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ANÁLISE COMPARATIVA DO EFEITO DO ESTRESSE INDUZIDO POR HORMÔNIO NA HEMOSTASE EM *CYPRINUS CARPIO*E *OREOCHROMIS NILOTICUS*

COMPARATIVE ANALYSIS OF THE EFFECT OF HORMONE-INDUCED STRESS ON HEMOSTASIS IN THE *CYPRINUS CARPIO* AND THE *OREOCHROMIS NILOTICUS*

СРАВНИТЕЛЬНЫЙ АНАЛИЗ ВЛИЯНИЯГОРМОНИНДУЦИРОВАННОГО СТРЕССА НА ГЕМОСТАЗ*CYPRINUSCARPIO*И *OREOCHROMISNILOTICUS*

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RESUMO

Introdução: A mortalidade de peixes de água doce devido ao estresse durante diversas manipulações da produção é um problema grave, que requer um conhecimento profundo dos mecanismos básicos envolvidos, incluindo o sistema de hemostasia. Portanto, no nível de aplicação, o estudo da coagulação do sangue pode realizar o diagnóstico de coagulopatias de peixes e desenvolver métodos práticos de anticoagulação preventiva e terapêutica para a piscicultura. **Objetivos:** O objetivo desta pesquisa foi avaliar comparativamente a reação de alguns parâmetros de hemostasia de duas espécies comerciais de peixes, a carpa (*Cyprinuscarpio)* e a tilápia (*Oreochromisniloticus)*, ao estresse de diferentes durações induzido por corticosteroides. **Métodos:** Os peixes foram divididos em três grupos: estressados cronicamente (indução por betametasona), estressados agudamente (indução por dexametasona) e animais controle com sangue retirado do canal hemal caudal antes do tratamento hormonal (por dexametasona e betametasona), novamente 7 e 21 dias após. **Resultados e Dicussão:** Foram estudadas alterações nos seguintes parâmetros: tempo de trombina, tempo de protrombina, tempo de tromboplastina parcial ativada, concentração de fibrinogênio, complexos de monômero de fibrina solúvel, antitrombina. Verificou-se que tanto o estresse induzido por hormônio quanto o estresse de manuseio associado à coleta de sangue aumentaram fortemente a capacidade de coagulação do sangue nas carpas (redução do tempo de protrombina em 78,5 a 86,1%, aumento do fibrinogênio em 12,7 a 100%, redução do tempo de trombina em 83,4 a 85%, e a antitrombina III diminui em 15,3 a 21,7%), enquanto nas tilápias, a aceleração da coagulação do sangue por vias intrínsecas e extrínsecas foi registrada ao final do experimento apenas em peixes com imitação de estresse crônico (redução do tempo de protrombina em 76,8%, redução do tempo de tromboplastina parcial ativada em 20,0% e 2,3 multiplicação dos complexos monômeros de fibrina solúveis). **Conclusões:** Concluiu-se que os mecanismos adaptativos do organismo da tilápia (*Oreochromisniloticus*) permitiram que a função de coagulação se recuperasse na maioria dos casos ao final do experimento em todos os grupos de peixes, ao contrário das carpas (*Cyprinuscarpio*).

Palavras-chave: *corticosteróides, sangue, coagulação, coagulograma, peixes.*

ABSTRACT

Background: The mortality of freshwater fish due to stress during various production manipulations is a severe problem, which requires a thorough understanding of the basic mechanisms involved, including the hemostasis system. Therefore, on the application level, the study of blood clotting can perform fish coagulopathies diagnostics and develop practical preventive and therapeutic anticoagulation methods for fish farming. **Aim:** The goal of this research was a comparative assessment of the reaction of some hemostasis parameters of two commercial fish species, carp *Cyprinus carpio* and tilapia *Oreochromis niloticus*, to the stress of different duration induced by corticosteroids. **Methods:** The fishes were divided into three groups: chronically stressed (induction by betamethasone), acutely stressed (induction by dexamethasone), and control animals with blood taken from the caudal hemal canal before hormone treatment(by dexamethasone and betamethasone), then 7 and 21 days after. **Results and Discussion:** Changes in the following parameters were studied: thrombin time, prothrombin time, activated partial thromboplastin time, the concentration of fibrinogen, soluble fibrin monomer

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complexes, antithrombin. It was found that both hormone-induced stress and handling stress associated with blood sampling strongly increased blood clotting ability in carps (prothrombin time decrease by 78,5-86,1%, fibrinogen increase by 12,7-100%, thrombin time decrease by 83,4-85%, and antithrombin III decreases by 15,3-21,7%), while in tilapias, acceleration of blood clotting by intrinsic and extrinsic pathways were recorded by the end of the experiment only in fishes with imitation of chronic stress (prothrombin time decrease by 76,8%, activated partial thromboplastin time decrease by 20,0%, and 2,3 multiplying soluble fibrin monomer complexes). **Conclusions:** It was concluded that the adaptive mechanisms of the tilapia (*Oreochromis niloticus*) organism allowed the clotting function to recover in most cases by the end of the experiment in all groups of fish, in contrast to carps (*Cyprinus carpio*).

Keywords: *corticosteroids, blood, coagulation, coagulogram, fish*.

