

Effect of cassava mill effluents on the osmotic and hematology of *clarias gariepinus* juveniles

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Abstract: *Clarias gariepinus* juveniles of mean weight $17.0 \pm 0.5g$ were allotted to aquaria at 10 fish per treatment (1-5) in two replicates based on the concentration of cassava mill effluent to be administered (100, 110, 120, 130 and 140 ml/l) respectively. Treatment 6 serves as the control. The different concentrations were administered to the various treatments and after 96 h, no mortality was recorded in the control (treatment 6), 100% mortality was recorded in concentrations 130 and 140 ml/l, 20% and 30% mortality was observed in concentrations 100 and 110 ml/l respectively while 60% mortality was recorded in concentration 120 ml/l during the 96 h experiment. Osmotic fragility of *Clarias gariepinus* revealed that cassava mill effluent has no significant effect on *Clarias gariepinus* juveniles.

Hematological changes observed include; anemia was significantly high, PCV, Hb, MCV values were significantly low in all the experimental treatments relative to the control ($P < 0.05$). MCH value was significantly low, while MCHC was significantly high ($P < 0.05$). The total white blood cell count (WBC) was significantly higher ($P > 0.05$) than the control. Other observations during the experiment includes reduced activities (swimming), haemorrhagic patches on the ventral surface of the fish, general discoloration and anoxia.

Keywords: Cassava, *Clarias gariepinus*, Hematological parameters, Mill effluent, Osmotic fragility,

I. Introduction

Aquatic animals in nature are constantly exposed to toxins. The type and concentrations of toxins in water are determined by geochemical processes and large scale releases into the aquatic environment by human activities (anthropogenic activities) [1]. Rapid industrial development, as well as the use of metals in production processes has led to the increased discharges of heavy metals into the environment [2]. According to [3], the harmful effects of heavy metals as pollutants result from incomplete biological degradation. Therefore, these metals tend to accumulate in the aquatic environment. Since heavy metals are non-biodegradable, they can be bio-accumulated by fish, either directly from the surrounding water or by ingestion of food [4,5]. In addition, [6] indicates that when metals reach sufficiently high concentrations in body cells they can alter the physiological functioning of the fish. Toxic substances cannot easily be defined due to a number of factors that can influence and modify the toxicity of these substances.

Human destructive influence on the aquatic environment is in the form of sub-lethal pollution, which results in chronic stress conditions that have negative effect on aquatic life [7]. The main source of freshwater pollution can be attributed to discharge of untreated waste, dumping of industrial effluent, and run-off from agricultural fields. Stress response is characterized by physiological changes and the effect of pollutants on fish is assessed by acute and chronic toxicity tests [8]. Normal physiological processes are affected long before death of an organism hence the need for physiological indicator of health and sub-lethal toxicant effects [9,10]. In recent years, hematological variables were used more when clinical diagnosis of fish physiology was applied to determine the effects of external stressors and toxic substances as a result of the close association between the circulatory system and the external environment [11, 12]. [13] also suggested that hematology, biochemical changes, growth rate and oxygen consumption of fish can be used in determining the toxicity of pollutants. The process of starch extraction from cassava tuber requires a large quantity of water, thereby resulting in the release of significant quantity of waste water (effluent). It is common for this effluent to be discharge into nearby rivers and streams. These effluents pose a serious threat to the environment and quantity of life in the receiving waters. [14] observed that the concentration of total cyanoglucosides in cassava effluents ranged between 12.9 – 66.6 mg/l in the case of initial sampling, whereas in the case of final wastewater samples, the concentration ranged between 10.4 – 274 mg/l.

