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Effects of combined vitamins C and E on the growth performance and some antioxidant production levels in *Clarias gariepinus* (Burchell, 1822) fingerlings

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Abstract

The demand for improved aquacultural practices is on constant increase to meet the demands of the teeming population of the human race at the lowest cost possible. The effects of combined vitamins C and E on the growth performance, catalase (CAT) and reduced glutathione (GSH) production levels in *Clarias gariepinus* fingerlings (10.00±0.00g) were evaluated for 12 week. 120 samples of the fish were assigned to 4 treatments thus: 00 (control), 200mg/L (T_1), 300mg/L (T_2) and 400mg/L (T_3). The total length (TL), standard length (SL) and weight of the fish were determined weekly. The percentage weight gain (%W), specific growth rate (SGR) were also calculated. The kidneys, liver and gills were excised, homogenized in phosphate buffer; and then assayed for CAT and GSH every 4th week of the experiment. From the results, the TL and SL increased significantly with increase in the concentration of the combined vitamins especially in T_2 and T_3 much better than the control. The weight increased significantly from T_1-T_3 all through the 12 weeks with 120.88±28.75g as the highest, 1109%WG and SGR of 5.686g/day obtained in T_3. The CAT and GSH in the kidneys, liver and gills decreased with increase in the concentrations of the combined vitamins and duration of the experiment with the control values significantly higher than all the treatments in most cases. The results from this research have shown significant improvements in growth and physiology of *C. gariepinus* and therefore, administration of these combined vitamins can serve as invaluable addition in catfish farming.

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Introduction

The demand for high quality fish and fishery products is growing significantly every year mostly due to their nutritional fact that they contain plentiful of beneficial healthy substances (FAO, 2018) that are acceptable to all age groups. The most important of these are fish lipids, which usually contains high amount of omega-3 fatty acids, mainly α-linolenic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). The omega-3 fatty acids have several beneficial impacts on human health which include decreasing the risk of myocardial infarction (Abdel Rahman *et al.*, 2019); lowering blood pressure and triglyceride concentration in blood enhancing the immune system (Damsgaard *et al.*, 2007) and sustaining proper brain function in human body. It is also known that, fish proteins have high biological values as they contain all essential amino acids in the right proportion and specially, lysine as well as sulphur containing amino acid such as methionine and cysteine which are absent in plant protein (Leduc *et al.*, 2018). The minerals present in fish include iron, calcium, zinc, phosphorus, selenium fluorine, iodine; these minerals are with high 'bioavailability' meaning that they are easily absorbed by the body (Han *et al.*, 2019). Fish and shellfish provide about 14% of the worlds need for animal proteins and 4% to 5% of the total protein requirement (Khalil *et al.*, 2017).

In recent years, the aquaculture industry has expanded dramatically compared to the other food production sectors, mainly to fulfill the increasing demand for fish as food, particularly in a few developing countries where fish is considered the main source of nutrition (FAO, 2018). *C. gariepinus* is a tropical hardy species belonging to the Phylum Chordata, class Actinopterygii and family *Claridae*. *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. It has been reported that apart from tilapia, *Clarias* specis is the most abundant cultivated fish species in Nigeria (FAO, 2003). The common species found are *Clarias gariepinus*, *Clarias anguillaris*, *Clarias buthupogon* and *Clarias lazera* (Samuel *et al.*, 2021).

Vitamins are organic compounds that are essential for life as they are required in trace amounts for normal growth, reproduction, and health (Gasco *et al.*, 2018; Gouda *et al.*, 2020). Fish lack L-gluconolactone oxidase enzyme, which is responsible for the *de-novo* synthesis of vitamin C, and therefore, fulfill their vitamin C requirements from an exogenous source (Fracalossi *et al.*, 2001). 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 reaction 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. 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.

Glutathione exists in two forms: reduced (GSH) and oxidized (GSSG). Under normal conditions the predominant form of glutathione is the GSH (almost 98%). Glutathione is used as a substrate for GPX during the reduction of H_2O_2 (Zelko *et al.*, 2002). Furthermore, glutathione is also involved in a process called "protein S-glutathionylation" modulating protein functions (Lu, 2013). Previous studies have indicated how GSH was utilized in the kidneys, liver and gills of *Clarias gariepinus* in combating the menace of toxicant (lead nitrate) in its environment (Samuel *et al.*, 2017); and how there was significant

differences in GSH, MDA, SOD and total protein in the gills of *Hemichromis fasciatus* and *Chrysichthys nigrodigitatus* collected from polluted Lagos lagoon (Ayoola *et al.,* 2014) indicating the relevance of the antioxidant in the presence of oxidative stress occasioned by the presence of the pollutants. Catalase is a common enzymatic antioxidant found in nearly all living organisms exposed to oxygen (such as bacteria, plants and animals) (Okoye *et al*., 2018). Catalase has one of the highest turnover number of all enzymes, one catalase molecule can convert approximately 5 million molecules of hydrogen peroxide to water and oxygen each second (Goodsell, 2004).

