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Brief Research Report

*Corresponding author
Francesco Fazio, DVM, PhD
Associate Professor
Department of Veterinary Sciences
Polo Universitario Annunziata
University of Messina
98168 Messina, Italy
Tel. +39 0903503516
Fax: +39 0903503975
E-mail: ffazio@unime.it

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Influence of Body Size on Blood Hemogram in Rainbow Trout *Oncorhynchus Mykiss* (Walbaum, 1792)

Francesco Fazio, DVM, PhD*; Concetta Saoca, BSc; Irene Vazzana, BSc; Giuseppe Piccione, DVM

Department of Veterinary Sciences, University of Messina, Polo Universitario dell' Annunziata, 98168, Messina, Italy

ABSTRACT

Aim: The aim of this study was to investigate the influence of body size on hematological parameters in Rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792).
Materials and Methods: For the purpose of the study, fifty Italian trout were divided into two groups: Group S1 (n=25) of weight (300-500 g) and length (25-32 cm); Group S2 (n=25) of weight (510-700 g) and length (33-38 cm). Blood samples were collected from each fish, to record the count of white blood cell (WBC), red blood cell (RBC), determine the hemoglobin concentration (Hb), hematocrit (Hct) value, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) mean corpuscular hemoglobin concentration (MCHC), and thrombocyte count (TC).
Results: The collected blood was analyzed using an automatic method to determine the hematological parameters. Statistical analysis (unpaired *t*-test) showed a significant effect of size ($p<0.05$) on the RBC count, Hb concentration and Hct value. No statistically significant differences were observed in the WBC count, MCV, MCH concentration, MCHC and TC.
Conclusion: The data reported in this study contributed towards the knowledge of hematological evaluation in farmed trout and showed that fish size was an important factor that helped establish the "physiological range" supporting the accurate interpretation of hematological parameters in examining the health status of this species.

KEY WORDS: Body size; Biometric indices; Hematological parameters; Trout.

ABBREVIATIONS: RBC: Red Blood Cell; Hct: Hematocrit; Hb: Hemoglobin Concentration; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; Mean Corpuscular Hemoglobin concentration; MCHC: Mean Corpuscular Hemoglobin Concentration; WBC: White Blood Cell; TC: Thrombocyte Count; EDTA: Ethylenediaminetetraacetic Acid; DO: Dissolved Oxygen.

INTRODUCTION

Global fish production has notably increased in the last five decades and intensive farming has contributed significantly towards the development of aquaculture. Rainbow trout is a major farmed species in Italy and the second most common to be farmed in Europe after the Atlantic salmon.[1] Together with commercial globalization, increasing trout farming consequentially leads to several fish diseases, thus rendering it necessary to prevent and cure related conditions while increasing the demand for veterinary drugs to treat the aquatic species. The evaluation of blood parameters assists in the culture of fish by facilitating the early detection of diseases and identification of sub-lethal conditions affecting the productivity.[2] Various literary references report about the blood parameters and their variations observed in fish living in different habitats.[3-5] In a previous study conducted to establish the reference range for haematological and biochemical values on *Clarias gariepinus*, a positive correlation was observed between the biometric and blood indices.[4] Variations with respect to the normal values of blood parameters

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exist in fish and can be attributed to some internal and external factors such as physiological homeostasis and environmental factors.[6] Body size alongside water quality and temperature, may contribute to the variability in the haematological parameters that are difficult to interpret. Considering that the changes in the blood parameters depend on the biometric parameters and the physicochemical conditions of the water, the aim of this study was to evaluate the influence of body size on some hematological parameters in the farmed trout.

