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Haematological and biochemical reference intervals for African catfish (*Clarias gariepinus*) reared in RAS

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Abstract

In Bulgaria the production of high-quality local species is insignificant and is represented mainly by carp, grass carp and trout. Recently, diversification of the produced species has included specie such as African catfish (*Clarias gariepinus*). The present study was conducted to establish reference normal range of some hematological and biochemical parameters in wels sharptooth catfish (*Clarias gariepinus*) being reared in RAS, Bulgaria. To reduce stress during the blood sampling fish were anesthetized with mobile electro stunning device and blood was drawn with a syringe containing heparin as anticoagulant, by the caudal vein puncture method. The collected blood (approximately 2.0 ml) has been centrifuged at 3000 rpm for 5 min (Ohaus FC5515, Ohaus Corp., Germany) at room temperature and plasma was immediately separated and stored at -20°C until analysis. The different parameters were determined using standardized clinical methods and haematological techniques. The haematological parameters include the erythrocyte sedimentation rate (17.9±0.94), haemoglobin (25.96±4.58) and haematocrit (27.47±3.10). The mean values for plasma biochemical parameters - total protein (TP), albumin (Alb), globulin (Glb), urea (Ur), creatinine (Cr), aspartate transaminase (AST), alanine transaminase (ALT), glucose (Gl), calcium (Ca) and phosphorus (P) were 15.93±2.70 g/l; 8.28±2.02 g/l; 7.90±1.83 g/l; 0.67±0.18 µmol/l; 73.61±21.33 µmol/l; 73.78±28.06 UI; 14.71±3.67 UI; 1.76±0.36 µmol/l; 0.94±0.22 µmol/l respectivity. The haematological and biochemical reference intervals established may allow clinically important decisions in the assessment of health and well-being of this economically important African catfish (*Clarias gariepinus*) in Bulgarian aquaculture.

Keywords: Biochemical indices, catfish, hematological indices, reference intervals.

1. Introduction

In Republic of Bulgaria during the past decade a tendency of cultivatied for mid-to-high-end of fish species. These changes are due, in part, to the changes in the Law on Fisheries and Aquaculture in August 2012 and as well as the introducion of modern technologies and exotic species, regardless of local natural climatic conditions. An example of exotic species are Tilapia, Nile perch (*Lates niloticus*), Iridescent shark catfish *(Pangasianodon hypophthalmus)* and African catfish (*Clarias gariepinus*)[8].The African catfish is widely cultured in Africa, Europe and some parts of Asia (Israel, Syria and Turkey) and South America (Brazil and Paraguay)[16]. Although African catfish (*Clarias gariepinus*) is one of the well known fresh water fish in world aquaculture, in Bulgaria is no data on blood hematological and biochemical reference intervals of this fish as far as we are aware. Information about this exotic fish for Bulgaria can be found in several ichthyological articles and national reports [8; 10; 11; 15]. Thus, this work aimed to establish the baseline information on haematological and biochemical

parameters of African catfish and to compare the recorded values with reference intervals reported by other researchers.

2. Material and Methods

The African sharptooth catfish were grown under superintensive system (Cell Aquaculture Netherlands Holdings BV) in private fish farm located near the town of Stara Zagora, Bulgaria. The rate of water flow was 36000 liters/day and the water exchange was continuous, with a stocking density of 200 fish/m^3. The investigation was performed according to the Guidelines of the European Union (2010/63/UE). For the purposes of this project, from 30 catfish (W = 152.31± 17.6 g, TL = 27.15 ± 3.61 cm) blood for analysis was obtained aseptically from the caudal vein (*v. caudalis*) using plain vacutainers. Prior to anaesthesia, food was withheld for 24 hours to minimize the risk of regurgitation. Fish were stunned electrically with mobile device consisting of a capacitor (condenser; low capacitance of 47 μF) and two copper plates (electrodes). The blood was collected (1.0-1.5 ml) and centrifuged at 2500 rpm for 15 min (Ohaus FC5515, Ohaus Corp., Germany). Plasma was immediately separated and stored at -20°C until analysis. Erythrocyte sedimentation rate (ESR) was performed Micro-Wintrobe method [4]. Haematocrit (Ht) was determined as Yildiz, [17]. Haemoglobin (Hb) was colorimetrical determined by haemometer (Superior Marienfeld, Lauda-Königshofen, Germany) according to Hermann Sahli. Plasma biochemical parameters were analysed using an semi-auto analyser (Mindray BA-88, Mindray Bio-Medical Electronics, Shenzhen, China) with commercial diagnostic kits (Giesse, Diagnostics, Italy). Concentration of globulins was calculated by distracting albumin from total protein. The data was compared with the reference values of hematological and biochemical parameters of African catfish (*Clarias gariepinus*). Statistical analysis was performed using one-way analysis of variance (ANOVA). The results were processed with software Statistica v.12 (StatSoft Inc., USA, 2002). All results were presented as mean and standard deviation of the mean (Mean±SD).