Little is known about the influence or effects of combined vitamins C and E supplements on the growth parameters, Glutathione and Catalase production level in *C. gariepinus* as morphological manifestations and the physiological changes taking place in the organism due to the presence of the vitamins. *C. gariepinus* is a hardy species known to be capable of surmounting various environmental challenges; and are omnivorous in nature. It tolerates difficult conditions in aquaculture (Ukwumi *et al*., 2019). However, adequate vitamins and other essential nutrients may not be readily available from their routine consumption activities. This study therefore, espouses the needs for improvement of the nutritional requirement of *C.gariepinus* by administering certain concentrations of the combined vitamins C and E; and determining their effects in terms of morphological differentiations and some antioxidant production levels over time.

Materials and methods

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 Laboratory of Old Research Farm of the Department of Water, Aquaculture and Fisheries Technology (WAFT), Federal University of Technology, Bosso Campus in a 25 litres container with water inside to reduce the risk of mortality. Sixty (60) fingerlings were carefully distributed into two rearing plastic Aquaria tank (19 cm x 13.5cm x 9.6) containing twenty (20) litres of Borehole water. During acclimatization, the fishes were fed twice daily to satiation with a commercial feed (Blue crown) after every 8 hours. Water exchange was done every 48 hours during the 14 days acclimatization period.

Experimental Setup

The vitamins C and E granules (500g each) were purchased from commercial chemical stores. Three treatments including control with replicate in each case were set-up for the experiment. The vitamins were administered as follows: (00 as control and 200mg/L, 300mg/L, 400mg/L for T1, T2 and T3 and their replicate, respectively) for a period of twelve (12) weeks.

Measurement of Growth Parameters

Standard Length (SL)

Two samples of fish were randomly selected from each aquarium every week of the exposure period. The fishes were carefully placed on an aluminium foil using a transparent metre rule graduated in centimetres (cm) between the mouth and the caudal peduncle of the fish. The measurement of the fish was done separately and the average taken to represent the SL of each sampling period.

Total Length (TL)

Two samples of fish were randomly selected from each aquarium every week of the exposure period. The fishes were carefully placed on an aluminium foil using a transparent metre rule graduated in centimetres (cm) from the head to the tail end. The fishes were measured individually, and the mean of the measurement was taken to represent the TL of the sampling period.

Measurement of Weight

Two samples were randomly selected from each aquarium every week of the exposure period. The fishes were carefully placed on plastic petri-dish, then the electronic pocket scale was taped to zero before the weight of the fish was determined in gram.

Weight gain

The weight gain (WG) was calculated as the difference between the final weight of fish and the initial weight in grams (Ahmed, 2012).

Percentage weight gain (%)

The Percentage weight gain was calculated as described by Ahmed (2012) thus:

Weight gain (%) =

$$\frac{\text{Final body weight} - \text{initial body weight}}{\text{Initial body weight}} \text{ X100}$$

Specific growth rate

The specific growth rate (SGR) was calculated using the formula;

$$\text{SGR} = \frac{(\ln W2 - \ln W1)}{(T2 - T1)} \text{ X100}$$

Where W1 = initial weight, W2 = Final weight, T_2-T_1 = Number of days of the experiment.

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.

Tissue harvesting and homogenization

Three fishes were randomly picked from each trough that is, T1-T3 including the control and their replicate in each case. These were dissected and the kidneys, gills and liver excised. Each organ extracted was put inside a labeled test tube containing sodium phosphate buffer solution. After this process the organs were homogenized using a mortar and pestle. After each homogenization the mortar and pestle was rinsed with distilled water.