II. Materials and Methods

2.1. Fish. 300 experimental *Clarias gariepinus* juveniles (mean weight, $17.0 \pm 0.5g$) were obtained from Training and Research farm of the Department of Fisheries and Aquaculture Technology, Federal University of Technology, Akure and acclimated to laboratory conditions for 24 hours prior to the toxicity test. Fish were

randomly distributed into 12 plastic aquaria containing 10 liters of fresh water at 10fish/aquarium. Fresh cassava mill effluents were collected from a small-scale cassava processing mill in Shagari village, Akure, Nigeria. The effluents were scooped from the same point into 25 L plastic containers from moulds in the mills in order to form a homogenous mixture, Five varying concentrations (100, 110, 120, 130 and 150 ml/l) in duplicate with a control were used on *Clarias gariepinus* juveniles.

During the 96h exposure period, temperature, pH and DO₂ of the experimental media were measured using standard laboratory methods as described by American Public Health Association [15].

Five fish from each of the experimental media were collected and blood was drawn from the caudal peduncle of the fish into some heparinized micro- haematocrit tubes. Osmotic fragility of the erythrocyte of the red blood cell was determined in fish hemolymph by measuring and analyzing with Hitachi atomic absorption spectrophotometer (Model 170). Haematocrit count (Hb), Packed Cell Volume (PCV), Red Blood Cell (RBC-erythrocyte), White Blood Cell (WBC), total leucocytes and thrombocytes were counted with a Neubauer hemacytometer with Dacies solution as a diluting fluid. The Mean Cellular Volume, Mean Cell Hemoglobin and Mean Cellular Hemoglobin Concentration were also calculated by standard formula [16]. The data collected from the five fish taken from each of the experiment media were subjected to statistical analysis using two way analysis of variance (ANOVA) at 0.05 level of probability using SPSS (Statistical Package to Computer Software)

III. Results

The physic-chemical parameters measured during the 96 h exposure period is presented in TABLE 1. The various parameters were not significantly different ($P>0.05$) from each other among the treatments. The hematological results of *Clarias gariepinus* exposed to different concentrations of neem is shown in Fig.1. The cassava mill effluent has no significant effect on the erythrocyte osmotic fragility of *Clarias gariepinus*. Results of osmotic fragility revealed that erythrocyte hemolysis occurred in phases as concentrations of test effluent increases. At the lowest concentration of 100ml/l, erythrocyte fragility values were 24.33%, 22.00%, and 29.00% in phases 1-3 respectively, phase 4 has a value of 25.00%, phases 5 and 6 at concentration 100ml/l were 23.00% and 20.00% (TABLE 2).

Phases 1,2 and 3 at concentration of 110ml/l have the value of 29.00, 32.66 and 32.33 % respectively. At Phase 4 of the same concentration hemolysis had reduced to a minimum value of 24.66%. Phases 5 and 6 of concentration 110 ml/l have minimum values of 24.00% and 23.00% respectively (TABLE 2). Osmotic fragility at Phase1 of concentration 120 ml/l was 25.00% while in Phase 2, 3 and 4 osmotic fragility had increased to 30.00 %, 30.33% was the maximum value at 120ml/l and 28.00% as minimum value at 120 ml. Phases 5 and 6 have osmotic fragility values of 25.66% and 21.00% respectively, both had minimum value of osmotic fragility (Table 2).

Osmotic fragility in Phases 1,2 and 3 were 27.00%, 28.00% and 29.66% respectively at concentration 130ml while Phases 4 ,5 and 6 were 27.00%, 24.00% and 21.33% minimum values. At concentration 140ml/l, osmotic fragility at Phases 1, 2 and 3 were 25.00% ,21.00% and 28.66% while in Phases 4 ,5 and 5, osmotic fragility were 29.00% ,23.00% and 18.00% respectively (TABLE 2, Fig.2).

