AEXTJ
AGRICULTURAL EXTENSION JOURNAL
ISSN 2521 – 0408

Available Online at www.aextj.com
Agricultural Extension Journal 2019; 3(1):21-25

RESEARCH ARTICLE

Effects of Soil Chemical Composition on the Hematology of Nile Tilapia, *Oreochromis niloticus* (Linnaeus, 1758) in Earthen Ponds

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Received: 25-09-2018; Revised: 23-10-2018; Accepted: 20-01-2019

ABSTRACT

Effects of soil chemical property on the hematology of *Oreochromis niloticus* in earthen ponds were investigated between March and August 2016. Three soil profile pits were dug side-by-side three existing earthen ponds in three different geomorphological locations of Imo state at Umuagwo, Ulakwo, and Uboma. Three homogenous soil horizons (0–20, 20–50, and 60–150 cm) were identified and evaluated for soil moisture, texture, and chemical composition using standard methods (USDA, 1971, Singer and Mum, 1996). 10 adults of *O. niloticus* of 250.0 ± 5.4 g average weight were randomly selected from each of the associated earthen ponds and chemically analyzed for nutrient composition in accordance with AOAC (2005). Soil texture was sandy at Umuagwo (sand: 798%, silt: 11.7%, and clay: 10.0%), sandy loam at Ulakwo (sand: 68.7%, silt: 16.0%, and clay: 15.3%), and clay loam at Uboma (sand: 32.8%, silt: 21.5%, and clay: 50.0%). The soil pH (4.0–4.5), organic carbon (0.4–0.6%), total nitrogen (0.04–0.08%), and exchangeable bases (Ca: 1.35–1.45, Mg: 0.05–1.60, and Na: 0.003–0.05 m/100 g) recorded for the clay soil at Uboma were significantly higher ($P < 0.05$) than in other test soils. Hematological parameters of fish from the sandy soil (PCV 39.5 ± 3.0%, red blood cell [RBC] [2.78 ± 0.5 × 10^6 cells/mm^3], white blood cell [WBC] [45.7 ± 0.2 × 10^3 cells/mm^2], and Hb [7.8 ± 0.8 g/dl]) did not significantly differ ($P > 0.05$) from those of sandy loam (PCV [43.6 ± 3.6%], RBC [2.70 ± 0.6 × 10^6 cells/mm^3], WBC [45.0 ± 2.7 × 10^3 cells/mm^3], and Hb [8.13 ± 0.6 g/dl]) and clay loam soils (PCV was 43.2 ± 2.1%, RBC [2.80 ± 0.01 × 10^6 cells/mm^3], and Hb [8.13 + 0.6g/dl]). There was thus no discernible impact ($P > 0.05$) in the blood of *O. niloticus* attributable to differences in soil chemical composition.

Key words: Earthen ponds, Hematology *Oreochromis niloticus*, Soil chemical composition

INTRODUCTION

Hematological characteristics are important parameters for the evaluation of the physiological status of fish in response to environmental conditions such as the underlying soil or water as a culture medium.[1,2] Hematological parameters have been effectively employed in monitoring the responses of fish to stressor conditions and their impact on the health of the fish in question.[3] These hematological indices provide substantial diagnostic information once reference values are known for comparison under standard conditions.[4]

Blood is important as a supplier of essential nutrients, ions, gases, and endocrine factors in the circulatory system of fish as well as a reservoir of excretory products of metabolism.[5] Alterations in blood parameters reflect the overall toxic impact of environmental contaminants. The desire of every fish farmer is to produce sizeable table fish with good market value within the shortest possible time. This can only be realized when culture medium (e.g., soil) does not impede the growth and well-being of the fish. The present study is aimed at ascertaining the impact of soils of different chemical composition on the hematology of the Nile tilapia, *Oreochromis niloticus* in earthen ponds.

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MATERIALS AND METHODS

Selection of fish ponds and experimental design

Three existing earthen fish ponds were selected for the trial at Umuagwo in Ohaji-Egbema LGA,[6-9] Ulakwo in Ngor-Okpala LGA, and Uboma in Ihitte-Uboma LGA of Imo state. Each pond measured approximately 30 m × 25 m × 1.5 m (750 m^2) and stocked with 3500 fingerlings of *O. niloticus* of 15.0 ± 0.8 g mean body weight at the rate of 10 fingerlings/m^2. The fish were fed with commercial feed concentrates at 5% body weight divided into two feeding regimes of mornings (0600 h) and evenings (18,000 h) and 6 days in the week.

