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Fishmeal replacement with *Spirulina Platensis* and *Chlorella vulgaris* in African catfish (*Clarias gariepinus*) diet: Effect on antioxidant enzyme activities and haematological parameters

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ABSTRACT

This study explored fishmeal replacement with two freshwater microalgae: *Spirulina Platensis* and *Chlorella vulgaris* in African catfish (*Clarias gariepinus*) diet. The effect of inclusion of the two microalgae on biomarkers of oxidative stress, haematological parameters, enzyme activities and growth performance were investigated. The juvenile fish were given 3 distinct treatments with isonitrogenous (35.01–36.57%) and isoenergetic (417.24–422.27 Kcal 100 g^{-1}) diets containing 50% *S. platensis* (50SP), 75% *S. platensis* (75SP), 50% *C. vulgaris* (50CL), 75% *C. vulgaris* (75CL) and 100% fishmeal (100% FM) was used as the control diet. The result shows that all the diets substituted with both *S. platensis,* and *C. vulgaris* boosted the growth performance based on specific growth rate (SGR) and body weight gain (BDWG) when compared with the control diet. The feed conversion ratio (FCR) and protein efficiency ratio (PER) was significantly influenced by all the supplementations. The haematological analysis of the fish shows a significant increase in the value of red and white blood cells upon supplementation with 50SP and 50CL but decrease slightly when increased to 75SP and 75CL. Furthermore, the value of haematocrit and haemoglobin also increased upon supplementation with 50SP and 50CL but decrease slightly when increased to 75SP and 75CL. The white blood cell (WBC), red blood cell (RBC) increased, while total cholesterol (TCL), and Plasma glucose levels decreased significantly upon supplementation of algae. This is a clear indication that *S. platensis* and *C. vulgaris* are a promising replacement for fishmeal, which is a source protein in the *C. gariepinus* diet.

1. Introduction

African catfish (*Clarias gariepinus*) is commonly patronized in Africa and Southeast Asia for human consumption (Taufek et al., 2016). Its popularity, as well as commercial importance, is due to its high palatability, ability to combat diseases and high fecundity (Erondu et al., 1993). Nevertheless, the increasing cost of feed because of the high cost of fishmeal is the major limitation in the culture of African catfish. Fishmeal is the most important source of protein in aquaculture (catfish inclusive). The cost of fishmeal is about 80% of aquaculture industry operating costs where protein is the controlling factor, which determines the cost of fish diet (Shepherd and Jackson, 2013). However, it is not only expensive but the supply is stagnated due to over-exploitation of the natural resources and competition from humans and other livestock ventures (Jabir et al., 2012). Hence there is need to find a suitable replacement for it in order to bring down production cost.

Recently, the use of microalgae is gaining the attention of several researchers as a promising alternative to fishmeal. Microalgae are essential in aquaculture and have been consumed as live feeds for finfish, larval or juvenile crustaceans, for all bivalve mollusks comprising mussels, clams, scallops, and oysters. They are used as feed for zooplankton used in aquaculture. Microalgae are rich in carotenoid pigments, essential fatty acids, essential amino acids, minerals, and vitamins for aquatic animals (Augustin et al., 2011). Several authors have

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reported the remarkable performance of various microalgae as a source of protein and carotenoid for shrimp (Patnaik et al., 2006; Regunathan and Wesley, 2006). (Mustafa and Nakagawa, 1995) reported that fishmeal replacement with microalgae enhanced carcass quality, feed utilization, disease resistance, physiological activity, starvation tolerance, stress response, and growth (protein accretion). The recent study of (Radhakrishnan et al., 2014) states that microalgae are viable sources of protein for *Macrobrachium culture*.

S. platensis is a fresh water microalgae with abundant fatty acids (gamma – linolenic acid (GLA), vitamins, antioxidant pigments like carotenoids, essential amino acids, protein, and minerals (Augustin et al., 2011). Several authors have used dried *S. platensis* as a feed supplement (Augustin et al., 2011; Kim et al., 2013; Radhakrishnan et al., 2014), and explored as a partial replacement. For instance in *Peneaus semisulcatus* feed (Ghaeni et al., 2011), Pacific white shrimp *Litopenaeus vannamei* feed (Hanel et al., 2007), Guppy fish feed (Dernekbasi et al., 2010) on growth and feed conversion in guppy) and white shrimp *Litopenaeus schmitti* feed (Jaime-Ceballos et al., 2005). Supercritical fluid obtained from *S. platensis* exhibits antimicrobial and antioxidant activity (Mendiola et al., 2007), it is safe and employed as a substitute for commercially available synthetic antioxidants.

C. vulgaris is a freshwater based single-celled algae, which contains the highest quantity of chlorophyll of all plants. It is a superfood with an abundant nutrient containing various vitamins and minerals, 18 amino acids (all with the entire essential amino acids inclusive), and 60% protein. *Chlorella* Growth Factor (CGF), which is a phytonutrient is one of its unique properties. CGF is abundant in the nuclei of algae, made up of vitamins, nucleic acid associated substances, amino acids, proteins, peptides and sugars of specific interest because of purification with the aid of peptide glutathione in the CGF (Nick, 2003). Over 20 minerals and vitamins are present in *C. vulgaris*. These include iron, potassium, calcium, phosphorous, magnesium, pro-vitamin A, vitamins B1, B2, B5, B6, B12, C, E and K, biotin, folic acid, inositol, plus vitamins C, E, and K. *C. Vulgaris* exhibits a better activity towards inhibiting peroxidation of lipid when compared with glutathione and also exhibit antioxidant properties (Bengwayan et al., 2010). Generation of reactive oxygen species (ROS) like HO, hydroxyl, and superoxide radical as cellular metabolism proceeds is the characteristic feature of all aerobic organisms. ROS promote the oxidation of biomolecules because they are highly reactive. They are commonly called pro-oxidants (Amin and Hashem, 2012). The antioxidant resistances/defense of the cells containing non-enzymatic antioxidants and antioxidant enzymes neutralized the pro-oxidant activities of ROS (Sies, 2015). In normal cells, there is a delicate balance called redox balance between the antioxidant defenses and pro-oxidant forces. Oxidative stress arises when ROS increase in the absence of oxidant defense. When the lipid content of the feed undergoes oxidation, the feed becomes tasteless, thereby reducing feed intake and fish growth (Taufek et al., 2016). The report of (Avanzo et al., 2002) shows that oxidative stress in aquatic organisms is on the extreme when exposed to xenobiotic, hypoxia and elevated temperature, nutritional deficiency. However, supplementation of a dietary antioxidant containing mineral, vitamins, fatty acid, and amino acid guards the aquatic animals against oxidative stress facilitated by hypoxia and elevated temperature (Hwang and Lin, 2002).

