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HEMATOLOGICAL GROWTH AND BLOOD METABOLITE RESPONSES IN THE MONOCULTURE OF JUVENILE CLARIAS GARIEPINUS TO INCREASING STOCKING DENSITY.

Ayoade, O. A[1], Olutimehin I.O[2] , O. A. Oyelese[3]
(1) Department of Agriculture and Fisheries management, University of Ibadan, Ibadan.
(2) Department of Animal Production and Fisheries management, Joseph Ayo Babalola University, P.O. Box5006, Ilesha, Osun state, Nigeria
(3) Department of Agriculture and Fisheries management, University of Ibadan, Ibadan.

ABSTRACT: *A ten (10) week feeding trial was undertaken to assess the growth performance, nutrient utilization and blood metabolites of eight week old Juvenile Clarias griepinus of average weight range 250gm — 300gm stocked under 5 different treatments (10 (Trt I), 15 (Trt 2), 20 (Trt 3), 25 (Trt 4) and 30 (Trt 5) Juvenile Clarias gariepinus/25O litre bowl (under laboratory conditions) with increasing stocking densities. The increasing stocking density caused a decrease in value of the final fish body crude protein (Trt 1 (75.18%) > Iii 2 (73.91%) > Trt 3 (72.71%) > Trt 4 (71.21%) > Trt 5 (70.19%). A similar trend was followed in the specific growth rate (SGR) pattern decreasing from 0.20 (Trt 1) to 0.015 (Trt 5), mean weight gain (MWG) from 6.94 (Trt I) to 3.03 (Tn 5), protein efficiency ration (PER) from 0.29 (Trt I) to 0.04 (Trt 5), productive protein value (PPV) from 0.24 (Trt I) to 0.04 (Tit 5) and Net protein utilization (NPU) from 32.25 (Trt 1) to 19.28 (Trt 5). However the food conversion ratio (FCR) kept increasing from treatment (Trt 1)—88.46 to 662.31 in (Trt 5) with increasing stocking density. FCR correlation coefficient is positive (r 1.2964). Other growth and nutrient utilization parameters had negative correlations respectively. The following haematological and blood parameters (MCV, Hb, RBC, MCV, MCHC, MCH, and Total WBC) also showed significant (P <0.05) pattern of variations with stocking density. For parked cell volume (PCV) significant (P <0.05) drop were noticed from Trt 3 (26.98) to Trt 5 (2.00). Haemoglobin count (Hb) dropped from 6.97 in Trt 4 to 6.18 in Trt 5., confirming reduced activity, stressful conditions noticed at higher stocking densities along poor growth noticed in juvenile fishes in Treatment 5. The gradual and steady significant (P < 0.05) increase in WBC count of 29.26 in Itt 1 (10- fish) to 37.23 in Trt. 5 (30 fish) with increasing stocking density is also a reaction of the fish to stress. However, the Red Blood Cell Count followed a narrow range between Trt 1 2.53 — Trt 5 — 2.99, with no significance (P> 0.05) difference. All diets fed the 5 treatments were isonitrogenous at 40% crude protein.*

KEYWORDS: Hematology, Growth, Metabolites, Monoculture, Stocking Density.

INTRODUCTION

A close monitoring of the stocking density and quantity/quality of feed fed on the haematological and biochemical parameters of the fish is required to ensure optimum growth within the shortest possible period especially with increasing stocking density as it is the case in this study.Haematological characteristics are widely used in clinical diagnosis and pathological conditions in human and domestic animals. The application of haematological

techniques is therefore valuable in fishery biology in the assessment of fish health and stress responses (Blaxhall and Daisley, 1973, Sovio and Oikari, 1976). Decreases in haemoglobin content and haematocrits value are said to cause reduction in fish activity (Wedemeyer and Yatsutake 1977 and Roscoe et al., 1983).The decrease in both haemoglobin and haematocrit are possibly the consequence of anemia and haemodilution caused by massive erythrocytosis. This contention is supported by the observation that haemoglobin and haematrocit values increased during the recovery period when fish were held in toxicant free water.Haematology is the science dealing with natural functions and diseases of blood. The haematological parameters include, packed cell volume (PCV), Haemoglobin (Hb), Red blood cell count (RBC), White blood cell count (WBC), plasma protein concentration, all these assess the blood volume status of an animal. After spinning on the centrifuge, the percentage of the total volume in the number of circulating red cells or reduction in the circulating plasma volume is calculated as follows:

$$\text{Blood Volume} = \text{plasma volume} \times \frac{100}{100 - \text{PCV}}$$