The trial was designed as a completely randomized experiment (CRD) with three replications. The mathematical model[10] is as follows:

$$CRD: xij = M + Ti + Eij$$

Where,

xij = Value of independent observation

M = Unknown population variable

Ti = Treatment effect

Eij = Error term

Soil profile pit selection and description

In each of the pond locations at Umuagwo, Ulakwo, and Uboma, a soil profile pit was dug. Each pit measured 1 m × 1 m × 1.5 m deep. The depth was chosen in line with the maximum permissible depth of fish ponds as 1.5 m (Njoku, 2000). The color of the various soil horizons in the soil profile was qualitatively determined on exposure by visual method and by the use of color chart.[11] Thereafter, soil samples were collected in triplicates from each of the homogenous horizons for separate analysis.

i. Soil moisture

Moisture content of the soil was estimated by gravimetric method.[12] Known weight of the soil sample was collected in Petri dish, dried to constant weight in an oven at a temperature of 105°C and reweighed. Moisture content (%) was computed, thus:

$$Wa=Wb/Wc$$

Where,

Wa = Weight of empty dish

Wb = Weight of dish + wet sample before oven drying

Wc = Weight of dish + dried sample.

ii. Soil particle size analysis and textural class determination

Standard method was adopted in soil particle size analysis using sodium hexametaphosphate as dispersant after treating the sample with hydrogen peroxide to remove the organic matter.[12,13] Thereafter, the soil textural classes were determined using the soil textural triangle.

iii. Soil chemical (Nutrient) analysis

Soil chemical analysis was carried out in accordance with standard analytical methods by USDA and Udoh and Lekwa.[14,15] Organic carbon was determined by the acid dichromate digestion method, total nitrogen by micro Kjeldahl digestion, and ammonia distillation method. Exchangeable bases (saturated extract) of calcium (Ca) and magnesium (Mg) by the ethylenediaminetetraacetic acid (EDTA) titrimetric method[16] and atomic absorption spectrophotometer, while sodium (Na) and potassium (K) were extracted using the flame photometric method. Total phosphorus (P) was by perchloric acid digestion method.[17] Cation exchange capacity was estimated by the centrifugation method using ammonium acetate (NH_4OAc, pH 7.0), sodium acetate (NaOAc, pH 8.2), and potassium acetate (KOAc, pH 7.0). Base saturation (B.S) was then estimated by dividing the sum of the extracted bases by the base exchange capacity.[3,18-20]

Hematological analysis of fish blood

Ten adult individuals of 6-month-old *O. niloticus*, with mean body weight of 250 ± 8.5 g randomly selected from each of the earthen ponds at Umuagwo (sandy soil), Ulakwo (sandy loam), and Uboma (clay loam) in Imo state were used for the study between March and August 2016. Blood samples were collected from the caudal peduncle using 2.0 ml heparinized hematocrit tubes. After collection, the blood samples were transferred into EDTA bottles for analysis. Blood parameters were determined in accordance with Blaxhall.[21] Values of parameters were estimated as follows:

(i) Packed cell volume (PCV)

The packed cell volume, PCV (%) also known as the hematocrit was read directly from the microhematocrit reader and expressed in percentage.

(ii) Hemoglobin concentration (Hb)

$$Hb(g/dl) = \frac{\text{OD of test sample} \quad \text{concentration of standard soil}}{\text{OD of standard solution}}$$

Where,
O.D = Optical density
(iii) WBC

$$WBC\ (\ \ 10^3\ cells/mm^3) = \frac{N\ \ DF}{A\ \ D}$$

Where,
N = Number of cells in the counting chamber
DF = Dilution factor
A = Area of the counting chamber (hemocytometer)
B = Depth of the counting chamber.
(iv) RBC

$$RBC\ (\ \ 10^6\ cells/mm^3) = \frac{N\ \ DF}{A\ \ D}$$

Where, N = Number of cells in the counting chamber
Df = Dilution factor of the blood sample
A = Area of the counting chamber
D = Depth of the counting chamber.
The red blood cell parameter was further partitioned into three indices of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and the mean corpuscular hemoglobin concentration (MCHC) as follows:

$$\text{(v) MVC (g/100pg cells)} = \frac{\text{Haemotocrit (\%)}}{RBC(cells/mm^3)} \times 10$$

$$\text{(vi) (g/100pg cells)} = \frac{\text{Haemotocrit (g/100ml)}}{RBC(cells/mm^3)}$$

$$\text{(vii) MCHC (g/100ml)} = \frac{\text{Haemotocrit (g/100ml)}}{\text{Harmoglobin (\%)}}$$

Statistical method

Data were analyzed with one-way analysis of various as described by Steel and Torrie (1980) and Njoku *et al.* (1998).[22,4] Significant differences in mean values of parameters were separated using the Duncan's multiple range test.[9] This statistical analysis employed the computer Statistical Package for the Social Sciences (SPSS), version 19, Window 8.

