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RETRACTION

Comparative physiological aspects of plasma hemostasis of some commercial fish species

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Abstract: The hemostasis system is designed to ensure the integrity of the body's internal environment, stop bleeding, and maintain a liquid state of blood in the vascular channel. Modern biological and veterinary science presents highly fragmented and scarce data containing clinical and diagnostic clotting characteristics in different fish species. An essential point emphasising the practical component of such studies is spontaneous thrombus formation in fish farming described in the literature. The present research is devoted to the study of the functional state of plasma hemostasis in some ray-finned commercial fishes: phylogenetically more ancient cartilaginous ganoids – sturgeon *Acipenser baerii* and hybrid of sterlet *A. ruthenus* and starred sturgeon *A. stellatus*, as well as bony fishes – carp *Cyprinus carpio* and tilapia *Oreochromis niloticus*. It should be noted that the current study was performed at the aquaculture development center "AquaBioCenter" of VSDFA from 2015 to 2020. Species-specific features of clotting were revealed: activation by common and extrinsic pathways, characterised by thrombin time (*TT*), prothrombin time (*PT*), and fibrinogen concentration, is several times faster in cartilaginous ganoids than in both bony fish species; hemostasis with activation of the intrinsic pathway, characterised by activated partial thromboplastin time (*APTT*), is faster in hybrids and tilapias, in contrast to carps and sturgeons. Content of soluble fibrin monomer complexes (*SFMC*) in all fish was higher than in dogs and humans but lower than in cattle. The highest amount of *SFMC* was detected in carps, the lowest – in cartilaginous ganoids.

Keywords: blood, carp, clotting, coagulogram, commercial fish species, plasma hemostasis, sturgeon, tilapia

INTRODUCTION

The clotting (blood coagulation) system appeared before the descent of tetrapods and bony fishes about 430 mln years ago. The hematological triad of roundworms, cartilaginous fishes, and cartilaginous ganoids formed the main evolutionary path of the blood system by natural selection. The intrinsic, extrinsic, and common pathways of the clotting system of marine and freshwater bony fishes have been described by colleagues [Lewis 1996; Obeta *et al.* 2019], including theoretical and experimental aspects. Along with this, the genes of multiple factors comprising the hemostatic response cascade have been characterised, and the ways of this essential function formation in phylogeny have been proposed [Jagadeeswaran *et al.* 2005].

Rapid blood clotting is very important for fish life, especially benthic fish. Studies carried out on bony fishes indicate that the coagulation process is fundamentally similar to other vertebrates, particularly mammals, with the only difference being that it is adapted to lower temperatures. The main thrombogenic protein components have been found in fish: thrombotropin, prothrombokinase and thrombokinase, prothrombin, thrombin, and fibrinogen [Blanco-Abad *et al.* 2018; Doolittle, Surgenor 1962; Schmid, Fent 2020]. The abovementioned genetic and routine laboratory screening tests used for human blood [Smit, Schoonbee 1988] also show that fish blood clotting factors are similar to those in mammalian or human blood, and the coagulation factors cascade also involves three classical processes (phases).

Enzymes involved in the blood clotting of fish can work in a wider range of temperature than in warm-blooded species. Blood clotting in fishes: loach (*Cobitidae*), perch (*Perca*), sterlet (*Acipenser ruthenus*), sturgeon (Acipenseridae), carp (*Cyprinus carpio*), and gudgeon *Gobio gobio*), is almost instantaneous, i.e., within 10–12 s, whereas in mammals and birds – within 2–12 min. Skin mucus, which is believed to contain a large amount of thrombokinase, serves as a process accelerator [Little *et al.* 2020; Perez-Ruzafa *et al.* 2018].

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. Platelets in fish play a central role in the internal conversion of prothrombin to thrombin and are responsible for clot retraction, although the nature of platelet factors promoting clotting is unknown [Doolittle, Surgenor 1962].

As for bony fish of fishery importance, 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 (*Cyprinus carpio*) [Kawatsu 1986], rainbow trout (*Onchorynchus mykiss*) [Ruis, Bayne 1997], and catfish (*Ameiurus nebuiosus*) [Langdell *et al.* 1965].

