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Can Vitamin C Influence Proximate Composition, Morphometric, Haematological and Some Antioxidants Indices of *Clarias Gariepinus* (Burchell, 1822) Fingerlings?

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ABSTRACT: *The impacts of vitamin C on morphometric and haematological parameters as well as proximate composition and production levels of Reduced glutathione (GSH) and Malondialdehyde (MDA) were evaluated for a period of 12 weeks. A total of 250 samples of Clarias gariepinus fingerlings were acclimatized for two weeks during which they were fed to satiation twice daily. These were then distributed into three treatments and replicates including control with each trough containing 15 samples thus; 350mg/L (T_1), 450mg/L (T_2) and 600mg/L (T_3). Three samples of the samples were picked at random bi-weekly; and the following measurements were made: Total Length (TL), Standard Length (SL) and Weight (W). The percentage weight gain (PWG), specific growth rate (SGR), condition factor (CF) and percentage hepatosomatic index (%HI) were also calculated at the end of the experiment. Three samples of the fish were also randomly selected and blood samples collected for hematological analyses. Three samples of fish were selected randomly at the end of the 12 weeks of the experiment and sacrificed; kidneys, livers and gills were excised and homogenized in phosphate buffer. These were then assayed for GSH and MDA following standard methods. Proximate compositions of the fish were determined from two randomly selected samples following standard procedures. The data generated were subjected to one-way analysis of variance and considered significant at $P<0.05$. From the results, the maximum SL (20.80±1.10cm),TL (23.90± 0.80cm) and W (82.65±0.40g) were obtained in T_1. The highest SGR and CF were also obtained in T_1. While T_2 had the peak PWG. The total white blood cells count, packed cell volume and blood haemoglobin in T_1 were significantly different from all other treatments. The blood platelets and red blood cells count increased significantly as the concentration of the vitamin increased. The proximate composition indicated peak protein content (20.58±0.26%) in T_3. The moisture content decreased with increase in the concentration of the vitamin with 75.04±0.12% as the maximum. The GSH production levels in the livers, kidneys and gills increased with increase in the concentration of the vitamin with T_3 mean values significantly higher than other treatments including the control. The MDA activities decreased with increase in the concentration of the vitamin in all the organs of interest, and the lowest mean values were*

obtained in T_3. The results of this research have indicated that vitamin C is an invaluable addition capable of improving the health and physiological status of the fish and hence, lead to improved fish farming.

KEYWORDS: vitamin C, *C. gariepinus*, GSH and MDA production levels, blood parameters, proximate composition and morphometric features

INTRODUCTION

Aquaculture is an enterprise that is growing rapidly and improving the economy worldwide. Fish farming has developed to the stage where the annual world production from aquaculture is over 22 million tons, which was over 20 per cent of the total aquatic harvest by fishing and farming in 2014 (FAO, 2014). In 2010, global aquaculture production reached 79 million tons, growing at an annual rate of 9.7 percent since 1998 (FAO, 2012). However, aquaculture global fish production stood at 66.6 million tons in 2012 (FAO, 2014). In 2014, world aquaculture production of fish accounted for 44.1% of total production from capture fisheries and aquaculture up from 42.1% in 2012 (FAO, 2016). This leaves with a huge gap for improvement in the practices of fish farming especially in developing countries of the world.

Clarias gariepinus is an important species for aquaculture in Sub-Saharan Africa as well as in some areas of Europe and Asia where it has been introduced. Most of these catfishes are cultured in tanks under semi-intensive and intensive conditions with high stocking densities. Cultivation of this species has taken different dimensions in producing quantities sufficient at least for local consumption at lowest cost possible without compromising quality. It is now a standard practice to supplement the diets of intensively grown fish with vitamins. The vitamin nutrition of catfish has been the subject of numerous research reports especially vitamin C (Ascorbic acid). Ascorbic acid is an indispensable and multifunctional micronutrient. It plays important roles in resisting stress (Henrique *et al*., 2011). Vitamins are organic substances that are generally classified as either fat soluble or water soluble. Fat-soluble vitamins (vitamin A, vitamin D, vitamin E, and vitamin K) dissolve in fat and tend to accumulate in the body. Water-soluble vitamins (vitamin C and the B-complex vitamins, such as vitamin B6, vitamin B12, and folate) must dissolve in water before they can be absorbed by the body, and therefore cannot be stored. Vitamin C or ascorbic acid (ASA) is an essential micronutrient for normal growth, antioxidant capacity and immunity of fish (Nasar *et al*., 2021). It has also been reported that vitamin C deficiency can cause serious issues for fish, including high mortality, stagnant growth rate, anorexia, low iron absorption, bulging of eyes, spine deformity, brain problems, skin damage, irregular pigmentation and depressed immunity (Council, 2011).