The control experiment had osmotic fragility in Phases 1,2 and 3 of 41.00% which increased to 42.00% and further increased to 46.66% in Phases 4,5 and 6 osmotic fragility reduced gradually from 32.66% in Phase 4 to 27.00% in Phase 5 and finally reduced to 25.00% in Phase 6 (Fig.2)

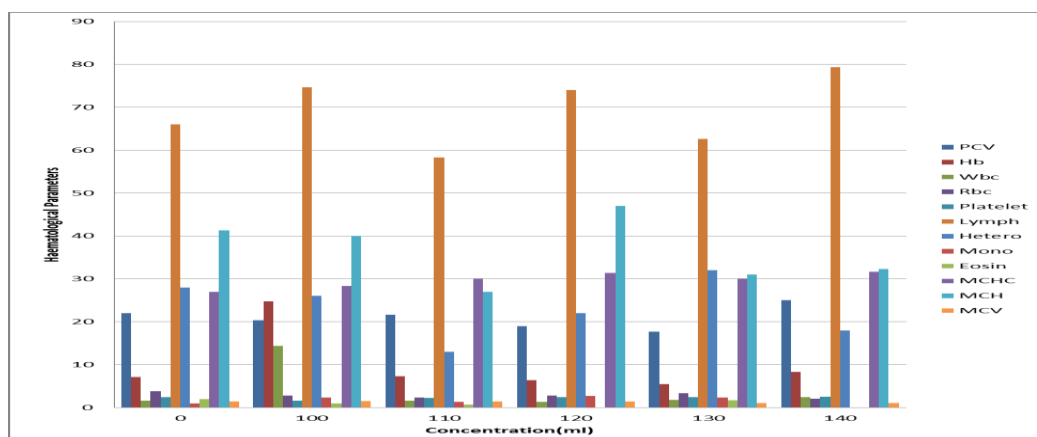


Fig 1. Effect of different Concentrations of Cassava Mill Effluent on Haematological Prameters of *C. gariepinus*

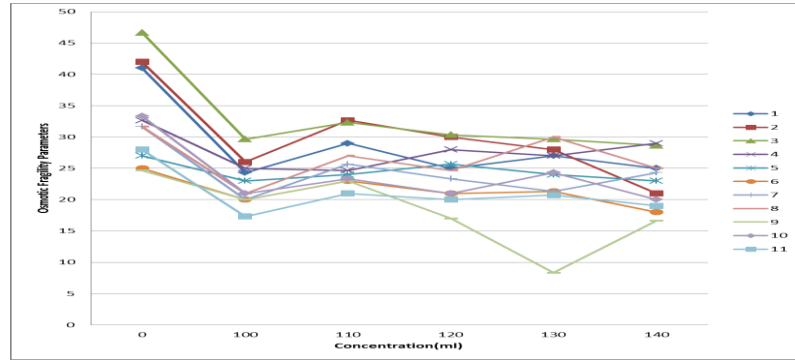


Fig 2: Osmotic fragility Parameters of *C. gariepinus* Juveniles Exposed to Different Concentrations of Cassava Mill Effluent.

Red blood cell (RBC) and Packed Cell Volume (PCV) counts were $2.81 \pm 160.72/\text{mm}^3$ and $20.33 \pm 1.52\%$ respectively at concentration of 100ml (TABLE 3, Fig.3). The values of Red Blood Cell and Packed Cell Volume (PCV) in concentrations 110 and 120ml/l were $2.35 \pm 57.73/\text{mm}^3$, $21.66 \pm 1.52\%$, $2.80 \pm 0.00/\text{mm}^3$ and $19.00 \pm 1.00\%$ respectively while in concentrations 130ml and 140ml, RBC and PCV were $3.32 \pm 50.00/\text{mm}^3$, $17.66 \pm 0.57\%$, $2.09 \pm 0.00/\text{mm}^3$ and $25.00 \pm 1.00\%$ respectively (TABLE 3, Fig.3). The control value for RBC and PCV were $3.84 \pm 493.25/\text{mm}^3$ and $22.00 \pm 1.00\%$ respectively (TABLE 3).