Mean Corpuscular volume (MCV) Volume of red cells in all per 100ml of blood. Mean Corpuscular haemoglobin count (MCHC)

$$= \frac{\text{Haemoglobin in grams per 100mI blood}}{\text{Volume of Red Cells in ml per 100ml blood}} \times \frac{100}{1}$$

$$= \frac{\text{Haemoglobin content}}{\text{Haecmotocrit}} \times \frac{100}{1}$$

The composition of blood may vary from the normal as a result of pathological condition and differential leucocytes have been suggested as an indication of stress in fish (Blaxhall and Daisley, 1973). Increases in the number and distribution of small lymphocytes have been recorded following stress, (Srivastava and Agrawal, 1977, Agrawal and Srivastava and Sahai, 1987). Stress may also result in hypocoagubility of blood thus rendering the fish susceptible to infectious or physiological disease measurable by metabolic, haernatologic and osmoregulatory disturbances according to Wedemayer and Wood (1974) and this could lead to market fish losses (Piper, 1970).Decreases in haemoglobin content and haematocrit value are said to cause reduction in fish activity (Wedemeyer and Yatsutake 1977, Roscoe et al., (1983). They further stated that the decrease in both haemoglobin and haematrocit are possible the consequence of anaemia and haemodilution caused by massive erythrocytosis. This contention is supported by the observation that haemoglobin and haemotocrit values increased during the recovery period when fish were held in toxicant-free water. It is therefore the objective of this study to:

(1) To determining the effect of increasing stocking density on the haematology and blood metabolities in the monoculture of juvenile Clarias gariepinus.
(2) To determine the best haematological blood metabolite level and stocking density that will promote optimum growth of monocultured juvenile Clarias gariepinus.

MATERIALS AND METHODS

Experimental Fish

Eight weeks old juvenile Clarias gariepinus (of average weight range 250gm - 300gm) were used for the 10 week experiment. The fishes were deprived of feed for 2 days in order to

acclimatize them to the new environment. Before the feeding commenced-the initial weight of the fishes were taken and recorded, and then distributed into five 250m1 bowls containing water i.e. treatment I contained 10 fishes, treatment 2 contained 15 fishes, treatment 3 - 20 fishes, treatment 4 - 25 fishes and treatment 5 - 30 fishes. That is a total of 100 juvenile Clarias gariepinus were used and feeding rates were adjusted weekly. Water was changed daily to avoid pollution, the volume of water in each bowl at any given time was 447.48cm^3.

GROSS COMPOSITION OF EXPERIMENTAL DIET

The fishes were fed with 40% crude protein. The ingredients used include yellow maize, palm kernel cake, soyabean meal, Groundnut cake, fish meal, bone meal, oyster shell, Grower premix and salt. All dry feed ingredients were properly weighed, grinded and mixed thoroughly before the addition of starch used for the pelleting with the aid of locally adapted pelleting tray. The pellets were later sundried and stored in air tight polythene bags.

Table 1: Gross Composition of Experimental Diet Using 40% Crude Protein

Ingredients	Quantity (kg)
Yellow Maize	10
Palm Kernel Cake	10
Soya beans meal	25
Groundnut Cake	40
Fish meal	10
Bone meal	3
Oyster shell	1.25
Growers premix	0.25
Salt	0.50
	100kg

Feeding Trials and Proximate Analysis of Fish and Feed

The fish were fed twice daily with the compounded 40% crude protein diet at 9.OOa.m. and 5.OOp.m. at 3% body weight (divided into two halves for each feeding). Daily feeding of fishes is usually carried out immediately after changing the water in each treatment.

The proximate analyses of fish were carried out before and after the experiments in each treatment while similarly the proximate analysis of the compounded feed were also determined.

Blood Analysis

The blood analysis was carried out bi-weekly by sacrificing two fishes from each treatment for the determination of the following haematological parameters:- Haemoglobin estimation (Hb), Packed Cell Volume (PCV), Red blood cell (RBC), white blood cell count (WBC), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC).