Fish require vitamins to survive because they act as enzyme cofactors (Drouin *et al.*, 2011). Furthermore, ascorbic acids have been reported as having the ability to act as a co- factor in protein synthesis (Chen *et al*., 2015). Fish show deficiency signs if they are fed diets deficient in vitamin C, most commercial feed ingredients are almost completely devoid of vitamin C and it has to be

supplemented in the diets (Lim *et al.*, 2009). Several features of the fish usually indicate whether there is improvement in the status of the fish or not. Some of these characteristics are morphometric parameters, proximate composition and physiological indicators such as effects on some common antioxidants such as malondialdehyde, superoxide dismutase, alanine amino and aspartate amino transferases, catalase, lipid peroxidase, glutathione, as well as haematological indices that would directly the effects of the supplements n major components of the blood. The morphometric characters have a key role in identification of a species and for detecting variations in the fish population (Kanwal, 2017). It is also known that, the morphometric relationships between various body parts of fish can be used to assess the well being of individuals and to determine possible difference between separate unit stocks of the same species (Brraich and Akhter, 2015; King, 2007). Proximate composition in fishes points directly to how the fish is fairing in terms of mineral component, protein, fibre, ash and carbohydrate contents especially determined by the environment and components of the feeds the fishes are subjected to and, or available to them. For instance, proximate composition has been reported to improve with an increase in dietary ascorbic acid supplementation (Nasar *et al*., 2021). In another development, thaobarbituric acid reactive substances or MDA, the most abundant aldehyde which is usually used to check flesh quality, are an important indicator of liver health, with its higher level indicating lipid peroxidation, bio-toxicity and cell damage (Dawood *et al*., 2017). This research therefore, attempted to supply vitamin C supplement in varying concentrations to see the impacts they would have on morphometric, haematological and proximate features as well as some physiological status in terms of production of reduced glutathione and malondialdehyde on *C gariepinus* under usual laboratory conditions and ascertain whether there would be improvements in these features.

MATERIALS AND METHODS

Sample Collection and Acclimatization

Two hundred and fifty (250) samples of *Clarias gariepinus* fingerlings (6 weeks old) were purchased from private fish farm in New Bussa, Niger State. These fishes were carefully transported in a 25 litres container with water to the Department of Animal Biology Laboratory, Federal University of Technology, Minna, Niger State. These were then distributed into five (5) containers with 50 fishes each, and acclimatized for two weeks (14 days) during which they were fed to satiation, twice daily at 08.00hr and 17.00hr. The water medium in each trough was changed every 48th hour. Vitamin C (500g) was purchased from commercial store and stored in cool dry place.

EXPERIMENTAL SETUP

After acclimatization, the fishes were further separated into eight plastic aquaria with 15 samples each in each plastic aquarium filled with 20 litres of borehole water. The plastic aquaria were labeled according to treatments and replicates. Three treatments were setup for the experiment, each treatment with its own replicate including control. The treatments had the following

concentrations of the vitamin: 350mg/L (T_1), 450mg/L (T_2) and 600mg/L (T_3). The set-up ran for 12 weeks and the water medium in each trough was changed every 72 hours. Fresh concentrations of the vitamin were measured into each of the troughs containing 20 litres of water.

DETERMINATION OF MORPHOMETRIC PARAMETERS

At the end of every two weeks during the course of this work, three fishes were picked randomly from each treatment and its replicate as well as the control for measurement of the following morphometric parameters; standard and total lengths with metre rule graduated in centimeters, and weight with weighing balance. The total length (TL) was determined from tip of the snout of the fish to its tail end while standard length (SL) was measured from the end of the snout to the caudal lobe. The following calculations were also made from the weight of the fish:

Specific Growth Rate (SGR) was determined thus:

$$\text{SGR (g/day)} = \frac{\text{Total weight}}{\text{Number of days}}$$

Percentage Weight Gain (PWG) was calculated as follow:

$$\text{PWG (\%)} = \frac{\text{Final weight - Initial weight}}{\text{Initial weight}} \times 100$$

