

Leukocyte count in fish – possible sources of discrepancy

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Abstract

The values obtained during leukocyte count (white blood cells; WBC) of the same group of common carp *Cyprinus carpio* were compared between three diluents and three persons. Furthermore, direct counting in a hemocytometer was compared with indirect values calculated from red blood cell counts (RBC) and the number of leukocytes in smears. The WBC values were significantly affected by rater and method of counting (direct vs. indirect) but not by diluent. The WBC values obtained using the indirect method were higher compared to the direct count. The visibility of leukocytes was better and easier to count in colorless isotonic Hayem's solution than when staining with Natt-Herrick's or Dacie's solutions. The values of RBC and thrombocyte count (TC) were also significantly rater-related.

Introduction

Hematological analysis is a noninvasive and easy to perform method used to evaluate fish physiological and health status (Svobodova et al., 1991; Arnold et al., 2014). Leukocyte count is an important indicator of fish immune status (Svobodova et al., 1991; Ishikawa et al., 2008). Blood cell count in fish is usually performed using manual methods (in a hemocytometer under microscope magnification) using blood diluted with various solutions, mostly containing dyes that stain cell nuclei blue (Blaxhall and Daisley, 1973; Svobodova et al., 1991). Analysis of data from 50 papers concerning common carp hematology revealed that Natt-Herrick's solution is the most frequently used diluent followed by Dacie's, while isotonic solution containing no dye is rarely used (Witeska et al., 2016). Counting of leukocytes in vertebrates that

have all blood cells nucleated is considered difficult by some authors (Walberg, 2001; Inoue et al., 2002; Aroch et al., 2013). Others point out the difficulty in discrimination between leukocytes and thrombocytes and reported significantly differing WBC values (Ishikawa et al., 2008; Tavares-Dias et al., 2008). Reports on the WBC values in healthy juvenile *Cyprinus carpio* show considerable differences ranging from $1.4 \pm 0.1 \times 10^3/\mu\text{L}$ (Ajani and Akpoilih, 2010) to $197 \pm 6.3 \times 10^3/\mu\text{L}$ (Wang et al., 2014). The problem of WBC accuracy concerns all vertebrates that have nucleated erythrocytes and thrombocytes that do not undergo complete lysis in diluents used for leukocyte counting. According to Walberg (2001), WBC values in the same avian blood obtained by different laboratories may differ 3-fold, while Tavares-Dias et al. (2002) reported

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