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Research Article

Impacts of Vitamin E on Some Growth, Haematological, Antioxidant Activities and Proximate Compositions of *Clarias Gariepinus* (Burchell, 1822) Under Laboratory Conditions

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Abstract

The assessment of the impacts of vitamin E on the growth, haematological parameters, proximate compositions, and production levels of reduced glutathione (GSH) and Malondialdehyde (MDA) of *Clarias gariepinus* (Burchell, 1822) fingerlings was evaluated for a period of twelve (12) weeks. A total of 300 fish were acclimated for two weeks, during which they were fed twice daily. These were then distributed into four treatments and replicates, including a control. Vitamin E was administered as 350mg/L for T_1, 450mg/L for T_2, and 600mg/L for T_3. Measurements were made from each trough randomly bi-weekly for the duration of the research. These measurements include total length (TL), standard length (SL), and body weight. The specific growth rate (SGR), percentage total weight gain (%TWG), condition factor (CF), and Hepatosomatic Index (%HSI) were also calculated. Blood collection was also made from three randomly selected samples at the end of the experiment. These blood samples were analysed for full blood count following standard procedures. 3 samples were also randomly selected, and the liver, kidney, and gills were excised; homogenized in phosphate buffer, and then assayed for GSH and MDA. 2 samples of the fish were also processed for proximate composition analyses. The data generated were subjected to one-way analyses of variance at $P<0.05$ level of significance. The results indicated that monocyte and neutrophil counts were significantly different in T_2 and T_3, respectively. The red blood cells, white blood cells, haematocrit, and blood platelet counts were not significantly different. The SL mean values increased with an increase in Vitamin E concentration, and the maximum value was obtained in T_3 at the end of the 12th week. At later stages of the research, the TL also increased with an increase in Vitamin E concentration and peaked in T_3 with a mean value of 18.17cm. Likewise, the highest weight value was also obtained in T_3. The peak SGR, %HSI, and %TWG were also obtained in T_3, while the maximum CF was obtained in T_1. The highest protein content (20.58±0.26%) was obtained in T3. The moisture content decreased with an increase in the concentration of the vitamin. The GSH production levels in the liver, kidney, and gills increased with an increase in the concentration of the vitamin, and the T3 mean values were significantly higher than those of other treatments, including the control. The MDA production levels, however, decreased with an increase in vitamin E concentration. The results in this research have displayed positive impacts of the addition of vitamin E in terms of physical and physiological features of *C. gariepinus* and can therefore serve as an invaluable addition in fish farming

Introduction

African catfish, *Clarias gariepinus*, is an important commercial fish due to its high growth rate, high consumer acceptability, and ability to withstand poor water quality and oxygen depletion [1]. Also, the African catfish, *Clarias gariepinus*, is the foremost warm water aquaculture species in Africa, Asia, and recently Europe and Latin America [2]. In Nigeria, catfish is one of the major stable foods affordable to many. The current demand for fish in Nigeria, for instance, stands at about 2.7 million metric tons, while the total domestic supply stands at about 0.7 million metric tons [3]. To bridge these gaps between supply and demand, and to sustain fish under culture, a supplementary diet must be provided to complement the natural feed supply [4].

A vitamin is an organic molecule or a set of molecules

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closely related chemically that are essential micronutrients that an organism needs in small quantities for the proper functioning of its metabolism. Essential nutrients cannot be synthesized in the organism, either at all or not in sufficient quantities, and therefore must be obtained through the diet. There are eight vitamers of vitamin E: four tocopherols and four tocotrienols. Some examples include vitamin A (as all-*trans*-retinol, all-*trans*-retinyl-esters, as well as all-*trans*-beta-carotene and other provitamin A carotenoids), vitamin B_1 (thiamine), vitamin B_2 (riboflavin), vitamin B_3 (niacin), vitamin B_5 (pantothenic acid), vitamin B_6 (pyridoxine), vitamin B_7 (biotin), vitamin B_9 (folic acid or folate), vitamin B_{12} (cobalamins), vitamin C (ascorbic acid), vitamin D (calciferols), vitamin E (tocopherols and tocotrienols), and vitamin K (phylloquinone and menaquinones). [5]. Vitamins have diverse biochemical functions. Vitamin A acts as a regulator of cell and tissue growth and differentiation. Vitamin D provides a hormone-like function, regulating mineral metabolism for bones and other organs. The B complex vitamins function as enzyme cofactors (coenzymes) or the precursors for them. Vitamins C and E function as antioxidants. Both deficient and excess intake of a vitamin can potentially cause clinically significant illness, although excess intake of water-soluble vitamins is less likely to do so [5]. Vitamin E is a generic term for all naturally occurring tocopherols and tocotrienols as well as their derivatives [6]. Since its hydroxyl moiety on carbon 6 can be easily oxidized, vitamin E has a strong reducibility, which protects important substances from oxidation *in vivo* and has an important role in the maintenance of normal metabolic processes and physiological functions in the body. Vitamin E is required to protect the cell membrane from peroxide damage, maintain immunity and enhance disease resistance, and is also associated with embryonic development, nucleic acid metabolism, ascorbic acid biosynthesis, as well as maintenance of tissue quality. Vitamin E deficiency impairs aquatic animal performance, including reduced weight gain, protein efficiency ratio, and feed coefficient [6].