Condition Factor (CF): this was calculated according to Biney *et al*. (1994) as reported by Ciftci *et al*. (2015) thus;

$$\text{CF(g/cm)} = \frac{\text{Total body weight}}{\text{Total length}}$$

Percentage Hepatosomatic Index (HIS): at the end of the experiment the liver of each treatment and replicate including the control was weighed and the following calculation was made according to Biney *et al*. (1994).

$$\text{HIS (\%)} = \frac{\text{Liver weight}}{\text{Total body weight}} \times 100$$

DETERMINATION OF HAEMATHOLOGICAL PARAMETERS

At the 12th and final week of the experiment two (2) 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.*, 2021) using plastic syringe, fitted with 21-gauge hypodermal needles. Each blood sample was collected in duplicate into heparinized (50 IU per mL of blood) sample bottles. These pre-iced collecting bottles were placed in the refrigerator before further handling and analyses within 30 minutes of the 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.

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

Two fishes were randomly picked from each trough that is, T_1-T_3 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.

Glutathione (GSH) Estimation

According to the methods described by Ell-man (1959) with slight modification, the GSH was determined from an aliquot of 100 µL of each of the samples was precipitated with 200 µL of 20% TCA and centrifuged at 2000rpm 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 412nm. The concentration of GSH was calculated from calibration curve.

Determination of MDA

Malondialdehyde assay was carried out using the method of Del-Rio *et al*. (2003) with slight modification. 200 µL of the samples in each case was 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 was 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). The flocculent precipitate was removed by centrifugation at 3500 rpm for 15 mins; then supernatant was obtained and absorbance reading of the supernatant was done at 532 nm against a blank that contains the reagents minus the samples. The malondialdehyde concentration of the sample was calculated using the adduct extinction coefficient of 1.56 x 105 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 fishes were taken randomly from each trough labeled appropriately and were used for the proximate test. It involved an assay for all the major constituents of the sample in each case. Each fish sample was 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 produced from morphometric and haematological parameters, antioxidant activities as well as proximate compositions of *Clarias gariepinus* 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

Morphometric parameters of *Clarias gariepinus*

The results of the total length (TL) of *C. gariepinus* subjected to different concentrations of vitamin C for a period of 12 weeks indicated that in weeks 2 and 10 there were no significant difference, and the highest mean values were obtained in T_1 and T_2, respectively at the end of the twelfth week. (Table 3.1). Likewise, the standard length (SL) exhibited no significant difference in all the treatments and in most of the weeks of the experiment. Just like in the TL of the fish, the SL increased as the duration of the experiment increases. (Table 3.2).

Table 3.1 Total length (cm) of *C. gariepinus* subjected to varying concentrations of vitamin C for a period of 12 weeks

Treatment	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
Control	12.85 ± 0.45^{a}	14.75 ± 0.55^{a}	15.10 ± 0.30^{a}	18.05 ± 0.35^{a}	20.55 ± 0.75^{a}	22.05 ± 0.45^{a}
T_1	13.35 ± 0.55^{a}	15.80 ± 0.30^{b}	21.03 ± 4.73^{b}	19.80 ± 0.20^{b}	21.55 ± 0.65^{a}	23.90 ± 0.80^{b}
T_2	12.75 ± 1.05^{a}	15.25 ± 0.65^{ab}	16.25 ± 0.15^{a}	17.95 ± 0.85^{a}	21.70 ± 1.60^{a}	23.45 ± 0.95^{ab}
T_3	12.44 ± 0.04^{a}	14.35 ± 0.45^{a}	15.40 ± 0.30^{a}	18.05 ± 1.45^{a}	21.00 ± 0.97^{a}	22.90 ± 0.80^{ab}

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 3.2 Standard Length (cm) of *C. gariepinus* subjected to varying concentrations of vitamin C for a period of 12 weeks

Treatment	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
Control	11.30±0.70^{a}	12.90±0.50^{ab}	13.15±0.35^{a}	15.55±0.35^{a}	17.75±0.65^{a}	19.40±1.00^{a}
T1	11.65±0.35^{a}	13.75±0.25^{b}	14.55±0.25^{b}	17.00±0.50^{a}	18.30±0.50^{a}	20.80±1.10^{a}
T2	10.85±0.95^{a}	13.25±0.55^{ab}	14.20±0.20^{b}	15.60±0.50^{a}	18.65±1.55^{a}	20.20±0.20^{a}
T3	10.85±0.25^{a}	12.65±0.55^{a}	13.45±0.35^{a}	16.10±1.50$_{a}$	18.40±0.90^{a}	19.55±0.85^{a}