Haematological indices are vital tools for evaluation of physio-pathological and physiological changes in tilapia fish (Hrubec et al., 2000). The ecological features that affect haematological activities are feeding habits and stock density (Turnbull et al., 2005). On the other hand, variations in haematological parameters could be caused by pollutant and other environmental elements.

To combat free-radical damage, terrestrial and aquatic animals utilize a number of defense mechanisms, which include antioxidant enzymes, such as glutathione S-transferase (GST) catalase (CAT), and superoxide dismutase (SOD), and compounds like glutathione, ascorbic acid, polyphenolics, carotenoids and a-tocopherol (Ahmed et al., 2017; Kruger and Mann, 2003). Nonetheless, the antioxidative defense can be reduced by a rise in the level of pollutants, thereby lowering fish production. This problem could be practically solved by lessening oxidative stress, as well as consequent damage via dietary supplementation using natural nutraceuticals and antioxidants (Jayakumar et al., 2011).

Therefore, the study was done to assess the suitability of both algae as FM replacement in African catfish nutrition without negatively affecting their feed efficiency and growth. Thus, this research explored the effect of *S. platensis* and *C. vulgaris* supplementation as a partial replacement for fishmeal on parameters such as growth, haemato-biochemical and antioxidant response in African catfish.

2. Materials and methods

2.1. Experimental diet

S. platensis and *C. vulgaris* powder used for this study were purchased from TST Bioceuticals (Perak, Malaysia). The powder was used to formulate 9 experimental diets based on the protein requirement of clariid fish fingerlings. The diets were formulated in sets of either *S. platensis* or *C. vulgaris* to replace fishmeal at various inclusion levels of 50 and 75% resulting in diet levels of SP50 and SP75 or CL50 and CL75. All the feeds were iso-nitrogenous at 35% crude protein. Diets were formulated using Pearson's Square and WINFEED 2.8 version software (Mirza, 2004). After milling, the ingredients, DCP, minerals, and vitamins were carefully mixed using water, then pelleted into 1 mm particle size in a small pelleting machine before oven drying at 70 °C and preserving in a feed cold room (4 °C) until use.

Table 1 presents the chemical composition formulating the feed ingredients in the experimental diets. Five different isoenergetic and isonitrogenous diets (35.01–36.57% crude protein and 417.243–422.27 Kcal 100 g^{-1} respectively) were formulated and used in the feeding

Table 1
Gross composition (g/100 g Dry Matter) of the experimental diets containing graded level of Spirulina and Chlorella.

	Dietary treatments				
	CTR	SP50	SP75	CL50	CL75
Fishmeal	25	12.5	6.25	12.5	6.25
SBM	43	43	43	43	43
Corn Meal	10	10	10	10	10
Spirulina		12.5	18.75		
Chlorella				12.5	18.75
Vitamin Premix[a]	1.5	1.5	1.5	1.5	1.5
Mineral Premix[b]	1.5	1.5	1.5	1.5	1.5
Methionine	1	1	1	1	1
Lysine	1	1	1	1	1
Fish Oil	1.8	1.8	1.8	1.8	1.8
Binder	15.2	15.2	15.2	15.2	15.2
Total	100	100	100	100	100
Nutrient level determined by as is basis (% dry matter basis)					
Crude protein	36.57	35.87	35.52	35.53	35.01
Crude lipid	8.21	7.71	7.85	7.73	7.87
Ash	9.55	9.94	9.98	9.95	9.87
Moisture	9.22	8.99	8.95	8.91	8.47
Fibre	1.94	1.99	2.02	1.95	2.52
NFE	34.51	35.5	35.68	35.93	36.26
Gross energy Kcal g^{1}	422.27	417.536	417.594	417.538	417.243

Each value is the mean of three replicates. Control (0% algae); SP50, SP75, CL50 and CL75–50% and 75% Spirulina and Chlorella meals respectively.
NFE: Nitrogen free extract, SBM: Soybean meal.

[a] Vitamin premix supplied: vitamins A,500 IU; B1,1.0 mg; B2,0.5 mg; B3,0.3 mg; B6, 0.2 mg; B12,0.001 mg; C,0.1 mg D3 100 IU; E,0.75 mg, K,0.02 mg; niacin,0.2 mg, folic acid,0.1 mg; biotin,0.24 mg; pantothenic acid,1.0 mg; inositol, 2.5 mg.

[b] Mineral premix provided the followings per kg diet: iron, 8.0 mg; selenium, 0.2 mg; magnesium oxide, 0.6 mg; manganese, 1.0 mg; zinc, 8.0 mg; copper, 0.15 mg; potassium chloride, 0.4 mg; sodium bicarbonate, 1.5 mg; iodine,1.0 mg; cobalt, 0.25 mg.

trials. Fishmeal was replaced at 50% and 75% either with *S. platensis* (SP50 and SP75) or *C. vulgaris* meal (CL50 andCL75) and the control is 100 fishmeal (100 FM). The formulated diets were selected based on the results of our previous growth experimental, which revealed the optimum growth of 68.5% and 69.41% for *S. platensis* and *C. vulgaris* respectively.

2.2. Fish and experimental set-up

The *C. gariepinus* were procured from Balakong hatcheries and conveyed in aerated plastic bags (filled with pond water and oxygen) to an aquarium. The juveniles were arbitrarily grouped into 5 each in triplicates of 15*C. gariepinus* (average weight 42.07 ± 0.3 g). All the *C. gariepinus* were adapted to the natural habitat for 14 days before the main experiment. They were fed with conventional diets, twice daily during the adaptation. The feed was given twice daily at 2% of their body weight as the trial period proceeds for over twelve weeks. The total feed supplied was documented and all leftover feed was recovered once the feeding ends, and later weighed to estimate the overall feed intake. The quality of water was frequently checked and mortality was documented. The feeding activity entails the use of fifteen 200 l plastic tanks in a closed re-circulatory system. The tanks were equipped in accordance with the setup of (Taufek et al., 2016) using 50% of water replacement quality assurance. The quality of water in all tanks was monitored according to the procedure described by (APHA, 1992).

2.3. Proximate and chemical scrutiny

The proximate composition of the experimental diets and ingredients were investigated according to the method reported by Association of Official Analytical Chemist (AOAC, 2005). Kjedahl method (method 981.10), was employed to analyzed crude protein after acid digestion Vapodest 50 (Gerhardt Germany). The dry matter and moisture content were obtained by drying to persistent weight at 105 °C in an oven (method 934.01). The ash content of the samples was evaluated by combustion in a muffle furnace (Memmert UFB500 and Carbolite Furnace Memmert CWF 11/13 Germany) at 600 °C (method 942.05). The amount of crude lipid in the diet was obtained by using petroleum ether extraction (Gerhardt Soxtherm) per Soxhlet method (method 945.18), while the Fibre content was obtained after alkali and acid digestion (method 962.09). The Nitrogen Free Extract (NFE) was estimated as = 100 - (% ash + % crude fat + % crude protein). The dietary body composition and gross energy were estimated using the following model: CP × 5.65 + lipid × 9.45 + carbohydrates × 4 (Jobling, 1983).

