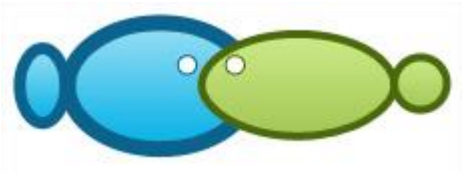

Dietary *Spirulina platensis* and *Chlorella vulgaris* effects on survival and haemato-immunological responses of *Clarias gariepinus* juveniles to *Aeromonas hydrophila* infection

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Abstract. This study investigated the effects of *Spirulina platensis* and *Chlorella vulgaris* on the growth, non-specific immunity and survival of *Clarias gariepinus*. *C. gariepinus* juveniles were infected with *Aeromonas hydrophila* after a 16-week feeding period with 5 different diets which served as replacements for fishmeal diet. The diets are 50% *C. vulgaris* (CL50%), 75% *C. vulgaris* (CL75%), 50% *S. platensis* (SP50%), 75% *S. platensis* (SP75%), and control (100% fishmeal). Fish (n=225; 41.85± 0.05 g) were randomly divided into fifteen tanks (150 L capacity each) with triplicate groups of 15 fish per tank. CL50% had the most significant ($P<0.05$) weight gain (233.72±0.02 g), relative growth rate (558.15±0.57%), feed conversion ratio (0.89±0.01) and protein efficiency ratio (5.81±0.03) compared to the control, while there were no significant differences ($P>0.05$) in growth performance among the algae treatments. Similarly, lysozyme and respiratory burst activity and post-challenge haemoglobin, haematocrit, red blood cells, serum total protein, albumin and higher density lipoprotein cholesterol were significantly higher ($P<0.05$) in CL50% treatment. The present study indicates that replacing 50%-75% of fishmeal with *C. vulgaris* or *S. platensis* could improve the growth and confer significant protection against *A. hydrophila* in the algae-fed *C. gariepinus*.
Key Words: microalgae, African catfish, growth, immunity, biochemical.

Introduction. The request for fish as a protein source has been increasing due to the growth of the world population. Aquaculture has witnessed continual increase in production over the last couple of decades, and it has been suggested to be the fastest growing food production (FAO 2018). Thus, continuous demand for fishmeal and shortfall in its supply has made it imperative to source for alternative replacement.

The health and immune responses of fish are strongly related to their nutrition (Kumari & Sahoo 2005; Priya et al 2004). The use of dietetic immune-stimulants rather than antibiotics in the prevention and control of fish diseases is of great importance to the aquaculture industry. Immuno-stimulants can activate the immune system of fish by stimulating the natural killer cells, raising the number of phagocytes, and enhancing lysozyme complement activities. The stimulation of these immunological functions can support the host against contagious diseases (Sakai 1999). Hence, there is a need to produce feed which does not only promote growth, but can also serve as immunostimulants.

An extensive review on alternative sources of feedstuffs by Romano (2018) has highlighted the use of feather meals, insect meals, algae and seaweeds. Although,

macroalgae are the most farmed and explored aquaculture organisms in the world, due to their ease of farming, technology and vast applications (Romano 2018; Thanigaivel et al 2016). The two most well-known microalgae are *Chlorella vulgaris* and *Spirulina platensis*, and have been reported as a potential alternative source to fishmeal due to their economic cost and environmental safety, and they also contain all nutrients required for fish growth (Andrade et al 2018). Agents that have been used as immunostimulant in aquaculture industry include: oligosaccharides, polysaccharides beta-carotene, chitosan FK-565, chitin, chitosan vitamins glucans, microorganisms, yeast, microalgae and herbal extracts, which are cost effective and environmental friendly (Andrews et al 2011; Choudhury et al 2005; Han et al 2002; Harikrishnan et al 2003; Şahan et al 2016; Sakai 1999; Salnur et al 2009; Supamattaya et al 2005).

S. platensis is a filamentous blue-green alga that is very rich in nutrients and antioxidant properties, which can be attributed to the presence of phycobili-proteins (Jian-Feng et al 2007; Lu et al 2006). *S. platensis* is also found to have immune enhancement effects on *Oreochromis niloticus* and *Cyprinus carpio* (Abdel-Latif & Khalil 2014; Watanuki et al 2006). On the other hand, *C. vulgaris* is a widely distributed chlorophyte in nature, especially in fresh water. It is very rich in vitamins, proteins, polysaccharides, minerals, lipids, essential fatty and amino acids (Becker 2004, 2007; Han et al 2002; Kang et al 2004). *C. vulgaris* possesses immune-stimulatory enhancement properties, aids pigmentation and digestion, and has scavenging and peroxidation properties (Khani et al 2017; Zhang et al 2014).

