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Research Article

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Effects of combined Vitamins C and E on haematological parameters and some antioxidant production levels in *Clarias gariepinus* (Burchell, 1822) fingerlings under laboratory conditions

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Abstract

The need for improved fish diet in ensuring overall healthy growth of the fish cannot be overemphasized. The effects of combined vitamins C and E on the blood parameters, alanine amino transferase (ALT) and aspartate amino transferase (AST) in *Clarias gariepinus* were evaluated for a period of 12 weeks. 120 samples of the fish were acclimated for two weeks during which they were fed twice daily. These were then distributed into three treatments and replicate including control with each trough containing 15 samples thus; 200mg/L (T_1), 300mg/L (T_2) and 400mg/L (T_3). Three samples were selected randomly every 4th week of the experiment and sacrificed for blood parameters analyses and the kidneys, liver and gills were excised and homogenised in phosphate buffer. The data generated were subjected to one way analysis of variance and considered significant at $P \leq 0.05$. From the results, the total white blood cell count (TWBC), parked cell volume (PCV) and blood haemoglobin (Hb) were significantly different in T_3; while the red blood cell count (RBC) and mean corpuscular haemoglobin (MCH) were significantly different in T_1 at the end of the 4th week. There were significant increases in the blood platelets (PLT) as the concentration of the combined vitamins increased in both 4th and 8th weeks of the experiment. The RBC was also significantly different in the 8th and 12th weeks mostly in higher concentrations. The ALT production levels in the kidneys increased with increase in the concentration and duration of the research from T_1-T_3 as from the 8th and 12th weeks; with T_3 mean values significantly higher in all the sampling periods. High production levels of the enzyme were also recorded in the liver and gills of the fish with T_2 mean values significantly different in most of the sampling periods. The AST production levels in the kidneys and liver were significantly different in T_2 and T_3, respectively in all the sampling periods. The production level in the gills was significantly different in T_2 in the 8th and 12th periods of sampling. The out-come of this research has proven that combined vitamins C and E can improve the physiological status of the fish and therefore, recommended in boosting the growth performance of *C. gariepinus*.

Keywords: Combined vitamins C and E, haematological parameters, Alanine amino transferase, Aspartate amino transferase and *Clarias gariepinus*

1.0 Introduction

Fish is a rich source of animal protein throughout the world. It also serves both subsistent and commercial purposes in many developing countries of the world. African catfish, *Clarias gariepinus* is an important commercial fish due to its high growth rate, high consumer acceptability, and aility to withstand poor water quality, and oxygen depletion (Abbes *et al*., 2016). *Clarias* species is a widely distributed fish in Asia and Africa; in these areas, the fish is extremely popular on account of its tasty flesh, its unparalleled hardness, its rapid growth and its somewhat acceptable market price (FAO, 2003). In Nigeria, *Clarias* species is an indigenous fish occurring in freshwater throughout the country.

Vitamins are usually organic compounds that are required in little quantities (in most cases) to enhance or stimulate biosynthesis of biomolecules necessary for survival of organisms. Samuel *et al*. (2021a) posited that two major types of vitamin are fat-soluble vitamins and water-soluble vitamins. The fat-soluble vitamins include the A vitamins, retinols (responsible for vision), D vitamins, calciferols (for bone integrity), E vitamins, tocopherols (antioxidant) and K vitamins, cobalamins. Water soluble includes the B vitamins such as thiamine, riboflavin, niacin, pyridoxine, pantothenic, phylloquinone and vitamin C (ascorbic acid). Vitamin C is known to play a crucial role in the immunological and antioxidant properties of vertebrates capable of maintaining the integrity, fluidity of membranes and capable of controlling the oxidizing reactions of fatty acids, thus keeping cellular respiration and avoiding cell death (Ai *et al*., 2004). Non-enzymatic antioxidants such as vitamins C and E can also act to overcome oxidative stress, being a part of the total antioxidant system. They prevent the increased production of free radicals induced by oxidative damage to lipids and lipoproteins in various cellular compartments and tissues (Gbore *et al*., 2010). The main biological function of vitamin E is its direct influence on cellular responses to oxidative stress through modulation of signal transduction pathway (Adewolu *et al*., 2008). Vitamins E and C supplementation can induce protective effects on certain conditions after free radical-mediated cellular damage or disruption (Ahmad *et al*., 2008). Vitamin E (α-tocopherol) is a fat soluble antioxidant that inhibits the production of reactive oxygen species formed when fat undergoes oxidation.