2.4. Analysis of amino acid (AA)

The AA profiles of the experimental diets were obtained with the aid of High-Performance Liquid Chromatography (HPLC). The samples were analyzed by matching the peak retention time to that of a recognized standard according to the technique described by Heinrikson and Meredith (1984), whereas the alkaline hydrolysis as described by (Nielsen and Hurrell, 1985) was used to determine the Tryptophan.

2.5. Fatty acid determination

The fatty acid (FA) composition of the experimental diets was determined by first measuring the lipid contents of dried feed samples gravimetrically (Bligh & Dyer, 1959). The resultant fats were trans-esterified using 1.2% HCl in MeOH, water, and toluene (100 °C, 1 h) to obtain fatty acid methyl esters (Nobakht et al., 2012) (Ichihara and Fukubayashi, 2010). The FAME composition of the individual samples was evaluated by using Agilent 7820, a gas chromatograph fitted with a capillary column (SLB-IL 100, 30 m × 0.25 × 0.2 Supelco, USA) in addition to a flame ionization detector (FID) using the temperatures of detector and injector at 260 and 250 °C correspondingly (Nobakht et al., 2012). Different FAMEs were recognized by matching their retaining times with those of authenticated standards from (Sigma-Aldrich®, USA). The FAs were quantified in milligram per gram of lipids, by the addition of an internal standard C7:0 Sigma®, USA (Vello et al., 2014).

2.6. Sample preparation

After 12 weeks of the experiment, fish samples were randomly collected from each tank five for liver analysis. Each of the liver samples was marked, noting their body weights and lengths, before dissecting them to their anatomical parts, weighed and recorded appropriately. The livers and gonads were removed and weighed to determine the hepatic and gonad somatic index (HSI' % and GS%). Homogenization of liver was performed in 6 ml buffer using 0.6 g sample each. The buffer solution consists of 0.1 mM phenylthiourea (PTU), 0.1 mM dithiothreitol (DTT), 1.0 mM EDTA, 0.1 mM protease inhibitor, and 25 mM sodium phosphate buffer (pH 7.4). The homogenates were centrifuged as described by (Taufek et al., 2016).

Prior to liver excision, blood samples were collected from the caudal vein of the fishes with the aid of a sterile 2 ml syringe, after anesthetizing with clove oil (40 mg/L of water). The blood was then separated into two parts, the first part was extracted into an EDTA tube and immediately used for haematological indices (CBC), while the second was collected in special serum tubes and used for serum biochemical analysis. The blood was then centrifuged at 5000 rpm for 10 min to collect the serum and used to determine serum protein, albumin, lipid profile (triglycerides, total, HDL and LDL cholesterol). CBC was estimated with automatic haematology analyzer; Sysmex XN (Germany), whereas serum biochemical parameters were evaluated with Advia 2400 Chemistry System Siemens Healthiness (Germany).

2.7. Liver protein concentration

The protein concentration of liver was obtained using the Bio-Rad DC colorimetric protein assay (Bio-Rad 500–01 16) on the basis reaction of protein with an alkaline copper tartrate in a two steps process leading to colour development.

1) the interaction of protein with copper in an alkaline solution and
2) the successive reduction of Folin reagent using copper-treated protein (Lowry et al., 1951; Peterson, 1977, 1979).

The reagents package for this assay contains Reagent A (alkaline copper tartrate solution), REAGENT B (dilute Folin Reagent), and reagent S. Bovine (serum albumin (Bio-Rad 500–0007 medium) were used as the standard. 200 µl of reagent S was added to 10 ml of reagent A (working reagent A'). A 10 mg/ml stock solution of Bovine serum albumin (BSA) was prepared, out of which eight standard solutions were formulated ranging from 0.2–1.6 mg/ml. 5 µl each of standards and samples were pipetted into each well of a clean, dry 96 well plate. This was followed by addition 25 µl of reagent A and finally 200 µl reagent B. The plate was then placed in a reader to mix the reagents for 5 s. The mixture was left to stand for 15 min before absorbance was observed at 650 nm. The quantity of BSA in the samples was plotted against their corresponding mean absorbance. The amount of protein in the samples was determined from the curve.

2.8. Oxidative stress assay

The CAT activity was assayed as described by (Claiborne, 1985). The reaction mixture comprises 50 mM Na_3PO_4 buffer at neutral pH and 19 mM H_2O_2 prepared by using a Na_3PO_4 buffer. 300 µl of H_2O_2, 50 µl of samples and 2.65 ml of Na_3PO_4 buffer were mixed to a cuvette in a 3 ml reaction mixture. The reaction was measured at 25 °C by recording the consumption of H_2O_2 at 240 nm. The CAT activity was

Table 2
Amino Acid Profile of the experimental diets containing graded level of Spirulina and Chlorella.

