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Reference intervals for some clinical biochemical parameters and certain markers of inflammation in a female paddlefish (*Polyodon spathula*) in Bulgaria

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Abstract. The American paddlefish, (*Polyodon spathula*), belongs to the order Acipenseriformes, along with the other sturgeon species. It has been cultivated in Bulgaria's aquaculture since 2003, but there is still a gap in knowledge about the biology of this species. Therefore, the current study aimed to establish reference normal ranges of basic clinical biochemical parameters and markers of inflammation in a healthy female mature paddlefish being reared in polyculture with carp in earthen fish ponds. For the present study, 8 summer old healthy female mature individuals were selected. The blood reference intervals were calculated according to the non-parametric method. The findings from the present study may not be accurate for large-scale clinical interpretation but can certainly be a starting point and guide practitioners in their actions and practice.

Key words: Biochemical parameters, inflammatory markers, *Polyodon spathula*

Introduction

The blood biochemistry profile can be used as a useful indicator of the physiological and health status of fish (Weber & Innis, 2007). Blood biochemistry parameter values are specific for any different species and depend on many abiotic (e.g. environment) and biotic (e.g. pathogens) factors. Of course, the correct interpretation of these parameters depends on the availability of reference intervals for comparison (Casanovas *et al.* 2021). In general, sturgeon fishes are divided into two main extant families, Acipenseridae and Polyodontidae. The family Polyodontidae is a family of ray-finned fishes that includes the genera *Psephurus* and *Polyodon* (Káldy *et al.* 2020). The paddlefish (*Polyodon spathula*) is an endemic species found in the wild only in North America. In Europe, the acclimatisation and aquaculture of paddlefish started in the last century in Eastern and Central Europe (Jarić *et al.* 2019). In Bulgarian aquaculture, this exotic sturgeon species was first introduced in polyculture with carp in 2003 (Hubenov *et al.*, 2004). However, Nikolova & Stoyanova (2022) observed that there is still a paucity of data on the morpho-physiological characteristics of paddlefish in Bulgaria when reared in polyculture with carp.

The aim of this study is to determine the reference values of the main clinical blood biochemical parameters and certain markers of inflammation in paddlefish (*Polyodon spathula*), in order to provide more detailed information on this species cultivated in polyculture with carp in earthen ponds.

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Material and Methods

This study was conducted in accordance with the Guidelines of the European Union (2010/63/UE) and Law on Veterinary and Medical Activities and Animal Welfare Act. A total of 80 female mature paddlefish, aged eight summers, were collected from two earthen fish ponds in fish farm Tundzha 73, situated near the city of Nikolaevo in Bulgaria. The fish were captured using large pond drag nets during the period May-June 2023. Disposable 18G needles and test tubes containing heparin were employed to procure 3ml of blood from the v. caudalis of each fish. The blood samples were transported to the laboratory in a cooler bag and centrifuged at 3000 rpm for 5 min at room temperature. The plasma biochemical concentrations were analysed using an automatic biochemical analyser (Mindray BS-120, China), with the use of standard laboratory kits (Biolabo SAS, France). The concentration of ceruloplasmin was determined spectrophotometrically by using Revin's method with p-phenylenediamine (Bestujeva & Kolb 1982). The high sensitive C- Reactive Protein analyses were conducted with ELISA kit (AFG Scientific, Northbrook, USA).

A one-way analysis of variance (ANOVA) was conducted to perform the statistical investigation (StatSoft Inc., 2002). All dates are presented as the mean and standard deviation of the mean (Mean±SD). The statistical significance of the blood indices was evaluated by the least significant difference (LSD) test at $P<0.05$ consumption.

