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# β-Carotene of *Arthrospira platensis* versus vitamin C and vitamin E as a feed supplement: Effects on growth, haemato-biochemical, immune-oxidative stress and related gene expression of Nile tilapia fingerlings

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## Abstract

Microalgae are one of the most important sources of natural bioactive compounds, especially those revealing antioxidant activity such as β-carotene. Thus, this study was to compare the effects of dietary Vitamin C (L-ascorbic acid) or Vitamin E (α-tocopherol) with β-carotene extracted from *Arthrospira platensis* on Nile tilapia (*Oreochromis niloticus*) on growth, feed utilization, blood indices, antioxidant activity, non-specific immunological indices and related gene expression. Three hundred and sixty apparent healthy Nile tilapia (5.80 ± 0.286 g) were randomly distributed into four groups. Four isonitrogenous and isoenergetic diets were performed. A control diet was compared against three experimental diets supplemented with Vitamin C (0.5 mg $kg^{-1}$ diet), Vitamin E (1 g $kg^{-1}$ diet) or β-carotene (0.5 g $kg^{-1}$ diet) for 10 weeks. According to the results, there was no significant difference in feed intake ($p > 0.05$) between experimental diets. Dietary Vitamins C and E and β-carotene significantly ($p < 0.05$) enhanced the weight gain, final body weight, protein efficiency ratio, specific growth rate and apparent protein utilization in all groups, whereas the best FCR ($p = 0.017$) and the highest weight gain ($p = 0.007$) were detected in the β-carotene diet. Fish survival rates differed significantly ($p < 0.05$) amongst treatments, whilst fish fed a diet supplemented with β-carotene recorded the highest survival rate. The supplemental diet with β-carotene boosted the values of the biochemical and haematological parameters ($p < 0.05$) compared with the control diet. The activities of catalase, superoxide dismutase (SOD) and total antioxidant capacity (T-AOC) in the liver ($p = 0.0560$; $p = 0.0032$; $p = 0.0353$ respectively) and tilapia muscles were higher in fish fed either β-carotene or Vitamin E ($p = 0.0579$; $p = 0.1494$; $p = 0.2145$ respectively) than other groups. The highest values of SOD, CAT and immune globulin M-2 gene expression ($p < 0.05$) were found in fish fed a diet enriched with β-carotene. These results suggested that the dietary incorporation of β-carotene had a superior impact on growth performance, haemato-biochemical and immune-oxidative stress biomarkers in addition to the associated gene expression of *Oreochromis niloticus*.

  

KEYWORDS
*Arthrospira platensis*, gene expression, Nile tilapia, vitamin C, vitamin E, β-carotene

## 1 | INTRODUCTION

Aquaculture is considered the main source of animal protein compared with other food production sectors in developing countries. Recently, the aquaculture industry has increased significantly to fulfil the growing need for fish as food, particularly in Asia where fish is the predominant protein source (FAO, 2018). Intensification of aquaculture production makes fish more susceptible to pathogen microbe infections, disease outbreaks and financial losses due to fish mortality and chemotherapeutic costs (Abdel-Gawad et al., 2020; Hassaan et al., 2018; Hoseinifar et al., 2017). Furthermore, antibiotic resistance is caused by the overuse of antibiotics in fish feed, which has an impact on the fish's overall health (Hassaan et al., 2019; Hassaan, El-Sayed, et al., 2021; Kraemer et al., 2019). Thus, disease risk in farmed fish could be controlled using better nutritional formulations, immunostimulants and vaccines to improve their infection resistance (Abdel Rahman et al., 2019; Al-Khalaifah et al., 2020; Amer et al., 2018; Hassaan et al., 2019; Hassaan, Mohammady, et al., 2021; Soltan et al., 2017; Van Doan et al., 2022).

Vitamins are organic chemicals that fish require in trace amounts to develop, survive, reproduce, maintain their health and maintain their immune system function (Ghafarifarsani et al., 2022; Guerriero et al., 2004; Kong et al., 2021; Lin & Shiau, 2004; Liu et al., 2019; Parisi & Guerriero, 2019). Most cultured fish species require antioxidants like ascorbic acid (Vitamin C), α-tocopherol (Vitamin E) and natural immunostimulants such as carotenoids. Vitamin C is considered a booster and stimulator of different immunological mechanisms through macrophage activity, natural killer cell activity, cell proliferation, lysozyme level, complement activity, leucocyte phagocytic activity, cytokine production and antibody formation (Abdel Rahman et al., 2018; Carr & Maggini, 2017; Harsij et al., 2020; Li & Lovell, 1985; Navarre & Halver, 1989; Tewary & Patra, 2008). Fish require Vitamin C from an external source because they lack the enzyme L-gulonolactone oxidase, which is essential for de novo Vitamin C synthesis (Fracalossi et al., 2001). The usage of Vitamin C in various fish such as juvenile gilthead bream (*Sparus aurata*), Mexican Silverside (*Menidia estor*), Nile tilapia (*Oreochromis niloticus*) and juvenile yellow catfish (*Pelteobagrus fulvidraco*) has been demonstrated in several studies (Amerio et al., 2000; Barros et al., 2014; Liang et al., 2017; Martinez-Palacios et al., 2007). Vitamin E is a fat-soluble antioxidant present in the membranes of immune cells, and it protects macrophage membranes from free radical peroxidation, making it crucial for fish immunity and growth (Beharka et al., 1997; Hassaan, Wafa, et al., 2014; He et al., 2017; Waagbo, 1994). Also, Vitamin E deficiency has been linked to a reduction in immunological responses in several different fish species (Gombart et al., 2020; Montero et al., 2001; Pan et al., 2017; Puangkaew et al., 2004; Scalici et al., 2017; Wise et al., 1993).

Moreover, carotenoids are natural fat-soluble pigments that can be found in plants and microalgae such as *Arthrospira platensis*. Carotenoids are pigments used as antioxidants, precursors of Vitamin A (retinol) and its derivatives retinol and retinoic acid. Because fish cannot produce carotenoids, they require a dietary source of these pigments (Fraser & Bramley, 2004; Von Lintig, 2012). Carotenoids can also be employed as natural immunostimulants to boost the immune system and antioxidant capacity of fish (Babin et al., 2015). Carotenoids were found to improve rainbow trout growth, survival and immunity (Amar et al., 2001; Keleştemur & Çoban, 2016), European bullhead (*Cottus gobio*) (Dorts et al., 2012) and Nile tilapia (*Oreochromis niloticus*) (Hassaan, Mohammady, et al., 2021) in addition to lipid peroxidation prevention (Waagbø et al., 2003).

There have been no studies that compare the efficiency of commercial immunostimulants and antioxidants (Vitamins C and E) with natural antioxidants extracted from *Arthrospira platensis* as β-carotene. In this perspective, the current study sought to assess the efficacy of dietary Vitamins C and E and β-carotene as a supplement in *O. niloticus* diets in improving growth performance, blood indices, lipid profile, and non-specific immune response, and liver antioxidant activity.

## 2 | MATERIALS AND METHODS

### 2.1 | Ethics statements

The Scientific Ethics Committee, Animal Production Department, Faculty of Agriculture, Benha University, EG, approved (BUAPD-20115) the study's experimental procedures. The experiment was carried out at the Fish Nutrition Research Unit, Benha University.