	Dietary treatments					**
	CTR	SP50	SP75	CL50	CL75	
Hydroproxiline	3.06 ± 0.01	3.54 ± 0.02	3.79 ± 0.02	3.29 ± 0.03	3.41 ± 0.01	
Aspartic acid	1.78 ± 0.01	1.89 ± 0.02	1.94 ± 0.04	1.76 ± 0.02	1.74 ± 0.01	
Serine	4.86 ± 0.01	5.15 ± 0.02	5.30 ± 0.17	4.88 ± 0.03	4.89 ± 0.03	
Glutamic acid	3.09 ± 0.02	3.40 ± 0.12	3.55 ± 0.02	3.39 ± 0.02	3.53 ± 0.01	
Glycine	0.95 ± 0.01	0.91 ± 0.02	0.90 ± 0.01	0.92 ± 0.01	0.91 ± 0.02	
Histidine*	2.47 ± 0.01^{c}	2.62 ± 0.01^{b}	2.69 ± 0.03^{a}	2.43 ± 0.02^{c}	2.41 ± 0.02^{c}	1.2**
Arginine*	1.54 ± 0.02^{b}	1.66 ± 0.03^{a}	1.72 ± 0.02^{a}	1.55 ± 0.02^{b}	1.55 ± 0.01^{b}	3.6**
Threonine*	1.53 ± 0.01^{c}	1.79 ± 0.03^{b}	1.92 ± 0.01^{a}	1.80 ± 0.06^{b}	1.94 ± 0.02^{a}	2.80**
Alanine	1.71 ± 0.03	1.74 ± 0.02	1.75 ± 0.02	1.80 ± 0.12	1.85 ± 0.02	
Proline	3.16 ± 0.02	3.65 ± 0.02	3.89 ± 0.02	3.40 ± 0.17	3.52 ± 0.01	
Cysteine	0.29 ± 0.02	0.27 ± 0.02	0.26 ± 0.02	0.22 ± 0.01	0.18 ± 0.01	
Tyrosine	1.11 ± 0.01	1.22 ± 0.01	1.28 ± 0.02	1.09 ± 0.02	1.08 ± 0.01	
Valine*	1.71 ± 0.02^{d}	1.94 ± 0.02^{b}	2.06 ± 0.02^{a}	1.85 ± 0.01^{c}	1.92 ± 0.01^{b}	2.40**
Methionine*	1.53 ± 0.02	1.50 ± 0.06	1.48 ± 0.02	1.48 ± 0.02	1.46 ± 0.02	2.3*
Lysine*	3.32 ± 0.02^{b}	3.27 ± 0.02^{b}	3.24 ± 0.02^{b}	3.41 ± 0.01^{a}	3.46 ± 0.02^{a}	4.80**
Isoleucine*	1.51 ± 0.01	1.74 ± 0.02^{b}	1.86 ± 0.01^{a}	1.56 ± 0.02^{c}	1.59 ± 0.02^{c}	2.0**
Leucine*	2.68 ± 0.02^{b}	2.90 ± 0.17^{ab}	3.01 ± 0.03^{a}	2.85 ± 0.02^{ab}	2.94 ± 0.02^{ab}	3.50**
Phenylalanine*	1.63 ± 0.02^{c}	1.73 ± 0.02^{a}	1.79 ± 0.02^{a}	1.68 ± 0.02^{b}	1.70 ± 0.03^{b}	4.00*a
Tryptophan*	0.35 ± 0.01	0.34 ± 0.02	0.34 ± 0.01	0.34 ± 0.01	0.33 ± 0.01	0.5[1]

Values are means of triplicates of five different feed samples. Mean values on the same row with unlike subscript are significantly different ($P < .05$). Control (0% algae); SP50, SP75, CL50 and CL75–50% and 75% *Spirulina* and *Chlorella* supplemented meals respectively.
*b Methionine + Cysteine.
a* Phenylalanine/TAA (Total Amino Acid).
* Essential amino acids.
** Essential amino acid requirement (Jimoh et al., 2014).
[1] Essential amino acid requirement of *C. gariepinus*. Source: (Uys, 1989); (Unprasert, 1994).

obtained as nmol H_2O_2 disappeared/min/mg protein (ε_{240nm} = 0.0436 m/M/cm).

The SOD activity was determined as adopted by (Taufek et al., 2016). In the reaction mixture, the final concentration comprises 0.005 mM xanthine oxidase, 0.05 mM xanthine, 0.01 mM cytochrome *c*, 0.1 mM EDTA and 50 mM sodium phosphate buffer. The reaction commenced upon addition of xanthine oxidase to the enzyme extract at 25 °C and 550 nm absorption. The SOD activity is a measure of its ability to prevent 50% cytochrome *c* reduction, and the results were given as nmol/min/mg protein.

The GST activity was determined by obtaining its response to 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm (Taufek et al., 2016). The assay comprises 60 mM CDNB (dissolved in ethanol), 60 mM glutathione (GSH), and 100 mM sodium phosphate buffer (pH 6.5). The activity of the GST was obtained as the quantity of enzyme that catalyzed the conjugate of GSH per min and 1 µmol of CDNB at 25 °C (ε_{340nm} = 9.6 mM^{-1} cm^{-1}), which was obtained as nmol/min/mg protein.

2.9. Growth performance indices

The feed supplied fish weights and was recorded every two weeks to determine the growth indices according to Oliva-Teles and Goncalves (2001).

Mean weight gains = $(W_f - W_i)/n$
where: W_f: final weight; W_i: initial weight; and *n*: number of fish.
Relative growth rate = $W_g \times 100/W_i$
where: W_g = weight gain and W_i = initial weight.
Specific growth rate = $(logW_f - logW_i) \times 100/t$
where: *t* = time.
Feed conversion ratio (FCR) = F_i/FW_g
where: F_i = dry feed fed and FW_g = fish wet weight gain.
Protein efficiency ratio (PER) = MWG/MPI
where: MWG = mean weight gain and MPI = mean protein fed.
Survival rate = Fn/In × 100
where Fn = final quantity of fish at the end of the experiment and In = initial quantity of fish at the beginning of the experiment.
Protein Productive Value (PPV) = FPE/FPB × 100
where FPE = total fish protein at the end and FPB = total fish protein at the beginning of feeding experiment.
Condition (K) factor = FW × 100/L^3
where W = the weight of fish (g) and L = standard length (cm) (Htun-Han, 1978).
Haematological parameters (RBC indices) were determined based on (Seiverd, 1983).
MCV (fl) = Hct/Hgb.
MCH (pg.) = (Hgb × 100)/RBC.
MCHC (gdl^{-1}) = (Hgb/Hct) × 100.
Where MCV = Mean corpuscular volume.
MCH = Mean corpuscular haemoglobin and.
MCHC = Mean corpuscular haemoglobin concentration.

2.10. Statistical analysis

All data were analyzed using SPSS version 21.0 based on one-way analysis of variance (ANOVA). The variances between means were obtained at 5% ($P < .05$) level of probability by using Duncan's post hoc test. The data were obtained as mean ± standard error of mean (SEM).

3. Results

Table 1 presents the gross composition of the experimental diet and nutrient, which reveals that all the diets are isonitrogenous (35%) and isoenergertic (417 Kcal 100 g^{-1}). The crude protein, crude lipid, and moisture content decreased, while the ash and fibre levels increased with increase in the level of supplementation of both *S. platensis* and *C. vulgaris*.

Table 2 presents the amino acid profile of the experimental diets containing a graded level of *S. platensis* and *C. vulgaris*. There is no significant increase in the tryptophan value of all the diets including the control diet. Supplementation with *C. vulgaris* has no significant effect

Table 3
Fatty Acid Profile of the experimental diets containing graded level of Spirulina and Chlorella.