Lewis [1996] studied the coagulation cascade of vertebrates, including cartilaginous fish, bony fish, and cartilaginous ganoids. This work quantitatively characterised the hemostasis functioning of valuable fish species like sturgeon (*Acipenseridae*), arctic trout (*Salvelinus alpinus*), flounder (*Paralichthys dentatus*), sea bass (*Dicentrarchus labrax*), mullet (*Mugil cephalus*), and others. These studies revealed differences in clotting time and content of certain clotting factors in different groups of fish. They emphasised the need to use validated and uniform procedures (e.g., nature of thromboplastin used, type of laboratory dishes) in hemostasis studies of these hydrobionts. Douda *et al.* [2017] suggest that interspecies differences in blood clotting in fish may well be the result of differences in the resistance of these fish to stress. We assume that the functioning of the plasma hemostasis system has species specificity regardless of stress resistance, especially in commercial fish belonging to different classes.

An important point emphasising the practical component of such studies is spontaneous thrombus formation described in milkfish (*Chanos chanos*), skipjack (*Katsuwonus pelamis*), yellowfin tuna (*Thunnus albacares*), and mullet (*Mugil cephalus*) [Smith 1980]. Also, it is quite common for fish in aquaculture to die unexpectedly a few days after traumatic manipulation, such as sorting.

The main target of our study was a comprehensive assessment of the plasma component of the hemostasis system of some common species of commercial fish. To achieve it, we had to solve the following tasks: (1) to study the functional state of the plasma component of hemostasis in commercial species of cartilaginous ganoids and bony fishes; (2) to compare the pathways of coagulation activation in fish of different classes.

MATERIALS AND METHODS

STUDY DESIGN

The work was performed at the aquaculture development center "AquaBioCenter" of Vologda State Dairy Farming Academy (VSDFA) named after N.V. Vereshchagin (Rus. Vologodskaya gosudarstvennaya molochnokhozyaystvennaya akademiya imeni N.V. Vereshchagina) from 2015 to 2020. Studies were conducted in aquarium conditions on important fishery species of bony fishes (*Osteichthyes*) belonging to two different classes. Animal units of the bony fish class (*Teleostei*) – common carp (*Cyprinus carpio* L.) and Nile tilapia (*Oreochromis niloticus* L.) were used in the study. Animal units of Siberian sturgeon (*Acipenser baerii* B.) and hybrid of sterlet (*Acipenser ruthenus* L.) and starred sturgeon (*Acipenser stellatus* P.) were taken as cartilaginous ganoids (*Chondrostei*).

The study of *Acipenseridae* is of particular interest because they are members of an ancient group closer to vertebrate evolution's main path than bony fishes [Benun Sutton, Wilson 2019]. All fish species had a large mass and were suitable for hemostasiological studies involving taking large volumes of biomaterial. Fish were farmed commercially in the fish company Diana LLC (Vologda Region) and AquaBioCenter. The fish were kept in aerated aquariums: cold-loving at 16–18°C, heat-loving at 28–30°C. The acclimatisation period before the study was 48 h. Before blood sampling, fish were anesthetised by adding clove oil in water at a dose of 0.033 $cm^3 \cdot dm^{-3}$ with subsequent exposure for 15 min. Blood sampling was performed into glass tubes by puncturing the caudal hemal canal with 3.8% sodium citrate with a plastic syringe. The study object was platelet-poor plasma (PPP) obtained by blood centrifugation at 3000 rpm for 15 min.

STATISTICAL ANALYSIS

The values of outcomes are presented as mean and standard error of the mean ($M \pm SE$). Reliability of differences of blood parameters for multiple independent samples was determined using Kruskal–Wallis one-way analysis of variance.

RESULTS AND DISCUSSION

Secondary hemostasis is mainly performed by plasma clotting factors and includes three phases, the functional state of which in the studied fish is presented in Table 1. Activated partial thromboplastin time (*APTT*) characterises the first phase of blood coagulation (prothrombinase formation). It describes the intrinsic pathway of coagulation activation, prothrombin formation. It is a multistep process that results in the accumulation of a complex of factors in the blood that can convert prothrombin into thrombin [Bonar *et al.* 2017]. According to the results of our study (Fig. 1), *APTT* was reliably different in sturgeon and carp from hybrid and tilapia. *APTT* of sturgeon was 20.6 times longer than that of the related hybrid and 16.8 times longer than that of tilapia. *APTT* of carp was 2.8 and 2.2 times longer, respectively.