### 2.2 | β-Carotene extraction

Dried *Arthrospira platensis* (5 g) was collected from the phytoplankton laboratory at the National Institute of Oceanography and Fisheries, NIOF, Egypt, using commercially modified Zarrouk's medium (Zarrouk, 1966) in a 25 $m^3$ open pond raceway with urea as a nitrogen source and commercial phosphoric acid as a phosphorus source. The inoculum was examined under the microscope (pure culture). In the pond, *A. platensis* was cultured at room temperature. After 14 days, the biomass was collected with a 20 μ mesh plankton net. After that, it was sun-dried for 24–48 h outside. After that, the dried *A. platensis* was suspended in 50 ml methylene chloride, refrigerated overnight at - 4°C and then dried overnight at 40°C. After that, the residues were dissolved in petroleum ether (10 ml). The

β-carotene pigment was measured using a tetrahydrofuran (THF) standard at 451 nm according to Herrero-Martínez et al. (2006).

## 2.3 | Experimental design and diet formulation

Five isonitrogenous (302.80 g $kg^{-1}$ of crude protein) and isoenergetic (18.64 MJ/kg gross energy) diets were formulated and their chemical composition was measured according to AOAC (1995) as shown in Table 1. The control diet was compared against three experimental diets enriched with Vitamin C or Vitamin E (Purchased from Sigma-Aldrich Company, Egypt) or β-carotene. The control diet was enriched with a 0.5 mg $kg^{-1}$ diet of Vitamin C (ascorbic acid), 1 g Vitamin E (DL-α-tocopheryl acetate) and 0.5 g $kg^{-1}$ diet of β-carotene (an experimental dose). The concentration of Vitamin E or C was chosen to fulfil the nutritional requirements of Nile tilapia, *Oreochromis niloticus* according to Hassaan, Goda, et al. (2014) and Soliman et al. (1994), whereas the level of extracted β-carotene from *Arthrospira platensis* used in this study was an experimental dose. All of the feed ingredients were carefully combined with Vitamin C or E, and the dough was formed with the appropriate amount of water. After pressing the dough through a 2 mm hand pelletizer, the pellets were sun-dried for 48 h and stored at 4°C. After drying the samples for 24 h at 105°C, the dry matter was measured. Ash using incineration at 550°C for 12 h. Crude protein was evaluated using a micro-Kjeldahl method with N% × 6.25 (using a Kjeltec autoanalyzer, Model 1030, Tecator, Höganäs, Sweden) and crude fat was determined using Soxhlet extraction with diethyl ether (40–60°C).

**TABLE 1** Formulation and proximate composition of the experimental diets (g $kg^{-1}$ diet)

| Ingredient | Control |
|---|---|
| Fish meal 65% | 130.0 |
| Soybean meal 44% | 350.0 |
| Corn gluten meal 62% | 30.0 |
| Yellow corn 8.5% | 220.0 |
| Wheat bran 14% | 100.0 |
| Rice polishing 13% | 100.0 |
| Fish oil | 40.0 |
| Premix[a] | 25.0 |
| Vitamin C | 5.00 |
| Chemical analysis (g $kg^{-1}$) | |
| Protein | 302.80 ± 0.98 |
| Lipid | 70.30 ± 0.52 |
| Ash | 56.69 ± 0.45 |
| Fibre | 54.00 ± 0.21 |
| Nitrogen free extract[b] | 516.21 ± 1.12 |
| Gross energy[c] | 18.64 ± 0.28 |

[a]Vitamin and mineral mixture $kg^{-1}$ of mixture contains: 4800 I.U. Vit A, 2400 IU cholecalciferol (Vit. D), 40 g Vit E, 8 g Vit K, 4.0 g Vit $B_{12}$, 4.0 g Vit B2, 6 g Vit B6, 4.0 g, Pantothenic acid, 8.0 g Nicotinic acid, 400 mg Folic acid, 20 mg Biotin, 200 gm Choline, 4 g Copper, 0.4 g Iodine, 12 g Iron, 22 g Manganese, 22 g Zinc, 0.04 g Selenium. folic acid, 1.2 mg; niacin, 12 mg; d-calcium pantothenate, 26 mg; pyridoxine. HCl, 6 mg; riboflavin, 7.2 mg; thiamine. HCl, 1.2 mg; sodium chloride (NaCl, 39% Na, 61% Cl), 3077 mg; ferrous sulphate ($FeSO_4.7H_2O$, 20% Fe), 65 mg; manganese sulphate ($MnSO_4$, 36% Mn), 89 mg; zinc sulphate ($ZnSO_4.7H_2O$, 40% Zn), 150 mg; copper sulphate ($CuSO_4.5H_2O$, 25% Cu), 28 mg; potassium iodide (KI, 24% K, 76% I).

[b]NFE (Nitrogen free extract) = 100−(crude protein + lipid + ash + fibre content).

[c]Gross energy calculated using gross calorific values of 23.63, 39.52 and 17.15 kj/g for protein, fat and carbohydrate, respectively, according to Brett (1973).

## 2.4 | Experimental system and feeding protocol

Healthy Nile tilapia fingerlings were obtained from the farm of the National Institute of Oceanography and Fisheries, NIOF, Egypt, and adapted in two tanks (1 $m^3$ for each) for 2 weeks and fed with commercial feed, 30% crude protein at a rate of 5% of the whole biomass three times daily before the start of the experiment. After the acclimation period, 360 Nile tilapia fingerlings (5.80 ± 0.286 g) were allocated at random into 12 plastic tanks (450 L) at a density of 30 fish per tank with three replicate tanks for each of the four dietary treatments. After removing the accumulated excreta, aerated fresh water was used to replace around 10% of the water volume in each tank daily. Aeration was provided to all tanks, and fish were fed the experimental diets three times a day until ad libitum at 9:00 a.m., 11:00 a.m. and 3:00 p.m., for 70 days. The total amount of feed consumed by fish throughout the trial period was calculated and expressed as feed intake.

## 2.5 | Water quality parameters

During the experimental trial, water quality parameters were evaluated every day from each tank at 15 cm depth. A portable oxygen meter (Jenway, London, UK) was used to determine temperature (°C) and the amount of dissolved oxygen (DO). The pH was determined using a pH meter (Digital Mini-pH Meter, USA). Special kits (HACH Co., Loveland, USA) were used to measure the total ammonia ($NH_4$-N). For water temperature, DO, pH and $NH_3$, the values ranged from 26.5 to 29.7°C, 6.2 to 6.5 mg/L, 7.6 to 7.8 and 0.135 to 0.242 mg/L respectively. These parameters are maintained at levels suitable for fish farming, according to Boyd and Tucker (2012).

## 2.6 | Growth indices, feed utilization and survival rate

At the beginning and termination of the feeding trial, the number of fish in each tank was counted and recorded. All of the equations used for estimating the parameters of growth indices and feed utilization efficiency are presented in the footnote of Table 2.