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) belong to the plasma non-functional enzymes which are normally localized within the cells of liver, heart, gills, kidneys, muscle and other organs. These enzymes are liberated into the blood in pathological situations and therefore are of clinical importance. Gaafar *et al*. (2019) reported that in *Oreochromis niloticus*, there was a marked reduction in AST in liver and muscle in response to the lower or higher level of ochratoxin. They attributed the reduced levels of aminotransfersase in various organs to tissue damage and consequently the reduction of enzyme biosynthesis for reasons related to the presence of ochratoxin. On the other hand, the ALT activities in liver and muscle were found to increase during the time course of endogenous cortisol elevation induced by ochratoxin intoxication. The ameliorative role of vitamins was evident when Vitamin E and metallothionein treatments protected against Cd-induced damage of liver in grass carp by decreasing AST and ALT content, repairing organelles, and maintained the antioxidant system by elevating CAT, SOD, and GSH-Px activity and regulating related mRNA transcript expression (Ahmed *et al*., 2020).

Haematological parameter is a good indicator to determine the health of an organism (Joshi *et al*., 2002). Fish haematology is gaining increasing importance in fish culture because of its importance in monitoring the health state of fish.

The need for improvement in fisheries and aquaculture is on the increase due to increasing human population. There is also high cost of fish production in Nigeria with constant need for minimal cost of production in order to maximize profit by both consumers and farmers themselves. Presently, there is dearth of information on the effects of vitamins C and E supplements on fish and how they can lead to improvement in some of the biochemical constituents of fishes as well as its hematological profile especially when not treated with one toxicant or the other. The nutritional status of organisms goes a long way in its ability to withstand stress and lead to a healthy life. The haematological parameters as well as antioxidants such as ALT and AST are important in diagnosing the functional status of organisms (fish) especially when treated with vitamins. This study therefore, examines how the physiological status of the fish can be improved upon to ensure increased productivity of the fish and increased profit to the fish farmers.

2.0 Materials and Methods

2.1 Sample collection and Acclimatization

One hundred and twenty (120) sample of *Clarias gariepinus* fingerling (6 weeks old) were purchased from Private Fish Farm in New Bussa, Niger State. These fishes were carefully transported to the Old Research Farm of Department of Water, Aquaculture and Fisheries Technology, Bosso Campus in a 25 litres container with water. Sixty (60) fingerlings were carefully distributed into the rearing plastic Aquaria (19 cm x 13.5cm x 9.6) containing twenty (20) litres of Borehole water for acclimatization for a period of 2 weeks. During this period, the fishes were fed to satiation twice a day. The water medium in each trough was changed every 48^{th} hour.

2.2 Experimental Setup

The vitamins C and E granules (500g each) were purchased from commercial chemical stores. Three (3) treatments including control with one replicate in each case were set-up for the combined Vitamins C and E. The vitamins were administered as (00, 0.2g, 0.3g, 0.4g for Control, T1, T2 and T3 and replicates, respectively) for a period of twelve (12) weeks. T1-T3 represents the various treatments and their replicates supplemented with combined vitamin C and E. Fifteen (15) samples of the fish were distributed into the troughs representing each treatment and replicate. The water media in each trough were changed every 72 hours.

2.3 Determination of Haematological parameters

At the end of every 4^{th} week of the experiment, three (3) fish were randomly selected from each treatment tank and blood samples of the fish were collected through the caudal vein; and in between the operculum and the pectoral fin on the ventral side of the fish (Samuel *et al*., 2021b) using plastic syringe, fitted with 21-gauge hypodermal needles. Each blood sample was collected in duplicate into heparinized (50 IU per mL of blood) bottles which were used for haematological analyses. The blood samples collected were stored in pre-iced collecting bottles and later put in refrigerator for further handling and analyses within 30 minutes of their collection. The haematological analyses of blood samples were carried out in the Laboratory Services of General Hospital, Minna, Nigeria. Evaluation of the haemogram involves the determination of the total erythrocyte count (RBC), total white blood cell count (WBC), haematocrit (PCV), haemoglobin (Hb) concentration and platelet using MindrayR Auto Haematology Analyser (3000 plus). Determinations were carried out in duplicate.

2.4 Preparation of Sodium Phosphate Buffer

Sodium phosphate buffer solution (0.2 M) was prepared from the mixture of sodium dihydrogen orthophosphate with 0.1 M and disodium hydrogen orthophosphate with 0.1 M. The pH was adjusted to 8.0.