Catalase (CAT) Bio-assay

The CAT activities in the plasma were measured following the protocol of Aebi (1984). The decomposition rate of hydrogen peroxide (H_2O_2) into O_2 and H_2O by the samples in 3 minutes time was tested at 240nm wavelength using a UV spectrophotometer (Shimadzu UV-Visible- 1800 series, spectrophotometer). The reaction was initiated by addition of 20µL of serum to the total 1ml reaction mixture containing 100µL of 100mM H_2O_2 and 880µL of 50mM potassium phosphate buffer (pH 7.0). The change in the absorbance at 240 nm was measured at 20 seconds interval until 3 minutes. The enzyme activity was calculated by 0.0436mM extinction coefficient of H_2O_2 at 240nm. The catalase activities units/ml of seminal plasma was calculated based on the conversion ability of number of µmol/min H_2O_2 at 25°C.

Calculations:

$$\frac{\text{Absorbance (sample) - Absorbance (blank)}}{\text{Change in Absorbance (sample)} - \text{change in Absorbance (blank}} \quad \text{0.0436 X volume of sample}$$

0.0436= millimolar extinction coefficient of H_2O_2 at 240 nm.

Reduced Glutathione Bioassay

The GSH (reduced glutathione) produced in each organ of the fish from each treatment and replicate were determined from their homogenates. The following reagents were used for the analysis: 0.2M phosphate buffer (8.40 g of NaH_2PO_4 and 9.94 g of Na_2HPO_4 was dissolved in distilled water and made up to 1000ml mark in a volumetric flask. The buffer was adjusted to pH8.0); 10% Trichloroacetic acid (10 g of TCA was dissolved in distilled water and made up to 100ml in the volumetric flask); and Ellman' reagent (19.8 mg of 5,5'-Dithiobis Nitro Benzoic acid (DTNB) in 100ml of 0.1% sodium nitrate).

To 150µL of the tissue homogenate (in phosphate-saline pH 7.4), 1.5ml of 10% TCA was added, and centrifuge at 1500 g for 5 min. 1.0ml of the supernatant was treated with 0.5ml of Ellman's reagent and 3.0ml of phosphate buffer (2.0 m pH 8.0). The absorbance was read at 412 nm. Estimation of Reduced Glutathione was determined by the method of Ellman (1959) as described by Rajagopalan *et al.* (2004). The amount of glutathione was calculated using a GSH standard curve and expressed as micron grams of GSH formed/mg protein in each case.

Data Analyses

Data generated from the bi-weekly morphometric parameters were represented in bar charts. Length-Weight regression analysis was used to establish relationship between the standard lengths and weights of the samples. One-way analysis of variance (Statistical Package version 20) was used to compare the means of data produced from the catalase and GSH production levels at $P \leq 0.05$ level of significance.

Results and discussion

Results

Mean± Standard Deviation of Standard and Total lengths (cm) of C. gariepinus subjected to varying concentrations of combined vitamins C and E for the period of 12 weeks

The result of standard length of *C. gariepinus* subjected to varying concentrations of combined vitamins C and E for a period of 12 weeks obtained in week 1 indicated that the T_1 and T_3 mean values were significantly higher than other treatments. In week 2 however, there was no significant difference in all the treatments including the control. However, in weeks 3, 4, 6, 7, 8 and 12 the mean values obtained indicated that T_2 and T_3 were significantly higher than other treatments including the control. In weeks 5, 9 and 11, the T_3 mean values were significantly higher than other treatments including the control. The mean value obtained at week 10 indicated that T_2 (18.70±0.62cm) was significantly higher than other treatments including the control. At week 12, the mean value obtained in T_2 (20.87±2.78cm) and T_3 (20.87±2.78cm) were significantly higher than T_1 including the control (Table 1).

The result of total length of *C. gariepinus* subjected to varying concentrations of combined vitamins C and E for a period of 12 weeks shows that the values obtained in weeks 1, 2, 3, 4, 9, 10, 11 and 12 indicated that T_3 mean values were significantly different from other treatments including the control. In week 5, 6, 7 and 8 the T_2 and T_3 mean values were significantly different from other treatment and control. There was general increase in total length from T_1-T_3 in all the weeks of the experiment. (Table 2).

Table 1. Standard length (cm) of *C. gariepinus* subjected to varying concentrations of combined vitamins C and E for the period of 12 weeks.