Fish cannot synthesize all biologically active forms of vitamin E and rely on the exogenous dietary sources for their supply [7]. The quantitative requirements of dietary vitamin E have been documented in various farmed fish, including Atlantic salmon, *Salmo salar*, rainbow trout, *Oncorhynchus*, grass carp, *Ctenopharyngodon idellus*, cobia, *Rachycentron canadum*, grouper, *Epinephelus malabaricus*, Rohu, *Labeo rohita*, mrigal, *Cirrhinus mrigala*, hybrid striped bass, *M. saxatilis*, *Pelteobagrus fulvidraco*, hybrid snakehead, *Channa argus* × *Channa maculata* and Japanese eel, *Anguilla japonica* [8], which ranged from 6 to 200 mg kg^{-1} *a*-tocopherol. Diets deficient or lacking in vitamin E may lead to reduced growth, impaired erythropoiesis, muscular dystrophy, darkened skin, exudative diathesis, skin depigmentation, liver fat degeneration, and even death [9]. On the other hand, excess levels of vitamin E could induce lipid peroxidation in rainbow trout, grass carp [10].

Haematological parameters are a good indicator to determine the health of an [11]. 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 the increasing human population. There is also a high cost of fish production in Nigeria, with a constant need for minimal cost of production in order to maximize profit for both consumers and farmers themselves. Presently, there is a dearth of information on the effects of vitamin E supplements on fish and how they can lead to improvement in some of the biochemical constituents of fish, as well as their haematological profile, especially when not treated with one toxicant or the other [12]. The nutritional status of organisms goes a long way in their ability to withstand stress and lead a healthy life. The haematological parameters, as well as antioxidants, are important in diagnosing the functional status of organisms (fish), especially when treated with vitamins. Little is known about the influence or effects of vitamin E supplements on the growth parameters of *Clarias gariepinus,* as morphological manifestations of the physiological changes taking place in the organism due to the presence of the vitamin. [13]. demonstrated that vitamin E could improve daily intake, body weight gain, and feed efficiency ratio of *Clarias gariepinus*.

The proximate composition of an organism usually depicts the important constituents as a result of the prevailing food nutrients the organism is fed with over a period of time. The protein, carbohydrate, ash, and moisture contents, as well as other components of the organisms, determine the rate of conversion of the food nutrient into body biomass. For instance, Nasr, et al. [14]. reported that there were no significant differences regarding growth performances and body composition among the other groups, except that the feed conversion ratio was improved in the highest treatment in an attempt to replace fish meal with a plant protein source (soybean and sunflower meal) in *C. gariepinus.* Antioxidant activities taking place within the fish go a long way in determining the physiological status of the fish due to the presence of one vitamin (or any other supplement) or the other. Several research findings have indicated alterations in one way or another. For instance, elevated levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), 53.49 – 59.29U/L, 65.38 – 73.26U/L, and 73.67 – 77.75U/L, respectively, were recorded as replacement of soya bean meal with sesame seed meal increased in the diets [15]. Also, the immunological responses and antioxidant status of *C.gariepinus* were not affected when they consumed a diet with fishmeal replaced by up to 50% with plant protein (SBM and SFM) with methionine and lysine supplementation, but total globulin, NO, and cumulative mortality were impaired with a diet containing a 100% fish meal replacement [16]. This research, therefore, was geared towards unraveling the major impacts of supplementation of the media of *C. gariepinus* with vitamin E in terms of morphometric and haematological parameters, proximate composition, and some antioxidant concentrations in the fish.