## 2.7 | Haematological, biochemical parameters and non-specific immune response

Blood was obtained from a fish's caudal vein and divided into two halves using clean syringes at the termination of the experiment. The first half was gathered using the anticoagulant ethylenediaminetetraacetate, EDTA, at a concentration of 10% to determine haematocrit (Htc), haemoglobin (Hb) and total count of white blood cells (WBCs) and red blood cells (RBCs) as described in the standard procedures of Rawling et al. (2009). The smears were stained with May–Grunwald–Giemsa for differential leucocyte counting (Rosenfeld, 1947) for the establishment of each cell count. Mean cellular haemoglobin (MCH), mean cellular haemoglobin concentration (MCHC) and mean cellular volume (MCV) were determined and calculated using the formulae (Jain, 1993) in the footnote of Table 3.

The blood sample's remaining part was then centrifuged for 10 minutes at 3000 rpm after clotting overnight at 4°C. A serum that had not been hemolyzed was collected and kept at 20°C until needed. The levels of alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) were measured using Reitman and Frankel (1957). Spectrophotometric measurements of serum uric acid, creatinine, phosphorus and calcium were made with standard Pasteur labs kits (Egyptian American Co. for Laboratory Services, Egypt). Standard Kits (Modern Laboratory Kits) were used to determine the serum lipid profile, which included triglycerides, cholesterol, high-density lipoprotein cholesterol (HDL–C) and low-density lipoprotein cholesterol (LDL–C). Total serum protein and albumin were assessed according to Henry (1964) and Wotton and Freeman (1974) respectively. By subtracting total serum albumin from total serum protein, total serum globulin was calculated (Coles, 1974). The total IgM levels in the blood were determined using an Enzyme-Linked Immunosorbent Assay (ELISA) kit (Cusabio, Wuhan, Hubei, China). The turbidimetric approach was used to determine lysozyme activity (Parry Jr. et al., 1965) modified by Siwicki (1993). According to the method of Cai et al. (2004), to calculate leucocyte phagocytic activity, no clotting blood samples were used.

## 2.8 | Antioxidant activity measurements in the liver and muscles

Fish ($n$ = 3) livers and muscles from each replicate were weighed, washed and ground-in glass homogenizer tubes with ice-cold saline (0.9 ml saline and 0.1 g of liver, pH 7.0) and after that, centrifuged for 10 min at 3000 g. The supernatant was collected and utilized to assess the superoxide dismutase (SOD) activity using Peskin and Winterbourn's (2000) method. The catalase (CAT) activity assay was done using a modified Beers and Sizer (1952) method. According to

**TABLE 2** Oligonucleotide name and sequence of qRT-PCR primers

| Gene | Forward 5–>3 | Reverse 5–>3 | GenBank Acc. |
|---|---|---|---|
| 18s rRNA | GGTTGCAAAGCTGAAACTTAAAGG | TTCCCGTGTTGAGTCAAATTAAGC | AF497908.1 |
| SOD | CATGCCTTCGGAGACAACAC | ACCTTCTCGTGGATCACCAT | AY491056.1 |
| Catalase | AGCTCTTCATCCAGAAACGC | GACGTCAGGCGTCACATCTT | JF801726.1 |
| IgM-2 | CCACTTCAACTGCACCCACT | TGGTCCACGAGAAAGTCACC | KC677037.1 |

**TABLE 3** Growth performance and feed utilization of Nile tilapia, *O. niloticus* fed diets supplemented with Vitamins C and E and β-carotene for 10 weeks

| Parameters | Control | Vitamin C | Vitamin E | β-carotene | $p$-values |
|---|---|---|---|---|---|
| Initial body weight (g fish$^{-1}$) | 6.05 ±0.21 | 5.80 ±22 | 5.70 ±0.30 | 5.65 ±0.27 | 0.772 |
| Final body weight (g fish$^{-1}$) | 23.30 ±0.70$^{c}$ | 26.82 ±0.71$^{b}$ | 28.80 ±0.69$^{b}$ | 34.15 ±0.68$^{a}$ | 0.006 |
| Weight gain (g fish$^{-1}$) | 17.25 ±0.76$^{c}$ | 21.02 ±0.72$^{b}$ | 23.10 ±0.75$^{b}$ | 28.50 ±0.65$^{a}$ | 0.007 |
| Specific growth rate (% day$^{-1}$) | 1.92 ±0.11$^{c}$ | 2.18 ±0.05$^{b}$ | 2.31 ±0.06$^{b}$ | 2.57 ±0.09$^{a}$ | 0.043 |
| Feed intake (g fish$^{-1}$) | 34.9 ±0.91 | 35.80 ±0.72 | 36.60 ±0.65 | 37.65 ±0.72 | 0.245 |
| Feed conversion ratio | 2.02 ±0.05$^{a}$ | 1.70 ±0.08$^{b}$ | 1.58 ±0.07$^{bc}$ | 1.32 ±0.05$^{c}$ | 0.017 |
| Protein efficiency ratio | 1.64 ±0.09$^{c}$ | 1.95 ±0.12$^{cb}$ | 2.10 ±0.06$^{b}$ | 2.52 ±0.09$^{a}$ | 0.018 |
| Apparent protein utilization | 41.24 ±2.1$^{c}$ | 48.97 ±1.96$^{cb}$ | 52.57 ±1.89$^{b}$ | 63.09 ±2.16$^{a}$ | 0.018 |
| Survival rate (%) | 94.24 ±0.51$^{c}$ | 96.50 ±0.43$^{bc}$ | 97.50 ±0.49$^{ab}$ | 99.50 ±0.52$^{a}$ | 0.021 |

*Note:* Means followed by different superscript letters in the same row are significantly different ($p < 0.05$).

Weight gain (WG) = final weight (g)–initial weight (g); Specific growth rate (SGR) = $LnW_2$–$LnW_1$/t (days), Where, Ln = the natural log; $W_1$ = initial fish weight, $W_2$ = the final fish weight in grams and t = Period in days; Feed conversion ratio (FCR) was calculated according to by the equation: FCR = Feed intake (g)/weight gain (g); Apparent protein utilization (APU) = Apparent protein utilization (APU) = 100× (protein gain/protein in diet); Survival rate (%) = 100× (total number of fish at the end of the experiment/total number of fish at the start of the experiment); number of replicate/treatment = 3.

Benzie and Strain (1996), the total antioxidant capacity (T-AOC) was estimated. Malondialdehyde (MDA) activity was determined according to Dogru et al. (2008).

### 2.9 | Gene expression

Using a tissue homogenizer (QIAGEN, Hilden, Nordrhein-Westfalen-40724, Germany), fish were anaesthetised with 3-aminobenzoic acid ethyl ester (MS 222, 100 mg/L, Sigma, St. Louis, MO). The manufacturer's procedure was followed to extract ribonucleic acid (RNA) from the tissues using the RNeasy® Mini kit (QIAGEN, Cat No. 74104). The high-capacity cDNA Reverse Transcription Kit (Applied Biosystems, MA, USA, Cat# no.4368813) was used to synthesize cDNA from 1000 ng of total RNA, and the cDNA was kept at 80°C for subsequent molecular investigates. The used primers to amplify the gene encoding Ig M-2, SOD, CAT and 18S ribosomal RNA (18S rRNA; as reference gene) were utilized to quantify the target gene expression using real-time PCR (qRT-PCR) approaches (Table 2). 2.5 µg/L cDNA, 12.5 µl SYBR Green PCR Master Mix (QuantiTect SYBR Green PCR Kit, QIAGEN), 0.3 µM forward and reverse primers and a final volume of 25 µl sterile double distilled water were used in quantitative PCR. The reaction was carried out using an Applied Biosystems 7500 Real-time PCR Detection System (Applied Biosystems) for 10 min at 95°C then 45 cycles for 20 s at 95°C, 20 s for 60°C and 20 s for 72°C. All experimentally made variations in the gene expression under study are expressed as n-fold differences compared with the control. $RQ = 2^{-\Delta\Delta CT}$ was used to calculate relative gene expression ratios (RQ) between the control and treatment groups (Livak & Schmittgen, 2001).