Estimation of Packed Cell Volume (Pcv) or Haematocrit

The packed cell volume (PCV) is expressed as the percentage volume of the blood which is occupied by the red blood cells. The capillary and centrifuging method was used in this study as described below

(a) Fill the capillary tube to ¾ of its length

(b) Put in the centrifuge, and allow to rotate 36,000 times, revolve for about 5 minutes.
(c) Put the capillary tube on the haematocrit reader and read the volume of red blood cell.

Additional Parameters

The haematocrit value or parked cell volume is then used to calculate

1. Mean Corpuscular Volume (MCV) MCV $\frac{\text{PCV XJ}}{\text{RBC}}$ (f1)
2. Mean Corpuscular Haemoglobin (MCH) MCH $\frac{\text{Hb X 100}}{\text{RBC}}$ pg.
3. Mean Corpuscular Haemoglobin Concentration (MC I IC)

$$\text{MCH} = \frac{\text{HB X 100gm}}{\text{PCV}}/100\text{ml}$$

Unit of measurement = % PCV

Determination of Red Blood Cell Count In The Experimental Fish

The method is based on counting the number of red blood cells in a known small volume of accurately diluted blood. Blood is diluted with Hayem's solution (HgC1 Na 2.50gm NaCl 0.5gm, H lOOm!) in a red cell pipette and the red blood in the small volume of the diluted blood is counted in a counting chamber as stated below:- -

(a) See that the pipette is clean and dry.
(b) After titling the blood downward then fill the pipette with blood to 0.5 mark.
(c) Withdraw pipette and keep it horizontally to prevent blood from shifting.
(d) Keeping the pipette nearly horizontal and draw the hayem's solution to the 101 mark, do not overshoot.
(e) Mix the blood thoroughly for 1 mm. after removing the rubger tubing without squeezing.
(f) See that counting chamber and cover slip are quite clean.
(g) Bring the tip of pipette in contact with exposed part of counting chamber and allow the fluid to run under the cover slip.
(h) Place the slide on the horizontal stage of the microscope and allow the corpuscles to settle for 2 mins. Examine using " x 10" objective.

Note that if distribution of cells is uneven, the slide must be cleared and dried and a fresh diluted blood run under the coverslip. But if evenly distributed, proceed to count using" X40" objective, count the number of corpuscles, in each of the small squares.

Calculation:-

Volume of diluted blood over each small square = $\frac{1}{400}$ cu.mm

(Since depth of chamber is 1/10mm and area is l/400sq mm). Volume of diluted blood over 80 small squares 80/4,000cu.mm x the number of corpuscles lying over 80 small squares. Then corpuscles are present in 80/4,000cu.mm of diluted blood. If blood is diluted 200 times, then 80/4,000 x 200 corpuscles are present in diluted blood.

Determination of White Blood Corpuscles in The Experimental Fish

The procedure is as the same as the determination of Red Blood Corpuscles count. The little differences are the diluting fluid in 2% Acetic acid with dye like methyl violete destroys the red cells membrane so that the red cells are not seen. The white blood corpuscles pipette is marked 1 below and 11 above its bulb contain a white bead and the dilution factor is I : 20.

The calculation is:-

(a) Size of square area is 1mm and depth is 0.1mm. Hence the number of W.B.C. counted is 0.lcu.mm. The dilution is 1 : 20 so the number oW.13.C. per cu.mm is equal to the number counted x 200.

(b)

Indices of Growth and Nutrient Utilization

(1) Mean Weight Gain (MWG) = $\frac{\text{Total Weight Gain (gm)}}{\text{No. of fish}}$

(2) Mean Weight Gain per day (MWGD) = $\frac{\text{Mean Weight Gain}}{\text{No. of days}}$

(3) Mean Weight Gain per week (MWG/W) = $\frac{\text{Mean Weight Gain per Week}}{\text{No. of Weeks}}$

(4) Total Feed Intake = This is obtained by calculating the amount of feed per week for the experimental period.