АННОТАЦИЯ

Введение: Смертность пресноводных рыб в результате стресса при различных манипуляциях на производстве представляет серьезную проблему, что требует глубокого понимания основных задействованных механизмов, в том числе системы гемостаза. На прикладном уровне проведение исследования свертывания крови имеет потенциал для диагностики коагулопатий рыб и разработки практических профилактических и терапевтических противосвертывающих методов для рыбоводства. **Цель:** Целью этого исследования была сравнительная оценка реакции некоторых параметров гемостаза двух видов промысловых рыб – карпа *Cyprinuscarpio* и тиляпии *Oreochromisniloticus* в условиях экспериментальной модели стресса различной продолжительности, индуцированного кортикостероидами. **Методы:** Рыбы были подразделены на три группы: рыбы с хроническим стрессом (индукция бетаметазоном), острым стрессом (индукция дексаметазоном) и контрольные животные, кровь у которых брали из хвостового гемального канала до обработки гормонами, и далее через 7 и 21 день после. **Результаты и Обсуждение:** Было исследовано изменение следующих параметров коагулограммы: тромбиновое время, протромбиновое время, активированное частичное тромбопластиновое время, концентрацию фибриногена, растворимых фибрин-мономерных комплексов, антитромбина. Было установлено, что как индуцированный гормонами стресс, так и хэндлинг-стресс, связанным с забором крови, сильно увеличивают свертывающую способность крови у карпов (сокращение протромбинового времени на 78,5-86,1%, увеличение фибриногена на 12,7-100%, сокращение тромбопластинового времени на 83,4-85% и антитромбинаIIIна 15,3-21,7%), тогда как у тиляпий ускорение свертывания крови по внутреннему и внешнему путям к концу эксперимента было зафиксировано только у рыб с имитацией хронического стресса (снижение протромбинового времени на 76,8%, снижение активированного частичного тромбопластинового времени на 20,0%, увеличение фибрин-мономерных комплексов в 2,3 раза). **Заключение:** Можно отметить, что адаптивные механизмы организма тиляпий позволили свертывающей функции в большинстве случаев восстановиться к концу эксперимента у всех групп рыб, в отличие от карпов.

Ключевые слова: *кортикостероиды, кровь, коагуляция, коагулограмма, рыба*.

1. INTRODUCTION:

As one of the dominant carp species, *Cyprinus carpio* (common carp) is cultivated in more than 100 countries worldwide and accounts for up to 10% of the global annual freshwater aquaculture production (Xu *et al.*, 2014; Moshayedi*et al.*, 2016). *C. carpio* is also a major ornamental fish species subject for modern veterinary medicine, which has recently moved to a new stage in evolving surgical care for these animals. Also, current aquaculture production statistics from the Food and Agriculture Organization show that about 125 countries practice tilapia (*Oreochromisspp*.) farming. It accounts for 5.1% of overall aquaculture production and 10.14% of total farmed fish (Abdel-Fattah, 2019), especially in Egypt (Mehanna *et al.*, 2020).

The mortality of freshwater fish due to stress under various manipulations is a severe problem, which requires a thorough understanding of the basic mechanisms involved. Furthermore, changes in the functioning of organism physiological systems under extreme conditions are primarily reflected in hematological indices (Wendelaar Bonga, 1997). Therefore, it was empirically established that these fish are readily available and suitable for hematological studies generally and hemostasiological in particular.

Hemocoagulation is of considerable interest from veterinary, medical, and evolutionary points of view. The hemostasis system is designed to ensure the integrity of the body's internal environment and stop bleeding in

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case of damage to the vascular wall, its permeability and resistance, and maintain the liquid state of blood in the vascular channel. Rapid blood clotting is of great importance for fish life, especially bottom fish. Studies conducted on bony fishes indicate that the coagulation process is fundamentally similar to that of other vertebrates, particularly mammals. The only distinction is that it is adapted to lower temperatures. Skin mucus, which is believed to contain a large amount of thrombokinase, serves as a process accelerator (Botiazhova, 2000; Golovina, 1996; Zhichkina *et al.*, 2017; B.A. Kudryashov *et al.*, 1958). The main differences between blood clotting in fish and that in mammals lie in the predominance of internal conversion of prothrombin to thrombin in the latter, while the extrinsic pathway is probably similar (Doolittle and Surgenor, 1962; Fomina *et al.*, 2015).

An important point emphasizing the practical component and relevance of such studies is spontaneous thrombus formation described in milkfish *Chanoschanos*, skipjack *Katsuwonuspelamis*, yellowfin tuna *Thunnusalbacares*, and mullet *Mugil cephalus* (Smit and Hattingh, 1980). Also, it is not uncommon for fish in aquaculture to die unexpectedly a few days after traumatic manipulation, such as sorting. This probably occurs due to trauma capable of causing spontaneous thrombosis (Smit and Hattingh, 1980). Therefore, on an applied level, the study of blood clotting has the potential to diagnose fish diseases (Blaxhall and Daisley, 1973), and further research of clotting is necessary to develop practical preventive and therapeutic anticoagulation methods for fish farming (Smith and Hattingh, 1980; Smith, 1980).