### 2.10 | Data analysis

Homogeneity and normality tests were made for data before analysis. Then, using the SAS ANOVA program, data were analysed using a one-way analysis of variance, and differences between means were determined using Duncan's multiple range test (SAS, version 6.03, Soft Inc., Tusla, OK, USA, SAS, 1993). Means and standard errors of the mean (±SEM) values are used to represent the data.

## 3 | RESULTS

### 3.1 | Growth indices and feed utilization

In comparison to the control diet, supplemental diets with Vitamins C and E and β-carotene boosted the growth indices and feed utilization efficiency ($p < 0.05$) (Table 3), whereas fish fed a supplemental diet containing β-carotene had the highest ($p < 0.05$) feed efficiency parameters and growth indices. On the other hand, the lowest feed utilization and growth performance ($p < 0.05$) were found in the group that was fed a control diet. The survival rate of fish fed a β-carotene-enriched diet was higher ($p < 0.05$) than in other treated diets (Table 3).

### 3.2 | Haematological and biochemical parameters

Table 4 displays the findings of haematological and biochemical parameters. Haemoglobin, mean corpuscular volume (MCV) and neutrophil levels did not differ significantly amongst treatments ($p > 0.05$). The lowest significant ($p < 0.05$) MCHC (mean corpuscular haemoglobin concentration) and MCH (mean corpuscular haemoglobin) values were found in the group fed the control diet. A fish-fed diet enriched with β-carotene had higher haematocrit and RBCs ($p < 0.05$) than other diets. Also, the differences in values of lymphocytes and monocytes amongst groups fed dietary Vitamins C and E and β-carotene were insignificant ($p > 0.05$). In terms of aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, urea and neutrophils, there were no differences between treatments ($p > 0.05$).

### 3.3 | Lipid profile

Table 5 shows the lipid profile, which includes cholesterol, triglycerides, HDL-C and LDL-C. Compared with the control diet, there was a decrease in cholesterol and triglyceride values in all supplemental diets, whereas the fish-fed dietary β-carotene had the lowest levels ($p < 0.05$). The addition of Vitamins E and C and β-carotene had an insignificant ($p > 0.05$) effect on HDL-C. In addition, there were no significant ($p > 0.05$) differences between fish-fed Vitamin E and β-carotene.

### 3.4 | Non-specific immune responses

The albumin, globulin and total protein contents were increased ($p < 0.05$) by supplementing fish diets with Vitamins C and E and β-carotene, compared with the control diet (Table 6). The highest value ($p < 0.05$) was noted in the β-carotene-rich diet offered to fish. In comparison to a control diet, adding Vitamins C and E and β-carotene boosted lysozyme, immunoglobulin M (IgM) and phagocytic activity (Table 6), whereas, a fish-fed diet enriched with β-carotene had the uppermost levels of IgM, lysozyme and phagocytic activity.

### 3.5 | Antioxidant enzyme response

The data on antioxidant enzyme response of fish liver and muscles are shown in Table 7. Compared with the control, diets supplemented with Vitamins C and E and β-carotene improved the oxidative enzymes in the liver and muscles of fish. However, the maximum ($p < 0.05$) values of superoxide dismutase (SOD), catalase

**TABLE 4** Haematological and biochemical parameters of Nile tilapia, *O. niloticus* fed diets supplemented with Vitamins C and E and β-carotene for 10 weeks

| Parameters | Control | Vitamin C | Vitamin E | β-carotene | *p*-values |
|---|---|---|---|---|---|
| Haematocrit (%) | 18.50 ± 0.52$^{c}$ | 20.50 ± 0.59$^{bc}$ | 22.50 ± 0.62$^{ba}$ | 23.50 ± 0.62$^{a}$ | 0.03 |
| Haemoglobin (g/L) | 8.00 ± 0.89 | 9.45 ± 0.87 | 9.45 ± 0.86 | 9.75 ± 0.85 | 0.55 |
| RBCs ($\times 10^4$ mm$^{-1}$) | 2.60 ± 0.11$^{c}$ | 2.95 ± 0.07$^{bc}$ | 3.30 ± 0.08$^{ba}$ | 3.45 ± 0.09$^{a}$ | 0.02 |
| MCV (fl) | 112.45 ± 4.43 | 118.65 ± 4.41 | 119.80 ± 4.45 | 129.00 ± 4.20 | 0.25 |
| MCH (pg) | 53.15 ± 4.70$^{c}$ | 57.90 ± 4.71$^{ba}$ | 78.80 ± 4.76$^{a}$ | 71.50 ± 4.72$^{ba}$ | 0.08 |
| MCHC (g dl$^{-1}$) | 43.90 ± 3.23$^{c}$ | 50.70 ± 3.59$^{bc}$ | 70.40 ± 3.31$^{a}$ | 64.50 ± 3.33$^{ba}$ | 0.03 |
| WBCs ($\times 10^4$ mm$^{-1}$) | 34.00 ± 2.19$^{b}$ | 41.80 ± 2.11$^{ba}$ | 44.00 ± 2.29$^{a}$ | 49.25 ± 2.26$^{a}$ | 0.06 |
| Neutrophil (%) | 2.50 ± 0.51 | 3.50 ± 0.48 | 3.50 ± 0.49 | 3.50 ± 0.51 | 0.50 |
| Lymphocytes (%) | 85.50 ± 0.65$^{b}$ | 91.00 ± 0.62$^{a}$ | 91.50 ± 0.60$^{a}$ | 91.50 ± 0.61$^{a}$ | 0.02 |
| Monocytes (%) | 1.50 ± 0.26$^{b}$ | 3.50 ± 0.27$^{a}$ | 3.50 ± 0.23$^{a}$ | 3.00 ± .024$^{a}$ | 0.03 |
| ALT (U/L) | 26.00 ± 1.23 | 21.00 ± 1.26 | 22.00 ± 1.29 | 20.50 ± 1.30 | 0.16 |
| AST (U/L) | 16.50 ± 1.20 | 17.00 ± 1.11 | 14.00 ± 1.18 | 12.50 ± 1.12 | 0.18 |
| Urea (mmol/L) | 25.00 ± 2.24 | 22.00 ± 2.25 | 21.50 ± 2.26 | 19.00 ± 2.21 | 0.44 |
| Creatinine (μmol/L) | 0.50 ± 0.14 | 0.35 ± 0.12 | 0.50 ± 0.13 | 0.30 ± 0.15 | 0.62 |

*Note:* Means followed by different superscript letters in the same row are significantly different ($p < 0.05$). Number of replicate/treatment = 3.
Abbreviations: ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; MCH, Mean corpuscular haemoglobinhemoglobin; MCHC, Mean corpuscular haemoglobinhemoglobin concentration; MCV, Mean corpuscular volume; RBCs, Red blood cells count; WBCs, White blood cell count.