Results and Discussion

Albumin (Alb) is a blood protein that plays a role in the transport of a range of endogenous chemical compounds, as well as foreign substances through the formation of non-covalent complexes at specific binding sites (Silva *et al.* 2015). The concentration of albumin is subject to variation according to the physiological and nutritional status of the individual, as well as their geographic location (Table 1). Glucose (Glu) is the source of energy for fish and may serve as indicator of physiological state. Furthermore, in contrast to higher vertebrate, glucose in sturgeons does not exhibit a consistent pattern. It is frequently observed that glucose level is higher during the summer months than during the winter period (Korabel'nikova 2009). The findings of Barton et al. (1998) indicated that the mean plasma glucose level in juvenile paddlefish is 62-75 mg/dL. Mims *et al.* (2006) reported lower values of 29-40 mg/dL in juvenile paddlefish (7 months). In the present study, the glucose level was found to be lower than in previous studies, which is attributed to the age of fish. Triglycerides (TG) represent the fat reserves stored in the body, which are normally transported into the blood to provide energy and nutrients to tissues and cells. Elevated cholesterol (Ch) levels, which are a constituent of cell membranes and the primary precursor of sexual hormones, have been observed to correlate with an increase in fish size and advancing age (Nabi *et al.* 2022). Moreover, the TG and Ch values were higher in females than in males during the maturation process. This is attributed to the fact that they are essential for the transport of energy resources to the oocytes (Chatzifotis *et al.* 2004). In general, creatinine (Cr) concentrations in the blood of fish are low and are primarily dependent on muscle metabolism. In the present study, the mean level was found to be several time higher than that observed in other sturgeon species (Nicula *et al.* 2010). The uric acid (UA), which functions as an antioxidant defense in both human and fish plasma, researchers have reported that the concentration was higher in males than in females in a number of representative ray-finned fish species (Nabi *et al.* 2022). This may be explained by the fact that the accumulation of uric acid in the seminal plasma of mature males serves as an antioxidant defense for fish sperm (Ciereszko *et al.* 1999). The determination of AST and ALT activities in the blood is one of the most widely used diagnostic tools in Pisces medicine (Shahsavani *et al.* 2010). As stated by Stojkovski (2001), their activities in healthy fish blood are within the range of 15-165 and 3-42 UI, respectively, which is comparable to the values observed in the present study. The values of specific ions can be utilised as

biomarkers of health in a diverse range of animal species, including fish. In the paddlefish exhibit physiological hypocalcemia, whereby low calcium levels are observed despite the capacity to regulate the metabolism of these ions (Ali *et al.* 2019). Moreover, it is crucial to acknowledge the observation that the composition of cations in paddlefish differs according to sex and stage of the life cycle. Notably, calcium ions exhibit a range of 0.66-0.95 mmol/L (Knowles *et al.* 2006). In general, studies related to ceruloplasmin (CP) and C-reactive protein in fish are less abundant than in mammals. A review of the literature reveals a dearth of data pertaining to these two proteins in paddlefish. It has been proposed that ceruloplasmin may be classified as an acute-phase protein in certain fish species, including those belonging to the Acipenseridae. Furthermore, it has been demonstrated that sturgeon exhibited no alteration in the values during the summer months, but demonstrated an increase during the autumn season (Kolman *et al.* 2000). It was demonstrated that the ceruloplasmin level in sturgeon increased in correlation with growth. This may be attributed, at least in part, to the larger liver mass that is associated with the growth of the fish (Kolman *et al.* 2000). CRP is a pentraxin that can be found in both vertebrates and invertebrates, and is frequently associated with the acute phase response (Bayne & Gerwick 2001). Nevertheless, it still unclear whether non-mammalian pentraxins can be classified as acute phase proteins and their levels can be increased during an acute phase reaction, or whether they can be decreased (Lund & Olafsen 1999).

The present study was conducted with a limited number of female mature paddlefish cultivated in polyculture with carp in Bulgaria. Consequently, the information collected does not reflect the prevalence of the general paddlefish population. Nevertheless, to the best of our knowledge, this is the inaugural study to report reference intervals of specific blood biochemical indices observed in paddlefish. It must be acknowledged that the accuracy of these reference data may be limited for large-scale clinical interpretation. However, they can serve as a foundation for guiding practitioners in their actions and practice.

Table 1. Mean reference levels in certain biochemical parameters in paddlefish.

Parameters	Reference	Mean±SD	Parameters	Reference	Mean±SD
Alb g/L	7.9 – 19.7	10.6±4.10	AST U/L	45 – 169	119.4±22.30
Glu mmol/L	1.37 – 3.05	2.355±0.47	ALP U/L	248 – 1134	662.7±271.37
Ch, mmol/L	3.99 – 18.22	7.388±3.73	Ca mmol/L	1.69 – 3.67	2.667±0.64
TG mmol/l	4.19 – 6.11	5.139±0.62	P mmol/L	2.37 – 5.88	3.491±1.02
Cr µmol/L	17 – 25	21±2.77	Cceruloplasmin mg/L	35 – 158	71.29±41.36
UA µmol/L	21 – 89	41.2±23.39			
ALT U/L	6 – 11	8.2±1.68	CRP pg/ml	84 – 378	197.19+101.75

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