Some data on secondary (plasmic) hemostasis in bony fish cover a small number of freshwater species, such as tilapia *Oreochromis mossambicus* (Smiley *et al.*, 2001), carp *Cyprinuscarpio* (Fujikata and Ikeda, 1985a; Fujikataand Ikeda, 1985b; Fujikataand Ikeda, 1985c; Jung andKawatsu, 1994; Kawatsu, 1986; Kawatsu *et al.*, 1991; Kawatsu and Kondo, 1989), rainbow trout *Onchorynchus mykiss* (Ruisand Bayne, 1997), and catfish *Ameiurusnebuiosus* (Langdell *et al.*, 1965). These studies revealed differences in clotting time and content of certain clotting factors in different groups of fish and emphasized the need to use validated and uniform procedures (e.g., nature of thromboplastin used, type of laboratory dishes) in hemostasis studies of these hydrobionts.

Stressors increase the clotting rate in fish. A major review of sources (Tavares-Dias and Oliveira, 2009) noted the activation of both primary and secondary hemostasis in stressed fish, including a decrease in clotting time (Ruisand Bayne, 1997), an increase in the number of platelets, a decrease in fibrinogen level (Bouckand Ball, 1966; Hattinghand Van Pletzen, 1974), a rapid reduction in plasma recalcification time, PT and APTT. According to A. A. Ivanov (Ivanov, 2021), the first stage of blood clotting, i.e., thromboplastin formation, is controlled by the hypothalamic-pituitary system and adrenaline. Cortisol, as expected, does not affect this process. This author also advises remembering that captured fish are acutely stressed fish and suggests that interspecies differences in blood clotting in fish may well be the result of differences in the resistance of these fish to stresses. Our past studies have also shown a hypercoagulation effect of hypoxic stress on carps (Berezina and Fomina, 2020).

The authors first suppose that different stress types lead to destabilization of coagulation mechanisms and activation of hypercoagulation in all fish, and second, chronic stress has the most pronounced effect on the acceleration of hemostatic reactions, not excluding the development of disseminated intravascular coagulation.

In biological practice, imitation of the stress of different duration by administration of exogenous cortisol is widely used (Espelid *et al.*, 1996; Pickering and Pottinger, 1989; Van Weerdand Komen, 1998; Vijayan *et al.*, 1997), cortisone (Mikryakov *et al.*, 2009; Mikryakov and Mikryakov, 2002; Mikryakov and Mikryakov and Mikryakov, 2005; Mikryakov *et al.*, 2007; Roth, 1972), or synthetic corticosteroids – dexamethasone (Balabanova *et al.*, 2009; Mikryakov, 2004; D. V. Mikryakov *et al.*, 2007), triamcinolone (Houghton and Matthews, 1986; Houghton and Matthews, 1990; Wechsler *et al.*, 1986), deoxycorticosterone (Mikryakov, 2004), betamethasone (Swift, 1982). Mainly, these are experiments devoted to studying the immunological function of fish blood, and all works in some way noted the suppressive effect of such hormonal treatments. A. K. Gamperl (1994) concludes that dexamethasone and betamethasone have cortisol-like effects, but their importance for assessing hormonal responses during stress may be limited.

1.1. Aim

Based on the above, the goal of this work was a comparative assessment of the reaction of

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plasma hemostasis of two species of commercial fish, carp and tilapia, to the effects of hormone-induced stress of different duration. To achieve it, the following objectives were set: (1) to study changes in coagulogram of carp and tilapia under the influence of dexamethasone, (2) the same under the influence of betamethasone, (3) to compare the dynamics of the revealed changes in the studied fish species.

2. MATERIALS AND METHODS:

2.1. Materials

The work was performed at the aquaculture development center "AquaBioCenter". The experiment was conducted on common carp *Cyprinus carpio L.* (n = 24) and Nile tilapia *Oreochromis niloticus L.* (n = 30). Fishes of each species were previously divided into three groups (Table 1). Dexamethasone phosphate (4 mg/ml) (L. V. Balabanova *et al*., 2009), which is metabolized within 4 hours, was used to simulate acute stress. Animals were treated once with dexamethasone (produced by Ellara, Russia) by parenteral injections at a dose of 0.2 ml or 0.8 mg of the active substance dexamethasone phosphate per animal unit. Betamethasone suspension (2.63 mg sodium betamethasone phosphate + 6.43 mg betamethasone dipropionate/ml) with a run-out period of more than ten days was used once as a glucocorticoid to simulate chronic stress. Diprospan (produced by Schering-Plough Labo N.V., Belgium) was injected into the fish at a dose of 0.5 ml per animal unit, corresponding to 3.5 mg of the active substance. These synthetic hormones have similar effects to corticosteroids (Gamperl *et al.*, 1994; Mazeaud *et al.*, 1977). The control group remained intact. The number of animals required for the experiment corresponded to Mead's resource equation (Van Zutphen *et al.*, 2001).

The fish were kept in a test setup with continuous water circulation between aquariums and forced aeration: carps at water temperature 18-20°C, tilapias – 28-30°C according to the rules of keeping adult fish as test subjects (Federal Agency for Fisheries, 2009). Blood sampling from animals participating in the experiment was performed immediately after acclimatization and further at 7 and 21 days after drug injection. Before blood sampling, fish were anesthetized by adding clove oil in water at a dose of 0.033 ml/l (Hamackova *et al.*, 2006) with subsequent exposure for 15 minutes. Then, blood sampling was performed into glass tubes by puncturing the caudal hemal canal with 3.8% sodium citrate. After blood sampling, individual labeling of fish was performed. Coagulation testing and separation of blood serum were performed in the first two hours after blood sampling.