Materials and methods

Materials sample collection and acclimatization

A total of three hundred (300) fingerlings of *Clarias gariepinus*

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were purchased from a commercial farmer in New-Bussa, Niger State, and transported in a 50-liter container filled with water to the Department of Animal Biology, Bosso Campus, Federal University of Technology, Minna, Nigeria. The fish were placed in troughs with water for acclimatization. They were fed twice daily (0800hrs and 1700hr) with a commercial feed (2mm) for two weeks of acclimatization. The holding water was changed every three (3) days during the period. The vitamin E granules (2kg) were purchased from commercial chemical stores. The vitamin E granules were used as supplements in percentages corresponding to the treatments. Hand gloves, 1ml syringe and needles, sampling bottles (EDTA bottles) were purchased from the pharmacy for hematological analysis.

Experimental setup/design

Four treatments, including control with replicates in each treatment, were set up with water, and three treatments with their replicate were exposed to a vitamin E supplement at different percentages corresponding to the treatment for a period of twelve (12) weeks. Each treatment was tagged Control (00 concentration of vitamin E), T_1 (350mg/L concentration of vitamin E), T_2 (450mg/L concentration of vitamin E), and T_3 (600mg/L concentration of vitamin E). Each treatment was replicated as Control (C1, C2); Treatment 1 (T_1R_1, T_1R_2); Treatment 2 (T_2R1, T_2R2) and Treatment 3 (T_3R_1, T_3R_2). Twenty-five (25) samples of the fish were used in a replicate. Random sampling was carried out using a hand-held basket to pick out samples for measurement, and all the analyses were carried out at the appropriate time designated for the set objectives. Measurement of the fish sample was made from each trough randomly every 14th day for twelve (12) weeks. Measurements made include the total length, standard length, and body weight.

Determination of haematological parameters

Two (2) fish were picked from each treatment, weighed, and laid on a spond sheet for blood collection. Blood was collected through the use of a 1ml syringe and needle. The samples were kept inside the sampling bottles (EDTA) to avoid coagulation. The syringe was inserted between the opercular-end and pectoral fin with the syringe held perpendicularly to draw out the blood through suction pressure [17]. This method was repeated for each treatment and replicate. The blood samples were labeled according to treatments and were transported to the Laboratory Services Unit of Ibrahim Badamasi Babangida (IBB) Specialist Hospital Laboratory for analysis.

Measurement of growth parameters

Total length was measured from the tip of the snout of the fish to the tail-end, while the Standard length was measured from the tip of the snout to the caudal curvatures or lobe of the fish using a metre rule graduated in centimetres. The weight of the fish was obtained using a weighing balance in grams.

The percentage weight gain was determined thus:

%WG = (Final Weight- Initial Weight/Initial Weight)× 100

The specific growth rate (SGR) was calculated using the formula stated below:

SGR(g/day) = lnWf-lnWi x 100/t

where:

lnWf = the natural logarithm of the final weight

lnWi = the natural logarithm of the initial weight

t = time (days) between lnWf and lnWi.

The Condition Factor of the Fish samples during the experiment was determined as follows:

CF(K_g/cm^3) = (100 x W)/L^3

Where: K is the condition factor

W is the average final weight of the fish

L is the fork length (average total length) of the fish

Percentage hepatosomatic index (%HIS)**:** The fish samples were randomly selected and dissected, and the liver was removed and weighed using an analytical balance. After the measurement, the %HSI was thus determined according to Biney, et al. [18]. as reported by Ciftci, et al. (2015).

%HSI= (Total Liver Weight/Total Body Weight)× 100

Phosphate buffer preparation

Eight hundred (800) ml of distilled water was prepared in a suitable container. 20.214g of Sodium Phosphate Dibasic Heptahydrate was added to the solution, which was followed by the addition of 3.394 g of Sodium Phosphate Monobasic Monohydrate. The mixture was adjusted to the final desired pH using HCL. Distilled water was then added to bring the volume up to 1000ml (1L) of the buffer.

Determination of antioxidant (GSH and MDA) activities

Two fish were taken from each trough, dissected, and the gills, liver, and kidney were removed, homogenized separately, and preserved in a test tube containing buffer solution and taken for antioxidant test.

Glutathione (GSH) determination: Following the method described by Ellman [19]. with slight modifications, the GSH content of the sera and tissues homogenates was estimated. An aliquot of 100 µL of each of the samples was precipitated with 200 µL of 20% TCA and centrifuged at 2000 rpm at 4 °C for 5 minutes. Thereafter, 50 µL of each of the supernatants was mixed with 100 µL and 50 µL of 1 M phosphate buffer and 5 mM DNTB, respectively. The reaction mixture was incubated at 37 °C for 10 minutes, and the absorbance was read at 412 nm. The concentration of GSH was calculated from the calibration curve.