(5) Food Conversion Ratio (FCR) = $\frac{\text{Food Consumed per Unit time}}{\text{Growth per unit time}}$

(6) Protein Intake (PT) = $\frac{\text{Total feed consumed x \% crude protein}}{100}$

(7) Protein Efficiency Ratio (PER) = $\frac{\text{Net Weight Gain}}{\text{Protein Intake}}$

(8) Total Percentage Weight Gain (%) (TPWG) T [PWG = $\frac{\text{Total Weight Gain x 100}}{\text{Initial Weight}}$

(9) Specific Growth Rate (SGR)% SGR = $\frac{\log_2 w_1 - \log_2 w_2}{T_2 - T_1} \times \frac{100}{1}$

Where W_1 = Initial weight of fish

W_2 = Fir weight of fish

T_2 = Time 2

T_1 = Time 1

(10) Gross Efficiency of Food Conversion (GEFC) GEFC = $\frac{1}{\text{FCR}} \times \frac{100}{1}$

(11) Productive Protein Value (PPV) PPV = $\frac{\text{Increment of Body Protein/day}}{\text{Protein Intake}}$

(12) Condition factor (K) = $\frac{100W}{L^3}$

L = Standard length

W = Body weight

K = Condition factor

K is the measure of the well being of a fish.

RESULTS

Table 2: Proximate Composition of Experimental Diet

Nutrient	**% Dry Weight**
Crude protein	41.59
Crude fat	15.03
Crude fibre	18.86
Ash Content	5.88
Moisture Content	16.89
NFE	1.75

Table 3: Proximate Composition of Experimental Fish

	Crude % Protein	**Fat %**	**Crude Fibre %**	**Ash % Content**	**Moisture Content %**	**NFE**
Before the Experiment	70.05	5.32	2.09	2.88	7.86	1.80
After the Experiment Treatment	Crude Protein	Fat %	Crude Fibre %	Ash % Content	Moisture Content %	NFE
TR1	75.18	4.64	1.36	10.51	8.11	0.20
TR2	73.91	4.56	1.49	10.84	7.96	1.24
TR3	72.71	4.45	1.64	10.98	7.85	2.39
TR4	71.21	4.34	1.73	11.13	7.70	3.89
TR5	70.19	4.27	1.82	11.31	7.64	4.77

As shown in Table 3 the crude protein of fish decreased from Treatment 1 (75.18%) to Trt 5 (70.19%) with increasing stocking density (i.e. from Trt 1 (10 fishes) to Trt 2 (30 fishes). Although crude protein of the final fish in each treatment was much higher than the initial crude protein of 70.05%.

Table 4: Growth and Nutrient Utilization of the Experimental Fish

	Experimental Fish				
TREATMENT	**I**	**II**	**III**	**IV**	**V**
Experimental period (days)	70	70	70	70	70
Number of fish stocked	10	15	20	25	30
Mean Initial Weight (gm)	2.09	2.00	1.93	1.65	1.62
Mean Final Weight (gm)	9.03	7.89	6.76	5.49	4.65
Mean Weight gain (gm)	6.94	5.89	4.83	3.84	3.03
Mean Daily Weight gain	0.009	0.084	0.069	0.055	0.043
Specific Growth rate(SGR)	0.02	0.19	0.018	0.017	0.015
Total Feed Intake	59.29	104.79	140.77	153.58	172.27
Mean Feed Intake	0.847	1.497	2.011	2.194	2.461
Food Conversion Ratio	88.46	185.91	325.95	448.54	662.31
Gross Feed Conversion Efficiency (%)	1.13	0.54	0.31	0.22	0.15
Protein Efficiency Ratio	0.29	0.14	0.09	0.06	0.04
Productive Protein Value	0.24	0.14	0.09	0.06	0.002
Nitrogen Metabolism	213.70	190.03	166.98	137.20	120.48
Net Protein Utilization	32.25	28.67	26.78	21.98	19.28
Protein Intake	68.91	61.40	56.30	41.90	23.90

Highest mean weight gain (6.94gm) specific growth rate (0.02), protein efficiency ratio (0.29), productive protein value (0.24), nitrogen metabolism (213.70), Net protein utilization (32.25) and protein intake (68.91gm) with least food conversion ratio of (88.46) was recorded for Diet treatment 1, the diet with the least stocking density of 10 juvenile Clarias per bowl. While the poorest growth and nutrient utilization pattern in terms of least mean (Table 4).