2.2. Methods

The parameters of plasma-coagulation hemostasis were determined on coagulometer "Thrombostat" produced by BehnkElektronik (Germany) (Fomina *et al.*, 2017) with additional use of medical kits. To assess the state of plasma-coagulation hemostasis, APTT, PT, TT parameters were determined using a medical set of reagents (Thrombo-test™, Techplastin-test™, APTT-test™, LLC Technology-Standard, Russia) and quantitative analysis of fibrinogen(Fibrinogen-test™, RPARENAM, Russia). In order to define TT per 0,1 ml of plasma analyzed, 0,1 ml of human thrombine solution was used. Plasma incubation was conducted under 37°C. In order to define PT, 0,2 ml of thromboplastin-calcium mixture was used (thromboplastin presented as extraction from the brain of a rabbit). To define APTT, 0,1 ml of kaolin-cephalin solution and 0,1 ml of calcium chloride solution were used. To define the fibrinogen level, 0.05 ml of human thrombine solution was used.

Anticoagulation properties of the blood were assessed by the content of antithrombin-like factor (AT III) in plasma (ChromotechAntithrombin™, LLCTechnology-Standard, Russia). Fibrinolytic activity in the plasma was measured using SFMC detection (SFMC-test™, pad variant, LLC Technology-Standard, Russia). Analysis was performed following the instructions of the manufacturers. Defining ATIII concentration involved 0,25 ml of analyzed plasma diluted with heparin, 0,5 ml of a chromogenic substrate, 0,25 ml of human thrombine solution, 0,5 ml of 30% acetic acid solution. The volume of FMC was defined under 18-25 °C using 0,1 ml of o-phenanthroline solution and 0,1 ml of analyzed plasma.

The values of outcomes are presented as Mean and Standard Error of the Mean (M ± SE). The reliability of differences of the blood parameters for multiple independent samples was determined using Kruskal-Wallis one-way analysis of variance; for paired dependent samples – Wilcoxon signed-rank test.

3. RESULTS AND DISCUSSION:

3.1. Results

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3.1.1. Carps

Coagulogram data obtained during the experiment are presented in Table 2. When assessing the changes, it can be observed that APTT of all carp groups significantly changed (Figure 1) synchronously and unidirectionally towards reliable reduction by 63.6-70.4% compared with the initial value.

Noteworthy is also a reliable PT severe reduction (Figure 2) by the last day of exposure to hormone-induced stress in all groups of carps by 78.5-86.1% compared with the initial value; in fish, with simulated acute stress, it was sharper. The quantitative content of fibrinogen in blood plasma in all groups of carps during the experiment has increased reliably by 12.7%, 43.4%, and two times respectively (Figure 3). The variation curve in the first and second experimental groups of animals treated with hormones has a similar shape to the control group. It should be noted that under the influence of betamethasone, the increase in fibrinogen was the most obvious.

Analyzing TT in dynamics (Figure 4), it is clear that this time, like PT, significantly decreased by the last day of the experiment almost synchronously by 83.4-85% relative to the initial time in all groups of carps.

AT III level changed reliably: in carps of the control group – more sharply (by 31.7%), in the first experimental group – more smoothly (by 22.8%), in the second experimental group – by 15.3% towards decrease by the middle of the experiment, which may be connected with blood intake, or with the increased need for compensatory processes of hemostasis associated with hypercoagulation. AT III content in carps of the second experimental group eventually has exceeded the value of the initial level (by 18.2%). There is a general excess of antithrombin in plasma in the experimental groups compared with the control animals during the days of the experiment.

During the study, the control and experimental groups of carps showed the same reliable SFMC fluctuations with a tendency to decrease by 6.6-13.3%. SFMC concentration of the second experimental group decreased more smoothly. If the coagulation processes are accelerated by the end of the experiment, so this indicator should increase. However, SFMC quantitative characteristics in fish blood and their role in the physiology of hydrobionts were not yet described in the literature. Therefore, any conclusions on this subject should be made cautiously.

Thus, the hemostasis of carps responded to the stress reactions by a high acceleration of clotting along the primary pathways in all groups studied, as evidenced by the decrease in TT, PT, APTT, and increase in fibrinogen level. Although the essential trend of changes was the same, the dynamics differed slightly in the treated fish – especially the fibrinogen level under the influence of betamethasone. At the same time, the antithrombin level in the latter decreased much less compared with the control, which also indicates a decrease in the activity of anticoagulation processes.

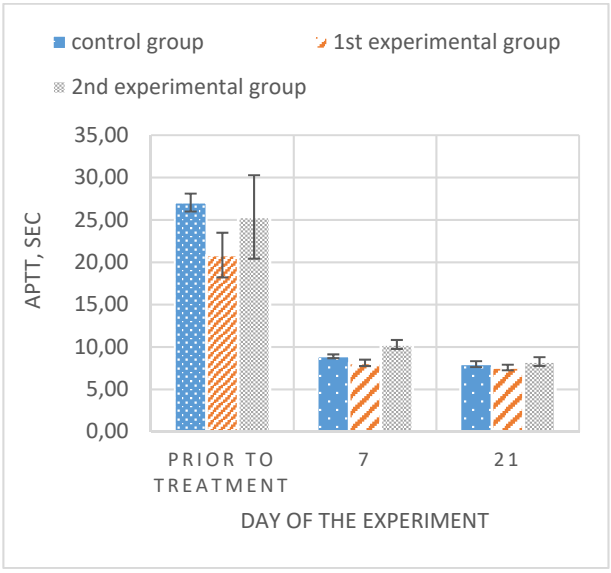

Figure 1. Dynamics of APTT in carps during the experiment. Vertical bars represent M ± SE.