	Dietary treatments					
	CTR	SP50	SP75	CL50	CL75	S.E.M.
SFA	42.55^{b}	40.73^{e}	42.76^{a}	41.56^{c}	41.06^{d}	0.989
MUFA	6.43^{c}	6.35^{bc}	6.79^{a}	6.67^{ab}	6.79^{a}	0.165
PUFA	20.02^{e}	21.92^{b}	23.86^{a}	20.78^{d}	21.15^{c}	1.218
Σn-6	9.99^{e}	11.17^{b}	12.07^{a}	10.25^{d}	10.38^{c}	0.788
C18:3 n-4	0.58^{c}	1.88^{b}	2.58^{a}	0.60^{c}	0.62^{c}	0.956
C20:5 n-3(EPA)	1.04^{c}	1.08^{bc}	1.16^{a}	1.11^{ab}	1.15^{a}	0.019
C22:6 n-3(DPA)	0.88^{d}	0.97^{c}	1.05^{c}	1.36^{b}	1.61^{a}	0.279
Σn-3	5.36^{b}	5.00^{b}	5.15^{b}	5.87^{a}	6.12^{a}	0.536
Σn-9	3.99^{a}	3.74^{b}	3.90^{a}	3.96^{a}	3.94^{a}	0.109
n-3/n-6 ratio	0.54^{a}	0.45^{b}	0.43^{b}	0.57^{a}	0.59^{a}	0.082

Values are means of triplicates of five different feed samples. Mean values on the same row with unlike subscript are significantly different (P < .05). Control (0% algae); SP50, SP75, CL50 and CL75–50% and 75% Spirulina and Chlorella meals respectively.
PUFA: Polyunsaturated fatty acids; MUFA: Monounsaturated fatty acids; SFA: Saturated fatty acids, EPA: Eicosapentaenoic acid and DPA: docosahexaenoic acid.
Σn-6 (comprising C18:2 n-6 t, C18:2 n-6C, C18:3 n-6 and C20:3 n-6).
Σn-3 (C18:3 n-3, C18:4 n-3, C20:3 n-3, C20:5 n-3 and C22:6 n-3).
Σn-9 (C18:1n-9c, C20:1n-9, C22:1 n-9 and C24:1 n-9).
Composition of major fatty acids of experimental diets.

on the value of arginine in the diet but it increased upon supplementation with *S. platensis*. Supplementation with *S. platensis* and *C. vulgaris* reduced the values of glycine, cysteine, and methionine in the diets. The value of aspartic acid, Histidine and Tyrosine decreased upon addition of *C. vulgaris* to the diet, while there values increased upon addition of *S. platensis*. Furthermore, the value of all other amino acid significantly increased upon the inclusion of *S. platensis* and *C. vulgaris* in the diet.

Table 3 presents the FA profile of the isonitrogenous and isoenergetic experimental diets containing a graded level of *S. platensis* and *C. vulgaris*. High level of *S. platensis* favored the high concentration of FA except for the sum of n-9 and n-3 FA. The fatty acid content was highest at 75% *S. platensis* supplementation. This indicates that high level of supplementation of *S. platensis* resulted in a higher total content of monounsaturated, PUFA, as well as saturated fatty acid. Similarly, high level of *C. vulgaris* engendered high concentration of FA except for saturated and the sum of n-3 FA. The FA content was highest at 75% *C. vulgaris* supplementation. This indicates that high level of supplementation of *C. vulgaris* resulted in a higher total content of monounsaturated and PUFA. Comparing the two supplements, SP75 has the highest content of PUFA, SFA, Σn-6, and C18:3 n-4, while CL75 has the highest amount of Σn-3, Σn-9, DPA (docosahexaenoic acid), as well as the n-3/n-6 ratio. They both have a similar amount of MUFA and EPA.

Table 4 presents the Liver protein, GST, CAT, and SOD activity of African catfish fed *S. platensis* and *C. vulgaris* enriched diets GST and liver protein in all the supplemented diet displayed slightly lower activities when compared with the control diets but with insignificant differences. The enriched diets showed significantly higher CAT activities when compared with the control diet with CL75 showing the highest activity but no significant difference between CL75 and SP75.

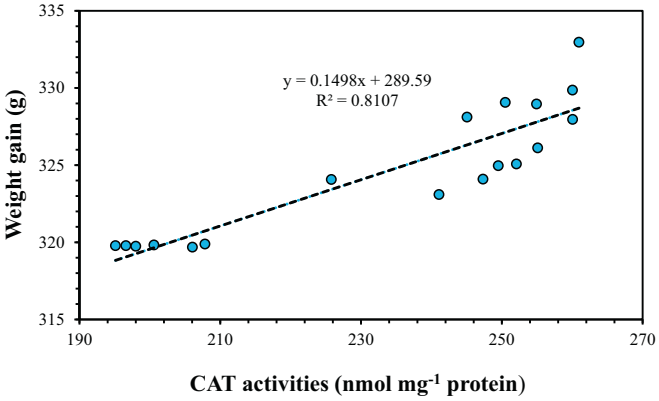

Fig. 1. Correlation between catalase activity and mean weight gain of fish fed Spirulina supplemented diets. The results mean ± standard error (SE) of five fish/tank (15 fish per treatment).

No significant difference was detected in GST activity with the level of diet supplementation for both *S. platensis* and *C. vulgaris* but SP75 shows the highest activity. However, there was a significant increase in the SOD activity. The activities of liver protein decreased upon incorporation of *S. platensis* and *C. vulgaris* but further increase in the level of incorporation yield no significant change.

Fig. 1 and Fig. 2 illustrates the correlation between liver catalase activities, and mean weight gain of African catfish fed *S. platensis* and *C. vulgaris* supplemented diets. The liver catalase activities of both *S. platensis* and *C. vulgaris* are characterized by increasing weight gain.

Table 5 presents the assessment growth performance of juvenile *C. gariepinus* fed graded levels of *S. platensis* and *C. vulgaris*. The outcomes show that *S. platensis* and *C. vulgaris* are good replacements for fishmeal as a source of protein in the diet of African catfish. The feed intake reduced, while the SGR increased upon supplementation with *S. platensis* and *C. vulgaris*. The body weight gain (BDWG) increased significantly as the level of supplementation increased. The highest BDWG was observed with CL75, which also exhibit the best FCR and PER. The best protein protective value (PPV) was obtained at 50% replacement for both *S. platensis* and *C. vulgaris*. A further increase to 75% led to a significant decrease in PPV for *S. platensis* but insignificant for *C. vulgaris*. The value of K factor and HIS increased significantly upon supplementation with *S. platensis* and *C. vulgaris*, and the highest values were observed with CL75.

Table 6 shows the haematological indices of *C. gariepinus* fed of graded levels *S. platensis* and *C. vulgaris* meals. The value of HGB, MCV, total protein and HDL cholesterol increased upon supplementation with *S. platensis* and *C. vulgaris*, and a further increase in the level of supplementation yield no significant change. MCH, MCHC, and LDL Cholesterol slightly decreased upon supplementation with *S. platensis* and *C. vulgaris*, and further increase in the level of supplementation yield no significant change. The value of HCT, RBC, WBC, globulins and albumin significantly increased upon supplementation with *S. platensis* and *C. vulgaris* but further increase in the level of supplementation yield no significant change. However, the level of albumin decreased, while that of globulins increased with increasing level of supplementation with *C.*

Table 4
Liver protein, Glutathione s-transferase Catalase (CAT) and Superoxide dismutase (SOD) activity of African catfish fed Spirulina and Chlorella diets.

