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Haematological and Biochemical Reference Intervals for *Heteropneustes fossilis*

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Abstract

This study presents reference intervals for haematological and biochemical variables in the Indian catfish, *Heteropneustes fossilis*. The haematological parameters include the primary and secondary blood cell indices - red blood cell (RBC), white blood cell (WBC), differential leucocyte count (DLC), neutrophils:lymphocytes (N:L), mean corpuscular haemoglobin (MCH), haemoglobin (Hb) and haematocrit (Hct), as well as the biochemical variables, plasma glucose and plasma chloride. The reference intervals for all the parameters were determined at the 25^{th} and 75^{th} percentiles and all the variables showed normal distribution. A new feature is the inclusion of reference intervals for N:L ratio, which is considered a potential stress biomarker. The study aims to provide a reliable database for haematological parameters, which can be clinically important in the assessment of health and well-being of this economically important catfish of the Asian sub-continent.

Introduction

Currently a lot of emphasis is being laid upon the general welfare and well-being of animals including fish. In fish farming and in laboratory experimentations, maintenance of the well- being and the health status of fishes are of prime concerns. Fish being aquatic animals, are highly sensitive to their environment. Slightest disturbance in the environment as well as those which may cause stress greatly affects the homeostatic mechanisms and the first of the physiological changes can be observed in the blood. Similarly, the first indications of the onset of a disease are reflected by the physiological and biochemical changes in the blood. It is now a well-established fact that haematological and biochemical profiles are good indicators of the health status of animals and proper haematological evaluation can provide important information for the onset of stress (Wedemeyer and Nelson,1975) and diagnosis of diseases in fish (Tavares-Dias and Moraes, 2007). It is therefore imperative that we have sufficient databases for the normal haematological values of fish species.

Reference intervals for variables assume importance as they are derived from healthy individuals out of a reference population which is defined as a set of individuals meeting certain criteria, chief among which is the absence of disease (Rehulka et al. 2004; Tavares-Dias and Moraes, 2007). Since reference intervals are defined by the upper and lower limits that cover the

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majority of the values obtained for healthy individuals in the reference population, they provide a good estimate of these variables as occurring in the natural populations. The normal range for various haematological and biochemical parameters in various fish species have been established by several workers in fish physiology and pathology (Shterman,1970; Wedemeyer and Chatterton, 1970; Houston,1971).However, reference interval values for haematological parameters have been obtained for only a few fishes, viz. cultured tilapias (Hrubec et al. 2000), channel catfish, *Ictalurus punctatus* (Bentinck-Smith et al. 1987; Tavares-Dias and Moraes, 2007) and koi carp, *Cyprinus carpio* (Tripathi et al. 2003, 2004).

Indian catfish, *Heteropneustes fossilis* (Saccobranchidae) is a popular fish species among fish farmers and research workers of India and other Asian countries. The fish is economically important and also thoroughly studied (Goswami et al. 1983; Anwar and Jafri, 1995; Borah and Yadav, 1996). However, no systematic attempt has been made to establish the normal values of haematological parameters in this fish. A single study that provides reference intervals for the various haematological and biochemical parameters can be highly useful as a ready reference, particularly while monitoring the health of the fish. The proposed study was carried out to serve this purpose in *H. fossilis.*

Materials and Methods

Experimental fishes

Adult *H. fossilis* (average length 10.0-25.0cm and average body mass 20-50g) were purchased from the local markets and maintained in the laboratory under natural photoperiod condition, 14L:10D. The aquaria were well aerated and maintained under the following natural conditions: dissolved oxygen levels 8-10 $mg{\cdot}L^{-1}$, pH 7.5-8.2 and temperature 26-30ºC. The fish were fed with chopped goat liver on alternate days *ad libitum* and acclimatized for 15 days before they were sampled for blood analysis.