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

The mean weight of the fish subjected to varying concentrations of the vitamin depicted that the weight increased with increase in the duration of the research in T_1 especially as from the 8th week to the 12th week. There were general increases in weight in all the treatments. (Table 3.3).The percentage hepatosomatic index (%HSI) obtained in this research indicated that there were high values with the exception of the control. The highest was obtained in T_3. The peak condition factor (CF) was gotten from T_1. The maximum total weight gain (TWG) was recorded in T_2. While the specific growth rates (SGR) were at their zeniths in T_2 and T_1, respectively. (Table 3.4).

Table 3.3 Mean Weight (g) of *C. gariepinus* subjected to different concentrations of vitamin C for a period of 12 weeks

Treatment	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
Control	16.80±2.70^{a}	26.20±1.50^{ab}	26.40±2.60^{a}	45.85±3.05^{a}	66.70±3.80^{a}	69.70±2.40^{a}
T1	20.40±2.70^{a}	31.05±0.95^{b}	37.55±4.65^{c}	60.70±3.30^{b}	76.10±0.20^{a}	82.65±6.05^{b}
T2	19.10±6.50^{a}	28.55±3.05^{ab}	33.40±1.60^{bc}	43.40±4.90^{a}	73.65±14.15^{a}	81.75±0.45^{b}
T3	16.85±1.25^{a}	24.60±2.20^{a}	29.70±1.80^{ab}	45.25±7.15^{a}	69.35±8.25^{a}	71.70±10.40^{ab}

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 3.4 Weight derivatives of *C. gariepinus* subjected to varying concentrations of vitamin C for a period of 12 weeks

Treatment	%HSI	CF	TWG	SGR
Control	2.9	130.7	314.9	1.65
T1	3.4	152.8	305.1	1.96
T2	3.2	149.8	328.0	1.95
T3	3.6	133.7	325.5	1.70

Haematological parameters of *Clarias gariepinus*

The haematological parameters examined in this research, indicated that there were increased significant mean values of white blood cells (WBCs) of the fish subjected to T_1 and T_3. The red blood cells (RBCs) in the treatments increased from T_1-T_3. The haemoglobin (HG) and the haematocrit (HCT) in T_2 and T_3 were significantly different from the other treatment including the control. There were no significance differences in the mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) in all treatments. The mean corpuscular haemoglobin (MCH) however, displayed significant differences in both control and T_1 over T_2 and T_3. The blood platelets (PLT) increased with increase in the vitamin supplement with the peak value obtained in T_3. The value obtained in the control was also significantly lower than other treatments. (Table 3.5).

Table 3.5 Haematological parameters of *C. gariepinus* subjected to varying concentrations of vitamin C for a period of 12 weeks

	Treatments			
Parameter	Control	T1	T2	T3
WBC	193.50 ± 6.50^{b}	205.50 ± 0.50^{c}	171.00 ± 9.00^{a}	239.50 ± 0.50^{d}
RBC	3.05 ± 0.25^{ab}	2.50 ± 0.20^{a}	4.20 ± 0.80^{bc}	4.30 ± 0.90^{c}
HGF	7.05 ± 0.75^{ab}	5.50 ± 0.80^{a}	10.95 ± 3.15^{b}	10.95 ± 3.15^{b}
HCT	22.25 ± 2.25^{ab}	17.60 ± 3.40^{a}	33.50 ± 8.50^{b}	33.35 ± 9.35^{b}
MCV	105.00 ± 5.00^{a}	106.00 ± 4.00^{a}	93.50 ± 11.50^{a}	92.00 ± 9.00^{a}
MCH	41.00 ± 1.00^{b}	42.50 ± 0.50^{b}	35.00 ± 4.00^{a}	34.00 ± 4.00^{a}
MCHC	35.00 ± 1.00^{a}	35.50 ± 0.50^{a}	34.50 ± 0.50^{a}	35.10 ± 0.10^{a}
PLT	150.00 ± 0.50^{b}	152.50 ± 2.50^{a}	161.00 ± 1.00^{a}	194.50 ± 45.50^{a}
LYM	95.50 ± 0.50^{b}	92.50 ± 0.50^{a}	96.00 ± 2.00^{b}	96.00 ± 2.00^{b}
MXD	2.50 ± 0.50^{b}	3.00 ± 0.00^{b}	1.50 ± 0.50^{a}	1.50 ± 0.50^{a}
NEUT	2.00 ± 0.00^{a}	4.50 ± 0.50^{b}	2.50 ± 1.50^{ab}	2.50 ± 1.50^{ab}