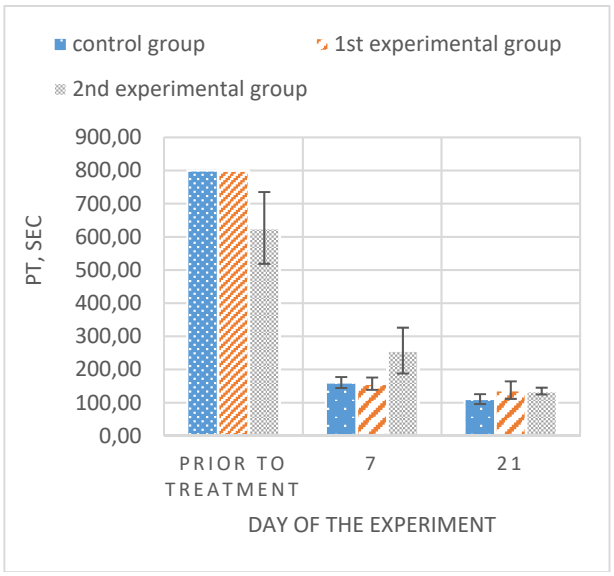

Figure 2. Dynamics of PT in carps during the

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experiment. Vertical bars represent M ± SE.

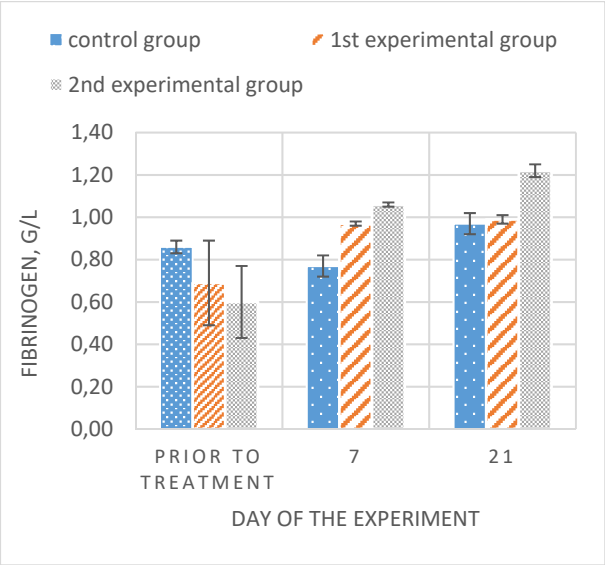

Figure 3. Dynamics of Fibrinogen in carps during the experiment. Vertical bars represent M ± SE.

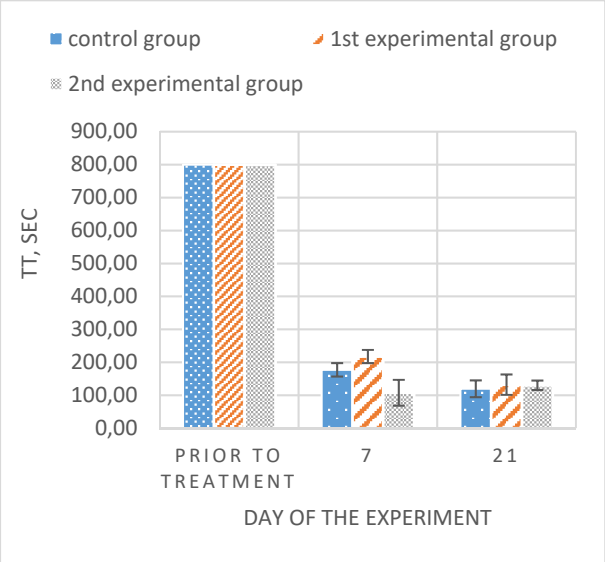

Figure 4. Dynamics of TT in carps during the experiment. Vertical bars represent M ± SE.

3.1.2. Tilapias

The experimental data are presented in Table 3. The APTT of tilapias (Figure 5) had a similar trend of change for carps only in the second experimental group, expressed as a short-term reduction by the 7th day by 41.5%, and by the last day remained 20% shorter relative to the initial value. In contrast, APTT of the first experimental and control groups extended by the 7th day of the experiment by 66.5% and 79.2%, respectively, and remained longer than the initial value by 34% and 98%, respectively.

The chronically stressed fish experienced a reliable sharp decrease in PT (Figure 6) to dangerously low by 76.8% of the initial value. The control and acutely stressed groups (the latter reliably) reacted similarly by an adaptive increase of time in the middle of the experiment (by 78.2-109.4%) and shortening to almost initial values by the end.