**TABLE 5** Lipid profile of Nile tilapia, *O. niloticus* fed diets supplemented with Vitamins C and E and β-carotene for 10 weeks

| Parameters | Control | Vitamin C | Vitamin E | β-carotene | ±SEM | *p*-values |
|---|---|---|---|---|---|---|
| Cholesterol (mg dl$^{-1}$) | 6.90 ± 0.42$^{a}$ | 5.65 ± 0.43$^{ab}$ | 5.20 ± 0.46$^{ab}$ | 4.70 ± 0.42$^{b}$ | 0.45 | 0.01 |
| Triglycerides (mg dl$^{-1}$) | 5.25 ± 0.08$^{a}$ | 5.05 ± 0.05$^{ab}$ | 4.90 ± 0.06$^{b}$ | 4.50 ± 0.06$^{c}$ | 0.07 | 0.02 |
| HDL-C (mg dl$^{-1}$) | 2.60 ± 0.25 | 3.10 ± 0.26 | 3.45 ± 0.29 | 3.70 ± 0.29 | 0.27 | 0.19 |
| LDL-C (mg dl$^{-1}$) | 0.45 ± 0.02$^{a}$ | 0.40 ± 0.01$^{ab}$ | 0.30 ± 0.04$^{b}$ | 0.30 ± 0.04$^{b}$ | 0.03 | 0.04 |

*Note:* Means followed by different superscript letters in the same row are significantly different ($p < 0.05$).
Abbreviations: HDL-C, High-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol.

**TABLE 6** Non-specific immune parameters responses of Nile tilapia, *O. niloticus* fed diets supplemented with Vitamins C and E and β-carotene for 10 weeks

| Parameters | Control | Vitamin C | Vitamin E | β-carotene | *p*-values |
|---|---|---|---|---|---|
| Albumin (U/L) | 1.75 ± 0.06$^{b}$ | 1.95 ± 0.08$^{ba}$ | 2.00 ± 0.06$^{ab}$ | 2.20 ± 0.05$^{a}$ | 0.03 |
| Globulin (U/L) | 1.25 ± 0.19$^{b}$ | 2.15 ± 0.16$^{a}$ | 1.95 ± 0.16$^{ab}$ | 2.05 ± 0.19$^{a}$ | 0.04 |
| Total Protein (U/L) | 3.00 ± 0.26$^{b}$ | 4.10 ± 0.25$^{a}$ | 3.95 ± 0.24$^{ab}$ | 4.25 ± 0.21$^{a}$ | 0.04 |
| Immunoglobulin M (IgM, μg/ml) | 22.71 ± 1.13$^{c}$ | 31.12 ± 1.15$^{b}$ | 32.6 ± 1.14$^{b}$ | 36.89 ± 1.10$^{a}$ | 0.03 |
| Lysozyme (μ/ml) | 134.70 ± 1.19$^{c}$ | 140.13 ± 1.19$^{b}$ | 147.12 ± 1.13$^{b}$ | 161.12 ± 1.13$^{a}$ | 0.02 |
| Phagocytic activity (%) | 65.70 ± 1.90$^{c}$ | 72.31 ± 1.83$^{b}$ | 71.12 ± 1.84$^{b}$ | 86.97 ± 1.87$^{a}$ | 0.03 |

*Note:* Means followed by different superscript letters in the same row are significantly different ($p < 0.05$).

(CAT) and total antioxidant capacity (T-AOC) in the liver were detected in the fish-fed diets supplemented with either Vitamin E or β-carotene with no differences that are statistically significant between them ($p > 0.05$). On the other hand, the lowest values of malondialdehyde (MDA) in the liver were noted in fish fed either Vitamin E or β-carotene. SOD, T-AOC and MDA levels were not affected by any supplemented diets in fish muscles ($p > 0.05$). In addition, it is noted that the activity of CAT in fish muscles was superior ($p < 0.05$) in fish that were fed a diet enriched with either Vitamin E or β-carotene.

### 3.6 | Gene expression

The gene of superoxide dismutase (SOD) was significantly upregulated ($p < 0.05$) in fish-fed diets supplemented with Vitamins C and

**TABLE 7** Antioxidant enzyme response (U/g protein) of Nile tilapia, *O. niloticus*, fed diets supplemented with Vitamins C and E and β-carotene for 10 weeks

| Parameters | Control | Vitamin C | Vitamin E | β-carotene | *p*-values |
|---|---|---|---|---|---|
| Liver | | | | | |
| MDA | 73.10 ± 0.41$^{a}$ | 57.50 ± 0.41$^{b}$ | 48.50 ± 0.42$^{c}$ | 49.00 ± 0.43$^{c}$ | 0.0001 |
| CAT | 5.40 ± 0.66$^{b}$ | 8.40 ± 0.67$^{a}$ | 9.10 ± 0.63$^{a}$ | 9.55 ± 0.62$^{a}$ | 0.0560 |
| SOD | 58.00 ± 1.92$^{c}$ | 78.20 ± 1.93$^{b}$ | 91.00 ± 1.96$^{a}$ | 91.10 ± 1.94$^{a}$ | 0.0032 |
| T-AOC | 19.40 ± 1.04$^{b}$ | 23.45 ± 1.03$^{ab}$ | 26.05 ± 1.07$^{a}$ | 27.65 ± 1.05$^{a}$ | 0.0353 |
| Muscles | | | | | |
| MDA | 27.95 ± 2.05 | 19.00 ± 2.09 | 21.75 ± 2.02 | 19.55 ± 2.09 | 0.1468 |
| CAT | 21.20 ± 5.45$^{b}$ | 32.25 ± 5.47$^{ab}$ | 54.30 ± 5.45$^{a}$ | 51.10 ± 5.68$^{a}$ | 0.0579 |
| SOD | 35.30 ± 12.41 | 53.90 ± 12.16 | 82.90 ± 12.12 | 85.50 ± 12.12 | 0.1494 |
| T-AOC | 23.90 ± 3.52 | 24.55 ± 3.69 | 27.40 ± 3.67 | 37.00 ± 3.71 | 0.2145 |

*Note:* Means followed by different superscript letters in the same row are significantly different ($p < 0.05$).
Abbreviations: CAT, Catalase; MDA, Malondialdehyde; SOD, Superoxide dismutase; T-AOC, Total antioxidant capacity.