2.5 Tissue harvesting and homogenization

Three fishes were randomly picked from each trough that is T1-T3 including the control. These were then dissected and the fish organs of interest (gills, liver and kidney) were excised and homogenized in sodium phosphate buffer using ceramic mortar and pestle. After each homogenization the mortar and pestle was rinsed with distilled water before usage for other tissues from other treatments.

2.6 Aspartate aminotransferase (AST) and alanine aminotransferase (ALT)

Fish tissues AST and ALT were determined as described by Reitman and Frankel (1957) from all the treatments and replicates. Spectrophotometric method was used for the assay of aspartate and alanine aminotransferase. The homogenates were prepared in the laboratories as follow: 100 µl (0.1 ml) of the tissue homogenate was added into test tubes with 500 µl (0.5 ml) of reagent 1(buffer). The mixture was incubated for 30 minutes at 37^{o}C. Subsequently, 500 µl (0.5 ml) of reagent 2 (2, 4- dinitrophenylhydrazine) was added and kept for 20 minutes at 25^{0}C. The reaction was terminated with the addition of 5000 µl (5.0 ml) of 0.4 Mol/L NaOH to the mixture. The blank was prepared with 500 µl (0.5 ml) of reagent1 and 0.1µl (100 µl) of distilled water. The absorbance was read at 546 nm.

2.7 Determination of Physicochemical Parameters

The water temperature, Electrical Conductivity, pH, Dissolved Oxygen and Total Alkalinity of the test media were measured with the aid of portable multiparameter analyzer (Model CT-3030) on weekly basis for a period of 12 weeks.

2.8 Data Analyses

Data generated from the haematological parameters of the *C. gariepinus* and AST and ALT produced from all the treatments were subjected to One-way Analysis of Variance (ANOVA) using SPSS version 20. Duncan multiple range test was used to separate the means where significant at P ≤ 0.05 level of significance.

3.0 Results and Discussion

3.1 Results

3.1.1 Heamatological parameters of *C. gariepinus* subjected to varying concentrations of combined vitamins C and E for a period of 4 weeks

The result of the haematological values of *C. gariepinus* subjected to combined vitamins C and E at varying concentration for a period of 4 weeks exhibited tremendous variations in the various blood parameters. The mean values of total red blood cell count (TWBC) showed that T_1 and T_3 were significantly higher than other treatments including control. The mean value obtained in Red blood cell (RBC) in T_1 was significantly higher than other treatments including the control. The mean values of packed cell volume (PVC) and haemoglobin (Hb) in T_1 and T_3 was significantly higher than T_2 including control. The Mean Corpuscular Volume (MCV) obtained in control was significantly higher than all the other treatments. The mean value obtained in mean corpuscular haemoglobin (MCH) in T_1 was significantly higher than other treatments including control. In the Mean corpuscular haemoglobin concentration (MCHC) there was no significant difference in all the treatments including the control. The mean values of lymphocytes (L), Neutrophil (N) and Eosinophils (E) and Basophil(B) indicated that there were no significant differences in all the treatments including control. The mean value of Red blood cell distribution width (RDWC) in the control was significantly higher than all the other treatments. High values of blood platelets were obtained in all the treatments including the control. (Table 1).

Table 1 Mean ± Standard Deviation of Heamatological parameters of *C.gariepinus* subjected to varying concentrations of Combined vitamins C and E for a period of 4 weeks

	CR	T_1	T_2	T_3
TWBC (10^9cell/L)	3.800 ± 000^a	13.550 ± 1.55^{ab}	8.600 ± 6.351^a	15.500 ± 0.693^c
RBC (mil/mm^3)	1.100 ± 000$_a$	3.600 ± 5.77^c	3.400 ± 0.115^b	1.900 ± 0.462^b
PLT (Cmm)	438.000 ± 0.000^a	1360.000 ± 0.026^a	369.600 ± 174.937^a	393.000 ± 50.807^a
PCV (%)	9.000 ± 0.000^a	19.000 ± 0.000^c	17.500 ± 0.577^b	18.500 ± 0.577^c
Hb (g/dl)	3.175 ±0. 171^a	6.375 ± 0.150^c	5.900 ± 0.294^b	6.450 ± 0.208^c
MCV (FI)	71.000 ± 0.000^b	64.500 ±2.887^a	63.00 ± 3.464^a	66.500 ± 0.577^a
MCH (pg)	14.000 ± 0.000^a	16.000 ± 1.155^b	15.00 ± 1.155^{ab}	14.000 ± 1.155^a
MCHC (g/dl)	27.000 ± 0.000^a	28.500 ± 0.677^a	27.500 ± 0.577^a	27.500 ± 1.732^a
N (%)	13.000 ± 0.000^b	5.500 ± 0.577^a	10.000 ± 5.773^{ab}	6.000 ± 1.155^a
L (%)	81.000 ± 0.000^a	88.000 ± 4.619^a	84.500 ±7.506^a	87.500 ± 5.196^a
M (%)	6.000 ± 0.000^a	6.500 ± 4.041^a	5.500 ± 0.1731^a	6.500 ± 4.041^a
E (%)	6.000 ± 0.000^a	6.500 ± 4.041^a	5.500 ± 0.1731^a	6.500 ± 4.041^a
B (%)	6.000 ± 0.000^a	6.500 ± 4.041^a	5.500 ± 0.1731^a	6.500 ± 4.041^a
RDWC (%)	15.80 ± 0.000^b	14.25 ± 1.091^a	14.200 ± 0.921^a	13.351 ± 0.171^a