Table 5: Linear Equation Coefficients of Determination (r^2) Relating Each of the Independent Variable (Stocking Density. (SD) and Nutrient Utilization Parameters

PARAMETERS X	STOCKING DENSITY Y	PREDUCTION EQUATION	R	R^2
SGR	SD	18.2884 + 0.9427X	- 0.1957	0.0382
PPV	SD	43.525 – 30.98X	- 0.9190	0.8447
FCR	SD	26.6577 – 0.1945X	1.2964	1.6808
PI	SD	17.1026 + 0.5743X	- 0.8106	0.6570
GEFC	SD	14.8949 + 0.9528X	- 0.8154	0.3870
PER	SD	0.59 + 14.90X	- 0.8899	0.7919
MWG	SD	-0.1528 + 0.4144	- 0.6693	0.4480

Weight gain (3.03gm), specific growth rate (0.043), protein efficiency ratio (0.04), productive protein value (0.002), nitrogen metabolism (120.48), net protein utilization (19.28), protein intake (23.90) and highest food conversion ratio (662.31) was recorded. Diet treatment 5, the diet with the highest stockng density of 30 juvenile Clarias per bowl.

Furthermore as shown in Table 5, there is a strong negative correlation between stocking density and SGR (r = - 0.1957), PPV (r = - 0.9 190), GEFC (R - 0.8154) PER (r = - .8890) MWG (r - 0.6693) all indicating growth and nutrient utilization of fish is not favoured with increasing stocking density, that is growth of Clarias is slowed down with increasing stocking density.

Table 6: Bi-Weekly of Haematological Parameters of the Experimental Fish

Treatment	Weeks	PCV	HB	RBC	MCV	MCHC	MCH	Total WBC
Initial	0	30.00	10.80	2.90	103.60	36.00	36.40	28.50
TR1	2	29.20	10.20	2.86	101.20	36.10	36.00	28.56
10	4	28.90	9.80	2.50	98.40	36.25	35.10	29.01
	6	26.70	9.40	2.45	95.30	36.40	34.20	29.55
	8	25.30	9.10	2.40	92.70	36.20	33.01	30.01
	10	24.60	8.50	2.36	90.00	36.30	33.12	30.40
	Mean	27.45	9.65	2.60	96.87	36.21	34.81	29.26
TR2	9	30.00	10.80	103.60	36.00	37.40	37.40	28.00
15	2	28.50	9.95	102.70	26.20	35.76	35.76	30.00
	4	27.20	9.30	95.30	36.35	35.02	35.02	31.50
	6	26.80	9.09	93.40.	36.21	34.51	34.51	31.78
	8	26.00	8.56	90.10	36.10	34.33	34.33	32.01
	10	25.40	8.23	89.50	36.33	33.21	33.21	32.51
	Mean	27.32	9.32	2.56	95.77	36.20	35.03	30.80
TR3	0	30.00	10.80	2.90	103.60	36.00	37.40	28.00
20	2	28.80	19.81	2.70	98.20	34.76	35.23	32.80
	4	27.20	9.40	2.50	97.80	35.09	34.81	33.01
	6	26.10	9.20	2.43	96.70	36.20	34.22	33.20

	8	25.60	8.89	2.38	96.30	33.25	33.90	34.15
	10	24.20	8.60	2.30	94.10	34.10	33.62	34.51
TR4	0	30.00	10.80	2.90	103.60	36.00	37.40	28.00
25	2	24.10	6.48	2.68	109.80	29.45	34.35	34.00
	4	23.20	6.20	2.51	98.70	28.01	3409	34.81
	6	31.80	9.03	2.44	97.40	30.02	33.66	35.20
	8	20.50	5.76	2.38	64.30	30.58	33.15	35.98
	10	19.60	5.36	2.29	92.90	31.21	32.05	36.53
	Mean	23.20	6.97	2.53	99.45	30.87	34.11	34.08
TR5	0	30.00	10.80	2.90	163.60	36.00	37.40	28.00
30	2	20.10	5.80	2.57	114.00	30.49	34.51	36.00
	4	19.70	5.61	2.50	113.10	30.69	34.10	37.98
	6	19.20	5.25	2.42	112.90	31.02	32.28	38.78
	8	18.70	4.91	2.38	111.20	31.51	32.28	40.01
	10	18.40	4.73	2.19	110.90	31.78	31.71	42.58
	Mean	21.61	6.18	2.99	110.90	31.90	33.23	37.23