Determination of MDA: Malondialdehyde assay was carried out using the method of DelRio, et al. [20]. with slight modification. Briefly, 200µL of the samples each combined with 0.2mL of 8.1 % SDS; 1.5 mL acetic acid and 1.5 mL TBA the solution made up to 4 mL with distilled water; then the solution is boiled for 60 minutes in a boiling water bath (95°C); after cooling, the reaction product (TBA–MDA complex) was extracted by adding 1 mL of n-butanol-pyrimidine (15:1; v/v).

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The flocculent precipitate was removed by centrifugation at 3500 rpm for 15 mins; then, the supernatant was obtained, and the absorbance reading of the supernatant at 532 nm was taken against a blank that contained the reagents minus the samples. The malondialdehyde concentration of the sample was calculated using the adduct extinction coefficient of 1.56 × 10^5 M^{-1} cm-1 for MDA.

Malondialdehyde concentration (M) = Abs532/155

MDA conc. (µM): M x 1000 = MDA conc. (µM/mL) of the sample.

Proximate composition of *C. gariepinus*

Two fish were taken randomly from each trough labeled accordingly and taken for the proximate test. It involves an assay for all the constituents of a sample apart from the major food constituents. Each fish sample was taken to the laboratory, sacrificed, and homogenized. Samples for the different analyses were then taken from the homogenized material. Triplicate determinations were carried out on each group. The proximate analysis of the sample was carried out by the methods of AOAC (1990). Following this standard procedure, the moisture, crude protein, ash, and carbohydrate contents were determined from the fish samples after 12 weeks of the experiment.

Data analyses

Data generated from haematological and morphometric parameters, antioxidant activities, and proximate composition of *Clarias gariepinus* produced from all the treatments and replicates were subjected to One-way Analysis of Variance (ANOVA) using SPSS version 26 after a period of 12 weeks, and the means were separated with Duncan Multiple Range Tests where significant. These were considered significant at $p < 0.05$ level of significance.

Results and Discussion

Proximate composition of *C. gariepinus* after 12 weeks of exposure to vitamin E

The result of the effect of different concentrations of vitamin E on the proximate composition (%) of *Clarias gariepinus* indicated that the fish exposed to 350mg/L (T_1) of vitamin E recorded the highest moisture content (72.70 ± 1.04). This is not significantly different from the moisture content recorded from the control (C) groups (72.20 ± 0.33). The lowest moisture content was recorded for the fish exposed to 600mg/L (T_3) of vitamin E. Although this is not significantly different from the moisture content obtained from the group exposed to 350mg/L (T_1) of vitamin E. (Table 1).

On the other hand, the ash contents of all groups of fish exposed to the different amounts of vitamin E (except T_1) were not significantly different from the control group. The Fat content (%) increased with an increase in the level of vitamin E. The proximate composition of protein was also observed to increase with increases in the levels of vitamin E added. The highest protein content was observed in T_3 (22.04 ± 0.30 %). However, carbohydrate content decreases with an increase in vitamin E. (Table 1).

GSH and MDA Production levels in *C. gariepinus* treated with vitamin E

The result of the effect of different levels of vitamin E on the antioxidant concentration in the liver, kidney and gills of *Clarias gariepinus* showed that there was significant difference in the antioxidant concentration recorded in the treatments when compared with the control that contain no vitamin; it was generally observed that GSH (µg/ml) mean values increased with increase in the concentration of vitamin E in all the organs of interest. On the other hand, when compared to the control, it was observed that in the liver, kidney, and gills, an increase in the concentration of vitamin E led to a decrease in the production of MDA. And as the concentration of vitamin E increased from T_1 to T_3, MDA production decreased, respectively (Tables 2-4).

Haematological characteristics of *clarias gariepinus* treated with different concentrations of vitamin E for a duration of three (3) months

From the samples of fish exposed to varying concentrations of Vitamin E for a period of 12 weeks, the mean values of white blood cells indicated that the highest concentration was obtained in T_3, the treatment with the highest concentration of the supplement, while the red blood cell count had its highest in T_2. The mean values of Haemoglobin and Haematocrit were lower in all the treatments, including the control. Mean Corpuscular Volume, Mean Corpuscular Haemoglobin, and Mean Corpuscular Haemoglobin Concentration had their maximum concentrations in T_2, T_3, and T_1, respectively. Blood platelet count recorded in T_1 and T_2 was high when compared to T_3. (Table 5,6).

