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Effect of Stocking Density Stress on the Hematological Profile of *Oncorhynchus mykiss*

Samina Qadir Charoo[1], Salman Rauoof Chalkoo[2] and T.A. Qureshi[3]

[1,3]Department of Applied Aquaculture and Zoology, Barkatullah University, Bhopal, Madhya Pradesh, India
[2]NHPC & NABARD Funded Fisheries Projects, Department of Fisheries, Government of J&K, India

Correspondence should be addressed to Samina Qadir Charoo, seemazahoor123@gmail.com

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Abstract The present research aimed at the investigation into the effect of stocking density on the hematological response of *Oncorhynchus mykiss* maintained in flow through condition. The stock having a weight of 520.22 ± 48.20 g and 580.25 ± 52.2 g were stocked in flow through FRP tanks at the stocking density of 38 kg/m^3 and 30 kg/m^3 respectively. In order to collect blood sample for analysis, the blood was collected through cardiac puncture. Red blood cell counts (RBCc), hematocrit values (Hct), hemoglobin concentration (Hb), were analyzed through haemoanalyser, while as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated through standard formulas. Differences in hematological parameters were statistically analyzed by Student's T-Test. The stocking density stress was evident from the significant increase ($p<0.05$) in RBC, Hb, Ht, MCV and insignificant decrease ($p>0.05$) in MCH and MCHC.
Keywords *Oncorhynchus mykiss, Stocking Density, Hematological Indices*

1. Introduction

Trout are the most commonly cultured fish in the world, and are a food staple in many parts of Africa, Asia and South America. Aquaculture of trout, as with other species of finfish, is adversely affected by production related disorders and infectious diseases, among which the most important physical factor is the stocking density. In this study, we determined reference intervals for hematologic analytes in cultured trout. We also evaluated clinical chemistry results from a small group of trout raised under different culture conditions. To our knowledge, this is the first study to determine complete hematologic and clinical chemistry results for trout from Kashmir province of Jammu and Kashmir State, India and to report the values as reference intervals suitable for diagnostic use.

There is sharp rise in pisciculture in inland waters all over the country. Farmers find it economically more profitable and physically less cumbersome and less demanding; than traditional agriculture practices. It is here, that with increase in pisciculture, problems of aquatic environmental management and that of fish disease management are posing a serious challenge to fish biologist, fish culturists and experts of the subject. The hematology offers one of the easiest, cheapest and most reliable methodologies to diagnose the status of fish health and treat them.

Stocking density is an important management issue for a good husbandry practice, higher growth, disease free stock and better economic returns. There has been little work on the exact stocking density modules based on the assessment of the impact of various physic chemical factors on the growth of rainbow trout. An exact stocking density of rainbow trout in fresh flowing waters depends on the water flow, water quality, physical characteristics, plankton biomass, and the artificial feeding schedules in rearing spaces. However the stocking stress analysis is a prerequisite to get an idea about the hematological variation due to stress, in turn depicting the health status of rainbow trout at lower and higher density.

2. Materials and Methods

2.1. Stocking Density

The rainbow trout table size fish weighing 520.22 ± 48.20 g and 580.25± 52.2 g were stocked in two different tanks in replicas in the stocking density of 38 kg/m^3 and 30 kg/ m^3 (Table 1), the treatments were named as R1 and R2 and the replicas as R3 and R4. The rainbow trout stock was 2 years old reared in fibre tanks with continue flow through system. The experiment lasted for 90 days. During the tenure of research, care was taken to clean the tanks through water jet systems, avoiding any physical stress to the livestock. The feeding of the fishes was done at the rate of 2% of the body weight. No chemical treatment or change in any physical feature of water was undertaken during the experimental period, ensuring complete effect of stocking density as the sole physical factor altering hematology.

2.2. Blood Sampling and Analysis

0.5 ml of blood was sampled from 10 fish of each tank by caudal venous puncture using lithium heparin as anticoagulant at the beginning and the end of the experimental trial. Blood was analysed with routine method used in fish hematology [4]. The red blood cell counts (RBC x 10^6/µl) were determined by counting the erythrocytes from 5 small squares of Neubauer hemocytometer using Vulpian diluting solution. The hematocrit (PCV, %) was determined by duplicate using heparinised capillary tubes centrifuged for 4 minutes at 13000 rpm in a micro hematocrit centrifuge. Red blood cell counts (RBCc), hematocrit values (Hct), hemoglobin concentration (Hb), were analysed through haemoanalyser, while as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated through standard formulas [5, 6].

2.3. Statistical Analysis

Student t-test was used to analyse the difference between the treatment groups and the results were expressed as mean±SD.

3. Results and Discussion

The present investigation into the effect of higher stocking density on the health status of rainbow trout (*Oncorhynchus mykiss*) revealed some interesting results. The same could be used to assess the appropriate quantity of fish biomass to be kept in each cubic metre of water for its better growth and stress free metabolic activity. The present finding is a baseline for further research in the field of fish management and husbandry practices. Same investigation could form a basic hematological profile for better understanding of stress, if any, to the livestock in the fish farm, by any of the physical or chemical parameters.