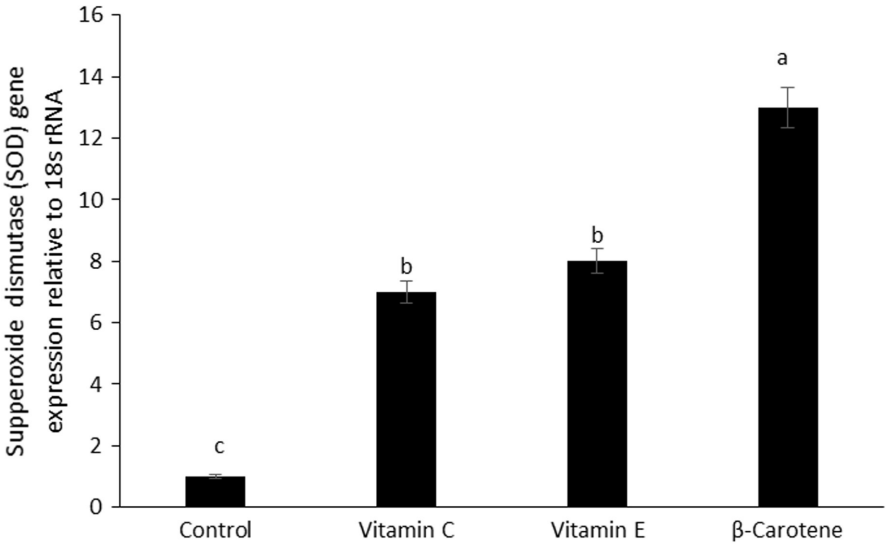


**FIGURE 1** Relative expression of SOD gene/18s rRNA of Nile tilapia, *O. niloticus*, after feeding experimental diets. Bars having different letters are significantly different at $p < 0.05$.

E and β-carotene extracted from *Arthrospira platensis* than in the control diet, whereas the higher relative gene expression of SOD was shown to be greater in diet enriched with β-carotene (Figure 1). The relative expression of the SOD gene did not differ significantly between Vitamin C and Vitamin E-fortified diets. Fish fed a β-carotene-fortified diet had the most upregulated catalase (CAT) gene expression (Figure 2). Fish fed a diet enriched with β-carotene had the highest upregulation of relative immunoglobulin M-2 (IGM-2) gene expression (Figure 3).

## 4 | DISCUSSION

### 4.1 | Growth response and feed efficiency

Using synthetic bioactive compounds like Vitamin C or Vitamin D or natural bioactive compound like β-carotene as a dietary supplement in Nile tilapia diets herein improved Nile tilapia growth and feed efficiency when compared with a control diet, whereas the highest values ($p < 0.05$) were noted in β-carotene fed fish extracted from natural sources (*Arthrospira platensis*). The current findings are in line with earlier research on other fish species including juvenile Japanese flounder (*Paralichthys olivaceus*), juvenile largemouth bass, *Micropterus salmoides* and meagre (*Argyrosomus regius*) (Gao et al., 2014; Chen et al., 2015; Rodriguez-Lozano et al., 2017). In this context, Hu et al. (2006) and Keleştemur and Çoban (2016) indicated that β-carotene has a growth-stimulating action for *Oreochromis niloticus* and rainbow trout (*Oncorhynchus mykiss*). Likewise, β-carotene enhanced ($p < 0.05$) the survival, specific growth rate and bodyweight of Indian carps, goldfish (*Carassius auratus*) and Nile tilapia (Aravindan et al., 2001; Goswami, 1993; Hu et al., 2006). Furthermore, black tiger shrimp (*Penaeus monodon*) fed diets supplemented with 200–300 mg $kg^{-1}$ of β-carotene from *Dunaliella salina* (Algro Nature®) had greater survival and growth performance (Supamattaya et al., 2005) (Table 7).

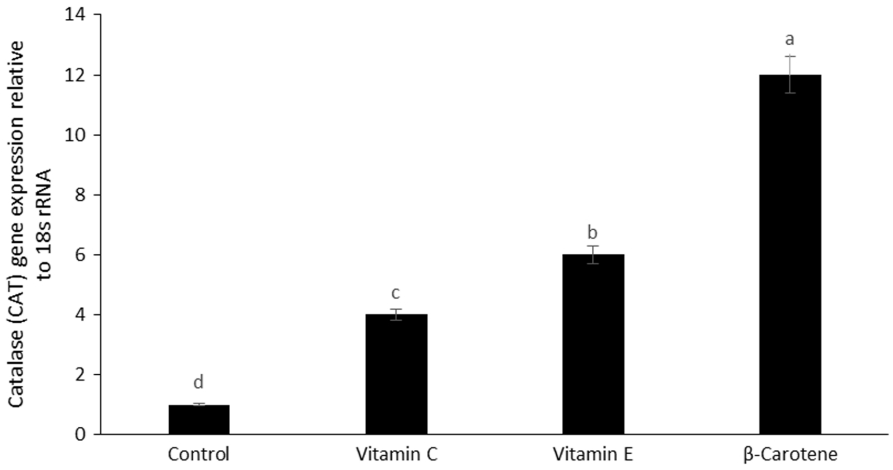


**FIGURE 2** Relative expression of CAT gene/18s rRNA of Nile tilapia, *O. niloticus*, after feeding experimental diets. Bars having different letters are significantly different at $p < 0.05$.

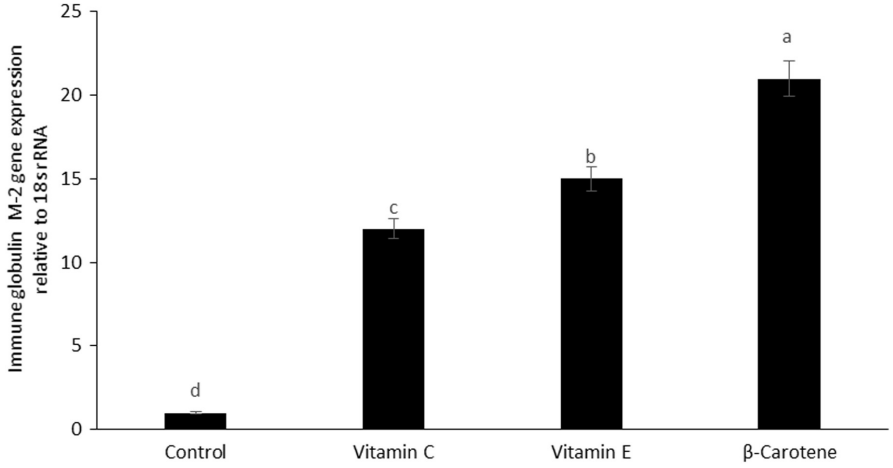


**FIGURE 3** Relative expression of Ig-M2 gene/18s rRNA of Nile tilapia, *O. niloticus*, after feeding experimental diets. Bars having different letters are significantly different at $p < 0.05$.

This improvement in growth indices could be attributed to different pathways as (i) β-carotene ability to stimulate and modify microflora in fish intestines to degrade indigestible feed components (Teimouri et al., 2013), which increases the secretion of digestive enzymes to transport fats for metabolism rather than storage, resulting in improved nutrient utilization, digestive efficiency, growth and protein synthesis in fish (James et al., 2006; Teimouri et al., 2013), (ii) β-carotene's antioxidant properties of free radical scavenging to minimize the oxidative damage, enhance immune system response and consequently improve growth performance, feed efficiency and health status; the bioactive compounds as Vitamins E and C and β-carotene improve growth hormone level in serum, (iii) improving the intestinal morphology, (iv) increase the intestinal absorptive surface, (v) enhance oxidative stability of feeds, fatty acid metabolism, protein synthesis and the immune and antioxidant function in fish (Abdel Rahman et al., 2018; Chagas & Val, 2003; Faramarzi, 2012) and v) the activity of anti-inflammatory mediators and antioxidant enzymes have greatly improved (Duquette et al., 2014; Hurnik et al., 2011; Kang et al., 2018), which is responsible for the favourable impacts on growth.