MATERIALS AND METHODS

A total of 50 Rainbow trout (*Oncorhyncus mykiss*) in excellent health status and supplied by a commercial farm in Palazzolo Acreide (Siracusa, Italy) were investigated in this study. All the fish were subjected to a natural day/night cycle (11L/13D) and were fed with a commercial dry food (crude protein 46%; crude fat 20%; ash 10%; fiber 1.5%) distributed twice daily for 7 days a week. The fish being studied were considered healthy on the basis of an external examination of any sign of abnormalities or clinical infestation. In the farm, the physical and chemical characteristics of water were recorded. Temperature, salinity, pH and dissolved oxygen (DO) were measured using a handheld multiparameter instrument (model YSI 556 MPS - Ohio, USA) and these values were recorded as has been shown in Table 1. After being captured, the fish were anaesthetized prior to blood sampling using 2-phenoxyethanol at a concentration of 200 mg L^{-1} and were immediately individually weighted using a balance (Kern 440-49 N, Germany) and their total length was measured using an ictiometer (Scubla SNC, 600 mm, Italy) in order to obtain two groups of different body size: Group S1 (n=25) with weight (300-500 g) and length (25-32 cm); Group S2 (n=25) with weight (510-700 g) and length (33-38 cm). Blood samples were collected between 8:00 a.m. and 10:00 a.m. and were obtained by puncturing the caudal veins using microtubes (Miniplast 0.6 mL; LP Italiana Spa, Milano) containing ethylenediaminetetraacetic acid (EDTA) (ratio 1.26 mg/0.6 mL) as the anticoagulant agent for the recording of hematological parameters. All the samples were analyzed immediately (T_0) by an automatic method using the blood cell counter HeCoVet C (SEAC, Florence, Italy) previously validated and used in this and the other species using a manual and an automatic system.[7,8] The procedure of analysis was performed to determine the following parameters: white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin concentration (Hb), hematocrit (Hct) value, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) concentration, mean corpuscular hemoglobin concentration (MCHC) and thrombocyte count (TC).

All haematological analysis were performed in triplicate by the same operator with a same instrument under the same conditions, and in a short period of time".

All the experimental procedures were carried out in accordance with the ethical considerations presented by the European legislation concerning the protection of animals used for scientific purposes (European Directive 2010/63). The recorded data was normally distributed (p>0.05; Kolmogorov–Smirnov test).

Statistical Analysis

The influence of body size on hematological parameters was determined by performing an unpaired t-test for two different groups. p values <0.05 were considered as statistically significant. Table 2 shows the descriptive statistics for haematological parameters obtained in the two different size groups of farmed Rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) showing a statistically significant difference.

RESULTS

The results of this study showed the influence of body size on some haematological parameters in farmed trout as reported in Table 2.

Statistical analysis (unpaired t-test) showed a significant effect of size (p<0.05) on some parameters (RBC, Hb and Hct). In particular, a significant increase in the RBC count, Hb concentration and Hct value was observed for the fish of Group 2 relative to that of Group 1. No statistically significant differences were observed in WBC, MCV, MCH, MCHC and TC.

DISCUSSION

Hematological parameters are closely linked with metabolism thus, these results could be attributed to the higher metabolic activity and high energy demand during growth. The increased metabolic demand during the growth of the trout could increase the volume of RBC, which could lead to a consequential increase in the Hct value. Some authors[9] observed that the values of RBC count, Hb concentration and Hct value increased with an increase in the fish size. Moreover, in fish, the hematological parameters are closely associated with the metabolic activity

Table 1: Water quality values (Mean±DS) of farmed Rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792).

Water Parameters	*Oncorhynchus mykiss*
Temperature (°C)	9.86±0.23
Dissolved Oxygen (mg/L)	8.13±0.01
pH	8.40±0.07

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Table 2: Statistical results for haematological parameters in farmed Rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792). Means without the same alphabetical characters within the same parameters represent statistically significant differences ($p<0.001$).