Prothrombin time (*PT*) indicates the activity of the extrinsic clotting pathway. This parameter characterises the first (prothrombinase formation) and second (thrombin formation) phases of plasma hemostasis and reflects the activity of the prothrombin complex [Bonar *et al.* 2017]. Literature data indicate *APTT* and *PT* values in carps much less than the values we obtained [Kawatsu 1986]. When evaluating the extrinsic clotting pathway, which is of predominant importance in fish [Doolittle, Surgenor 1962; Sheehan *et al.* 2001], it may be noted that cartilaginous ganoids have 1.9–2.6 times reliably more rapid clot formation when tissue factor is added than bony fish.

Table 1. Parameters of the plasma-coagulation component of hemostasis in commercial fish

Parameter	*Acipenser baerii* (*n* = 12)	*Acipenser ruthenus* × *A. stellatus* (*n* = 12)	*Cyprinus carpio* (*n* = 30)	*Oreochromis niloticus* (*n* = 14)
TT (s)	28.02 ±10.23^{ct}	24.94 ±5.19^{ct}	500.36 ±59.42^{sh}	648.38 ±38.70^{sh}
PT (s)	241.28 ±34.99^{c}	176.85 ±42.67^{c}	457.31 ±58.32^{sh}	316.96 ±54.67
APTT (s)	148.06 ±54.75^{ht}	7.18 ±1.40^{sc}	20.12 ±2.04^{ht}	8.88 ±1.52^{sc}
Fibrinogen (g·dm^{-3})	3.02 ±0.38^{t}	3.79 ±0.79^{ct}	1.99 ±0.33^{ht}	0.62 ±0.01^{shc}
SFMC (mg·(100 cm^3)$^{-1}$))	17.63 ±2.51	7.53 ±1.61^{c}	24.90 ±1.61^{ht}	14.93 ±2.17^{c}

Explanations: *n* = number of samples, *TT* = thrombin time, *PT* = prothrombin time, *APTT* = activated partial thromboplastin time, *SFMC* = soluble fibrin monomer complexes; differences are reliable ($p \leq 0.01$): ^{s} = with sturgeon (*Acipenser baerii*); ^{h} = with a hybrid (*Acipenser ruthenus* × *A. stellatus*); ^{c} = with carp (*Cyprinus carpio*); ^{t} = with tilapia (*Oreochromis niloticus*).
Source: own study.