Values are presented in Mean ± Standard Deviation. Values with different superscript across the row are significantly different at P≤ 0.05.

3.1.2 Heamatological parameters of *C. gariepinus* subjected to varying concentrations of combined vitamins C and E for a period of 8 weeks

The result of the haematological values of *C. gariepinus* subjected to combined effects of vitamins C and E at varying concentrations after 8 weeks indicated that the TWBC showed no significant difference in all treatments including control. However, the RBC was significantly higher in T_3 than in other treatments including control. The mean value obtain in platelets (PLT) showed an improvement in T_1-T_3 with T_3 significantly higher than other treatments including the control. The mean values of PCV, haemoglobin (Hb), MCV, MCH and MCHC in the control were significantly higher than other treatments. The mean values of Neutrophil (N) and lymphocytes (L) showed no significant difference in all the treatments including control. The mean value of Monocytes (M), Eosinophils (E), and Basophil (E) in control was significantly higher than other treatments. The mean values of Basophil, Monocyte and Eosinophil in T_2 were significantly higher than T_1 and T_3. The RDWC mean value in T_3 was significantly higher than other treatments including control. (Table 2).

Table 2 Mean ± Standard Deviation of Heamatological parameters of *C. gariepinus* subjected to varying concentrations of combined vitamins C and E for a period 8 weeks

Hematological parameters	CR	T_1	T_2	T_3
TWBC (10^9cell/L)	18.1000 ± 0.000^a	14.650 ± 8.025^a	11.250 ± 10.450^a	14.450 ± 8.25^a
RBC (mil/mm^3)	1.000 ± 0.000^a	1.300 ± 0.000^{ab}	1.200 ± 0.000^{ab}	1.800 ± 0.924^b
PLT (Cmm)	165.000 ± 0.000^{ab}	141.000 ± 1.155^a	144.500 ± 0.577^a	256.000 ± 122.398^b
PCV (%)	21.000 ± 0.000^c	12.000 ± 3.464^b	8.000 ± 2.309^a	9.500 ± 0.577^{ab}
Hb (g/dl)	7.000 ± 0.000^c	4.050 ± 1.212^b	2.800 ± 0.808^a	3.300 ± 0.231^a
MCV (FI)	154.000 ± 0.000^c	137.000 ± 3.464^b	125.000 ± 3.464^{ab}	120.500 ± 16.743^a
MCH (pg)	69.000 ± 0.000^b	24.000 ± 10.392^a	20.500 ± 10.970^a	26.500 ± 19.053^a
MCHC (g/dl)	45.000 ± 0.000^b	21.000 ± 10.392^a	24.500 ± 2.887^a	22.500 ± 10.970^a
N (%)	3.000 ± 0.000^a	5.500 ± 5.196^a	7.000 ± 3.464^a	2.500 ± 0.577^a
L (%)	89.000 ± 0.000^a	89.000 ± 6.928^a	87.500 ± 5.196^a	94.000 ± 0.000^a
M (%)	9.000- ± 0.000^c	5.500 ± 1.732^{ab}	6.000 ± 2.300^{ab}	3.500 ± 0.577^a
E (%)	9.000- ± 0.000^c	5.500 ± 1.732^{ab}	6.000 ± 2.304^b	3.500 ± 0.577^a
B (%)	9.000- ± 0.000^c	5.500 ± 1.732^{ab}	6.000 ± 2.304^b	3.500 ± 0.577^a
RDWC (%)	15.900 ± 0.000^{ab}	14.525 ± 0.608^{ab}	13.850 ± 1.212^a	16.050 ± 2.520^b

Values are presented in Mean ± Standard Deviation. Values with different mean values across the rows are significantly different at $P \leq 0.05$.