Parameters	Groups (body size)	Mean±SD	95% confidence interval (CI)	2.5th-7.5th percentile range
WBC (x10³/µL)	***Group 1 (n=25)***	19.89±0.74[a]	19.59± 20.20	19.10±20.18
	Group 2 (n=25)	20.12±0.92[a]	19.74± 20.45	18.57±20.70
RBC (x10⁶/µL)	***Group 1 (n=25)***	1.53±0.14[a]	1.47±1.59	1.46±1.62
	Group 2 (n=25)	1.64±0.12[b]	1.59±1.69	1.59±1.74
Hb (mmol/L)	***Group 1 (n=25)***	1.64±0.15[a]	1.57±1.70	1.57±1.75
	Group 2 (n=25)	1.73±0.11[b]	1.68±1.78	10.00±11.70
Hct (%)	***Group 1 (n=25)***	29.00±3.07[a]	27.73±30.27	27.60±30.44
	Group 2 (n=25)	31.62±1.78[b]	30.88±32.36	30.00±32.77
MCV (fL)	***Group 1 (n=25)***	189.2±8.57[a]	185.70±192.70	183.8±193.70
	Group 2 (n=25)	193.4±16.48[a]	186.6±200.20	181.00±200.00
MCH (pg/cel)	***Group 1 (n=25)***	10.76±1.34[a]	10.20±11.31	10.10±11.54
	Group 2 (n=25)	10.60±0.96[a]	10.20±10.99	10.00±10.85
MCHC (%)	***Group 1 (n=25)***	5.70±0.82[a]	5.37± 6.04	5.24±6.21
	Group 2 (n=25)	5.49±0.42[a]	5.32±5.66	5.12±5.79
TC (x10³/µL)	***Group 1 (n=25)***	59.20±10.60[a]	54.83±63.57	50.00±57.25
	Group 2 (n=25)	59.40±10.76[a]	54.96±63.84	66.50±65.00

related to size. Previously our researchers[10] have shown that the values of RBC count and Hb concentration were higher in mullet compared to other species with the highest biometric parameters. It should be noted that the differences recorded in blood parameters between the fish of various sizes are genetically determined,[11] even though these differences might be due to the higher metabolic rate of bigger fish compared to smaller ones.[12] In a research conducted on Nile tilapia *Oreochromis niloticus*, there was an effect of fish size on all the parameters except for MCV.[13] In a study on *Mugil platanus,* it was verified that the mean values for Hct, Hb, MCV, MCH and MCHC showed a slight tendency to increase as the fish continued to grow larger.[14] In addition, larger individuals present higher mean values for MCV, MCH and MCHC for Dolphin fish *Salminus maxillosus*.[15] WBCs, the cells responsible for the body's defence system, are considered an important parameter to evaluate the health status of the fish; the recorded values of WBC count are indicative of their immune responses and determine the ability of the fish to fight infections. According to some authors,[16,17] the WBC levels depend on various factors such as water temperature, environmental stress, fish age and sex. Species with higher counts of WBC were able to fight infections more effectively than other species. Among the defense cells of fish, the thrombocytes have a phagocytic capacity besides their functions in coagulation. Our findings did not suggest the influence of body size on WBC and TC, but it is probably on account of a small number of fish being studied.

CONCLUSION

In conclusion, our study suggests that the determination of hematological parameters require a correct interpretation in relation to the body size. Body size is an important factor, not only from the commercial point of view, but also for the accurate determination of hematological parameters, that many veterinarians demand to effectively control and manage the health status of the aquaculture fish.

AUTHORS' CONTRIBUTIONS

All authors have made substantial contributions to each step of the experimental procedure and article preparation. The idea for this study was conceived by Francesco Fazio and Giuseppe Piccione.
The experiment was designed by Francesco Fazio.
The experiment was performed by Concetta Saoca and Irene Vazzana
The data was analyzed by Francesco Fazio.
The article was written by Francesco Fazio and Concetta Saoca.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. Associazione Piscicoltori Italiani. 2005. La piscicoltura Italiana. Web site. http://www.api-online.it. 2004.

2. Yavuscan YH, Bekcam S, Karasu Benli AC, Akan M. Some blood parameters in the eel (*Anguilla Anguilla*) spontaneously infected with *Aeromonas hydrophila. Aquacult Res*. 2005; 60: 1388-1390.

3. Nilza LR, Ligia MM, Dense OS, et al. Haematological and biochemical values for Nile tilapia (*Orechromis niloticus*) cultured in semi-intensive system. *Acta Sci Biol Sci Maringa*. 2003; 25: 385-389.