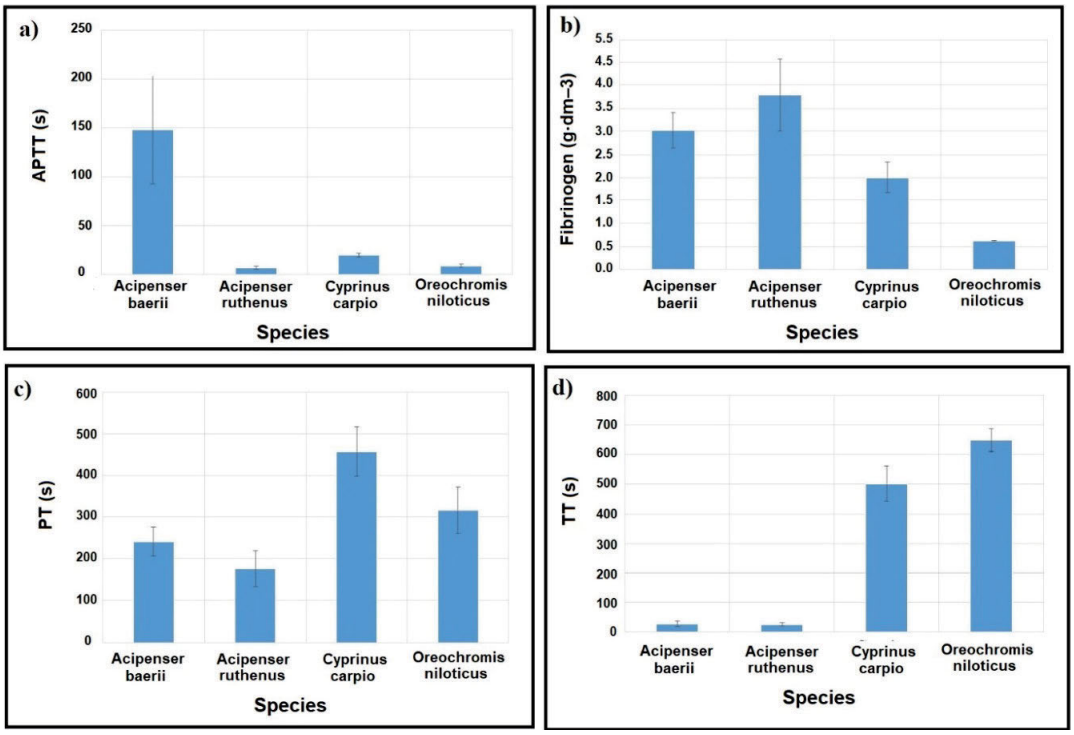

Fig. 1. Parameters of plasma hemostasis in fish: a) activated partial thromboplastin time (*APTT*), b) fibrinogen concentration, c) prothrombin time (*PT*), d) thrombin time (*TT*); vertical bars represent ±1 *SE*; source: own study

The third phase of blood coagulation (fibrin formation) was assessed using fibrinogen and thrombin time (*TT*) values. The fibrinogen quantitative content in blood plasma providing clot formation was the lowest in tilapias, 4.8–6.11 times lower than in hybrids, and 3.2 times lower than in carps. *TT* is a screening test of the last phase of blood clotting, reflecting the rate of fibrinogen to fibrin conversion [Bonar *et al.* 2017]. Analysing obtained characteristics of the plasma-coagulation component of the bony fish clotting system and comparing them with the corresponding ones of cartilaginous ganoids, it can be said that the rate of fibrin clot formation (*TT*) in the former is reliably higher by 17.8–26.0 times than in the latter.

We also assessed the soluble fibrin monomer complexes (*SFMC*) content in fish blood plasma. *SFMC* are small fragments of blood clots formed during massive thrombosis as a result of the thrombus breaking after the healing of the vascular wall. Interesting that they are markers of thrombinemia in human intravascular clotting (DIC syndrome) [Dubova *et al.* 2020; Rauch *et al.* 2020] and usually do not exceed 3 ±0.1 mg·(100 cm^3)$^{-1}$. The analysis of the obtained data shows that carps have *SFMC* in 3.3 and 1.7 times reliably more than hybrids and tilapia, respectively. Meanwhile, this is 1.3–4.3 times higher than *SFMC* in dogs and 2.5–8.3 times higher than humans have. However, except for carps which *SFMC* is 1.4 times higher, other fish species have 1–2.4 times lower *SFMC* than cattle.

CONCLUSIONS

The data obtained confirm the vast variability of coagulation parameters in fish of different classes and fishery importance. It is worth considering the identified species specificity when developing and implementing diagnostic and therapeutic methods. Also, by the basic clinical diagnostic tests, it is possible to conclude the functioning of the clotting cascade in commercial fish. Its activation by the common pathway is several times faster

in cartilaginous ganoids than in both species of bony fish. This observation also finds its confirmation in the difference in the level of fibrinogen – its highest amount was detected in cartilaginous ganoids, the lowest – in tilapias. Carp also have slower clotting by the extrinsic pathway. Hemostasis with activation of the intrinsic pathway is faster in hybrids and tilapias, unlike carp and sturgeon. The highest amount of fibrin degradation products were detected in carp, which, along with the slowing of prothrombin time (*PT*) and activated partial thromboplastin time (*APTT*), may indicate the activity of the processes of thrombosis and fibrinolysis with the revealed signs of hypocoagulation due to stress. The lowest soluble fibrin monomer complexes (*SFMC*) was in hybrid, which can be explained by the coagulation processes activity and fibrinolysis inactivity with the rest of the data.

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