The opposite pattern of changes is in the fibrinogen concentration in all groups of fish (Figure 7). A decrease of fibrinogen by 29.5 to 70% occurred by the 7th day, especially in the second group. Then the level of this protein increased in all fish close to the initial level. TT of tilapias (Figure 8) had similar dynamics to fibrinogen level: adaptive time decrease in the middle of the experiment by 9% to 22.5% and time recovery by the end in all groups (except the control), the strongest and most reliable in the fish of the first experimental group.

AT III level in all tilapias fluctuated insignificantly. SFMC concentration in fish of the second experimental group doubled (2.3 times) by the end of the experiment, while in other groups, it decreased similarly to carps amounting to 33.5-44.4% less than the initial.

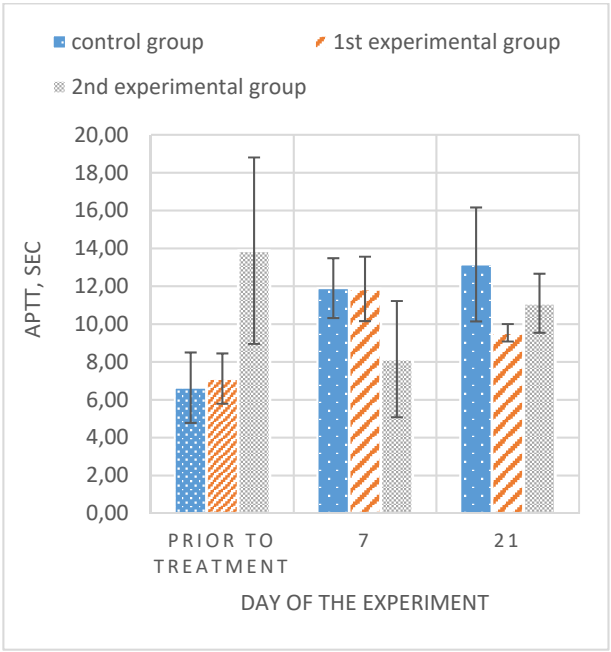

Figure 5. Dynamics of APTT in tilapia during the experiment. Vertical bars represent M ± SE.

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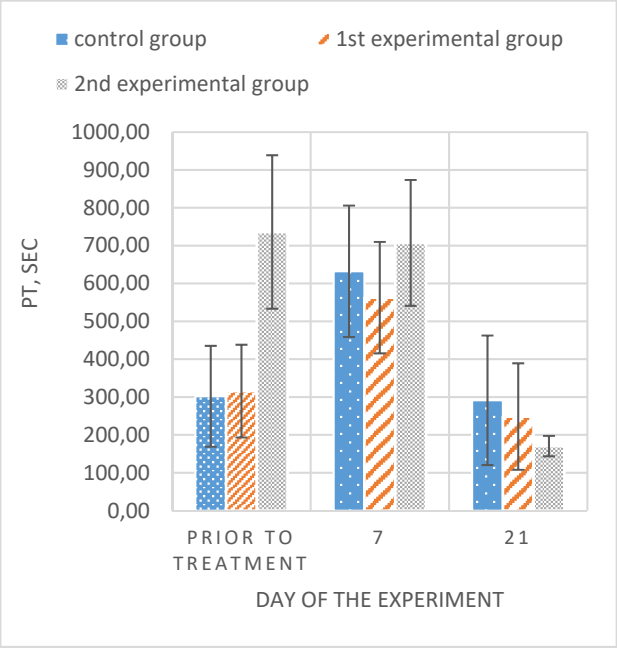

Figure 6. Dynamics of PT in tilapia during the experiment. Vertical bars represent M ± SE.

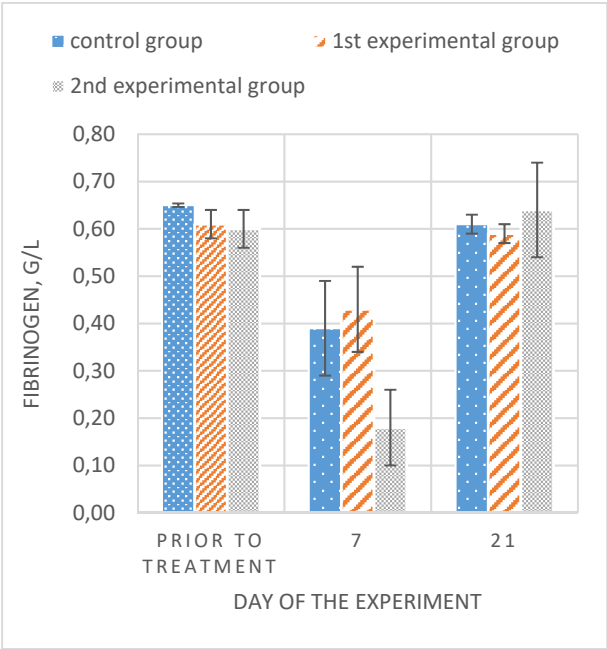

Figure 7. Dynamics of Fibrinogen in tilapia during the experiment. Vertical bars represent M ± SE.