Morphometric features of *Clarias gariepinus* treated with different concentrations of vitamin E for a duration of three (3) months

The various measurements obtained in this research indicated that the highest standard lengths were obtained mostly in T_3 in all the weeks of sampling, except week 2, where

Table 1: Proximate Composition of *C. gariepinus* treated with varying Concentrations of Vitamin E for a duration of 12 weeks

Sample	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Carbohydrates (%)
Control	72.20 ± 0.33^b	2.64 ± 0.22^b	0.81 ± 0.02^a	17.91±0.23^a	6.46 ± 0.34^b
T_1	72.70 ± 1.04^b	2.07 ± 0.08^a	0.82 ± 0.06^a	18.38±0.17^a	6.05 ± 0.86^b
T_2	70.65 ± 0.63^a	2.55 ± 0.32^b	1.09 ± 0.11^b	20.22±0.77^b	5.50 ± 0.57^b
T_3	70.06 ± 0.49^a	2.79 ± 0.04^b	1.64 ± 0.19^c	22.04±0.30^c	3.48 ± 0.34^a

Values are presented as mean ± standard deviation (SD). Values with different superscripts in a column are significantly different at $p < 0.05$.

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Table 2: GSH and MDA Activities in the Liver of *C. gariepinus* treated with different concentrations of Vitamin E for a period of 12 weeks

Sample	GSH (µg/mL)	MDA (µM)
Control	32.35 ± 1.07^{a}	12.28 ± 0.65^{d}
T_1	33.76 ± 1.37^{a}	10.78 ± 0.46^{c}
T_2	38.08 ± 1.63^{b}	8.20 ± 0.72^{b}
T_3	47.49 ± 1.63^{c}	7.02 ± 0.31^{a}

Values are presented as mean ± standard deviation (SD). Values with different superscripts in a column are significantly different at $p < 0.05$.

Table 3: GSH and MDA production levels in the kidney of *C. gariepinus* subjected to varying concentrations of Vitamin E for a duration of 12 weeks

Sample	GSH (µg/mL)	MDA (µM)
Control	23.48 ± 0.92^{a}	16.60 ± 0.92^{d}
T_1	26.24 ± 1.06^{a}	13.01 ± 0.83^{c}
T_2	34.42 ± 2.30^{b}	10.13 ± 0.31^{b}
T_3	47.03 ± 1.28^{c}	7.79 ± 0.96^{a}

Values are presented as mean ± standard deviation (SD). Values with different superscripts in a column are significantly different at $p < 0.05$.

Table 4: GSH and MDA Concentrations in the gills of *C. gariepinus* exposed to different levels of Vitamin E for a period of twelve weeks

Sample	GSH (µg/mL)	MDA (µM)
Control	15.65 ± 1.07^{a}	9.50 ± 0.71^{d}
T_1	22.10 ± 1.36^{b}	8.40 ± 0.59^{c}
T_2	24.19 ± 1.30^{b}	7.03 ± 0.31^{b}
T_3	31.16 ± 1.31^{c}	5.88 ± 0.25^{a}

Values are presented as mean ± standard deviation (SD). Values with different superscripts in a column are significantly different at $p < 0.05$.

Table 5: Haematological Parameters of *C. gariepinus* Treated with Different Concentrations of Vitamin E for a period of 12 Weeks

Parameters	Treatments			
	C	T_1	T_2	T_3
WBC	215.00±5.00^{c}	180.00±10.00^{a}	194.00±2.00^{b}	201.00±9.00^{b}
RBC	5.45±0.45^{a}	3.65±1.45^{a}	7.75±1.85^{a}	5.90±3.70^{a}
HGB	14.85±0.75^{b}	9.40±4.70^{ab}	10.95±4.65^{ab}	5.50±0.80^{a}
HCT	44.70±2.30^{b}	28.45±14.25^{ab}	34.10±12.70^{ab}	17.80±3.50^{a}
MCV	82.00±1.00^{a}	88.50±6.50^{a}	90.50±8.50^{a}	69.00±27.00^{a}
MCH	30.50±0.50^{a}	35.60±5.40^{a}	32.00±1.00^{a}	36.50±3.50^{a}
MCHC	34.60±0.40^{a}	34.65±0.65^{a}	34.50±0.50^{a}	33.50±1.50^{a}
PLT	155.00±5.00^{b}	150.00±8.00^{ab}	150.50±2.50^{ab}	144.00±4.00^{a}
LYM	94.50±2.50^{a}	95.00±2.00^{a}	94.50±0.50^{a}	94.50±0.50^{a}
M	2.50±0.50^{b}	1.50±0.50^{a}	2.00±0.00^{ab}	2.50±.50^{b}
N	2.50±1.50^{a}	3.50±2.50^{a}	3.50±0.50^{a}	3.00±0.00^{a}