Sampling and analysis

Three batches, each of 25 healthy fish of average body mass 25-50g and length 12.0-25.0 cm were selected as the reference population for the evaluation of haematological parameters. The fish were regularly checked for any sign of physical/ behavioural indications of disease/ stress. Only those fish which were healthy and showing normal behaviour were selected. Blood sampling was always done on the next day of feeding. Fish were anesthetized with ethyl-p-aminobenzoate (Acros, Germany) at a concentration of 0.35 $g{\cdot}L^{-1}$ and blood was drawn with a syringe containing EDTA (di-sodium ethylenediamine tetra acetate), by the caudal vein puncture method. Utmost care was taken to ensure a smooth sampling of blood without causing stress to the fishes. Blood smears were prepared and stained with Wright's and Leishman's stain for differential leucocyte counts (DLC) and neutrophil:lymphocyte (N:L) ratio was determined as an indicator of stress. DLC were done by

counting a total of 200 white blood cells and expressing the different leucocytes as a percentage. Identification of the leucocytes was done according to Williams and Warner (1976) and Ellis (1977). Total erythrocyte (RBC) and total leucocyte (WBC) counts were done optically on Neubaur's haemocytometer. Secondary blood cell indices (MCH) were determined according to Dacie and Lewis (1975).

Haemoglobin (Hb) was determined spectrophotometrically (540 nm) using the standard cyanohaemoglobin method as described by Blaxhall and Daisley (1973). An aliquot of the blood was centrifuged in Wintrobe's tube for determining the haematocrit value (Hct) and the plasma was utilized for evaluating the biochemical parameters. Plasma glucose was determined using the o-oxidase method and chloride by the thiocyanate method using Siemens Diagnostics Kits, India. All the counts (RBC,WBC and DLC) as well as other parameters were performed in triplicate for each batch of fish and then averaged for agreement within a 15% difference.

Statistical analysis

All the variables - RBC, WBC, DLC, Hb, Hct, plasma glucose and plasma chloride showed normal distribution. The data were tested using Kolmogorov-Smirnov test. Reference interval for the blood variables were determined using the non- parametric methods.

Results

The reference intervals values for the primary and secondary blood cell indices and the biochemical parameters of *H. fossilis* are presented in Table 1.

Table 1. Reference interval values for the haematological variables of *H. fossilis* (n=25).

Variables	Median	$25^{th} – 75^{th}$ percentile	Range
Plasma glucose ($mg \cdot dL^{-1}$)	62.075	47.97-73.05	37.39-88.76
Plasma chloride ($mEq \cdot L^{-1}$)	85.46	65.56-105.25	31.09-141.5
Haemoglobin ($g \cdot dL^{-1}$)	19.78	18.56-21.74	11.66-25.47
Haematocrit (%)	31.5	29.55-36.5	22.0-43.0
RBC count ($X10^6 \cdot \mu L^{-1}$)	6.11	5.14-6.69	4.51-8.85
WBC counts ($X10^3 \cdot \mu L^{-1}$)	17.33	13.86-25.12	8.16-33.03
DLC counts			
Lymphocyte (%)	69.21	60.05-72.04	27.87-74.6
Thrombocyte (%)	9.01	7.68-12.30	5.86-34.16
Neutrophils (%)	6.995	5.88-7.93	3.92-11.66
N : L ratio	0.095	0.08-0.12	0.05-0.32
MCH (pg)	33.2	26.6-40.85	19.424-49.532

Discussion

This study presents the reference intervals for the haematological and biochemical variables in Indian catfish, *H. fossilis* which inhabits the muddy freshwaters of India. Reference intervals for the physiological and biochemical parameters have been estimated at the 25^{th} and 75^{th} percentiles. The study becomes reliably significant as the batches, selected for the study and constituting the reference population comprised of healthy, unstressed and disease free individuals.