RESULTS

Soil textural property

Tables 1-3 show the particle size distribution of the soil profiles developed at Umuagwo (recent alluvium), Ulakwo (coasted plain sands), and Uboma (sandstone and marine shale), respectively. The result shows that the soil at Umuagwo [Table 1] is dominantly sandy (sand: 79.8 ± 6.4%, silt: 11.7 ± 0.8%, and clay 10.0 ± 0.7%), Ulakwo [Table 2] was dominantly sandy loam (sand: 68.7 ± 7.0%, silt:16.0 ± 1.5%, and clay: 15.3 ± 1.3%), and clay loam at Uboma [Table 3] (sand: 32.8 ± 2.5%, silt: 21.5 ± 0.8%, and clay: 50.0 ± 4.5%). The third horizon was clay (sand fraction: 22.0%, silt: 14%, and clay: 64%).

Hematological characteristics of fish

Results of the hematological characteristics of *O. niloticus* reared in three different geomorphological ponds of Imo state are presented in Tables 4-6. For fish reared in sandy soil [Table 4], PCV was 39.5 ± 3.0%, RBC (2.78 ± 0.5 × 10^6 cells/mm^3), WBC (45.7 ± 0.2 × 10^3 cells/mm^3), Hb (7.8 ± 0.8 g/dl), MCV (1.85 ± 0.05g/100 pg cells), MCH (0.54 ± 0.08 pg cells), and MCHC (66.0 ± 4.5 g/100 ml). Table 5 shows the hematology of fish from the sandy loam ponds, PVC was 43.6 ± 3.6%, RBC (2.70 ± 0.6 × 10^6 cells/mm^3), WBC (45.0 ± 2.7 × 10^3 cells/mm^3),

Table 1: Particle size distributing of soil profile developed at Umuagwo, Imo state (recent alluvium)

Soil depth (cm)	Soil particle size (%)			Soil texture
	Sand	Silt	Clay	
0–20	95.0	0.6	4.9	Sandy
20–60	94.4	0.4	5.2	Sandy
60–150	50.0	34.0	20.0	Sandy loam
$\bar{X}$±SE	79.8±6.4	11.7±0.8	10.0±0.7	Sandy

SE: Standard error

Table 2: Particle size distribution of soil profile developed at Ulakwo, Imo state (coastal plain sands)

Soil depth (cm)	Soil particle size (%)			Soil texture
	Sand	Silt	Clay	
0–20	80.7	14.5	4.8	Sandy
20–60	65.0	15.5	19.5	Sandy loam
60–150	60.5	18.0	21.5	Sandy loam
$\bar{X}$±SE	68.7±7.0	16.0±1.5	15.3±1.3	Sandy loam

SE: Standard error

Table 3: Particle size distribution of soil profile developed of Uboma, Imo state (sandstone and marine shales)

Soil depth (cm)	Soil particle size (%)			Soil texture
	Sand	Silt	Clay	
0–20	47.8	24.0	28.8	Clay loam
20–60	28.5	26.5	45.0	Clay loam
60–150	22.0	14.0	64.0	Clay
$\bar{X}$±SE	32.8±3.0	21.5±2.8	50.0±4.8	Clay loam

SE: Standard error

Hb (8.13 ± 0.6 g/dl), MCV (1.8 ± 0.06 g/100 pg cells), MCH (0.53 ± 0.7g/100 pg cells), and MCHC (71.3 ± 8.0 g/100 ml). For the clay loam pond [Table 6], hematological parameters of fish include PCV (42.2 ± 2.1%), RBC (2.80 ± 0.01 × 10^6 cells/mm^3), Hb (8.13 + 0.6g/dl), MCV (1.8 + 0.06g/100 pg cells), MCH (0.53+ 0.07g/100 pg cells), and MCHC (65.83 + 8.0g/100ml).

Comparative analysis [Table 7] shows that there is no marked statistical difference ($P > 0.05$) in almost all the hematological parameters of *O. niloticus* from the three different geomorphological ponds. However, the PCV in fish from the sandy pond (39.5 ± 3.0%) was significantly lower ($P < 0.05$) than those of the sandy loam (43.6 ± 3.6%) and clay loam (43.2 ± 2.1%) ponds.