3.1.3 Haematological parameters of *C. gariepinus* fingerlings subjected to varying concentrations of combined vitamins C and E for a period of 12 weeks.

The result of hematological parameters of *C. gariepinus* subjected to combined vitamins C and E at varying concentrations for period of 12 weeks revealed that TWBC was not significantly different in all treatments including control. RBC was significantly higher in T_2 and T_3 than T_1 and control. The highest mean value of PLT was obtained in T_2. The mean values of PCV and Haemoglobin (Hb) were significantly higher in T_2 and T_3 than T_1 and CR. The MCV mean values in T_1 and T_2 were significantly higher than T_3 and CR. The mean values obtained for MCH and MCHC in T_3 were significantly higher than other treatments including the control. There was no significant difference in mean values recorded for N and L in all treatments including control. The mean values obtained for Monocytes, Eosinophil and Basophil in T_1 were significantly higher than other treatments including the control. The mean values obtained for RDWC were significantly higher in control and T_2 than T_1 and T_3. (Table 3).

Table 3 Mean ± Standard Deviation of Heamatological parameters of *C. gariepinus* subjected to varying concentrations of combined vitamins C and E for a period 12 weeks

Hematological parameters	CR	T_1	T_2	T_3
TWBC (10^9cell/L)	6.8000 ± .00000^a	6.1500 ± 5.72756^a	8.0500 ± 3.74767^{ab}	9.0000 ± 1.27279^b
RBC (mil/mm^3)	1.000 ± .00000^a	0.750 ± 0.919^a	1.600 ± 0.565^b	1.350 ± 0.495^b
PLT (Cmm)	152.000 ± 0.000^a	169.500 ± 74.246^a	261.5000 ± 163.341^b	217.500 ± 51.618^b
PCV (%)	11.000 ±0.000^a	13.5000 ± 9.192^a	18.5000 ± 4.949^b	17.000 ± 8.485^b
Hb (g/dl)	3.700 ±0.000^a	4.600 ± 2.969^a	6.300 ± 1.555^b	5.750 ± 12.021^b
MCV (FI)	1.4200 ± 0.000^b	143.000 ± 2.828^b	141.5000 ± 6.364^b	134.500 ± 12.021^a
MCH (pg)	47.000 ± 0.000^{bc}	26.000 ± 9.899^b	9.500 ± 0.707^a	100.500 ± 98.287^c
MCHC (g/dl)	33.000 ± 0.000^{bc}	18.000 ± 7.071^b	7.0000 ± 1.414^a	78.000 ± 79.195^c
N (%)	3.000 ± 0.000^a	5.000 ± 2.828^b	4.500 ± 3.535^b	3.000 ± 1.414^a
L (%)	93.000 ± 0.000^a	89.000 ± 5.656^a	90.000 ± 7.0710^a	94.0000 ± 1.414^a
M (%)	4.000 ± 0.000^{ab}	6.0000 ± 2.828^b	4.1500 ± 5.444^{ab}	3.000 ± .000^a
E (%)	4.000 ± 0.000^{ab}	6.0000 ± 2.828^b	4.1500 ± 5.444^{ab}	3.000 ± .000^a
B (%)	4.000 ± 0.000^{ab}	6.0000 ± 2.828^b	4.1500 ± 5.444^{ab}	3.000 ± .000^a
RDWC (%)	15.000 ± .000^b	14.750 ± 0.494^a	15.100 ± 1.555^b	13.800 ± .141^a

Values are presented in Mean ± Standard Deviation. Values with different mean values across the rows are significantly different at P≤ 0.05.

3.1.4 Mean ± Standard Deviation of ALT (nM/mL) production levels in kidney, Liver and Gills of *C. gariepinus* subjected to varying concentrations of combined vitamins C and E for a period of 12 weeks.

The results of ALT production levels in the kidney of *C. gariepinus* subjected to varying concentrations of combined vitamins C and E for a period of 12 weeks showed that at week 4 and 8, the mean values obtained in the control and T_3 were significantly higher than other treatments. However, at the 12th week, the mean value obtained in T_3 was significantly higher than other treatments including the control. (Table 4).

The result of ALT production in the Liver of *C. gariepinus* subjected to varying concentration of combined vitamins C and E for a period of 12 weeks showed that at weeks 4 and 8, the mean values obtained in T_2 were significantly higher than other treatments including the control. However, at week 12, the mean value obtained in T_3 (44.13±8.28nM/mL) was significantly higher than the other treatments including the control. (Table 5).