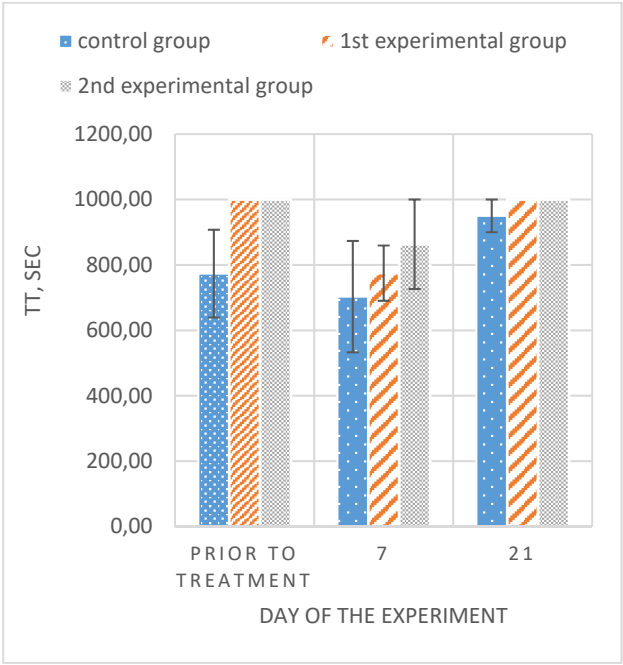

Figure 8. Dynamics of TT in tilapia during the experiment. Vertical bars represent M ± SE.

Thus, acceleration of blood coagulation in tilapias by intrinsic and extrinsic pathways by the end of the experiment was recorded only in fish with imitation of chronic stress (reduction in PT, APTT, and increase in SFMC). In other groups, coagulation by the common pathway (reduction in TT and fibrinogen) first increased, then slowed down by intrinsic and extrinsic pathways, and later all parameters became close to the initial values.

3.2. Discussion

From few literature sources, we can learn that in carps, APTT is 5.1 ± 0.7 sec (Kawatsu, 1986), which is significantly less than what we obtained all the time. The literature suggests a PT in carps equal to 23.5 ± 3.4 sec (Kawatsu, 1986), which is also lower than in our studies.

PT increase indicates insufficiency of the extrinsic hemostasis component, APTT – intrinsic component, TT– general component, and shortening indicates their activation and development of hypercoagulation and thrombogenesis, which may be caused by corticosteroid exposure. As shown in the experiments on the effect of stress on clotting involving mice (Polidanov *et al.*, 2020), the coagulation mechanisms were accelerated similarly. In the same work, the authors note a stress-induced increase in the level of blood plasma fibrinogen, which, as we also showed earlier (Berezina and Fomina, 2018), turned out to be true for carps as well.

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Although according to literature sources, antithrombin is not detected in hydrobionts (Jordan, 1983), we recorded the content of antithrombin-like factors in both carps and tilapias using the traditional medical test. However, the nature of this factor should be clarified. An excess of AT III in blood plasma indicates hypocoagulation processes in the plasma-coagulation hemostasis component, and deficiency leads to a lack of thrombolysis.

Our hypothesis about the clotting accelerating under stress was confirmed only partially because the clotting accelerating was detected even in the control group of carps. It remains a debatable question if the control group fish should be considered as relevant blood sampling in the context of handling stress. But if control group results can be acknowledged as relevant, it would appear that even handling stress, taking place in various manipulations with fish, can cause destabilization of clotting mechanisms in carps, leading toward hypercoagulation for quite a long time. In tilapias, these processes are also activated, but not for a long time.

The hypothesis about the impact of chronic stress caused by betamethasone injection is also partly confirmed since the dynamic pattern of some parameters in the carps of the 1st test group is explained by a higher increase in blood clotting. Furthermore, chronic stress affects tilapias at its peak: hemocoagulation is accelerated by intrinsic and extrinsic pathways at the end of the experiment, in contrast to other groups.

4. CONCLUSIONS

It can be concluded that all types of stress (including handling stress associated with blood sampling) strongly increase blood clotting ability in carps (Latin name of the species here), while intrinsic and extrinsic pathways of hemostasis activation in tilapias are more resistant under short-term and handling stress. It was noted that the adaptive mechanisms of the tilapia (Latin name of the species here) organism allowed the coagulation function to recover in most cases by the end of the experiment in all groups of fish, in contrast to carps.

Also, given the coagulogram characteristics of intact carps and tilapias, it is difficult to reliably detect interspecies differences in clotting and not just the effect of stress tolerance since handling stress is based on the results is a similar short-term stressor.

The author advises to take care when analyzing the conclusions obtained and remember that, at the moment, there are no developed standardized techniques and reagents for the study of coagulogram parameters of poikilothermic animals in general and fish in particular. Therefore, this topic requires further research in this direction.

5. DECLARATIONS

5.1. Study Limitations

No limitations were known at the time of the study.

5.2. Funding source

The Russian Foundation for Basic Research funded the reported study (RFBR), Project Number 19–34–90109.

5.3. Competing Interests

The authors declare no conflict of interest.

5.4. Open Access

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6. HUMAN AND ANIMAL-RELATED STUDIES

6.1. Ethical Approval

This research was conducted in strict accordance with ethical principles established by the European Convent on the protection of the Vertebrata used for experimental and other scientific purposes (adopted in Strasbourg on March 18, 1986, and confirmed in Strasbourg on June 15, 2006) and approved by the local Ethics Committee of the Vologda State Dairy Farming Academy (VSDFA) named after N.V.