N.B: Unit of Measurement: PCV = %; 1-TB g/100ml; REC Count x 10^6/UL, WBC = X 10^3/UL; MCV = Fe, MCHC = %(gm/100ml), MCH pg

As shown in Table 6, the haematological parameters measured in this study showed significant patterns (P <0.05) of variations.Packed Cell Volume (PCV) was almost constant in trt 11 x 2, but dropped significantly (P < 0.05) from trt 3(26.98) to trt 5(21.00).
Haemoglobin count (Hb) followed a narrow range of 9.32 to 9.65 in trt 1 to trt 3 but dropped significantly (P <0.05) to 6.97 in Irt 4 ad 6.18 in trt S.Red Blood Cell Count (RBC) followed a narrow range of 2.53 — 2.99 in trt 1 to trt 5.Mean Corpuscular Haemoglobin Concentration (MCHC) was constant for Trt I and 2 (36.21 and 36.20) but a significant drop (P <0.05) was noticed from Trt 3 (34.90) to trt 5 (31.90).Mean Corpuscular haemoglobin (MCH) dropped significantly (P <0.05) from trt 1 (34.81) to tn 5 (33.23).The White Blood Cell Count showed a gradual and steady significant (P <0.05) increase from 29.26 (trt 1) to 37.23 in trt 5.

DISCUSSION

Protein is one of the most essential nutrient for growth of fish. Therefore from the result obtained in the growth and nutrient utilization parameters monitored in the study, environmental stress as a result of increasing stocking density imposed a negative effect on the growth rate of the fish, level of protein intake and utilization.The assertion is confirmed by the highest crude protein percentage recorded in Treatment I (10 fishes) of 75.18% compared to 70.19% in Treatment 5 (30 fishes) in the final proximate analysis. Also the highest SGR 0.02 (trt 1) compared to 0.015 (trt 5), mean weight gain 6.94gm (trt 1) compared to 3.03gm (trt 5), protein efficiency ratio of 0.29 (Trt 1) compared to (0.04) in treatment 5 of much higher stocking density.The various blood parameters of the fish (PCV, fib, RBC, MCV, MCHC, MCH and RBC) measured in this study are affected by the increasing stocking density and consequently inducing poor physiological status on the fish especially at higher stocking density as it is the case in treatment 5. However the variation pattern is determined by the blood parameter monitored.

The significant (P <0.05) drop in PCV from trt 3 (26.98) to trt 5 (21.00), and haemoglobin count from treatment 4 (6.97) to 6.18 in treatment 5 with the highest stocking density is inline with Wedemeyer and Yatsuke (1977), and Roscoe et a!., (1983) findings that reduced haemoglohin content causes reduced fish activity, anaemia and haemodilution caused by massive erythrocytosis.

The gradual and steady significant (P <0.05) increase in WBC count of 29.26 in treatment I (10 fish) to 37.23 in treatment 5 (30 fish), that is with increasing stocking density is a reaction of the fish stress. The findings is also in line with Blaxhall and Daisley (1973), Savastava and Agrawal (1977), Agrawal and Srinvastava (1978). They found total and differential leucocyte counts as an indication of stress in fish which showed increase in the number and distribution of small lymphocyte counts as an indication of stress in fish which showed increase in the number and distribution of small lymphocytes during stress.The Red Blood Cell Count followed a narrow range with no significant differences (P > 0.05) among the treatments between treatment I — 2.53 to treatment 5 — 2.99. This tendency to maintain the RBC count pool is further enhanced since all diets fed the 5 treatments were isonitogenous at 40% crude protein even with increasing stocking density.The MCHC was constant for Treatment I and 2 (36.21 and 36.20), a significant drop was noticed from Trt 3 (34.90) to trt 5 (31.90). MCH dropped significant1y (P < 0.05) from treatment 1 (34.81) to treatment 5 (33.23). The MCH dropped significantly (P <0.05) from treatment 1 (34.81) to treatment 5 (33.23). All these are consequences of increasing stocking density of the fish.

CONCLUSION

Increasing stocking density of juvenile Clarias gariepinus under monoculture conditions promotes poor fish growth, reduced activities due to overcrowding and poor haematological status of the fish. There is increased agitation of the fish resulting into stressful conditions causing an increased production of white blood cells in the blood and a steady drop in Parked Cell Volume and haemoglobin count.

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