The result of ALT production levels in gills of *C. gariepinus* subjected to varying concentrations of combined vitamin C and E for a period of 12 weeks showed that at week 4 the mean values obtained in T_2 and control were significantly higher than other treatments. At week 8 the mean values obtained in T_1 and control were significantly higher than other treatments. However at week 12, the mean value obtained in T_2 (27.24±5.18 nM/mL) was significantly higher than other treatments including the control. (Table 6).

Table 4 ALT (nM/mL) production level in kidney of *C.gariepinus* subjected to varying concentrations of combined vitamins C and E for period of 12 weeks.

Treatments	4th week	8th week	12th week
CR	18.14± 0.00^{b}	20.73± 0.00^{b}	19.64 ± 0.00^{a}
T1	14.36± 3.64^{a}	14.96± 5.53^{a}	14.54 ± 4.11^{a}
T2	12.46± 3.37^{a}	16.02± 2.88^{b}	19.57 ± 4.02^{b}
T3	18.66± 2.05^{b}	21.87± 8.39^{b}	30.37 ± 1.49^{c}

Values are presented in Mean ± Standard Deviation. Values with different superscript in the same column are significantly different at P≤ 0.05.

Table 5 ALT (nM/mL) production levels in the liver of *C .gariepinus* subjected to varying concentrations of combined vitamins C and E for period of 12 weeks

Treatments	4th	8th	12th week
CR	29.73± 0.00^{ab}	32.94± 0.00^{c}	34.98 ± 0.00^{b}
T1	23.67± 4.91^{a}	21.86± 2.36^{a}	23.73 ± 2.96^{a}
T2	36.92± 6.91^{b}	35.97± 8.13^{c}	32.08 ± 5.16^{b}
T3	25.54± 4.14^{a}	28.62± 6.85^{b}	44.13 ± 8.28^{c}

Values are presented in Mean ± Standard Deviation. Values with different superscript in the same column are significantly different at P≤ 0.05.

Table 6 ALT (nM/mL) production levels in the gills of *C. gariepinus* subjected to varying concentrations of combined vitamins C and E for a period of 12 weeks.

	4^{th}	8^{th}	12^{th}
CR	23.92 ± 0.00^{b}	21.02 ± 0.00^{b}	16.87 ± 0.00^{a}
T1	14.80 ± 3.42^{a}	20.29 ± 1.88^{b}	23.25 ± 3.81^{ab}
T2	23.03 ± 4.50^{b}	17.90 ± 8.26^{a}	27.24 ± 5.18^{b}
T3	16.19 ± 3.72^{a}	17.53 ± 3.47^{a}	25.53 ± 2.71^{ab}

Values are presented in Mean ± Standard Deviation. Values with different superscript in the same column are significantly different at $P \leq 0.05$.

3.1.5 Mean ± Standard Deviation of AST (nM/mL) production levels in kidney, Liver and Gills of *C. garieapinus* subjected to varying concentrations of combined vitamins C and E for the period of 12 weeks.

The results of Aspartate amino transferase (AST) production levels in the Kidney of *C. gariepinus* subjected to varying concentrations of vitamins C and E for a period of 12 weeks showed that the mean values at the 4th, 8^{th} and 12^{th} week in T_2, respectively were significantly higher than other treatments including the control. (Table 7).

Similarly, the mean values of AST production levels in the Liver of *C.gariepinus* subjected to varying concentrations of combine vitamins C and E for a period of 12 weeks showed that at the 4th, 8^{th} and 12^{th} in T_3 were significantly higher than other treatments including the control. (Table 8).

Furthermore, AST production mean values obtained in T_1 of the gills of *C. gariepinus* subjected to varying concentrations of combined vitamins C and E for a period of 12 weeks at the 4^{th} week was significantly higher than other treatments including the control. However at the 8^{th} and 12^{th} weeks, the mean values obtained in T2 were significantly higher than other treatments including the control. (Table 9).

Table 7 AST (nM/mL) production level in kidney *C. garieapinus* subjected to varying concentrations of combined vitamins C and E for a period of 12 weeks

Treatment	4^{th}	8^{th}	12^{th} week
CR	21.34 ± 0.37^{a}	22.93 ± 0.00^{a}	21.74 ± 0.00^{a}
T1	40.93 ± 5.55^{c}	36.98 ± 3.10^{b}	31.70 ± 6.52^{b}
T2	52.66 ± 4.94^{d}	51.02 ± 2.08^{c}	42.43 ± 3.62^{d}
T3	27.99 ± 4.78^{b}	23.55 ± 4.37^{a}	36.83 ± 5.55^{c}

Values are presented in Mean ± Standard Deviation. Values with different superscript in the same column are significantly different at $P \leq 0.05$.