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Vereshchagin (Record No. 12 dated December 3, 2015).

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Table 1. *Characteristics of fish groups in the experiment on the effect of hormone-induced stress*

Group	Effect	Model
Control group carps n = 8 tilapias n = 10	Intact	Control
1st experimental group carps n = 8 tilapias n = 10	Single-injection of dexamethasone phosphate solution (4 mg/ml) at a dose of 0.2 ml/animal unit	Acute stress
2nd experimental group carps n = 8 tilapias n = 10	Single-injection of betamethasone suspension at a dose of 0.5 ml/animal unit	Chronic stress

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Table 2. *Dynamics of coagulogram parameters of carps during the experiment (n = 24) (#– Differences with the performance of the first experimental group on the same day of the experiment are reliable (p ≤ 0,05); * – Differences with the performance of the second experimental group on the same day of the experiment are reliable (p ≤ 0,05); b– Differences with the similar performance of the similar group on the 7th day of the experiment are reliable (p ≤ 0.05); c– Differences with the similar performance of the similar group on the 14th day of the experiment are reliable (p ≤ 0.05); d– Differences with the similar performance of the similar group on the 21st day of the experiment are reliable (p ≤ 0.05)*

Parameter name	Before treatment			7th day			21st day		
	Control group	1st experimental group	2nd experimental group	Control group	1st experimental group	2nd experimental group	Control group	1st experimental group	2nd experimental group
TT, sec	>800^{bcd}	>800^{bcd}	>800.00^{bcd}	144.9 ± 20.4^{d}	128.75 ± 19.98^{c}	184.58 ± 39.30^{c}	119.70 ±25.50	132.23 ±30.92	130.33 ±14.39
PT, sec	>800^{bcd}	>800^{bcd}	626.93 ± 108.12^{bcd}	160.75± 16.45^{d}	157.18 ± 18.5*	257.10 ± 69.11^{cd}	110.90 ±14.81	137.90 ±26.45	134.88 ±10.24
APTT, sec	27.05± 1.06^{bcd}	20.85± 2.63^{bcd}	25.35± 4.93^{bcd}	8.90± 0.23^{cd}	8.13± 0.38*^{c}	10.30± 0.53^{cd}	7.98±0.35	7.58±0.33	8.28±0.52
Fibrinogen, g/l	0.86± 0.03^{bd}	0.69± 0.20^{bcd}	0.60± 0.17^{bcd}	0.77± 0.05*^{cd}	0.97± 0.01	1.06± 0.01^{cd}	0.97±0.05*	0.99±0.02*	1.22±0.03
AT III, %	29.00± 0.41^{cd}	28.00± 0.71*^{d}	30.00± 0.71^{bcd}	28.50 ± 0.29^{cd}	29.00± 0.58^{cd}	27.75± 1.31	25.50± 1.26*	24.25± 1.18*	28.00± 0.00
SFMC, mg/100 ml	95.54± 1.99^{bcd}	101.38 ± 4.63^{bcd}	90.57± 13.52	65.17 ± 3.91#^{c}	78.21± 2.91^{cd}	76.69± 9.08^{d}	75.64± 10.41*	95.15± 2.83	107.04 ±3.67

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Table 3. *Dynamics of coagulogram parameters of tilapias during the experiment (n = 30) (#– Differences with the performance of the first experimental group on the same day of the experiment are reliable (p ≤ 0,05); * – Differences with the performance of the second experimental group on the same day of the experiment are reliable (p ≤ 0,05); b– Differences with the similar performance of the similar group on the 7th day of the experiment are reliable (p ≤ 0.05); c– Differences with the similar performance of the similar group on the 21st day of the experiment are reliable (p ≤ 0.05)*

Parameter name	Before treatment			7th day			21st day		
	Control group	1st experimental group	2nd experimental group	Control group	1st experimental group	2nd experimental group	Control group	1st experimental group	2nd experimental group
TT, sec	773.46± 134.21	>1000 b	>1000	703.00± 170.32	774.86 ± 84.53c	863.28 ± 136.73	950.00 ± 50.00	>1000	>1000
PT, sec	301.94± 133.31b	315.70± 122.56b	736.08 ± 202.81	632.30± 173.65	562.60 ±147.26c	707.25 ± 166.24c	291.35 ± 171.12	248.56 ± 140.69	170.40± 26.97
APTT, sec	6.64± 1.86*bc	7.12± 1.33*bc	13.88± 3.82	11.90 ± 1.58	11.86± 1.70	8.15± 3.07	13.15± 3.01	9.54± 0.46	11.10± 1.56
Fibrinogen, g/l	0.65± 0.004bc	0.61± 0.03b	0.60± 0.04b	0.39± 0.10c	0.43± 0.09*c	0.18± 0.08c	0.61± 0.02	0.59± 0.02	0.64± 0.10
AT III, %	126.60± 31.83	175.00± 42.42	165.25 ± 53.67	119.50± 33.05	167.60 ± 44.23	178.00 ± 108.03	122.00 ± 34.35	187.20 ± 12.80	167.33± 141.35
SFMC, mg/100 ml	15.80 ± 2.18bc	18.00 ± 2.98	10.00± 6.04	5.13± 0.92*	13.40± 4.30	17.00± 3.24	10.50± 4.97	10.00± 3.49*	22.67± 0.67

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