Cultured fish are susceptible to numerous diseases which may be bacterial, viral, fungal or other parasites (Wise et al 2004; Toranzo et al 2005). *A. hydrophila* can be described as a Gram-negative bacterium which is motile, rod-shaped, oxidase-positive and is catalytic in nature (Sabur 2006). It has been identified to be the cause of motile aeromonas septicaemia (MAS) which is characterized by a distended abdomen, red mouth, and haemorrhage in exterior surfaces, mostly around the anus (Alain 2009). Commonly reported symptoms of infection with this bacterium include skin lesions and haemorrhagic septicaemia (Ahamad et al 2013; Anyanwu et al 2015). However, growth performance and disease resistance of *C. gariepinus* has been reported being enhanced by fenugreek seed meal (Sheikhlar et al 2018), *Euphorbia hirta* extract (Sheikhlar et al 2017) and probiotic *Bacillus* strains (Reda et al 2018).

In Africa and other parts of the world catfish are commercially important valuable fish species that has gained prominence in aquaculture. The effects of *S. platensis* supplements on growth performance of catfish (Promya & Chitmanat 2011; Sayed & Fawzy 2014) and other fishes (Hudaidah et al 2019; Khanzadeh et al 2016; Perez-Velazquez et al 2019) have been reported. Similarly, addition of *C. vulgaris* meal in the diet of catfish (Enyidi 2017), carps (Khani et al 2017; Kopp et al 2019; Shi et al 2017; Zhang et al 2014) and other fishes (Badwy et al 2008; Metsoviti et al 2018) have also been reported. However, most of these reports only make use of the microalgae as a supplement (0-5%) or partial replacement (5-30%) of fishmeal, while the present study replaced substantial part of the fishmeal (50 and 75%) with the microalgae. Although the effects of *S. platensis* and *C. vulgaris* on the immuno-haematological response of *C. gariepinus* to *A. hydrophila* challenge are limited in the literature. Therefore, the present study evaluated the effect of replacing 50 and 75% of fishmeal with dietary *S. platensis* and *C. vulgaris* on the growth, haematology and innate immune response of the *C. gariepinus*.

Material and Method

Experimental diets. A total of five experimental diets were formulated using Pearson's square and WINFEED software (Mirza 2004) version 2.8 (Cambridge, UK). The diets were prepared in sets of either *C. vulgaris* (CL) (Taiwan Chlorella Manufacturing Co., Taiwan) or *S. platensis* (SP) (Earthrise Nutritional, CA, USA) powder to replace fishmeal at various inclusion levels of 0%, 50%, and 75% designated as control, SP50% and SP75% or CL50% and CL75%, respectively. The proximate compositions of the microalgae were analysed and presented (Table 1).

Table 1

Proximate, amino acids and fatty acids compositions of *Spirulina platensis* and *Chlorella vulgaris*

Composition	*Spirulina platensis*	*Chlorella vulgaris*
Proximate composition		
Dry matter (%)	92.61	95.18
Crude protein (%)	66.44	58.84
Crude lipid (%)	14.68	18.29
Ash (%)	6.67	5.82
Fiber (%)	4.82	1.97
Amino acids (g 100 g^{-1})		
Hydroxyproline	0	0
Aspartic acid	11.45	9.89
Serine	5.34	4.39
Glutamic acid	13.34	11.85
Glycine	8.8	10.26
Histidine	0	0
Arginine	7.72	6.42
Threonine	5.43	4.75
Alanine	7.15	8.65
Proline	3.84	5.41
Cysteine	0.66	0
Tyrosine	4.13	2.97
Valine	6.56	6.41
Methionine	1.58	1.62
Lysine	5.39	8.41
Isoleucine	5.84	4.3
Leucine	8.64	9.48
Phenylalanine	4.81	4.92
Fatty acids (%)		
Total fatty acids (TFA)	59.62	58.57
Saturated fatty acids (SFA)	35.76	42.33
Monounsaturated fatty acids (MUFA)	6.89	4.18
Poly unsaturated fatty acids (PUFA)	54.53	53.49
Butyric acid (C4:0)	2.81	0
Lauric acid (C12:0)	0.34	0.21
Mystic acid (C14:0)	0.26	0.2
Myristoleic acid (C14:1)	0.15	0.25
Palmitic acid (C16:0)	31.17	38.58
Palmitoleic acid (C16:1)	1.36	0.4
Stearic acid (C18:0)	3.15	3.08
Oleic acid (C18:1n9)	4.8	3.23
Linoleic acid (C18:2n6)	47.91	15.38
Gamma-linolenic acid (C18:3n6)	0	36.56
Alpha-linolenic acid (C18:3n3)	4.92	0.08
Arachidic acid (C20:0)	0.4	0.26
Eicosadienoic acid (C20:2)	0.66	0.9
Eicosenic acid (C20:1n9)	0.58	0.3
Lignoceric acid (C24:0)	0.44	0
Homo-y-linolenic acid (C20:3n6)	0	0.17
Eicosatrienoic acid (C20:3n3)	0.47	0
Eicosapentaenoic acid (C20:5n3)	0.56	0.41