The Mean Cell Volume (MCV) and Mean Cell Haemoglobin (MCH) were $1.48 \pm 1.00\text{ft}$ and $40.00 \pm 0.00\text{pg}$ respectively at concentration 100ml /l while MCV and MCH were $1.44 \pm 1.00\text{ft}$ and $27.00 \pm 4.35\text{pg}$, $1.43 \pm 1.52\text{ft}$ and $47.00 \pm 1.00\text{pg}$ respectively at concentrations 110 and 120ml, (TABLE 3, Fig. 4). At concentrations 130 and 140ml, MCV and MCH were $1.01 \pm 1.15\text{ft}$ and $31.00 \pm 1.00\text{pg}$, $1.04 \pm 2.00\text{ft}$ and $32.33 \pm 1.52\text{pg}$ respectively (Table 3). Control value for MCV and MCH were $1.40 \pm 1.00\text{ft}$ and $41.33 \pm 1.52\text{pg}$, respectively (Table 3, Fig.4). Mean Corpuscular Haemoglobin Concentration values at concentration 100-140ml ranged from 28.33 ± 1.53 - $31.66 \pm 1.52\%$. Control value of MCHC was 27.00 ± 1.00 (TABLE 3).

In the erythrocyte osmotic fragility result, when comparing the osmotic fragility of unaffected fish (control) with the affected fish (polluted fish), the minimum and maximum hemolysis stages of fish at different effluent differ significantly ($P < 0.05$). Hemolysis in all the stages decreased with increase in effluent concentration, showing that the osmotic fragility of fish from polluted waters may not show any fragility in the presence of harmful substance such as cassava mill effluent.

There was an intra species variation in hematological values as indicated by the wide ranges of some parameters in this study which have been attributed to many reasons. These findings agreed with that of [17] who reported changes in blood parameters of fresh water fish exposed to various handling procedure before experiment.

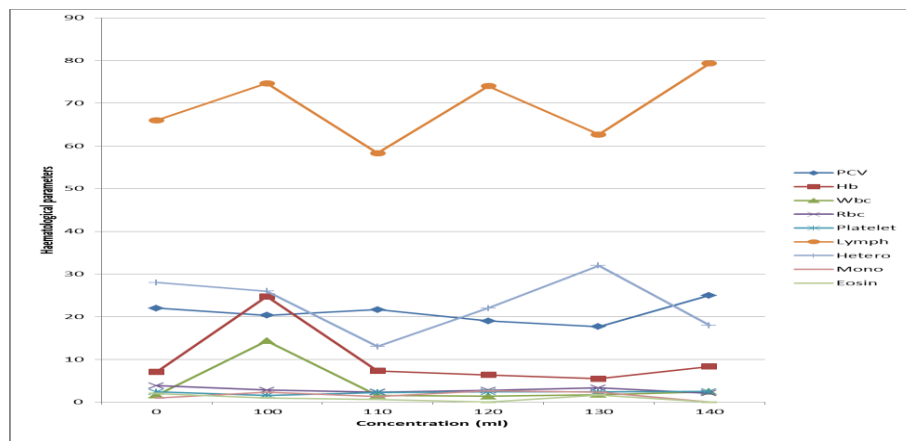


Fig 3. Haematological Parameters of *C. gariepinus* Juveniles Exposed To Different Concentration of Cassava Mill Effluent.