ISSN 2475-1286 http://dx.doi.org/10.17140/VMOJ-2-121

4. Adam HM, Agab H. Haematological and biochemical indices of *Clarias garipeinus* collected from River Nile, Sudan. *Sudan Acad Sci J*. 2007; 21: 67-90.

5. Caruso G, Denaro MG, Caruso R, Mancari F, Genovese L, Maricchiolo G. Response to short term starvation of growth, haematological, biochemical and non-specific immune parameters in European sea bass (*Dicentrarchus labrax*) and blackspot sea bream (*Pagellus Bogaraveo*). *Mar Environ Res*. 2011; 72(1-2): 46-52. doi: 10.1016/j.marenvres.2011.04.005

6. Ighwela KA, Ahmad AB, Abol-Munafi AB. Haematological changes in nile tilapia (*Oreochromis niloticus*) fed with varying dietary maltose levels. *World J Fish Mar Sci*. 2012; 4(4): 376-381.

7. Faggio C, Casella S, Arfuso F, Marafioti S, Piccione G, Fazio F. Effect of storage time on haematological parameters in mullet, *Mugil cephalus*. *Cell Biochem Funct*. 2013; 31(5): 412-416. doi: 10.1002/cbf.2915

8. Fazio F, Saoca C, Casella S, Fortino G, Piccione G. Relationship between blood parameters and biometric indices of *Sparus aurata* and *Dicentrarchus labrax* cultured in onshore tanks. *Mar Freshw Behav Physiol*. 2015; 48: 289-296. doi: 10.1080/10236244.2015.1041239

9. Jawad LA, Al-Mukhtar MA, Ahmed HK. The relationship between haematocrit and some biological parameters of the Indian shad, *Tenualosa ilisha* (Family Clupeidae). *Animal Biodivers Conserv*. 2004; 27: 47-52.

10. Fazio F, Faggio C, Marafioti S, Torre A, Sanfilippo M, Piccione G. Effect of water quality on hematological and biochemical parameters of *Gobius niger* caught in faro lake. (SICILY). *Iranian J Fish Sci*. 2013; 12 (1): 219-231.

11. Raizada MN, Jain KK, Raizada S. Monthly variations in the hematocrit values (PCV) in a teleost, *Cirrhinus mrigala* (Ham.). *J Comp Anim Physiol*. 1983; 8: 196-198.

12. Chaudhuri SH, Pandit T, Benerjee S. Size and sex related variations of some blood parameters of *Sarotheriodon mossambica*. *Environ Ecol*. 1986; 4(1): 61-63.

13. Tran-Duy A, Schrama JW, Van Dam AA, Verreth JAJ. Effects of oxygen concentration and body weight on maximum feed intake, growth and haematological parameters of anile tilapia, *Oreochromis niloticus*. *Aquaculture*. 2008; 275(1-4): 152-162. doi: 10.1016/j.aquaculture.2007.12.024

14. Ranzani-Paiva MJT. Caracteristicas hematológicas de tainha *Mugil platanus* Günther, 1880 (*Osteichthyes, Mugilidae*) da regiao estuarino-lagunar de Cananeia-SP (lat. 25 °00'S-long. 47 °55'W). *Bol Inst Pesca*. 1995; 22: 1-22.

15. Ranzani-Paiva MJT, Rodrigues EDL, Veiga MLD, Eiras ACD. Association between the haematological characteristics and the biology of the "dourado" *Salminus Maxillosus Valenciennes*, 1840 from Mogi-Guaçu River, state of São Paulo, Brazil. *Maring*. 2001; 23: 527-533.

16. Luskova V. Annual cycles and normal values of hematological parameters in fishes. *Acta Sc Nat Brno*. 1997; 31(5): 70.

17. Modrá H, Svobodová Z, Kolářová J. Comparison of differential leukocyte counts in fish of economic and indicator importance. *Acta Vet Brno*. 1998; 67(4): 215-226.