All data represent mean of triplicate samples.

Experimental diets were chosen based on the results obtained in an earlier growth study (Raji et al 2018) where the optimum growth was achieved at 68.5% and 69.4% for SP

and CL, respectively. After milling with a hammer mill (Disk Mill, FFC 454), ingredients such as dicalcium phosphate (DCP), fish oil, vitamins and minerals premix were added and mixed using water to form a stiff dough. The dough was then pelleted (KCM, Y132M-4) into 1 mm diameter pellets. The pellets were later oven dried at 60°C for 24 h, cooled to ambient temperature, packaged in airtight bags and preserved at 4°C until use. The formulation and proximate compositions of the experimental diets are presented in Table 2.

Table 2

Gross composition (g 100 g^{-1} dry matter) of the experimental diets containing graded levels of *Spirulina platensis* and *Chlorella vulgaris*

Ingredients	*Control*	*Dietary treatments*			
		SP50%	*SP75%*	*CL50%*	*CL75%*
Fishmeal	250	125	62.5	125	62.5
Soybean Meal	440	440	440	440	440
Corn Meal	100	100	100	100	100
S. platensis	-	125	187.5	-	-
C. vulgaris	-	-	-	125	187.5
Vitamin premix[a]	15	15	15	15	15
Mineral premix[b]	15	15	15	15	15
Methionine	10	10	10	10	10
Lysine	10	10	10	10	10
Fish Oil	18	18	18	18	18
Starch	142	142	142	142	142
Proximate composition (%)					
Crude protein	36.11	36.4	36.06	36.07	35.56
Crude lipid	8.67	8.25	8.39	8.27	8.42
Ash	9.95	9.94	9.98	9.95	9.87
Moisture	9.32	8.99	8.95	8.91	8.47
Fibre	1.94	1.99	2.02	1.95	2.52
NFE	34.01	34.42	34.60	34.85	35.16
Essential amino acids g 100 g^{-1}					
Histidine	2.47	2.62	2.69	2.43	2.41
Arginine	1.54	1.66	1.72	1.55	1.55
Threonine	1.53	1.79	1.92	1.80	1.94
Valine	1.71	1.94	2.06	1.85	1.92
Methionine	1.53	1.50	1.48	1.48	1.46
Lysine	3.32	3.27	3.24	3.41	3.46
Isoleucine	1.51	1.74	1.86	1.56	1.59
Leucine	2.68	2.90	3.01	2.85	2.94
Phenylalanine	1.63	1.73	1.79	1.68	1.70
Tryptophan	0.35	0.34	0.34	0.34	0.33
Fatty acid fractions (FA %)					
SFA	43.4	41.73	41.76	41.56	41.06
MUFA	11.5	8.85	8.47	8.67	8.79
PUFA	22.92	23.8	23.86	23.78	23.75
∑n-6	10.33	11.17	11.27	10.25	10.38
C183n4	0.58	2.48	2.58	0.6	0.62
C205n3	1.04	1.15	1.16	1.21	1.25
C226n3	0.88	1.03	1.05	1.56	1.61
∑n-3	5.36	5.9	5.95	8.41	8.60
∑n-9	3.99	3.89	3.9	3.96	3.94

All data represent mean±standard error, N=3. SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acid, EPA: Eicosapentaenoic acid, DHA: Docosahexaenoic acid.

[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.