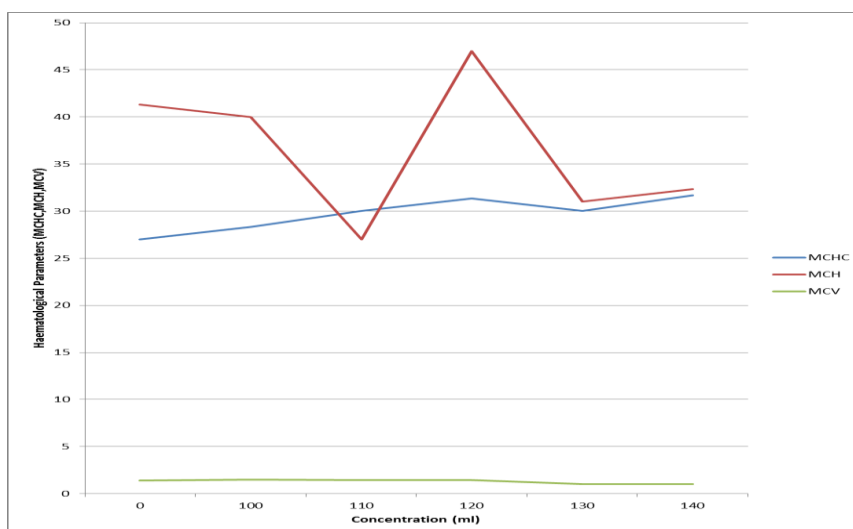


Fig.4.Haematological Parameters of *C. gariepinus* Juveniles Eexposed to Different Concentration of Cassava Mill Effluent

Table 1.Water quality of *Clarias gariepinus* juveniles exposed to sud- lethal concentration of cassava mill effluent during the 96 h exposure

Concentrations()	DO ₂	Temperature	p ^H
0.0	5.85±0.28 ^a	27.70±0.58 ^a	6.67±0.19a
100	4.14±0.07 ^a	27.73±0.58 ^a	7.71±0.19d ^c
110	4.14±0.07 ^a	27.75±0.58 ^a	8.72±0.19c
120	4.31±0.07 ^b	27.73±0.58 ^a	7.14±0.19b
130	4.51±0.07 ^c	27.75±0.58 ^a	7.69±0.19d
140	4.81±0.07 ^d	27.76±0.58 ^a	7.30±0.19c ^a

Mean ±SE of different superscripts signifies significant difference (P≤ 0.05)

Table 2. Osmotic fragility values of *Clarias gariepinus* juveniles exposed to sub-lethal concentrations of cassava mill effluent during 96 h exposure.

Concentrations()	Phase 1	Phase 2	Phase 3	Phase 4	Phase 5	Phase 6
0.0	41.00±1.00 ^b	42.00±2.00 ^a	46.66±1.52 ^a	32.66±1.52 ^b	27.00±1.00 ^a	25.00±1.00 ^a
100	24.33±1.52 ^b	22.00±1.00 ^c	29.00±0.57 ^c	25.00±1.00 ^a	23.00±1.00 ^b	20.00±1.00 ^c
110	29.00±1.00 ^a	32.66±1.52 ^b	32.33±1.15 ^b	24.66±1.52 ^c	24.00±1.00 ^a	23.00±1.00 ^b
120	25.00±1.00 ^b	30.00±1.00 ^c	30.33±0.57 ^a	28.00±1.00 ^b	25.66±1.52 ^a	21.00±1.00 ^b
130	27.00±1.00 ^c	28.00±1.00 ^a	29.66±0.57 ^c	27.00±1.00 ^b	24.00±1.00 ^b	21.33±1.00 ^a
140	25.00±1.00 ^b	21.00±1.00 ^c	28.66±0.57 ^a	29.00±1.00 ^b	23.00±1.00 ^c	18.00±1.00 ^a

Mean ±SE of different superscripts signifies significant difference (P≤ 0.05)