DISCUSSION

Values of hematological parameters recorded in *O. niloticus* reared

In soils of different chemical composition conformed to reference values[23,24] for healthy fish and did not differ due to soil chemical property. This implies that the chemical nature of the three soils has no observable effects on the blood of *O. niloticus*. Comparative analysis revealed that no particular

Table 4: Hematological parameters of *Oreochromis niloticus* from sandy ponds at Umuagwo

Sample	PCV (%)	RBC (×10^6 cells/mm^3)	WBC (×10^6 cells/mm^3)	HB (g/dl)	MCV (g/100 pg cell)	MCH (g/100 pg cell)	MCHC (g/100 ml)
1	35.0	2.38	38.5	7.5	1.75	0.50	58.00
2	38.5	3.10	48.6	8.0	1.80	0.48	65.20
3	45.0	2.85	50.0	7.8	2.00	0.65	75.00
$\bar{X}$±SE	39.5±3.0	2.78±0.5	45.7±0.2	7.8±0.5	1.85±0.05	0.54±0.08	66.0±4.5

RBC: Red blood cell, PCV: Packed cell volume, WBC: White blood cell, HB: Hemoglobin, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, SE: Standard error

Table 5: Hematological parameters of *Oreochromis niloticus* from sandy loam ponds at Ulakwo

Sample	PCV (%)	RBC (×10^6 cells/mm^3)	WBC (×10^6 cells/mm^3)	HB (g/dl)	MCV (g/100 pg cell)	MCH (g/100 pg cell)	MCHC (g/100 ml)
1	33.8	2.14	37.25	6.80	2.15	0.55	55.00
2	44.0	2.80	51.00	9.15	1.40	0.60	60.50
3	3.15	3.15	45.60	7.10	2.00	0.58	81.00
$\bar{X}$±SE	43.6±3.6	2.70±0.6	44.6±3.7	7.68±0.8	0.85±0.06	0.58±0.04	65.0±7.2

RBC: Red blood cell, PCV: Packed cell volume, WBC: White blood cell, HB: Hemoglobin, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, SE: Standard error

Table 6: Hematological parameter of *Oreochromis niloticus* from clay loam ponds at Uboma

Sample	PCV (%)	RBC (×10^6 cells/mm^3)	WBC (×10^6 cells/mm^3)	HB (g/dl)	MCV (g/100 pg cell)	MCH (g/100 pg cell)	MCHC (g/100 ml)
1	38.0	2.50	38.65	7.75	1.80	0.45	60.00
2	40.8	3.10	49.50	8.20	1.60	0.50	62.50
3	51.00	2.80	47.00	8.45	2.0	0.65	75.00
$\bar{X}$±SE	39.5±3.0	2.70±0.01	45.00±2.7	8.13±0.6	1.8±0.06	0.53±0.07	65.83±8.0

RBC: Red blood cell, PCV: Packed cell volume, WBC: White blood cell, HB: Hemoglobin, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, SE: Standard error

Table 7: Comparison of the hematological parameters of *Oreochromis niloticus* from the three earthen ponds with different soil chemical properties

Soil/pond location	Hematological parameters of *Oreochromis niloticus* ($\bar{X}$±SE)						
	PCV	RBC	WBC	Hb	MCV	MCH	MCHC
Umuagwo (sandy soil)	39.5±3.0^a	2.78±0.5^a	45.7±0.2^a	7.8±0.5^a	1.85±0.05^a	0.54±0.08^a	66.0±4.5^a
Ulakwo (sandy loam)	43.6±3.6^b	2.70±0.6^a	44.60±3.7^a	7.68±0.6^a	1.85±0.06^a	0.58±0.54^a	65.5±7.2^a
Uboma (clay Loam)	43.2±2.1^b	28.80±0.01^a	45.0±2.7^a	8.13±0.6^a	1.8±0.06^a	0.53±0.07^a	65.83±8.0^a

$^{a, b, c}$Mean values of parameter in the same column with different superscript are significantly different at $F = 0.05$. RBC: Red blood cell, PCV: Packed cell volume, WBC: White blood cell, HB: Hemoglobin, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, SE: Standard error

soil type impacted negatively on the hematology of the test fish. This claim is further justified by the fact that growth response of *O. niloticus* was not interrupted in any of the three soil locations during the culture period. The reduction in the value of PCV recorded in fish from the sandy soil, though not below normal, may be attributed to the fact that different fishes have different blood parameters, unlike in humans where it is constant.[25] According to Blaxhall (1972), changes in hematological indices of fish not only depend on the fish species but also on the age and the reproductive stage of the fish.

CONCLUSION AND RECOMMENDATION

Soil chemical (nutrient) composition has no effect on the blood parameters of *O. niloticus*. It is, therefore, recommended that site selection of earthen ponds should consider the ability of the soil to retain water rather than the effect of the chemical composition of the soil on cultured fish.

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