In contrast to the current findings, weight gain in *Oreochromis niloticus*, and Atlantic halibut, *Hippoglossus hippoglossus*, was not significantly different, when Vitamin E was increased from 4 mg $kg^{-1}$ to 47 mg $kg^{-1}$ (Lewis-Mccrea & Lall, 2007; Navarro et al., 2010). Similarly, supplemental diets with Vitamins E and C did not affect the growth performance of turbot (*Scophthalmus maximus*) (Tocher et al., 2002) or large yellow croaker (*Larimichthys polyactis*) (Ai et al., 2006). The discrepancy in results could be related to differences in cultured species, diet type, experimental conditions, source and doses of β-carotene, Vitamin C and Vitamin E.

## 4.2 | Haematological and biochemical parameters

Haematological indices are displayed the physiological and health status of fish as well as disease and metabolic disorders (Fazio, 2019; Fazio et al., 2012). Because erythrocytes are a major source of free radicals, and some of them can cause saturated fatty acid peroxidation in their membrane phospholipids, resulting in changes in their quality (integrity, size), red blood cell indices are employed as an indicator of oxidative status (Kiron et al., 2004; Pearce et al., 2003). This study's findings revealed that supplemental diets enriched with synthetic bioactive compounds such as Vitamin C or E, as well as a bioactive component that is naturally produced such as *A. platensis*-derived β-carotene, increased Hb, Htc, RBCs and WBCs, where, the group receiving dietary β-carotene have the highest values. In

line with our study, a considerable increase in Htc, RBC and Hb was shown in pirarucu, *Arapaima gigas* (de Menezes et al., 2006), matrinxa, *Brycon amazonicus* (Affonso et al., 2007) and *Cirrhinus mrigala* (Zehra & Khan, 2012). Htc and RBC were not significantly altered ($p > 0.05$) by the Vitamin C diet in catfish (*Pelteobagrus fulvidraco*), whereas Hb was greatly increased (Liang et al., 2017). Yu et al. (2020) found a significant increase in Htc, RBC and Hb in starry flounder, *Platichthys stellatus*, when they were given Vitamin C (ascorbic acid). Similarly, RBCs and WBCs counts have increased by supplementation of 10% *Spirulina* in the fish diet (Abdel-Tawabe et al., 2008). In contrast to our findings, Baker and Davies (1996) stated that African catfish, *Clarias gariepinus* fed a high dosage of α-tocopheryl acetate dose (500 mg $kg^{-1}$ dry feed) exhibited a lower haematocrit than those fed a basal diet. Furthermore, introducing carotenoids-rich *Spirulina platensis* to the *Oreochromis niloticus* diet-lowered haematocrit (Moe, 2011).

Furthermore, Vitamin C or E supplementation in fish diets improved the haematological parameters of Nile tilapia, including RBC, Ht and Hb levels. This enhancement may be attributed to (i) their role as antioxidants by preventing oxidation of red blood cell membranes, which is necessary for cell respiration (Narra, 2017). (ii) these vitamins promoted iron absorption by lowering ferric ion to ferrous ion, released iron from transferrin, which improved haemoglobin functions, and oxygen delivery to tissue, and inhibited anaemia in fish by decreasing ferric ion to ferrous ion (Affonso et al., 2007; Zafar & Khan, 2020). (iii) β-carotene has potential impacts to improve fish health by enhancing the immune system's ability to fight infection and stress by boosting the immunological parameters such as WBSs and their derivatives as basophils, monocytes, neutrophils and lymphocytes (Chow et al., 2016; Nakono et al., 2003).

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes in the serum of the blood are involved in cellular nitrogen metabolism, oxidation of amino acids, hepatic gluconeogenesis and hepatic status and their high levels in fish plasma could cause liver dysfunction (Abdel-Tawwab, 2016; Coz-Rakovac et al., 2005; Moazenzadeh et al., 2018). No significant ($p > 0.05$) differences were found in the levels of ALT and AST of fish, indicating normal liver function and good nutritional status, as well as circulatory system integrity (Schaperclaus et al., 1992). These findings confirmed that Vitamins C and E and β-carotene did not have any negative impacts on liver morphology and histology when they were used within the recommended level and this result could be attributed to the following factors: (i) Vitamins E and C and natural immunostimulants as carotene promote the production of cytokine, which consider an essential part of the inflammatory process that protected liver cells., (ii) the role of Vitamins E and C as antioxidant vitamins and anti-inflammatory agents that protect the liver fibrosis and dysfunction that aid to make the liver act within the normal function., (iii) the protective effects on liver damage using β-carotene may be due to avoid steatosis, fat accumulation and haemorrhages (Eddowes et al., 2019). Hassaan, El-Sayed, et al. (2021) confirmed these findings, demonstrating significant ($p < 0.05$) protective benefits of β-carotene extracted from *Spirulina* on liver enzymes; ALT and AST.

### 4.3 | Lipid profile

The lipid profile, which includes cholesterol and triglycerides, can change depending on nutritional status (Regost et al., 2001). Triglycerides (TG) are measured to monitor lipid metabolism. High TG levels can cause glycogen storage disease, nephritic syndrome and liver failure (Coz-Rakovac et al., 2005; Osman et al., 2010). The total lipid profile, which included cholesterol, triglycerides and LDL-L, was shown to be lower in Nile tilapia-fed diets containing Vitamin C, Vitamin E or β-carotene in this study. These findings may be due to the importance of Vitamins E and C in improving lipid profiles in the blood, inhibiting lipid oxidation, high-density lipoprotein cholesterol (HDL-C) and lowering the risk of atherosclerosis of low-density lipoprotein cholesterol (LDL-C) (Ashor et al., 2016; Karakilcik et al., 2014), whereas the lowest values of lipid profile were recorded in groups fed β-carotene indicating their role in strengthening and preventing the tilapia liver tissue injuries. These findings matched those obtained by Velasquez et al. (2016) when Nile tilapia fed *Spirulina* (*Arthrospira platensis*). Similarly, values of fish blood parameters fed *Arthrospira* revealed no negative effects on the health of juvenile Nile tilapia (Velasquez et al., 2016). Likewise, blood triglycerides and cholesterol were lowered in yellow croakers, *Pseudosciaena crocea* fed *Haematococcus pluvialis* supplemented diets (Li et al., 2014) and in olive flounder (*Paralichthys olivaceus*) fed *Eucheuma denticulatum* supplemented diets (Ragaza et al., 2015).

### 4.4 | Non-specific immune responses

The transfer of exogenous and endogenous metabolites, as well as the metabolism, is all dependent on serum albumin levels (Baker, 2002). Globulin is an important carrier and transporter for living organisms (Preeti & Seema, 2014). The addition of Vitamin C, Vitamin E or β-carotene increased total albumin, protein and globulin levels in blood serum, with the highest values recorded in β-carotene-fed fish derived from *Arthrospira platensis*. The reason for this finding might be ascribed to the significant antioxidant capacity of β-carotene derived from *Spirulina* in enhancing tilapia immunity and health (Abu-Elala et al., 2016; Hassaan, Mohammady, et al., 2021). Likewise to our results, Akbary et al. (2018) found that dietary supplementation with *Ulva rigida* methanolic extracts at various levels significantly improved albumin, total serum protein and globulin of grey mullet (*Mugil cephalus*). Similarly, the serum albumin, total serum protein and globulin of giant gourami (*Osphronemus gourami*) were enhanced in fish-fed diets supplemented with *S. platensis* (Simanjuntak et al., 2018). Parallel to the existing data, the supplementation with *S. platensis* and *Sargassum ilicifolium* extract enhanced serum globulin and total protein of Beluga (*Huso huso*) and *O. niloticus* diets (Yeganeh & Adel, 2019; Zeinab et al., 2015).