Enzymes and liver protein	Control	SP50	SP75	CL50	CL75	*P* Value
Liver protein (mg/ml)	0.67 ± 0.01^{a}	0.60 ± 0.03^{b}	0.61 ± 0.01^{b}	0.61 ± 0.01^{b}	0.62 ± 0.01^{b}	0.044
CAT (nmol mg^{-1}protein)	200.71 ± 0.01^{c}	244.41 ± 0.52^{b}	256.00 ± 0.58^{a}	243.51 ± 0.40^{b}	256.81 ± 0.50^{a}	0.000
GST (nmol mg^{-1}protein)	175.87 ± 0.03^{a}	175.80 ± 0.06^{a}	175,82 ± 0.05^{a}	175.81 ± 0.06^{a}	175.83 ± 0.05^{a}	0.874
SOD (nmol mg^{-1}protein)	33.54 ± 0.06^{b}	42.33 ± 0.11^{a}	42.35 ± 0.06^{a}	42.32 ± 0.06^{a}	42.34 ± 0.06^{a}	0.000

Mean ± SE of fifteen fish per dietary treatment, five fish/tank. Mean values in the same row with different superscript are statistical different ($p < .05$).

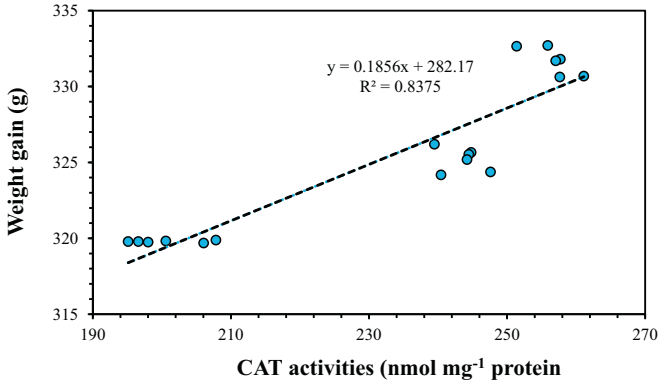

Fig. 2. Correlation between catalase activity and mean weight gain of fish fed Chlorella supplemented diets. The results mean ± standard error (SE) of five fish/tank (15 fish per treatment).

vulgaris. On the other hand, plasma glucose, albumin-globulin ratio and total cholesterol (TCL) significantly decreased upon supplementation with *S. platensis* and *C. vulgaris* but further increase in the level of supplementation yield no significant change. The amount of triglyceride decreased significantly as the level of supplementation increased for both *S. platensis* and *C. vulgaris*.

4. Discussion

This study explored the antioxidant activities and growth performance of African catfish using *S. platensis* and *C. vulgaris* as a substitute for fishmeal being the source of protein in the fish diet. A comparative study was carried out for all the experimental diet. Although the usage of *S. platensis* and *C. vulgaris* as a source of protein for other aquatic animals have been previously investigated (Radhakrishnan et al., 2014; Wu et al., 2016), little has been reported about the relationship between Spirulina and antioxidant defense of African catfish. Radhakrishnan et al. (2014) previously reported that *S. platensis* and *C. vulgaris* could be used as a substitute for fishmeal in the diet of *Macrobrachium rosenbergii*. They claimed that the supplemented diet improved vitamins C and E, and lowered the activities of enzymatic antioxidants (LPx, SOD, and CAT), which indicated that the formulated diets are not toxic and produce no stress to post-larvae.

In this study, an increase in the level of *S. platensis* and *C. vulgaris* supplementation boost the nutrient efficiency and growth of fishes. Although the reduction in feed consumption is not significant, the feed efficiency increases significantly upon supplementation, indicating positive growth response in the juvenile fishes. This is could be because of the high digestibility of *S. platensis* and *C. vulgaris* because of stimulation of the intestinal flora of fish thereby increasing the activity of digestive enzymes resulting in efficient diet utilization (James et al., 2006; Khani et al., 2017). A considerable increase in weight gain was also observed at 75% supplementation for both fresh algae, leading to a higher value of SGR in the fishes fed with supplemented diets. Furthermore, the observed increase in the value of HSI in fish fed with the supplemented diets could be because of high lipid and accumulation of glycogen in the liver (Cazenave et al., 2006). This shows the availability of a large amount of food at a favourable aquatic environment for growth for the samples fed supplemented diet. Fishes with higher HIS values are more energetic because HIS value is related to the performance and size of the liver. The favourability of the environmental condition is also confirmed by the increase in the value of the K factor, which increases significantly upon diet supplementation. The higher growth rate experienced with the fishes fed supplemented diet is also attributable to the digestibility and the nutritional value of the two fresh microalgae, which are higher when compared with that of the fishmeal.

S. platensis and *C. vulgaris* are better sources of amino acids for African catfish meal than fishmeal. All the essential amino acids (EAA) are higher in the supplemented diets except methionine and tryptophan. However, the presence of other amino acids like cysteine is a viable alternative, which could replace ~60% of methionine needed in the fish diet. Moreover, lysine content decreased upon supplementation with *S. platensis* (Lovell, 1989).

The physiological status of the experimented catfish was investigated by using haematological parameters. These parameters are also used for assessment of nutritional status and feed composition relative to the environmental conditions that affect the fish (Svobodová et al., 2005).

White blood cell, red blood cell, and haematocrit (HCT) are employed in checking feed toxicity and fish health (Ozovehe, 2013). The increase in RBC and HCT without corresponding increase in HGB the supplemented diet is capable of regulating the amount of protein in the red blood cell, thereby maintaining the physiology of the fish. The insignificant change in the values of HGB, MCH, and MCHC, observed throughout the levels of supplementation implies that replacement of fishmeal with neither of the two freshwater algae did not penalize the health status of the fishes. The obtained values in all the experimental diets agree with haematological parameters of healthy catfish (Dienye and Olumuji, 2014; Erhunmwunse and Ainerua, 2013; Taufek et al., 2016). Although there was no significant increase in the level of albumin with an increase in the level of supplementation, the level of albumin slightly increased upon supplementation with *S. platensis*, while supplementation with *C. vulgaris* results in a significant decrease in albumin level. Albumin and globulins help to sustain the osmotic pressure to maintain a healthy immune system and serve as a plasma

Table 5
Growth performance of *C. gariepinus* juveniles fed graded levels of Spirulina and Chlorella.