Weeks	Control	T1	T2	T3
1	6.50± 1.08^{a}	7.72± 1.20^{b}	7.00± 0.91^{a}	8.32± 1.27^{c}
2	11.00± 0.82^{a}	10.17± 0.66^{a}	10.42± 1.89^{a}	11. 25± 1.04^{a}
3	11.07± 0.15^{a}	11.20± 1.94^{a}	11.70± 1.23^{ab}	13.37± 0.85^{b}
4	12.50± 1.35^{ab}	11.95± 1.93^{a}	13.95± 1.62^{ab}	15.02± 2.12^{b}
5	13.20± 1.24^{a}	12.87± 1.54^{a}	14.03± 2.75^{a}	15.57± 2.38^{b}
6	13.00± 0.91^{a}	13.32± 1.47^{a}	15.75± 1.04^{b}	16.45± 1.72^{b}
7	14.30± 1.26^{a}	14.52± 1.57^{a}	16.12± 1.75^{b}	16.97± 2.55^{c}
8	15.37± 1.75^{a}	15.90± 1.34^{b}	17.25± 1.04^{c}	17.75± 2.46^{d}
9	16.00±1.58^{a}	15.50±0.91^{a}	17.12±1.04^{a}	18.50±2.16^{b}
10	17.75±1.19^{a}	17.37±0.85^{a}	18.70±0.62^{b}	17.75±1.19^{a}
11	18.75±1.19^{a}	18.75±0.64^{a}	19.25±0.64^{a}	21.07±1.62^{b}
12	17.62±1.10^{a}	18.37±1.25^{a}	20.13±0.85^{b}	20.87±2.78^{b}

Values are presented as Mean ± Standard Deviation. Values with different superscript in the same rows are significantly different from each other at P≤ 0.05.

Table 2. Total length (cm) of *C. gariepinus* subjected to varying concentrations of combined vitamins C and E for the period of 12 weeks.

Weeks	Control (cm)	T1 (cm)	T2 (cm)	T3 (cm)
1	9.42± 1.64^{a}	10.67± 2.07^{b}	10.30± 1.50^{a}	11.55± 1.70^{c}
2	9.85± 1.71^{a}	10.47± 1.40^{b}	13.75±2.79^{c}	15.50± 2.12^{d}
3	13.10± 0.27^{a}	12.87± 2.44^{a}	13.97± 0.98^{ab}	15.60± 1.14^{b}
4	14.15± 1.56^{a}	14.32± 1.27^{a}	14.55± 1.10^{a}	18.07± 1.72^{b}
5	15.22± 1.43^{a}	15.75± 1.55^{b}	17.77± 1.15^{c}	17.82± 2.53^{c}
6	15.25± 0.95^{a}	15.97± 1.50^{a}	18.42± 2.16^{b}	18.92± 2.16^{b}
7	16.95± 1.26^{a}	17.12± 1.79^{a}	19.10± 1.35^{b}	19.25± 2.78^{b}
8	17.82± 2.09^{a}	18.00± 2.61^{a}	20.77± 0.87^{b}	20.62± 3.14^{b}
9	17.86±2.02^{ab}	17.27±2.00^{a}	18.20±2.15^{ab}	22.25±2.61^{b}
10	18.00±2.11^{a}	19.13±2.11^{b}	18.38±2.16^{a}	24.00±2.84^{c}
11	19.95±2.32^{a}	19.75±2.13^{a}	22.12±2.55^{b}	25.87±3.02^{c}
12	20.50±2.32^{a}	20.75±2.42^{a}	22.82±2.58^{b}	24.37±2.90^{c}

Values are presented as Mean ± Standard Deviation. Values with different superscript in the same row are significantly different from each other at P≤ 0.05

Mean± Standard Deviation of weight (g) of C. gariepinus subjected to varying concentrations of combined vitamins C and E for the period of 12 weeks

The result of weight (g) of *C. gariepinus* subjected to varying concentrations of combined vitamins C and E for a period of 12 weeks showed tremendous improvement in all the treatments with the control having lower mean values. With the exception of week 2 the T_3 mean values in all the remaining weeks of the experiment were significantly higher than other treatment including the control. The mean value obtained in week 2 showed that T_2 (23.95±5.54) was significantly higher than other treatments including the control. At the end of the experiment (week 12), the highest mean value was obtained in T_3 (120.88±28.75g) which was almost twice the weight obtained in the control (66.33±5.80g) within the same time frame. (Table 3).The highest percentage weight gain (1109%) and specific growth rate (5.686g/day) were also obtained in T_3. (Table 4).

Table 3. Mean Weight (g) of *C. gariepinus* subjected to varying concentrations of combined vitamins C and E for the period of 12weeks.