Values are presented as Mean±Standard deviation of three replicates. Values with different superscripts in a row are significantly different at P<0.05.

the T_1 samples had the peak value. (Table 6). Meanwhile, at later stages of the experiment in weeks 8, 10, and 12, the maximum values of total lengths were recorded in T_2, T_1, and T_3, in that sequence. (Table 7).

Furthermore, there were variations in the weight of the samples at one point of sampling or another. The weight of the samples increased with an increase in the concentration of the vitamin in week 6. The highest weight values were obtained in T_2 and T_3 and in weeks 8 and 12, respectively. (Table 8). Other parameters determined indicated that the Specific Growth Rate (SGR) and Total Weight Gain (TWG) peaked at T_3, followed by the control in each case. The Condition Factor (CF) was high in T_1 and Control. The percentage Hepatosomatic Index increased and peaked in T_2 and T_3. (Table 9).

Discussion

The proximate composition of the samples of *C. gariepinus* exposed to a higher concentration of vitamin E (T_3) had the highest protein values (22.04±0.30) when compared to the control (17.91±0.23). This is probably due to the presence of the vitamin that ensured an increase in the nutritional values of the fish. This correlates with the result of Diyaware, et al. [21], who reported that there was an increase in protein content with an increase in vitamin E in the diet of *C. gariepinus*. The protein content of fish is also important when considering the quality and texture of the fish meat. In this research, the protein contents increased with an increase in the concentration of the vitamin, with a corresponding decrease in the carbohydrate contents. This probably depicts the fact that as the feed with the supplement is being converted to protein, it builds up much more than the proportion being converted to carbohydrate. Fish generally have very low levels of carbohydrates. The average carbohydrate content in fish in this research ranged from 0.04 to 0.36%. The low carbohydrate values could be because glycogen does not contribute much to the reserves in the fish's body tissue, and a larger chunk of the food has been converted to protein. In another development, partial or total replacement of Fish Meal with a plant protein source (soybean and sun-flower meal with high percentage of vitamin E) showed similar growth performance and body composition with greater economic efficiency; and that there were no significant differences in growth performances and body composition among the groups, except that the feed conversion ratio was improved in the group with the highest percentage of the plant protein [14].

Growth rate and weight gain are directly associated with the capability of an animal to ingest and digest and absorb nutrients present in the feed [22]. The standard and total lengths, as well as the weight of the fish in this research, also indicated that at the end of the twelfth week, the T_3 mean values were higher than all other treatments, including the control. Again, this is probably a result of the presence of a vitamin that ensured increased growth measurable by these morphometric parameters. In addition to the foregoing, the Specific Growth Rate (SGR) and Total Weight Gain (TWG) peaked at T_3, most likely buttressing the significance of the vitamin. The Condition Factor (CF) was high in T_1 and Control. The weight of the fish was probably proportional to the length of the fish in treatments with little or no vitamin E concentration. The percentage Hepatosomatic Index increased and peaked in T_2 and T_3. This probably indicated how the weight of the liver of the fish increased with an increase in vitamin concentration. In another development, Ukonze, et al. [23]. indicated that protein synthesis and increased tissue production in the treated catfish implied that fish growth was not due to the increase in weight alone and that the high moisture content might likely be responsible for the higher weight gained recorded by the fish fed with the commercial feed.

The haematological parameters exhibited lower values of

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Table 6: Standard Lengths of *C. gariepinus* Subjected to varying concentrations of Vitamin E for a duration of 12 Weeks

Treatments	Standard Lengths (cm)					
	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
C	11.35±0.19^{a}	12.65±0.62^{a}	14.08±0.28^{a}	16.13±1.20^{a}	13.64±0.04^{a}	16.12±1.09^{b}
T_1	11.39±0.82^{a}	12.21±0.25^{a}	14.90±0.20^{a}	15.57±1.27^{a}	13.80±0.30^{a}	14.10±0.13^{a}
T_2	10.58±0.05^{a}	12.38±0.25^{a}	14.73±1.20^{a}	18.69±0.86^{a}	13.57±0.54^{a}	14.02±0.65^{a}
T_3	10.68±0.38^{a}	12.98±0.38^{a}	14.67±0.37^{a}	14.94±0.27^{a}	13.70±0.17^{a}	18.17±0.54^{c}

Values are presented as Mean±Standard deviation of three replicates. Values with different superscripts in a row are significantly different at P<0.05.