Parameters	Concentrations (ml)					
	0.0	100	110	120	130	140
PCV (%)	22.00 ± 1.00 ^b	20.33 ± 1.52 ^b	21.66 ± 1.52 ^b	19.00 ± 1.00 ^b	17.66 ± 0.57 ^b	25.00 ± 1.00 ^b
HB(g/dl)	7.1 ± 0.10 ^a	24.76 ± 31.37 ^a	7.33 ± 0.49 ^a	6.40 ± 0.10 ^a	5.50 ± 0.10 ^a	8.33 ± 0.05 ^a
WBC(mm ³)	1.61 ± 0.35 ^b	14.33 ± 0.05 ^b	1.56 ± 0.02 ^b	1.75 ± 0.03 ^b	1.75 ± 0.03 ^b	2.42 ± 0.02 ^b
RBC(g/dl)	3.84 ± 493.25 ^b	2.81 ± 160.72 ^b	2.35 ± 57.73 ^b	2.80 ± 0.00 ^b	3.32 ± 50.00 ^b	2.09 ± 0.00 ^b
MCHC	27.00 ± 1.00 ^a	28.33 ± 1.53 ^a	30.00 ± 1.00 ^a	31.33 ± 1.52 ^a	30.00 ± 1.00 ^a	31.66 ± 1.52 ^a
MCH	41.33 ± 1.52 ^b	40.00 ± 1.00 ^b	27.00 ± 4.35 ^b	47.00 ± 1.00 ^b	31.00 ± 1.00 ^b	32.33 ± 1.52 ^b
MCV	1.40 ± 1.00 ^b	1.48 ± 1.00 ^b	1.44 ± 1.00 ^b	1.43 ± 1.52 ^b	1.01 ± 1.15 ^b	1.04 ± 2.00 ^b
MONOCYTE	1.00 ± 0.00 ^a	2.33 ± 0.57 ^a	1.33 ± 0.57 ^a	2.66 ± 0.56 ^a	2.33 ± 1.15 ^a	1.00 ± 0.00 ^a
EOSINOPHIL	2.00 ± 1.00 ^a	1.00 ± 0.00 ^a	0.66 ± 0.57 ^a	0.00 ± 0.00 ^a	1.66 ± 0.57 ^a	2.00 ± 1.00 ^a
PLATELET	2.40 ± 0.00 ^b	1.60 ± 57.73 ^b	2.26 ± 57.73 ^b	2.14 ± 13856.6 ^b	2.40 ± 1154.70 ^b	2.5 ± 115.47 ^b
LYMPHOCYTE	66.00 ± 1.00 ^a	74.66 ± 10.96 ^a	58.33 ± 34.9 ^a	74.00 ± 1.00 ^a	62.55 ± 0.57 ^a	79.33 ± 0.57 ^a
HETEROPHIL	28.00 ± 1.00 ^a	26.00 ± 1.00 ^a	13.00 ± 9.35 ^a	22.00 ± 1.00 ^a	32.00 ± 1.00 ^a	18.00 ± 0.00 ^a

Mean ± SE of different superscripts signifies there is significant difference (p ≤ 0.05)

Table 3. Mean values of Haematological Parameters of *C. gariepinus* Juveniles Exposed to Different Concentrations of Cassava Mill Effluent during 96h Exposure

Mean ± SE of different superscripts signifies there is significant difference (p ≤ 0.05)

IV. Discussion

The effect of stress on fish, such as fish transportation, low oxygen, high ammonia results in changes in haematological picture of fish [17]. The result of haematology on fish used for this study agreed with this finding. Speirs (1955) reported that eosinophil which is a white blood cell precursor disappears from circulation during stress. Laboratory acclimation of Channel catfish transported from the field to the laboratory displayed different haematological value from those considered to be normal and had lost their ability to respond in vitro to both nitrogenic and antigenic stimuli [18]. This finding was observed in the result of haematological parameter of *C. gariepinus* used in this experiment.[19] stated that sampling stress caused an increase in haematocrit value and decrease in mean cellular haemoglobin concentration in rainbow trout (*Salmon gairdineri*). This is an indication that red cell swelling, due to sampling stress caused haemoconcentration in the pike, *Exos Lucius*. Haemoglobin concentration and haematocrit of the fish blood decreases after the stress due to capture, handling and sampling procedure. The findings of [20] agreed with the result of haemoglobin of *C. gariepinus* used in this study. The various haematological response showed that cassava mill effluents like other toxicants led to physiological impairment in aquatic organisms as observed in this study and as reported by [21,22]

V. Conclusion

Responses of the test organisms in the various concentrations of the cassava mill effluent showed that cassava mill effluent is toxic to the fish. The dosage of each concentration and time of exposure influenced the fish opercula movement. The opercula movement increased with increased concentrations and the opercula movement gradually reduced.