Week	Control (g)	Treatment1	Treatment 2	Treatment 3
1	11.64± 1.69^a	16.55± 5.63^b	11.22± 4.46^a	18.95± 6.05^c
2	14.62± 1.52^a	16.00± 7.28^b	23.95± 5.54^d	19.29± 2.85^c
3	17.94± 0.90^a	17.04± 7.84^a	20.02± 4.86^b	25.04± 5.58^c
4	23.32± 3.95^a	22.98± 7.96^a	28.06± 8.66^b	40.12± 11.37^c
5	25.90± 3.32^a	28.45± 9.54^b	38.78± 7.49^c	45.67± 17.52^d
6	23.63± 4.19^a	30.18± 11.70^b	40.47± 7.31^c	50.57± 17.20^d
7	23.63± 4.19^a	32.85± 11.70^b	47.59± 10.48^c	56.19± 19.21^d
8	38.34± 10.59^b	34.50± 11.78^a	56.70± 7.21^c	72.81± 30.23^d
9	43.15±8.98^b	36.86±11.78^a	51.19±15.46^c	84.11±28.57^d
10	42.51±9.12^a	41.61±10.41^a	58.74±7.26^b	95.21±25.77^c
11	62.81±46.14^b	46.10±10.71^a	78.88±9.23^c	106.37±22.92^d
12	66.33±5.80^b	52.23±8.98^a	85.21±7.24^c	120.88±28.75^d

Values are presented as Mean ± Standard Deviation. Values with different superscript in the same rows are significantly different from each other at p≤ 0.05.

Table 4. Weight derivatives of *C. gariepinus* subjected to varying concentrations of combined vitamins C and E for a period of 12 weeks.

Treatments	Final Weight (g)	Initial Weight (g)	Weight gain (g)	%Weight gain	Specific Growth Rate (g/day)
Cr	66.33±5.80	10.00±0.00	56.33±5.80	563	4.952
T_1	52.23±8.98	10.00±0.00	42.23±8.98	422	4.655
T_2	85.21±7.24	10.00±0.00	75.21±7.24	752	5.259
T_3	120.88±28.75	10.00±0.00	110.88±28.75	1109	5.686

Mean ± Standard Deviation of Catalase (u/ml) production level in the kidney, Liver and gills of C. gariepinus subjected to varying concentrations of combined vitamins C and E for the period of 12 weeks

The result of catalase (CAT) production level in the kidney of *C. gariepinus* subjected to varying concentrations of combined vitamins C and E for a period of 12 weeks indicated that the mean value in T_1 was significantly different from other treatments including the control at the end of the 4th week of the experiment. The mean values obtained in the control at the end of both 8th and 12th weeks of the experiment were significantly higher than other treatments. The highest CAT production level in the kidney was 26.82± 0.00u/ml obtained in the control at the end of the 12th week. There were general increased production levels in the control when compared to lower values obtained in T_3 through-out the period of the experiment. (Table 5).

The result of CAT production levels in the liver of *C. gariepinus* subjected to varying concentrations of combined vitamins C and E for a period of 12 weeks showed that at week 4, the mean values obtained in the control and T_1 were significantly higher that T_2 and T_3. At week 8 the values obtained in T_1 (38.81±

2.41u/ml) was significantly higher than other treatments including the control; and this was also the highest value of CAT obtained in the liver. At the end of week 12, the mean value obtained in the control was significantly higher than all the treatments. (Table 6).

The result of CAT production level in the gills of *C. gariepinus* subjected to varying concentrations of combined vitamins C and E for a period of 12weeks indicated that at the end of weeks 4 and 8 the mean values obtained in the control were significantly higher than all the treatments. However, at the end of week 12 the mean value obtained in T_1 (21.12±2.553u/ml) was significantly higher than the other treatments including the control. (Table 7).

Table 5. Catalase (u/ml) production level in the kidney of *C. gariepinus* subjected to varying concentrations of combined vitamins C and E for the period of 12 weeks.

Treatments	4th week	8th week	12th week
Cr	14.66 ± 0.01^{b}	23.89 ± 0.00^{d}	26.82 ± 0.00^{c}
T_1	15.61 ± 2.38^{c}	17.89 ± 2.32^{c}	13.82 ± 1.26^{b}
T_2	12.39 ± 2.77^{a}	16.08 ± 2.47^{b}	13.95 ± 1.61^{b}
T_3	12.46 ± 2.24^{a}	13.33 ± 3.00^{a}	10.02 ± 0.00^{a}

Values are presented as Mean ± Standard Deviation. Values with different superscript in the same column are significantly different from each other at p≤ 0.05.

Table 6. Catalase (u/ml) production level in the liver of *C. gariepinus* subjected to varying concentrations of combined vitamins C and E for the period of 12 weeks.