3. Results and Discussion

The serum levels of measured blood parameters of farmed African catfish are shown in Table 1.

Table 1. Levels of selected blood plasma components in farmed sharptooth catfish (χ±SD), n=30

Parameteres	Measure unit	Mean values	Reference
Erythrocyte sedimentation	(mm/hour)	17.9±0.94	19.00 - 43.50
Hematocrit	(%)	27.47±3.10	32.64 - 45.74
Hemoglobin	(g/dL)	15.96±2.58	10.02 - 18.64
Urea	μmol/L	0.67±0.18	0.37 - 2.96
Creatinine	μmol/L	73.61±21.33	45.08 - 234.88
AST	UI	73.78±28.06	61.85 - 159.48
ALT	UI	14.71±3.67	6.44 - 36.43
Total protein	g/L	15.93±2.70	9.90 - 69.40
Albumin	g/L	8.28±2.02	8.3 - 20.8
Globulin	g/L	7.90±1.83	1.6 - 54.1
Albumin/Globulin ration		1.07/1	0.28 - 5.39
Calcium	mmol/L	1.76±0.36	1.42 - 3.01
Phosphorus	mmol/L	0.94±0.22	0.84 - 1.55

Heamatological and biochemical idices are often used to monitor the health status and stress indicators in fish. Several factors have been reported to affect hematological parameters of catfish; these include sex [3], age [13]

and culture conditions [6]. The Erythrocyte sedimentation rate is useful as a screening test for the presence of any chronic or acute condition which is marked by alteration in protein concentrations [2]. The mean value of ESR was slightly lower in compare with results obtained by Aderolu et al., [2] and we attributed it to alteration in total protein concentration. The median values for hematocrit (27.47±3.10) which is the percentage of total blood volume, determined in current investigation were much at par with those given by Dai et al., [6] and Akinrotimi et al., [3] at the same time were close to the mean values for collected African catfish from River Nile, Sudan [1]. Hemoglobin may show wide variability and can be attest to good transport of oxygen and removal of carbon oxide from fish tissues. The hemoglobin concentration (15.96±2.58 g/dL) in studied fish was similar to those for female catfish reported by Akinrotimi et al., [3], but was contradict those reported for African catfish reared in recirculating systems [6]. However, the high levels did not have negative effect on the fish and the oxygen carrying capacity of the blood was sufficient. The serum urea values were equated the values obtain by Adam and Agab [1] but were lower than those of Omitoyim, [14]. The levels found here were also close to the reference intervals obtained for *Clarias gariepinus* [13]. By comparison with serum urea, however, creatinine is the preferred test for assessment of kidney function. The creatinine concentration in this investigation fell within the reference range (45.08-234.88 μmol/L) as reported by Okoye et al., [13]. We have not found values that fall outside the reference range in both blood serum parameters which are main markers declining kidney and/or gill dysfunction [12]. The assessment of liver function includes a mandatory analysis of blood liver enzymes concentrations [5]. The values obtained for AST and ALT activities in the present examination were also similar to the reference intervals for *Clarias gariepinus* and Heterobranchus longifilis reported by Okoye et al., [13] and Omitoyin, [14]. The ALT level (14.71±3.67) of wels catfish was lower to reference standard values reported by Dorcas and Solomon [7] and this can be attributed to the different hydrochemical parameters such as temperature, pH, dissolved oxygen, alkalinity or hardness. Total protein concentration in African catfish juveniles range from 17.4 to 67.2 g/l estimated throught the biuret metod [1; 9; 14] which is similar than the levels of our study (15.93±2.70). Changes in concentration of total protein in blood plasma, may be used as an index for the health and stress status. In this study the observed reduction in total plasma protein may be related to the stress as a result of accomodation and cultivation in new environment. Furthermore, the blood samples were taken winter season between November and December, which might confirm this interpretation. The difference in A/G ration (1.07) with reference values mainly due to an decrease in the globulin fraction (7.70±0.46) and to a lesser extend the albumin fraction (8.28±2.02). In this study electrolyte levels, such as calcium (1.76±0.36) and phosphorus (0.94±0.22) for wels catfish were consistent with those of African catfish juveniles (*Clarias gariepinus*)[14] and sharptooth catfish (*Clarias gariepinus*)[9].

4. Conclusions

Haematological and biochemical parameters contribute to an understanding of the relationship between blood characteristics and the habitat and the adaptability of the fish to the environment, so they will help of veterinarins and aquacultural engineers to interpret the laboratory data appropriately and establishing normal values in different species of fish. The data obtained from the current study are the first reference range values published for African catfish in Bulgaria. This reference intervals can be used for monitoring health status, diagnosing diseases and improving the management.

5. References

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