Lysozyme, which has a link with leucocytes and is produced mostly by macrophages, is the most important sign of the immune response as a result of many immune stimulants and microbiological components (Ringø et al., 2012; Siwicki & Anderson, 1993).

Immunoglobulin M (IgM) is also the first immunoglobulin found in phylogeny, ontogeny and as an antibody in the immune response (Pilstrom & Bengten, 1996). In this study, IgM, lysozyme activity and phagocytic activity were positively influenced ($p < 0.05$) by synthetic bioactive compounds; Vitamin C or Vitamin D or natural bioactive compound, β-carotene. These findings could explain why fish-fed supplemented diets have a higher level of immunity than those on a control diet. β-carotene-rich diet may also improve the intestinal bifacial microbiota by improving fish digesting enzymes, so encouraging the immunological response (Tarkhani et al., 2020). Administration of ulvan polysaccharide extract from *Ulva clathrata* to *O. niloticus* diet has improved their immune function, which is consistent with the current findings (del Rocío Quezada-Rodríguez & Fajer-Ávila, 2017). Moreover, microalgae (*Phaeodactylum tricornutum*)-enriched diets boosted phagocytic activity in gilthead seabream (*Sparus aurata*) (Cerezuela et al., 2012) which is in line with our results.

## 4.5 | Antioxidant enzyme responses

The antioxidant enzymes revealed the body's antioxidant system's functional status, which reflected the body's ability to break down oxygen-free radicals and to protect the tissues of the fish from oxidative damage (Shan et al., 2019). However, there is a link between antioxidant defence and fish responsiveness in aquaculture (Guerriero et al., 2002). In this trial, nutritional supplementation with Vitamin C, Vitamin E or β-carotene from *Arthrospira platensis* dramatically increased the antioxidant enzyme activities of Nile tilapia in the liver and fish muscles, such as total antioxidant capacity, superoxide dismutase and catalase, as compared with the control diet, whereas those diets that were supplemented with either β-carotene or Vitamin E had stronger antioxidant activity. Several mechanisms might be responsible for these beneficial benefits: (i) lipid peroxidation inhibition, (ii) natural antioxidants have a strong capacity to scavenge free radicals from the antioxidant system within the body, (iii) reduced oxidative stress protects both natural body molecules and cells, and clean oxygen reactive species are formed (Oguzkan et al., 2018; Romay et al., 2003; Soni et al., 2008). Following the same patterns, Amar et al. (2000) noted that the oxygen radical generated by peripheral blood leucocytes in *Oncorhynchus mykiss* was significantly decreased in fish fed a 400 mg $kg^{-1}$ β-carotene-enriched diet. Damage to antigen-recognition receptors and cell membranes could be prevented by β-carotene-mediated peroxidation processes in membrane cells (Bendich, 1991). However, MDA is a lipid peroxide by-product (Devi et al., 2010) which might cause damage to cytomembranes and cells (Garg et al., 2009). In this study, higher levels of antioxidant enzymes including T-AOC, SOD, CAT and lower levels of MDA in the liver and muscles could be related to Vitamin C's and E's potent antioxidant activity. These results were similar to those stated by Liang et al. (2017) for juvenile yellow catfish, *Pelteobagrus fulvidraco* fed a diet enriched with 156.5 mg $kg^{-1}$ Vitamin C and Hu et al. (2013) for juvenile black carp, *Mylopharyngodon piceus* fed a diet supplemented with 63.0 mg $kg^{-1}$ vitamin C. However, Vitamin E improved oxidation resistance in yellow catfish and meagre, *Argyrosomus regius*, by increasing the activity of antioxidative enzymes and lowering lipid peroxidation (Lozano et al., 2017; Lu et al., 2016). Furthermore, unlike the steady amounts in muscle, the MDA of Atlantic halibut was dramatically altered when compared with liver MDA (Lewis-Mccrea & Lall, 2007) which concurred with this study. In contrast to the present data, the addition of 400 mg $kg^{-1}$ of Vitamin E to the diet of Atlantic salmon, *Salmo salar* did not affect SOD and MDA or GPx in the liver, but it did lower the levels of MDA levels in the fillet (Faizan et al., 2013). Moreover, Vitamin E in the highest dose (198 mg $kg^{-1}$) had a deleterious impact on tissue oxidation resistance. The explanation for this could be that too much Vitamin E in the diet can lead to a build-up of prooxidant Vitamin E radicals (Lu et al., 2016; Niki, 1987). The pattern was the same in hybrid snakeheads, *Channa argus*×*Channa maculata*, and grass carp (Pan et al., 2018; Zhao et al., 2018).

## 4.6 | Gene expression

In the present trial, the oxidative enzyme gene expression (CAT, SOD and Immune globulin M-2 genes) were selected as a significant illustration of the influence of various studied diets on gene transcriptomes in the liver. These genes were chosen due to their significance as stress indicators and inflammatory markers (Dawood et al., 2020). The current results are in accordance with Teimouri et al. (2019), who revealed that after administration of microalgae as a source of β-carotene, the expression leve8ls of catalase (CAT) and superoxide dismutase (SOD) genes in the fish liver have tremendously increased, with fish fed with 10% *S. platensis* in their diet having the highest level compared with the other groups. However, further research is needed to figure out how *A. pl8atensis* and its active components, such as β-carotene, modulate and regulate the antioxidative enzyme gene expression. Following the same pattern, in white shrimp, *S. platensis* extract concentration-boosted GPx and SOD enzyme activity and gene expression (Lin et al., 2010; Tayag et al., 2010). *S. platensis* supplementation in *O. niloticus* diets also increased CAT and SOD enzyme activities according to Abdelkhalek et al. (2015). Consequently, the current research findings suggest that beta-carotene derived from *Arthrospira platensis* offers complete immunological and antioxidant defence. However, more research is needed to understand whether Vitamin E or C can effectively affect the molecular mechanism, as well as whether diets rich in β-carotene can influence the expression of antioxidant enzyme genes.

# 5 | CONCLUSION

It can be concluded that the supplementation of the Nile tilapia diet with either natural (β-carotene) or synthetic bioactive compounds (Vitamins E and C) had a favourable impact on the health and growth of the fish, whereas the dietary incorporation of β-carotene had a superior impact on growth performance, haemato-biochemical,

immune-oxidative stress biomarkers and the associated gene expression of *Oreochromis niloticus*. Moreover, the study is needed to completely comprehend the molecular mechanisms of β-carotene's effect on antioxidant enzyme gene expression.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## AUTHOR CONTRIBUTIONS

The first author collected the samples and data as well as make the first draft of manuscript, while the contribution of other authors is equal.

## DATA AVAILABILITY STATEMENT

Data generated during the study are subject to a data-sharing mandate and available in a public repository that does not issue data sets with DOIs.

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