Treatment	4th week	8th week	12th week
Cr	30.12 ± 0.00^{c}	31.92 ± 0.00^{c}	29.07 ± 0.00^{d}
T_1	31.09 ± 3.96^{c}	38.81 ± 2.41^{d}	25.91 ± 2.81^{c}
T_2	22.49 ± 3.03^{b}	19.98 ± 1.22^{b}	23.59 ± 1.98^{b}
T_3	15.65 ± 1.93^{a}	9.95 ± 0.65^{a}	15.37 ± 3.45^{a}

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

Table 7. Catalase (u/ml) production level in the Gills of *C. gariepinus* subjected to varying concentrations of combined vitamins C and E for the period of 12 weeks.

	4th week	8th week	12th week
Cr	14.63 ± 0.06^{c}	17.93 ± 0.00^{b}	20.54 ± 0.00^{c}
T_1	10.17 ± 1.96^{a}	14.01 ± 1.06^{a}	21.12 ± 2.53^{d}
T_2	10.32 ± 2.95^{a}	14.38 ± 1.68^{a}	9.34 ± 1.56^{a}
T_3	12.36 ± 3.01^{b}	14.66 ± 1.98^{ab}	13.18 ± 3.25^{b}

Values are presented as Mean ± Standard Deviation. Values with different superscript in the same column are significantly different from each other at p≤ 0.05.

Mean ± Standard Deviation of Glutathione (µg/l) production level in the Kidney, liver and gills of C. gariepinus subjected to varying concentrations of combined vitamins C and E for the period of 12weeks

The result of reduced Glutathione (GSH) production level in the kidney of *C. gariepinus* subjected to varying concentrations of combined vitamins C and E for a period of 12 weeks showed that the mean value obtained in the T_2 (46.06± 2.39µg/l) at the end of the 4th week was significantly higher than other treatments including the control, and this was also the highest GSH produced in the kidney of the fish. At weeks 8 and 12 however, the mean values obtained in the control, respectively were significantly higher than all the other treatments. (Table 8).

The result of GSH in the liver of *C. gariepinus* subjected to varying concentrations of combined vitamins C and E for a period of 12 weeks indicated that at the end of weeks 4 and 8, the mean values obtained in T_2, respectively were significantly higher than other treatments including the control. However, at week 12 the mean value obtained in the control was significantly higher that all the treatments (Table 9).

The result of GSH in the gills of *C. gariepinus* subjected to varying concentrations of combined vitamins C and E for a period of 12 weeks indicated that at weeks 4 and 8, the mean values obtained in the control were significantly higher than other treatments. At the end of week 12, the mean value

obtained in T_1 was significantly higher than the other treatments including the control. (Table 10).

Table 8. Glutathione (µg/l) production level in the Kidney of *C. gariepinus* subjected to varying concentrations of combined vitamins C and E for the period of 12 weeks.

	4th weeks	8th week	12th week
Cr	40.24 ± 0.29^c	39.89 ± 0.00^c	26.86 ± 0.00^c
T_1	36.19 ± 4.08^b	35.85 ± 2.18^b	13.82 ± 1.26^b
T_2	46.05 ± 2.39^d	36.27 ± 4.08^b	13.95 ± 1.61^b
T_3	13.16 ± 3.23^a	11.67 ± 0.79^a	10.02 ±0.00^a

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

Table 9. Glutathione (µg/l) production level in the liver of *C. gariepinus* subjected to varying concentrations of combined vitamins C and E for the period of 12 weeks.

Treatment	4th week	8th week	12th week
Cr	28.30 ± 0.00^b	29.07 ± 0.00^b	29.07 ± 0.00^d
T_1	25.13 ± 5.40^b	30.40 ± 5.26^c	25.91 ± 2.81^c
T_2	38.81 ± 4.73^c	33.52 ± 3.93^d	23.59 ± 1.98^b
T_3	14.85 ± 2.43^a	16.59 ± 1.84^a	15.37 ±3.45^a

Values are presented as Mean ± Standard Deviation. Values with different superscript in the same columns are significantly different from each other at P≤ 0.05.

Table 10. Glutathione (µg/l) production level in the gills of *C. gariepinus* subjected to varying concentrations of combined vitamins C and E for the period of 12 weeks.