Variables	Control	SP50	SP75	CL50	CL75	*P* values
FW	361.89 ± 0.03^{c}	367.16 ± 0.03^{b}	371.01 ± 0.05^{ab}	367.26 ± 0.43^{b}	374.08 ± 0.29^{a}	0.001
IW	42.11 ± 0.18^{a}	42.07 ± 0.03^{a}	42.05 ± 0.16^{a}	42.08 ± 0.04^{a}	42.03 ± 0.03^{a}	0.988
BDWG	319.78 ± 0.02^{c}	325.09 ± 1.54^{b}	328.96 ± 2.31^{ab}	325.18 ± 0.41^{b}	332.05 ± 0.59^{a}	0.001
FI	249.66 ± 0.66^{a}	249.14 ± 0.06^{a}	249.42 ± 0.07^{a}	249.21 ± 0.03^{a}	249.01 ± 0.01^{a}	0.582
FCR	0.80 ± 0.01 ^{a}	0.77 ± 0.01^{b}	0.76 ± 0.01^{b}	0.77 ± 0.01^{b}	0.75 ± 0.01^{b}	0.022
SGR	2.56 ± 0.01 ^{b}	2.58 ± 0.01^{ab}	2.59 ± 0.01^{ab}	2.58 ± 0.01^{ab}	2.60 ± 0.01^{a}	0.013
PER	3.03 ± 0.01 ^{c}	3.11 ± 0.02^{b}	3.13 ± 0.02^{b}	3.11 ± 0.01^{b}	3.18 ± 0.01^{a}	0.000
PI	105.51 ± 0.36 ^{a}	104.64 ± 0.04^{bc}	105.18 ± 0.07^{ab}	104.64 ± 0.04^{bc}	104.51 ± 0.04^{c}	0.007
PPV	10.81 ± 0.05 ^{e}	13.11 ± 0.01^{a}	12.59 ± 0.01^{b}	12.37 ± 0.01^{c}	12.35 ± 0.01^{c}	0.000
PR	11.41 ± 0.02 ^{e}	13.72 ± 0.02^{a}	13.24 ± 0.02^{b}	12.94 ± 0.02^{c}	12.80 ± 0.02^{d}	0.000
K Factor	1.46 ± 0.07 ^{b}	1.55 ± 0.04^{ab}	1.56 ± 0.06^{ab}	1.55 ± 0.01^{ab}	1.62 ± 0.01^{a}	0.268
HSI	1.43 ± 0.01 ^{c}	1.59 ± 0.03^{b}	1.58 ± 0.02^{b}	1.59 ± 0.02^{b}	1.70 ± 0.01^{a}	0.000

Values displayed represents the means ± SE of 5 fish/tank with a total of fifteen fish per diet. Mean values within the same row with different superscripts are significantly different. FCR: Feed efficiency ratio; SGR: Specific growth rate; PER: Protein efficiency ratio; PI: Protein intake; PPV: Protein productive value; PR: Protein retention; K Factor: Fulton K condition factor; HIS: Hepatosomatic index.

Table 6
Haematological characteristics of *C. gariepinus* fed of graded levels Spirulina and Chlorella meals.

Variables	Initial	Control	SP50	SP75	CL50	CL75	P value
HGB(gdl^{-1})	7.20 ± 0.40 ^{b}	11.21 ± 0.01 ^{a}	11.60 ± 0.03 ^{a}	11.59 ± 5 ^{a}	11.59 ± 0.01 ^{a}	11.55 ± 0.03 ^{a}	0.000
HCT%	31 ± 0.58 ^{c}	41.00 ± 0.04 ^{b}	43.35 ± 0.02 ^{a}	43.30 ± 0.01 ^{a}	43.32 ± 0.03 ^{a}	43.28 ± 0.02 ^{a}	0.000
RBC (10^{6} cells mm^{-3})	2.27 ± 0.01 ^{d}	3.11 ± 0.01 ^{c}	3.26 ± 0.01 ^{a}	3.23 ± 0.01 ^{ab}	3.24 ± 0.01 ^{ab}	3.22 ± 0.02 ^{ab}	−000
MCV (fl)	134.22 ± 2.19 ^{a}	131.83 ± 0.12 ^{a}	132.98 ± 0.40 ^{a}	134.06 ± 0.57 ^{a}	133.71 ± 0.57 ^{a}	134.41 ± 0.58 ^{a}	0.468
MCH (pg.)	31.55 ± 1.76 ^{b}	36.05 ± 0.08 ^{a}	35.58 ± 0.14 ^{a}	35.85 ± 0.05 ^{a}	35.77 ± 0.07 ^{a}	35.87 ± 0.12 ^{a}	0.006
MCHC(gdl^{-1})	23.18 ± 0.87 ^{b}	27.34 ± 0.04 ^{a}	26.76 ± 0.18 ^{a}	26.74 ± 0.13 ^{a}	26.75 ± 0.16 ^{a}	26.69 ± 0.12 ^{a}	0.000
WBC (10^{3}cells mm^{-3})	90.00 ± 0.06 ^{c}	93.00 ± 0.58 ^{b}	94.96 ± 0.06^{a}	94.93 ± 0.08 ^{a}	94.85 ± 0.06^{a}	94.80 ± 0.05 ^{a}	0.000
Plasma glucose $mgdl^{-1}$	78.38 ± 0.02 ^{a}	78.01 ± 0.06 ^{b}	77.88 ± 0.01 ^{c}	77.81 ± 0.04 ^{c}	77.82 ± 0.03 ^{c}	77.79 ± 0.01 ^{c}	0.000
Total Protein gdl^{-1}	2.60 ± 0.06 ^{c}	3.82 ± 0.06 ^{b}	4.06 ± 0.02 ^{a}	4.05 ± 0.02 ^{a}	4.05 ± 0.05 ^{a}	4.03 ± 0.01 ^{a}	0.000
Albumin gdl^{-1}	1.30 ± 0.06 ^{c}	1.65 ± 0.03 ^{a}	1.65 ± 0.03 ^{a}	1.66 ± 0.03 ^{a}	1.60 ± 0.03 ^{ab}	1.50 ± 0.02 ^{b}	0.000
Globulin gdl^{-1}	1.30 ± 0.06 ^{d}	2.17 ± 0.02 ^{c}	2.41 ± 0.0 ^{b}	2.39 ± 0.03 ^{b}	2.45 ± 0.03 ^{ab}	2.53 ± 0.01 ^{a}	0.000
Albumin/globulin ratio	1.00 ± 0.10 ^{a}	0.76 ± 0.12 ^{b}	0.68 ± 0.02 ^{bc}	0.69 ± 0.03 ^{bc}	0.65 ± 0.02 ^{bc}	0.57 ± 0.02 ^{c}	0.001
Triglyceride $mgdl^{-1}$	88.50 ± 0.06 ^{a}	80.98 ± 0.02 ^{b}	80.76 ± 0.02 ^{b}	80.67 ± 0.02^{b}	80.68 ± 0.01^{b}	80.61 ± 0.04^{b}	0.000
TCL $mgdl^{-1}$	183.40 ± 1.11 ^{a}	117.02 ± 0.01 ^{b}	114.89 ± 0.06 ^{c}	114.68 ± 0.05 ^{c}	114.69 ± 0.06 ^{c}	114.53 ± 0.03 ^{c}	0.000
HDL Cholesterol $mgdl^{-1}$	51.74 ± 0.05 ^{b}	71.98 ± 0.02 ^{a}	72.07 ± 0.01 ^{a}	72.11 ± 0.03 ^{a}	72.08 ± 0.03 ^{a}	72.13 ± 0.02 ^{a}	0.000
LDL Cholesterol $mgdl^{-1}$	112.74 ± 0.22^{a}	90.68 ± 0.04 ^{b}	90.45 ± 0.03 ^{b}	90.41 ± 0.06 ^{b}	90.42 ± 0.05 ^{b}	90.40 ± 0.06 ^{b}	0.000