Due to the toxicity of the test effluent, osmotic fragility of the erythrocyte of the fish revealed decreased hemolytic values having/showing no significant effect on osmotic fragility of the fish. The result of this study showed that cassava mill effluents disposed into the water body directly or through drainage channels by local cassava mill industries is toxic to aquatic life including fish ,therefore this should not be encouraged.

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Table 4: mean value of haematological parameters of *c. Gariepinus* juveniles exposed to different concentration of cassava

Parameters	Concentrations (ml)					
	0.0	100	110	120	130	140
PCV (%)	22.00 ± 1.00 ^b	20.33 ± 1.52 ^b	21.66 ± 1.52 ^b	19.00 ± 1.00 ^b	17.66 ± 0.57 ^b	25.00 ± 1.00 ^b
HB(g/dl)	7.1 ± 0.10 ^a	24.76 ± 31.37 ^a	7.33 ± 0.49 ^a	6.40 ± 0.10 ^a	5.50 ± 0.10 ^a	8.33 ± 0.05 ^a
WBC(mm ³)	1.61 ± 0.35 ^b	14.33 ± 0.05 ^b	1.56 ± 0.02 ^b	1.75 ± 0.03 ^b	1.75 ± 0.03 ^b	2.42 ± 0.02 ^b
RBC(g/dl)	3.84 ± 493.25 ^b	2.81 ± 160.72 ^b	2.35 ± 57.73 ^b	2.80 ± 0.00 ^b	3.32 ± 5.00 ^b	2.09 ± 0.00 ^b
MCHC	27.00 ± 1.00 ^a	28.33 ± 1.53 ^a	30.00 ± 1.00 ^a	31.33 ± 1.52 ^a	30.00 ± 1.00 ^a	31.66 ± 1.52 ^a
MCH	41.33 ± 1.52 ^b	40.00 ± 1.00 ^b	27.00 ± 4.35 ^b	47.00 ± 1.00 ^b	31.00 ± 1.00 ^a	32.33 ± 1.52 ^b
MCV	1.40 ± 1.00 ^b	1.48 ± 1.00 ^b	1.44 ± 1.00 ^b	1.43 ± 1.52 ^b	1.01 ± 1.15 ^b	1.04 ± 2.00 ^b
MONOCYTE	1.00 ± 0.00 ^a	2.33 ± 0.57 ^a	1.33 ± 0.57 ^a	2.66 ± 0.56 ^a	2.33 ± 1.00 ± 0.00 ^a	1.00 ± 0.00 ^a
EOSINOPHIL	2.00 ± 1.00 ^a	1.00 ± 0.00 ^a	0.66 ± 0.57 ^a	0.00 ± 0.00 ^a	1.66 ± 0.00 ± 2.00 ± 1.00 ^a	2.00 ± 1.00 ^a
PLATELET	2.40 ± 0.00 ^b	1.60 ± 57.73 ^b	2.26 ± 57.73 ^b	2.14 ± 13856.6 ^b	2.40 ± 11.25 ± 115.47 ^b	2.5 ± 115.47 ^b
LYMPHOCYTE	66.00 ± 1.00 ^a	74.66 ± 10.96 ^a	58.33 ± 34.9 ^a	74.00 ± 1.00 ^a	62.55 ± 0.00 ± 79.33 ± 0.57 ^a	79.33 ± 0.57 ^a
HETEROPHIL	28.00 ± 1.00 ^a	26.00 ± 1.00 ^a	13.00 ± 9.35 ^a	22.00 ± 1.00 ^a	32.00 ± 1.00 ± 18.00 ± 0.00 ^a	18.00 ± 0.00 ^a