Table 1: *Biometric and Statistical Data*

Experimental	**R1**	**R2**	**R3**	**R4**
Trial	**T1**	**T2**	**T2**	**T1**
Total Biomass (g)	10400	10600	12000	11800
Number of Individuals	20	20	20	20
Average Weight (g/ex)	520.00	530.20	600.00	590.00
Standard Deviation	44.51	36.22	24.26	29.85
Coefficient of Variation	0.22	0.16	0.12	0.08

R1 – experimental version of the stocking density was 38 kg/m^3
R2 – experiment version of the stocking density was 30 kg/m^3

Table 2: *Changes in Hematological Parameters of Rainbow Trout during the Experiment*

Experimental Trial		Hematological Parameters (Average ± Standard Deviation)					
		Ht (%)	Hb (g/dl)	RBC x10^6/µl	MCV (µm^3)	MCH (pg)	MCHC (g/dl)
R1	T1 i	40±6.8	7.45±1.00	1.09±0.13	361.20±28.21	68.41±3.13	19.00±0.8
	T1 f	52±2.6	8.85±0.38	1.24±0.07	420.64±20.36	71.35±4.74	16.99±1.2
R1	T1 i	35±8.6	7.75±0.75	0.99±0.15	354.88±97.16	79.6±11.27	23.42±4.91
	T1 f	50±4.5	9.75±1.59	1.30±0.14	389.40±58.70	76.13±18.26	19.35±1.59
R2	T2 i	33±4.9	8.13±0.40	1.10±0.15	343.33±49.60	77.17±7.42	22.88±3.4
	T2 f	55±3.4	11.13±0.26	1.42±0.07	364.81±54.61	78.44±6.46	22.04±4.22
R2	T2 i	33±6.3	7.42±1.39	1.06±0.14	313.40±26.39	69.54±4.93	22.35±2.53
	T2 f	50±8.2	11.32±0.88	1.46±0.062	342.93±47.76	77.22±5.23	23.08±4.36

i, f – beginning and the end of experiment

The ANOVA of haemoglobin in fish exposed to stocking density stress revealed the following:

The quantity of Hb for the trout in R1 had an average of 8.85 and 9.75 g/dl, recording a slight increase as opposed to the initial moment, when it was 7.45 and 7.75 g/dl.

- In the case of the second experimental value, R2, the increase of the quantity of Hb was higher ($p<0.05$), recording an average of 11.13 and 11.32 g/dl, as opposed to the initial moment, when the average was 8.13 and 7.42 g/dl respectively (Table 2).

Nicula (2004) reported that the physical or environmental stress causes the rapid increase in the concentration of Hb, due to the erythrocytes collection from the spleen and the hemoconcentration due to the loss of plasmic water [9]. The quantity of hemoglobin from the blood of trout in experimental variant T2f, greatly decreased ($p<0.05$) in contrast to T2f. The important reduction of hemoglobin can modify the oxygen quantity from tissues and can thus lead to the slowing of the metabolic activity and hence the meager production of energy [10]. The important decrease of the hemoglobin can also be caused by the increase in the destruction rate of Hb or the reduction of its synthesis rate [11].

Haematocrit showed the same trend of increase as in case of haemoglobin. In contrast to initial value, when there were average values of 40±6.9 and 35±8.6 %, the trout from T1 and T2 registered an average 52±2.7% and 50±4.6%, augmenting from a statistic point of view ($p<0.05$). In the case of the second variant (R2), the increase in the value of Ht ($p<0.05$) was similar to that in the high density variant (R1), with an average of 55±3.5 and 50±8.2 %, while at the initial moment it had an average of only 33±4.9%. When the two experimental variants were compared, the value of the hematocrit was constant, with unimportant statistic differences.

The increase in hematocrit value is attributable to the higher viscosity of blood [9]. The number of erythrocytes also showed an increase pertinent to the increase in haemoglobin concentration in higher stocking density. There was an increase in hematocrit in case of both experimental values in contrast to the initial value, related to the increase in quantity of haemoglobin and hematocrit.

Similar results were obtained by Valenzuela A.E. [12] in his work concerning the physical stress, caused by the increase in temperature and the continuous use of light, on the physiology of the blood for *Onchorhynchus mykiss*. The spleen is considered as the depositing place for erythrocytes [13], being able to contract by adrenergic stimuli [14]. When the two experimental variants were compared, a noticed reduction ($p=0.01$) of the number of erythrocytes in the blood of the trout was observed under the influence of density, related to the reduction of haemoglobin quantity. The reduction of the haemoglobin blood concentration has an impact on the cardiac function because the circulating needs and the cardiac rhythm, necessary in order to deliver O_2 to tissues, grow significantly while the hematocrit decreases [9].

The erythrocyte constants (MCV, MCH, MCHC) for the blood of the trout were calculated using the values of Hb, RBC and PCV and putting the values in the standard formulas. During the present experiment, MCV significantly increased in high stocking density group as compared to the low stocking density. In case of high density group, MCV varied between 420.64 ± 20.36 μm^3 and 389.4±58.70 μm^3 as compared to the variant with a smaller density and with an average of 364.81 ± 54.61 and 342.93 ± 47.76 μm^3.

In contrast to MCV, the value of MCH was relatively constant as compared to the initial value, but insignificantly decreased ($p>0.05$) in high stocking density group, with a value of 71.35 ± 4.74 pg. MCHC had a similar value to MCH, insignificantly decreasing ($p>0.05$) with 23% in high stocking density, with a value of 71.35 ± 4.74 pg. Even if the erythrocytes from the blood of the trout in R1 significantly decreased, the adaptation response of the blood to the stocking density was promptly concretized into MCV. But this reaction was not efficient because MCH, as well as MCHC, decreased insignificantly ($p>0.05$). It is possible that modifications are the result of the stressing effect of stocking density above the optimal limit.

4. Conclusion

The results of the present experiment revealed that blood is an important component which reflects/adopts the stress response and projects it through the changes in various parameters. In case of high stocking density group, a significant ($p<0.05$) increase in the number of erythrocytes, hematocrit and the quantity of hemoglobin was recorded. Conclusively blood parameters are the best indices of stress in fish, which give the easy and best estimation of the stress due to various physical or chemical factors.

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