	4th	8th	12th week
Cr	21.99 ± 0.00^d	23.78 ± 0.00^d	20.54 ± 0.00^c
T_1	21.20 ± 2.33^c	21.92 ± 1.97^c	21.12 ± 2.53^d
T_2	10.72 ± 2.54^a	11.93 ± 4.97^a	9.34 ± 1.56^a
T_3	20.53 ± 2.67^b	15.53 ± 1.94^b	13.18 ± 3.25^b

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

Discussion of results

The use of vitamins as feed additives is becoming an important aspect in improving fish nutrition and enhancement of fish growth. Vitamins C and E have important roles as immuno-stimulants and as antioxidant scavengers. Vitamin C is chemically adept of reacting with most of the physiologically vital radicals and oxidants and acts as an established hydro-soluble antioxidant (Eboh, 2014). Vitamin E is the most important lipophilic antioxidant and is residing mainly in the cell membranes, and thus helps to maintain membrane stability (Sun *et al.*, 2012). In this research, the standard lengths increased with increase in the concentrations of the combined vitamins as well as the duration of the experiment increased. T_3 samples supplemented with 400mg/L concentration of the combined vitamins C and E had highest growth performance in terms of standard length, total length and weight. This could be attributed to the role of vitamins C and E in increasing the serum levels of growth hormone, enhancing the intestinal morphology, and improving the absorptive surface of the intestine in fish (Abdel Rahman *et al.*, 2018). In a related development, enhancement of growth rate and weight gain observed in *Cyprinus carpio* upon vitamin C supplementation was attributed to the vitamin C-induced stimulation of protein synthesis (Faramarzi, 2012). The same trend of improved total length and weight were also witnessed in all treatments and weeks of the experiment from T_1-T_3 in comparison to lower values in the control. The highest weight obtained in T_3 was 120.88±28.75g at the end of week 12 while the value obtained in the control was 66.33±5.80g. This improvement was possible probably because of the succoring effects and immune bolstering capacity provided by the presence of the combined vitamins C and E.

The success of catfish in terms of growth also probably depends on quality of meal, dietary protein and vitamin requirements. Also, the SGR obtained for *C. gariepinus* was 5.686g/day and high percentage weight gain (1109%) in T_3 all probably point to the same reasons adduced earlier; and that improved immune status provided by the combined vitamins may have led to improved physiology of the fish which was then manifested in improved morphological differences in the fish especially in higher concentrations. In a similar finding, Abdel-Rahmn *et al.* (2019) reported that the dietary incorporation of vitamin C significantly increased the final body weight, total weight gain (TWG), specific

growth rate, and daily weight gain (DWG) in all groups and the serum levels of vitamin C and growth hormone increased in the highest supplementation level group (400 mg kg–1). In another development, lower SGR values were obtained when *C. gariepinus* was exposed to cadmium chloride and supplemented with vitamin E (Samuel *et al.*, 2021).

In the present study at 4th, 8th and 12th weeks, catalase production levels decreased as the concentrations of combined vitamins C and E supplementation increased in the kidney and liver of *C. gariepinus*. There were also significant differences in the control and T_1 samples at one point or the other in all the organs of the fish. These lower values obtained in the higher concentrations of the combined vitamins were probably because there was no need to increase the production of the antioxidant as the reactive oxygen species which are natural by-products emanating from oxidative stress were already doused by the presence of the combined vitamins. This is also probably because excessive reactive oxygen species (ROS) that cause damaging effects are eliminated by the action of antioxidant enzymes, such as CAT and GSH (Liang *et al.*, 2017). This could also be attributed to the fact that Vitamin C has been shown to be an effective scavenger against oxygen and nitrogen oxide species, such as superoxide radical ion, hydrogen peroxide, the hydroxyl radical, and singlet oxygen; this property of vitamin C has vital processes in protection of cellular components from free radical-induced damage and that vitamin C is effective in regenerating the antioxidant form of vitamin E by reducing tocopheroxyl radicals (Fadime, 2017).