Table 8 AST production level in the liver of *C. gariepinus* subjected to varying concentrations of combined vitamins C and E for a period of 12 weeks

Treatment	4^{th}	8^{th}	12^{th} week
CR	13.58 ± 0.32^{a}	15.38 ± 0.00^{a}	17.90 ± 0.00^{b}
T1	22.48 ± 3.28^{c}	15.38 ± 0.54^{a}	15.02 ± 6.21^{a}
T2	14.76 ± 2.97^{b}	16.36 ± 0.54^{b}	15.76 ± 3.93^{a}
T3	26.33 ± 4.57^{d}	26.15 ± 7.81^{c}	32.42 ± 3.45^{c}

Values are presented in Mean ± Standard Deviation. Values with different superscript in the same column are significantly different at $P \leq 0.05$.

Table 9 AST (nM/mL) production levels in the gill of *C. gariepinus* subjected to varying concentrations of combined vitamins C and E for the period of 12 weeks

Treatment	4^{th}	8^{th}	12^{th} week
CR	10.35 ± 0.21^{a}	9.89 ± 0.00^{a}	12.39 ± 0.00^{a}
T1	31.28 ± 3.44^{c}	17.29 ± 7.85^{b}	15.42 ± 2.21^{b}
T2	19.37 ± 7.56^{b}	30.63 ± 8.94^{d}	31.71 ± 4.46^{d}
T3	20.97 ± 2.89^{b}	19.39 ± 8.70^{c}	27.86 ± 4.04^{c}

Values are presented in Mean ± Standard Deviation. Values with different superscript in the same column are significantly different at $P \leq 0.05$.

3.2 Discussion of Results

Heamatological parameters are becoming increasingly important diagnostic indicator in fish biology. It has been reported that haematological indices are a reflection of the effects of dietary treatments on animals in terms of the type, quality and amounts of feed ingested and nutrients available to an animal to meet its physiological and metabolic requirements (Gbore and Akele, 2010). It is considered as a good indicator of fish physiological and health state (Fazio *et al*., 2012). In this study varying concentrations of combined vitamins C and E, the Total White Blood Cell count (TWBC), Parked Cell Volume (PCV) and Blood Haemoglobin (Hb) were significantly different at the end of the 4^{th} week of the study in the lowest and highest concentration. This is probably because at the early stage of the experiment the fishes were still adapting to the changes in their environment though not substantial. This may also have accounted for the increase recorded in the Blood Platelets (PLT) with increase in the concentration of the treatments which were however, lower than what was obtained in the control. This is in conformity with the findings of Gbore *et al*. (2020) who reported significant increase in PCV, RBC, Hb and platelets in fish fed diets supplemented with vitamin C or E at 7.5mg FB1/Kg, and the WBC counts were significantly higher in diets containing 5.0 and 7.5mgFB1/Kg than the control of the *Clarias gariepinus* used in the experiment. The gradual decrease in Red Blood Cell (RBC), Mean Corpuscular Haemoglobin Concentration (MCHC) and Red blood cell Distribution Width (RDWC); and the non-significance of Mean Corpuscular Haemoglobin (MCH), Lymphocytes, Neutrophil, Eosinophil nd Basophil probably indicate that there is no direct linkage between these parameters and the combined vitamins which were only significant in T_1 at the end of the 12^{th} week of the experiment. Similarly, Liang *et al*. (2017) reported that RBC and Ht in juvenile yellow catfish, *Pelteobagrus fulvichraco* was not significantly affected by vitamins A and C diet. Also, Oladele and Ogini (2010) reported the minimal level of L, N and E in the blood of *C. gariepinus*. However, the PLT increases were recorded in T_1, T_2 and T_3 better than the control with zero vitamins supplementation at week 4. This could be attributed to the presence of the combined vitamins C and E at varying concentrations that probably protected the red blood cell membranes from oxidation and enhanced the transport ability. Salah *et al*. (2010) reported that Vitamin C and E are important for optimal functioning of energy system. Platelet (PLT) shows general improvement from T_1-T_3 as the duration increased and significant increase in T_3 at 8^{th} and 12^{th} weeks. These increased values of PLT may have arisen from the immune boosting effects of the combined vitamins C and E in *C. gariepinus*. Gbore and Akele (2010) reported how the haematological parameters in *Clarias gariepinus* fingerlings fed graded levels of dietary fumonisin B1 were improved upon.