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

GSH and MDA production levels in organs of *Clarias gariepinus*

The reduced glutathione (GSH) activity in the liver of *C. gariepinus* increased with increase in the concentrations of the vitamin from control to T3. Conversely, the malondialdehyde (MDA) levels decreased with increase in the concentrations of vitamin C. (Table 3.6). Similar trends were also exhibited in the kidneys and gills of the fish supplemented with different levels of vitamin C for both GSH and MDA. In addition to these, the production mean values for GSH were higher in the Liver than the other organs of interest. (Tables 3.7 and 3.8).

Table 3.6 GSH (µg/mL) and MDA (µM) Production Levels in Liver of *C. gariepinus* subjected to varying concentrations of vitamin C for a period of 12 weeks

Treatments	GSH (µg/mL)	MDA (µM)
Control	26.71±2.04^{a}	12.78±1.07^{b}
T_1	33.68 ±0.20^{b}	11.44±1.07^{b}
T_2	40.89±2.32^{c}	8.04±0.29^{a}
T_3	52.84±1.55^{d}	6.39±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 3.7 GSH (µg/mL) and MDA (µM) Production Levels in Kidneys of *C. gariepinus* subjected to varying concentrations of vitamin C for a period of 12 weeks

Treatments	GSH (µg/mL)	MDA (µM)
Control	19.60±0.62^{a}	10.08±0.77^{c}
T_1	28.45 ±1.29^{b}	8.02±0.46^{b}
T_2	29.39±0.73^{b}	8.37±0.96^{b}
T_3	36.96±1.28^{c}	5.25±0.59^{a}

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

Table 3.8 GSH (µg/mL) and MDA (µM) Production Levels in Gills of *C. gariepinus* subjected to varying concentrations of vitamin C for a period of 12 weeks

Treatments	GSH (µg/mL)	MDA (µM)
Control	17.99±0.48^{a}	8.38±0.37^{c}
T_1	25.28 ±0.90^{b}	7.96±0.07^{c}
T_2	37.00±1.52^{c}	5.85±0.37^{b}
T_3	38.22±0.95^{c}	4.99±0.12^{a}

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

Proximate composition of *Clarias gariepinus* treated with Vitamin C

The proximate composition of the samples of *C. gariepinus* subjected to varying concentrations of vitamin C showed that the control mean values of moisture contents were significantly higher than other treatments. The fat and protein contents increased with increase in the concentration of the vitamin. The peak ash and carbohydrate contents were obtained in T_3 and T_1, respectively. (Table 3.9).

Table 3.9 Proximate Composition of *C. gariepinus* treated with vitamin C for a duration of 12 weeks

Treatments	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Carbohydrate (%)
Control	75.04±0.12^{b}	2.43±0.38^{ab}	0.82±0.04^{a}	17.27±0.31^{a}	4.44±009^{ab}
T_1	71.24±1.12^{a}	2.26±0.08^{a}	0.88±0.05^{a}	18.10±0.23^{b}	6.04±0.29^{c}
T_2	71.46±0.54^{a}	2.39±0.07^{ab}	1.29±0.03^{b}	19.97±0.20^{c}	4.91±0.25^{b}
T_3	71.12±0.02^{a}	2.75±0.23^{b}	1.36±0.06^{b}	20.58±0.26^{d}	4.19±0.41^{a}