Table 7: Total Lengths of *C. gariepinus* Subjected to varying concentrations of Vitamin E for a duration of 12 Weeks

Treatments	Total Lengths (cm)					
	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
C	13.30±0.00^{a}	14.97±0.74^{a}	16.10±0.37^{a}	18.43±1.43^{ab}	14.67±0.64^{a}	19.07±0.56^{b}
T_1	12.32±1.79^{a}	13.95±0.35^{a}	17.17±0.07^{a}	19.03±0.20^{b}	15.78±0.22^{b}	16.03±0.17^{a}
T_2	12.33±0.27^{a}	14.75±0.15^{ab}	16.95±1.39^{a}	20.62±0.69^{c}	15.62±0.62^{b}	15.98±0.75^{a}
T_3	12.44±0.47^{a}	14.97±0.47^{b}	17.05±0.12^{a}	16.97±0.24^{a}	15.70±0.17^{b}	20.47±0.47^{c}

Values are presented as Mean±Standard deviation of three replicates. Values with different superscripts in a row are significantly different at P<0.05.

Table 8: Weight of *C. gariepinus* Subjected to varying concentrations of Vitamin E for a duration of 12 Weeks

Treatments	Weight (g)					
	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
C	19.98±0.02^{b}	25.27±4.14^{a}	35.10±5.37^{a}	51.49±7.9^{ab}	30.24±0.74^{a}	52.45±2.08^{b}
T_1	19.50±2.64^{ab}	23.62±2.59^{a}	36.30±2.67^{a}	43.05±1.39^{a}	40.65±6.75^{b}	32.70±2.04^{a}
T_2	16.67±1.04^{a}	24.76±1.50^{a}	37.50±4.20^{a}	65.85±8.22^{b}	30.78±5.78^{a}	32.13±2.27^{a}
T_3	17.08±1.55^{ab}	27.75±2.05^{a}	40.2±2.00^{b}	36.60±1.97^{a}	32.30±1.47^{ab}	61.09±0.12^{c}

Values are presented as Mean±Standard deviation of three replicates. Values with different superscripts in a column are significantly different at P<0.05.

Table 9: Percentage Hepatosomatic Index, Condition Factor, Total Weight Gain, and Specific Growth Rate of *C. gariepinus* treated with Varying Concentrations of Vitamin E for a period of 12 weeks.

Treatments	%HSI	CF (g/cm)	TWG (g)	SGR (g/day)
C	0.01	15.0	3.29	0.65
T_1	0.01	15.8	1.49	0.38
T_2	0.02	13.5	1.50	0.38
T_3	0.02	13.7	3.76	0.72

HSI stands for Hepatosomatic Index, CF indicates Condition Factor, TWG represents Total Weight Gain, and SGR stands for Specific Growth Rate.

the parameters tested for in most cases when compared to the values obtained in the control treatments. This probably indicates that the fish were in good condition and not undergoing many physiological perturbations. Hence, there was probably no need to engage the white blood cells and blood platelets in combating the effects of xenobiotics as a result of the succor provided by the presence of vitamin E. In line with this assertion, Diyaware, et al. [24]. demonstrated that the high platelet values observed were an indication that the fish likely withstood and healed from bruises that might have been acquired during fighting and the prevention of excessive bleeding via the enactment of rapid clotting at the injury site. Osuigwe, et al. [25]. also reported that fish hematological indices are influenced by a variety of issues that encompass fish mass, age, physiological condition, environmental situations, and feeding procedure. In a related development, the different diet types of the substitution of fish meal did not affect haematological parameters and blood indices [14] of *C. gariepinus*. In another development, Ukonze, et al. [23] reported that the experimental groups fed with 25% black bean substituted feed had more average weight gained and grew longer, had more RBC, MCV, WBC and PCV when compared with other graded levels of substitution and the control; and that the ash, fat and protein contents of the fish carcass ranged from 2.21-2.66, 1.14-1.62, 18.51-20.44%, respectively with fish fed supplemented black bean meal recorded higher mineral concentrations in their carcass as compared to the control. However, Ispir, et al. [26] observed that weight were not affected by dietary vitamin E concentrations, an increased red blood cells count and high hemoglobin concentration was obtained in treatments with 80 and 160 mg vitamin E kg^{-1} relative to control; Mean corpuscular volume and the number of leucocytes presented a significant increase in treatment with 240 mg vitamin E kg^{-1} when compared to control and that Mean corpuscular hemoglobin concentration was significantly decreased in treatment with 240 mg vitamin E kg^{-1}. This probably indicated that the effect of the right concentration of the vitamin must be above a certain threshold for its effect to be felt by the animal, as evident in the outcome of this research.