The alanin aminotransferase (ALT) production levels in *C. gariepinus* subjected to varying concentrations of combined vitamins C and E indicated that the production level increases with increase in the concentration of the combined vitamins especially at 8^{th} and 12^{th} weeks of the experiment in the kidney of the fish. There were significance differences in mean values of T_3 samples in the 4^{th}, 8^{th} and 12^{th} weeks of the experiment. This probably connotes the bolster effects of the combined vitamins on the immune system of the fish that led to the improved physiological status of the fish, hence the elevated production levels. In line with this position, Ispir *et al*. (2011) has posited that Vitamin E is one of the most important nutrients influencing the fish immune system. It is also known that Alanine aminotransferase and Aspartate amino transferase are non-plasma specific enzymes that are localized in tissues cells of liver, heart, gills, kidneys, muscles and other organs, and their presence in the blood (plasma) may give specific information about organ dysfunctions (Gabriel and George, 2005). In another development, the ALT production levels in the liver of the fish indicated significant differences in T_2 at the 4^{th} and 8^{th} weeks; and T_3 at the 12^{th} week of the experiment with 44.13 ± 8.28nM/mL. This is probably because both kidney and liver are the major determinants of the physiological status of the fish with the succoring presence of the combined vitamins since it is known that, vitamin C is involved in a number of metabolic processes in the human body, including those that are important for the optimal functioning of the oxygen energy system (Femi-Oloye *et al*., 2019). In addition to this, Gbore *et al*. (2020) posited that vitamin C helps vitamin E to return to its active form in the cell membrane; and due to this regeneration process, the combination of vitamins C and E can provide better antioxidant protection than vitamin C or vitamin E alone. Slightly similar trend also took place in the gills of the fish. Also, Gbore *et al*.(2020) in their work on *C. gariepinus* reported that the ALT levels in fish fed diet containing 5.0 mg FB1/kg but supplemented with vitamin C or E were statistically lower (P<0.05) than in those fed diets containing higher concentration of FB1.

AST production level in the kidney of *C. gariepinus* showed significant increase in T_2 at the 4^{th}, 8^{th} and 12^{th} weeks of the experiment. The highest AST value was 52.66± 4.94nM/mL. Similarly, there was significant increase in the production level in the liver of the fish in T_2 in all the sampling periods of the experiments. The AST production level in the gill was obtained in T_2 at weeks 8 and 12. These increases in production levels probably indicate the roles that combined vitamins C and E has played on the enzymatic activities taking place in *C. gariepinus* since, aminotransferases are normally intracellular enzymes, with low levels found in the plasma representing the release of cellular contents during normal cell turnover (Harvey and Ferrier, 2011). The varying production levels in the various organs also probably depict how relevant these organs are in the enhancement of the health of the fish. Gbore *et al*. (2020) reported that fish (*C. gariepinus*) fed diet containing the highest concentration of 7.5 mg FB1/kg without supplementation or with vitamin E supplementation recorded significantly highest serum AST level than those fed the control diet. Also, Samuel *et al.* (2021a) reported how vitamins A, C and E ameliorated the deleterious effects of Cd toxicant and improved the physiological status of *C.gariepinus* in dealing with the prevailing conditions.

Conclusions and Recommendation

This study has shown the effects of combined vitamins C and E on the haematological parameters and some antioxidant production levels in *C.garipinus* for a period of 12 weeks. There were significant differences in the total white blood count (WBC), Parked cell volume (PCV), haemoglobin (Hb), red blood cell and mean corpuscular haemoglobin concentration (MCH) especially at the early stage of the experiment. The blood platelets (PLT) increased with increase in concentration of the combined vitamins in most cases.

The Alanin aminotansferase (ALT) and Aspartate aminotransferase (AST) production levels varied from one organ to the other. The ALT and AST production levels increase with increase in the concentration of the combined vitamins especially at the later stages of the experiment. The kidneys and liver of *C. gariepinus* showed significant increase in the production levels of both enzymes in the highest concentration of the combined vitamins C and E (T_3) in all the sampling periods of the research.

The use of combined vitamins C and E supplements can serve as an invaluable addition to fish diets that can improve the health and physiology of fishes and hence, lead to improved growth and yield to the farmer.

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