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

DISCUSSION

Morphometric parameters have been used over the years to understanding how the physical features (morphology) correlate with the physiological status of the fish for a specified period of time. In this research, there were no significance differences and the fishes increased in growth in terms of Standard Lengths (SL) and Total Lengths (TL) in all the treatments including the control. The impacts of the vitamin at its varying concentrations were probably not manifested morphologically; since, morphometric characters respond to changes in environmental factors and these responses differ from species to species (Turan *et al.*, 2011). However, the weight of the fish increased in T1 relatively to other treatments including the control as from the 8-12th weeks of the

research. This concentration of the vitamin (350mg/L) is probably the optimal concentration for the normal functioning of the fish since vitamins and other supplements are usually required in little quantity. This is also probably why the peak condition factor of the fish was obtained in T1. The high percentage hepatosomatic index (%HSI) obtained in this research with the exception of the control probably indicates that as the concentration of the vitamin increased, the weight of the livers of the fish also increased in conjunction with the weight of the fish. The maximum total weight gain (TWG) was recorded in T_2 and the specific growth rates (SGR) were at their zeniths in T_2 and T_1, respectively. These were probably as a result of the succor provided by vitamin C. In a related development, results reported by Nasar *et al*. (2021) showed a linear increase in growth as ascorbic acid (ASA) supplementation increased from 0 to 100 mg/kg. They also indicated that, a further increase in dietary ASA supplementation from 200 to 400 mg/kg resulted in a slight decrease in growth parameters. Similarly, Abdel-Rahman *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). Likewise, improvements in growth parameters were also 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. Also in a related research on *C. gariepinus*, TL and SL increased significantly with increase in the concentration of the combined vitamins C and E especially in T_2 and T_3 much better than the control and that the weight increased significantly from T_1-T_3 (200, 300 and 400mg/L, respectively) through-out the period of the experiment with 120.88±28.75g as the maximum, 1109%WG and SGR of 5.686g/day recorded in T_3 (Samuel and Wada, 2022). Also in line with the findings of this research, Rowida *et al*. (2020) 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, the serum levels of vitamin C and growth hormone increased in the highest supplementation level group (400 mg kg^{-1}); and in a concentration-dependent manner, vitamin C markedly enhanced the serum and hepatic antioxidant activities, the levels of lysozyme, nitric oxide, and interleukin-10, the phagocytic percentage, and the relative percentage survival (RPS%) of the fish against *Aeromonas sobria*.

The haematological parameters investigated showed that there were increased significant mean values of white blood cells (WBCs) of the fish subjected to T_1 and T_3. The red blood cells (RBCs) in the treatments increased from T_1-T_3. The blood platelets (PLT) increased with increase in the vitamin supplement with the peak value obtained in T_3. The value obtained in the control was also significantly lower than other treatments. These increases and significances were probably occasioned by the presence of the vitamin and may have indicated the healthy state of the fish to combat any stress or challenges in the immediate environment of the fish. These findings are in consonance with Gbore *et al*. (2010) 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 research. In addition to this, Samuel and Kolo (2022) reported that when *C. gariepinus* was subjected to varying concentrations of combined vitamins C and E (200, 300 and 400mg/L), the total white blood cell count (TWBC), parked cell volume (PCV) and blood haemoglobin (Hb) were significantly different in the highest concentration; and RBC and MCH were significantly different in the lowest concentration at the end of the 4th week. They also reported 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 antioxidants (GSH and MDA) determined in this research indicated that the reduced glutathione increased with increase in the vitamin concentrations in all the organs tested while the MDA decreased with increase in the concentration of the vitamin. These phenomena probably depict the capacity of the vitamin to mitigate the inherent environmental stress, improve the physiological and health statuses of the fish; and ensure less utilization of GSH and less production of MDA since, reduced glutathione (GSH) is very essential enzymes in fishes as its primary line of defense in fish oxidative stress (Aluta *et al*., 2021). In a related research reported by Samuel and Kolo (2022), AST and ALT produced in the kidneys, livers and gills of *C. gariepinus* when combined varying concentrations of vitamins C and E were administered were significantly different at one point or the other as the concentrations of the vitamins increased through-out the sampling periods. Likewise, the CAT and GSH mean values in the kidneys, liver and gills decreased with increase in the concentrations of the combined vitamins and duration of the experiment; and that the liver catalase decreased with increase in the concentration of combined vitamins C and E throughout the 12 weeks (Samuel and Wada, 2022). Also in line with the findings of this research, other researchers reported how vitamin C reduced the malondialdehyde (MDA) content in the liver of juvenile largemouth bass (Chen *et al*., 2015), juvenile yellow catfish (Liang *et al*., 2017), juvenile Chu's croaker (Zou *et al*., 2020), juvenile cobia (Zhou *et al*., 2012) and hybrid striped bass (Sealey and Gatlin III, 2002). Likewise, the TBARS (MDA) values were negatively correlated with an increase in dietary ascorbic acid supplementation in the liver and muscles of grass carp (Nasar *et al*., 2021).