The outcome of this research indicated that GSH (µg/ml) mean values increased with an increase in the concentration of vitamin E in the liver, kidney, and gills. And MDA, on the other hand, decreased with an increase in the concentration of the vitamin. This probably indicates that when there was less stress more of the antioxidant, reduced glutathione was produced; and that the concentration produced was not utilized in combating the effects emanating from oxidative stress if there were xenobiotics in the environment of the fish; since, reduced glutathione (GSH) is an essential enzyme in fishes as its primary line of defense in fish oxidative stress [27]. Likewise, since there was less stress due to the presence of

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the vitamin, probably the concentration of malondialdehyde needed to be generated was minimal. In a similar research, Samuel and Uwada [11] reported that combined vitamins C and E led to a decrease in concentration of reduced glutathione as the concentration of the combined supplements increased. Also, Alkaladi [28] reported that the mixture of vitamin E and C was highly effective in alleviating the toxic effect of ZnONPs (zinc nano particles) and that the vitamin E and C mixture modulated the oxidative stress induced with ZnONPs in the liver and gills of *Oreochromis niloticus*. In a related development, serum growth hormone and amylase levels had no significant differences among the groups, while serum lipase levels decreased significantly due to partial or complete substitution of fishmeal with plant protein [14]. Also, hepatic antioxidant activities (TAC, SOD, CAT, and GSH-Px) of *Pseudobagrus ussuriensis* decreased with an increase in fish meal substituted with cotton meal, especially at 60% replacement after 8 weeks of feeding [29]. Similarly, SOD activity decreased significantly in the hepatopancreas of *L.vannamei* fed a diet with 25% of fish meal substituted with soya bean meal for 12 weeks [30]. The continual decrease in MDA activities as the concentration of vitamin E increased was probably because there were no external perturbations. In line with this position, Ahamefula, et al. [31] attributed high levels of MDA and low activity of SOD as marked effects of possible fish species exposure to environmental stress. It is also known that MDA is usually produced in large quantities when elicited by the presence of toxicants [32]. Furthermore, Sissein, et al. [33,34] observed that the polluted Gbarantoru swamp in the Niger Delta, Nigeria, had higher levels of heavy metals as well as high levels of MDA in liver cells of *C. gariepinus* and low levels of vitamins and glutathione as compared to the levels in the *C. gariepinus* harvested from the Niger Delta University Agricultural Farm (Control).

Conclusions

This study has demonstrated the effects of vitamin E on the growth and haematological features, proximate composition, and some antioxidant production levels in *C. gariepinus* reared for a period of 12 weeks. The results obtained indicated that haematological indices varied slightly with higher concentration values in the control samples in most cases. There were significant differences in the moisture, protein, fat, ash, and carbohydrate contents of the fish. The protein content increased with an increase in the concentration of vitamin E.

The reduced glutathione (GSH) and Malondialdehyde (MDA) production levels varied from one organ to another. The GSH production levels increased with an increase in the concentration of the vitamin, especially at the highest treatment (T_3) of the experiment, while the MDA production levels decreased with an increase in the concentration of the vitamins, especially in T_3. The kidneys and liver of *C. gariepinus* showed a significant increase in the GSH production levels, with the highest concentration of the vitamin, and a decrease in the MDA production levels, with the highest concentration of the vitamin.

Recommendations

The use of vitamin E supplements is an important addition to the diet of fish capable of enhancing or improving the health and physiological status of the fish, which can serve as an invaluable addition in fish farming and improve the quality yield for fish farmers. Based on the outcome of this study, it is recommended that fish farmers should employ the addition of a supplementary vitamin diet to fish feed so as to enhance their growth and make them more resistant to diseases while ensuring that this practice is cost-effective.

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