Normal range of some of the haematological parameters in *H. fossilis* have been ascertained in some of the earlier studies viz. Tandon and Joshi (1975), Pandey et al. (1976) and Goel et al. (1984) but reference intervals for the haematological values were never attempted before. Besides the earlier studies were conducted for the purpose of establishing normal database and no specific attention was paid to the stress factor. The utility of such studies remains poor if they have to be used for reference purpose. A single study that estimates most of the parameters at the 75^{th} and 25^{th} percentiles, as in the present case can serve as a database for the normal values representing those of the population, and therefore can play important clinical roles in ascertaining the health status and general well-being of farmed fishes and those maintained in laboratories. All the haematological parameters determined in the present study are health indicators as well as important stress markers. Fish blood variables like primary cell indices, haematocrit values, haemoglobin and plasma biochemistry have been found to be important in the assessment of stress (Ellsaesser and Clem, 1986; Barton and Iwama, 1991; Wendelaar Bonga, 1997; Barcellos et al. 2004). Plasma glucose has been considered an important stress indicator (Hattingh, 1976; Donaldson, 1981; Wedemeyer and McLeay, 1981), leucocyte profiles have recently been considered significant in assessing stress (Davis et al. 2008).

The reference interval values for haematocrit, plasma glucose and chloride in *H. fossilis*, as determined in this study (Table 1) are much at par with those given by Tavares-Dias and Moraes (2007) for channel catfish. The median values for plasma glucose (62.075 $mg \cdot dL^{-1}$) in *H. fossilis* are higher, but the upper limit of the range for glucose is the same in both the catfishes. The median values for haematocrit and chloride levels of *H. fossilis* are close to the mean values for channel catfish and jundia catfish *Rhamdia quelen* (Barcellos et al. 2004). Plasma chloride levels are important as corticosteroids and are known to stimulate ion-transporting mechanisms.

The median values for RBC (6.1 $x10^{6} \cdot \mu L^{-1}$) for *H. fossilis* as given in Table 1 are much higher than those reported in the pike - 1893000· mm^{-3} and the salmon - 1180,000· mm^{-3} (Lagler et al. 1977). It is also considerably higher as compared to jundia catfish (Barcellos et al. 2004) and channel catfish (Breazile et al. 1982) respectively, whereas the WBC numbers were lower in jundia catfish and much higher in channel catfish when compared to *H. fossilis*. The haemoglobin values for *H. fossilis* (19.9 $g \cdot dL^{-1}$) are considerably higher than that for both jundia catfish (Hb-9.02 $g \cdot dL^{-1}$) and channel catfish (7.08 $g \cdot dL^{-1}$), and the MCH values are also higher than that of channel catfish (21.5 pg) (Breazile et al. 1982). The considerably higher values of RBC and Hb (19.9 $g \cdot dL^{-1}$) and

MCH (33.2 pg) in *H. fossilis* may be attributed to the fact that the catfish inhabits low-oxygenated waters and is an air breather which requires increased demand for oxygen carrying capacity of blood. The high values of haemoglobin are also in accordance with the view of Larsson et al. (1976) that higher concentrations of haemoglobin are found in predatory, fast moving fishes as the catfish is predatory in habit and shows active surfacing activity.

Currently, leucocyte profiles have received emphasis in relation to stress (Espelid et al. 1996; Davis et al. 2008) and changes in the ratio of N:L are seen as responses to stress in vertebrates (Espelid et al. 1996; Davis et al. 2008). Determination of N:L ratio should be given prominence while evaluating the health and welfare status of fishes and hence the inclusion of reference values for N:L ratio in the present study. It is hoped that this endeavour which has not been attempted earlier for any fish species, will prove clinically useful in fish health.

An attempt has been made in this study to provide reliable database of reference interval values of the blood variables of *H. fossilis* using standard protocols in obtaining and analysing blood samples. Utmost care has been taken in making the RBC and WBC counts, and differential leucocyte counts in particular, to minimise the manual errors. To avoid other interfering factors such as environmental condition, nutritional requirements, sampling methods, the values were obtained from a well-defined reference population of healthy fishes, maintained under identical environmental conditions and so the reference ranges obtained in this study can be considered as a reliable database of normal haematological values for this economically important fish species.

Conclusion

This single study investigation is important because it provides database for reference intervals for the normal haematological and biochemical values of an Indian farm catfish, *H. fossilis* for the first time, for clinically ascertaining the health status or well-being of the fish. The nine haematological parameters used are health indicators as well as stress markers. Reference intervals for the blood variables were determined by the standard methods and statistically estimated at the 75^{th} and 25^{th} percentiles.

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