It has also been reported 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, Liang *et al*. (2017) posited that excessive reactive oxygen species (ROS) that cause damaging effects were eliminated by the action of antioxidant enzymes, such as CAT and GSH. Vitamin C has been reported as enhancing 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). Ascorbic acid is an indispensable and multifunctional micronutrient; it plays important roles in improving immune function (Fletcher *et al.*, 2011), resisting stress (Henrique *et al.*, 2011).

The knowledge of the carcass composition of fish feed is important because of the dietary and medical emphasis on the role of these nutrients in human health (Oyegbile *et al*., 2017). The proximate compositions of the fish in this research indicated that the fat and protein contents increased with increase in the concentration of the vitamin. These increases were probably because the fishes are in good physiological status as a result of the boost from the supplement leading to decrease in the carbohydrate content in comparison to control whose the carbohydrate contents increased with increase in the duration of the experiment. These improvements were more evident in the higher concentrations (T_2 and T_3). This is also probably why the peak ash and carbohydrate contents were obtained in T_3 and T_1. Vitamin C has probably improved on the up-take of other essential materials necessary for building up the protein and fat contents due to the fact that, fish feeds in sustainable fish culture system account for 40 to 60% total production cost and need to be supplied in the right proportions with proper nutritional composition as it determines the effectiveness of fish growth and survival rate (Toutou *et al*., 2018; Dorothy *et al*., 2018). In a related development, protein efficiency ratio was higher in the group fed 0mg/kg AA (ascorbic acid) than the other groups; an Liver AA was significantly higher in groups fed 92 mg/kg AA (Okhinkpamwonyi and Edema, 2017). In a related development, increasing trend was recorded for the whole body, proximate composition, antioxidant enzymes and organ indices against ASA supplementation in grass carp; and that the highest values of FW, WG, WG% and SGR were obtained in the fish fed with 100 mg/kg of ASA supplemented diet (Nasar *et al*., 2021). They also observed that proximate composition (DM, CP, CL and CA) was improved with an increase in dietary ASA supplementation and that; high CP and CL in the whole body were utilized in muscle formation and energy supply.

From the weight of the liver of the fishes in this research, the percentage hepatosomatic index (%HSI) obtained indicated that there were high values with the exception of the control. The highest was obtained in T_3 (the highest concentration). This probably indicates the influence of the vitamin on the fish that led to increased weight of the liver being the major determinant of the physiological status of the fish. In closely related findings, Nasar *et al*. (2021) reported that HSI and VSI improved with an increase in dietary ASA supplementation in grass carp, and posited that increased ASA supplementation resulted in improvement against lipid accumulation in the liver; improved HSI in tiger puffer (Eo and Lee, 2008) and juvenile largemouth bass (Chen *et al*., 2015).

CONCLUSION

The impacts of vitamin C on the growth parameters have indicated that the maximum Standard Length (20.80±1.10cm), Total Length (23.90± 0.80cm) and Weight (82.65±0.40g) were obtained in T_1, the lowest concentration. The highest specific growth rate (SGR) and condition factor (CF) were also obtained in T_1.While T_2 had the peak PWG.

The total white blood cells count (TWBCs), packed cell volume (PCV) and blood haemoglobin in T_1 were significantly different from all other treatments. The blood platelets (PLT) and red blood cells count (RBCs) increased significantly as the concentration of the vitamin increased. The proximate composition indicated maximum protein content (20.58±0.26%) in T_3, the highest concentration. The moisture content decreased with increase in the concentrations of the vitamin with 75.04±0.12% as the maximum.

The GSH production levels in the livers, kidneys and gills increased with increase in the concentrations of the vitamin with T_3 mean values significantly higher than other treatments including the control. The MDA activities on the other hand, decreased with increase in the concentrations of the vitamin in gills, liver and kidneys of the fish, and the lowest mean values were obtained in T_3.

Recommendations

The results of this research have indicated that vitamin C is an invaluable addition capable of improving the health and physiological status of the fish and can lead to improved fish farming. The incorporation of the vitamin into the feed in the right proportion since it is usually required in little quantity would go a long way in improving quality yield at low cost to the farmer.

Conflicts of Interest

The authors declare that to the best of their knowledge there is no conflict of interest whatsoever.

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