These findings are in agreement with the results reported by Liang *et al.* (2017) for juvenile yellow catfish (*Pelteobagrus fulvidraco*) that received a diet supplemented with 156.5 mg kg–1 vitamin C and those by Hu *et al.* (2013) for juvenile black carp (*Mylopharyngodon piceus*) that received a diet supplemented with 63.0 mg kg–1 vitamin E. Higher phagocytic percentage as well as higher levels of lysozyme were observed, in a concentration-dependent manner, in the fish that received vitamin C and E supplemented diet; and that vitamin C is reported to enhance various immune parameters, such as macrophage infiltration, complement activity, lysozyme levels, phagocytic activity of leucocytes, cytokine production, and antibody concentrations (Abdel Rahman *et al.*, 2018). Moreover, vitamin E is one of the major antioxidants present in plasma and cell membranes, which may serve as a potent scavenger of free radicals and increase the levels of available nitric oxide via oxidation defense and endothelial nitric oxide synthase activity (Huang *et al.*, 2000; Padayatty *et al.*, 2003). The use of vitamin C alone or in combination with *Echinacea purpurea* (EP) successfully enhanced the immune parameters in Nile tilapia (*Oreochromis niloticus*) (Abdel Rahman *et al.*, 2018). Furthermore, highest CAT production levels of 26.82±0.00u/ml and 38.81±2.41u/ml were obtained in the kidneys of the control and liver of the T_1, respectively. These organs are probably the major production sites of the enzyme. Okoye *et al.* (2018) reported that the highest production mean values of 149.55±43.65 and152.80±40.40 U/mgprotein were obtained in the kidney of the fish exposed in 57 and 43 mg/L of lead nitrate, respectively; and posited that the kidney of the fish exhibited a better control of the toxicant and as such, catalase production level in this organ should be used in assessing the level of physiological changes in the fish. In addition to this, Sumit *et al.* (2014) attributed the high CAT level (386.6±10.64µmol/mg protein) in the kidney of the fish to effective antioxidant system in this tissue where there is higher metal bioaccumulation and related to metal binding protein synthesis. In addition to this, the general lower values of the CAT enzyme obtained in this research are probably due to the absence of toxicants in the test media.

Reduced glutathione (GSH) is very essential enzymes in fishes as its primary line of defense in fish oxidative stress (Aluta *et al.*, 2021). In this study, GSH production in the kidney of the control samples was significant in both 8th and 12th weeks; but in the 4th week T_2 samples had 46.06±2.39µg/L in the kidney as the highest which decreased with increase in the duration of the experiment. Likewise, the gills of the control samples had significance in the 4th and 8th

weeks. This is probably because more of the antioxidant was needed in combating the free radicals produced from the normal respiratory activities of the fish. This was probably taken care of in treatments with combined vitamins C and E due to the succor or immunity provided or elicited by the vitamins. In line with this observation, Stanic *et al.* (2011) posited that deleterious effects of free radicals can be prevented or counterbalanced by antioxidant systems. Furthermore, Alkaladi (2019) 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 liver and gills of *Oreochromis niloticus*. In another development, T_2 mean values in the liver were significantly higher than other treatments in the 4th and 8th weeks of the experiment which also decreased with increase in the duration of the experiment. The immune status were probably altered at the early stages of the experiment and needed to be up-regulated. The immunity and antioxidant ability are the most important physiological functions in fish for avoiding diseases (Bebianno *et al.*, 2014); and changes in antioxidant defences and oxidative damage are used as biomarkers of oxidative stress (Romeo *et al.*, 2015). Also, Kadry *et al.* (2012) reported how using dietary vitamin E reduced lipid peroxidation in liver tissues of female African catfish (*Clarias gariepinus*) dealing with chronic atrazine exposure. In like manner, the presence of $CuSO_4$ led to increased oxidative stress as the concentrations of antioxidant endogenous enzymes - GPx, GST and GSH were depleted while potentiating lipid peroxidation and hydroxyl radical generation; and the changes in the haematological, biochemical and antioxidant parameters were restored in the fish fed with vitamin E-supplemented feed (Azeez and Braimah, 2020).

Conclusion and recommendation

The effects of combined vitamin C and E on the growth performance and catalase and reduced glutathione production levels in *C. gariepinus* displayed varying levels of improvement during the 12 weeks of the experiment. There were general improvements in the standard and total lengths as well as the weight of the fish as the duration and concentrations of the combined vitamins C and E increased. These improvements were more evident in the highest concentration (T_3: 400mg/L) far better than the control. The highest weight (120.88±28.75g), Specific Growth Rate, SGR (5.686g/day) and percentage weight gain (1109%) were also obtained in T_3 at the end of the experiment.

The catalase production levels in the kidneys, liver and gills decreased with increase in the concentration of the combined vitamins with significant differences mostly in the control samples. The GSH production levels in the kidneys and gills also decreased with increase in the concentration of the combined vitamins. Significantly higher values in the liver of the fish were obtained in T_2 (300mg/L).

This research has demonstrated how combined vitamins C and E has led to improved growth performance and physiology of the fish and can therefore, serve as invaluable addition in fish farming.

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