrinogen level, SFMC, mg/100 ml – soluble fibrin-monomeric complexes level; AT III, % - antithrombin III level

Figure 1. Dynamics of coagulogram indicators in fish of the control group during the experiment. Source: the authors

Figure 2. Dynamics of coagulogram indicators in fish of the first experimental group during the experiment. Source: the authors

Figure 3. Dynamics of coagulogram indicators in fish of the second experimental group during the experiment. Source: the authors