Values displayed represents the means ± SE of triplicate groups of 5 fish/tank with a total of fifteen fish per diet. Mean values within the same row with different superscripts are significantly different.

carrier (Nya and Austin, 2009). The rise in the level of albumin and total protein for the fishes fed *S. platensis* diet in this study corroborates with the report of (Yeganeh et al., 2015). The decrease in albumin level observed for Chlorella supplemented diet could be ascribed to reduction in the level of certain essential amino acid (histidine) upon supplementation Albumin-globulin ratio helps in the evaluation of different kidney and liver diseases. The observed decrease in the albumin/globulin ratio corroborates with the report of (Andrews et al., 2011).

The level of WBC and RBC differ significantly in all the experimental diets, which indicate the presence of physiological properties, and increasing quantities of antigens in the circulating system (Taufek et al., 2016). An increase in RBC also improves the fish wellbeing by facilitating oxygen transportation capacity. Increase in WBC indicates anti-infection properties and immunostimulatory effects of *S. platensis* and *C. vulgaris* (Yeganeh et al., 2015). The phycocyanin in Spirulina and the β-1,3-glucan in Chlorella have been linked with the aforementioned properties as seen in rainbow trout (Yeganeh et al., 2015) and Koi carp (Khani et al., 2017). Furthermore, none of the values across the experimental diets falls below the normal range peculiar to healthy catfish (Musa and Omoregie, 1999). Therefore, it is tenable to say that the haematological parameters revealed that catfishes fed supplemented diet had better health status than the catfishes fed fishmeal.

The CAT activities, which correlate with rising H_2O_2 concentration were found to increase the level of supplementation. Positive relationships were observed between CAT and weight gain of the catfish fed with diets supplemented with *S. platensis* and *C. vulgaris* (R2 = 0.8107 and 0.8375, respectively), which indicated that improved CAT activities were stimulated by an increase in the growth response because of the high rate of metabolism. This corroborates with the report of (Taufek et al., 2016), which stated that the CAT activity increased in African catfish and, thereby increasing their weight.

The inclusion of *S. platensis* and *C. vulgaris* in the diets have no significant effect on the GST activity but reduced slightly. This implies that the fishmeal diet and the supplemented diets have a similar amount of compounds capable of stimulating xenobiotics biotransformation. GST helps to detoxify products of oxidative stress via catalysis of conjugation of a variety of metabolites, which include lipoperoxidation products and xenobiotic metabolites, with GSH and by converting the toxic complexes into substances that can be discharged more easily.

The significant increase in SOD upon diet supplementation with microalgae is due to the presence of SOD in microalgae. Microalgae such as *S. platensis* and *C. vulgaris* have the capacity to express several enzyme antioxidants such as ascorbate peroxidase, SOD, CAT, and a non-specific peroxidase. The activities of SOD slightly increased with increase in the level of supplementation with SP75 demonstrating the highest activity. CAT–SOD enzyme mechanism represents the first line of defense against ROS. The defense against ROS production by the Mehler reaction is due to the presence of FeSOD in microalgae, in connection with photosystem I. Therefore, SOD catalyzes superoxide anions reduction to H_2O_2, which was subsequently disintegrated by CAT at extra- and intracellular levels (Taufek et al., 2016). The studies of (Han et al., 2011) and (Sahin et al., 2014) agree with this trend. SOD is a major antioxidant defense that protects tissues and cell against oxidative stress, and also ensure immunity against bacterial infection (Xu et al., 2014). The observed increase in the level of SOD indicates that both *S. platensis* and *C. vulgaris* have some bioactive substances capable of regulating the immune response of catfish. Moreover, the significant increase in the CAT activity with increase in the level of supplementation has a positive impact on the weight gain for both micro algae species. This is confirmed by the enhanced feed efficiency observed with increase in the supplementation level.

It was revealed that the FA profile in the two freshwater microalgae was distinctively different (Table 3). This shows the differences in the quality of food provided by different species of microalga, which is consequential on the consumption responses in the aquatic food web. Microalgae have the highest potential to produce long-chain PUFA when compared with other organisms in the aquatic food web (Krienitz and Wirth, 2006). On the other hand, most animals are unable to produce essential FAs. The presence of PUFA in the supplemented diet will boost the life cycle of consumer populations, for instance, the development and ontogenetic cycle of catfish. Diet supplementation with *S. platensis* and *C. vulgaris* also boost the EPA (eicosapentaenoic acid, C20:5) content, which is responsible for the formation of the membrane. Apart from being a protein source, *S. platensis* and *C. vulgaris* are also capable of performing a therapeutic function including anti-inflammatory, immunomodulatory, and antioxidant activities, which could play a vital role in animal health. They boost the activity of SOD and CAT, prevent DNA damage and lipid peroxidation, scavenges free radicals, and stimulate cellular antioxidant enzymes (Wu et al., 2016).

5. Conclusion

The results of this study reveal that two freshwater microalgae, *S. platensis,* and *C. vulgaris* are capable of improving the feed efficiency, as well as the growth performance of African catfish. The antioxidant responses of the supplemented diet increased the activity of CAT, while no significant change was observed in the activities of GST. However, there was a significant increase in the SOD activity. These reveal that *S. Vlatensis* and *C. vulgaris* are potential antioxidants booster for African

catfish. Furthermore, the obtained haematological parameters show a significant increase in the value of red and white blood cells upon supplementation but decrease slightly when in excess. This also established that appropriate dietary formulations of *S. platensis* and *C. vulgaris* are suitable diets for African catfish. This indicates that two freshwater microalgae are suitable and sustainable alternative source of protein in aquaculture industry.

Further studies are needed to study the influences of fishmeal replacement with *S. platensis* and *C. vulgaris* on other haematological parameters and oxidative enzymes in various physiological conditions. This will make a significant contribution to the aquaculture industry, making *S. platensis* and *C. vulgaris* more acceptable as sustainable sources of protein.

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Declaration of interest

None.

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