Fishes and experimental design. About 400 *C. gariepinus* juveniles (41.86±0.02 g) were purchased from a reputable hatchery in Balakong, Malaysia. They were acclimatized at the freshwater aquarium laboratory of the Institute of Biological Sciences, Faculty of Science, University of Malaya, for 14 days in 500 L aerated holding tanks. They were fed commercial diets (40% crude protein). Prior to stocking, 225 juveniles were starved for 24 hours, and were randomly stocked into 15 different tanks of 150 L capacity with a close re-circulation system equipped with top filter pumps (H6350, Shanda Aquarium, China) and aeration diffuser (Sonic P85, China). Water qualities were monitored weekly. Dissolved oxygen was measured with the aid of an Extech DO700 meter (Extech FLIR Systems, USA) and it ranged between 4.0–5.5 mg L^{-1}, while temperature ranged at 26-27°C. The pH was measured with a HI-98190 pH meter (HANNA instruments, Rhode Island, US) and ranged between 6.0-6.8. Total ammonia nitrogen and nitrite were measured with HI-96700 ammonia photometer and HI-96707 nitrite photometer (HANNA instruments, Rhode Island, US), respectively while ammonia averaged 0.2±0.08 mg L^{-1} and nitrite averaged 0.03±0.01 mg L^{-1}.

Experimental fish were stocked at fifteen fish per tank with 3 tanks per treatment and fed twice daily between 900 h and 1600 h at 3% body weight for 16 weeks. Fish sampling and weighing were done fortnightly. The quantities of feed administered to each tank were adjusted per the new weight. The tanks were monitored closely for mortality, while dead fishes were removed and recorded to determine survival rate.

Proximate investigation of trial diets. Proximate analysis (crude protein, lipid, dry matter, fibre and ash) was done on the ingredients and formulated diets in accordance with AOAC (2005). Crude protein analysis was done according to the Kjeldahl method N x 6.25 (method 981.10), after acid processing with Vapodest 50 (Gerhardt, Germany). Dry matter and moisture were determined by oven (Memmert 500, Germany) drying at 105°C for 24 h (method 934.01). Ash content was determined by means of combustion in a muffle furnace (Memmert UFB500, Germany) and Carbolite Furnace (Memmert CWF 11/13, Germany) at 600°C overnight (method 942.05). Crude lipid analysis for *S. platensis* and *C. vulgaris* was estimated following Bligh & Dyer (1959) methods. This involved dissolving the sample in a mixture of 2:1, chloroform: methanol in Soxhlet apparatus (Gerhardt Soxtherm, Germany) for 8 h, while petroleum ether extraction was performed for other samples for 1.5 h (method 945.18). Fibre contents were obtained by alkali and acid digestion of crude lipid remnants (method 962.09). The samples' Nitrogen Free Extract (NFE) was estimated as = 100 - (% crude lipid-+ % crude fibre + % crude protein +% ash). Gross energy (kJ/ g) was obtained using the physiological values, CP x 23.9 + lipid x 39.8 + carbohydrates x 17.6 (Schulz et al 2005).

The essential amino acid compositions were analyzed by high-performance liquid chromatography (HPLC) equipped with a fluorescence detector (Taufek et al 2016) and the contents were quantified using the Pico-Tag technique by Heinrikson & Meredith (1984). The protocol for this method has been described elsewhere by Raji et al (2018).

Growth performance indices. Fish weight, as well as feed supplied were measured and recorded fortnightly and used to compute the growth indices as follows:

$$\text{Mean weight gain} = (Wf - Wi)/n$$

Where: Wf - final weight, Wi - initial weight, and n = number of fish.

$$\text{Relative growth rate} = (Wf - Wi)/n \times 100$$

Where: Wf - final weight, Wi - initial weight, and n = number of fish.

$$\text{Specific growth rate} = (\log Wf - \log Wi)/t \times 100$$

Where: logWf - log of final weight, Wi - log of Initial weight and t= time.

$$\text{Feed conversion ratio} = Fi\ (g)/FWG\ (g)$$

Where: Fi - Feed ingestion and FWG - Fish weight gain

$$\text{Protein efficiency ratio} = MWG\ (g)/MPI\ (g)$$

Where: MWG - Mean weight gain and MPI - Mean protein ingestion.

$$\text{Survival rate} = \frac{Fn}{In} x100$$

Where: Fn - final quantity of fish and In - initial quantity of fish.

$$\text{Condition (K) factor} = W \times 100/L^3$$

Where: W - weight of fish (g) and L - Standard length (cm).

Haematological and biochemical analysis. Prior to sampling, the fish were starved for 24 h. They were then anaesthetised in 10% clove oil and were bled immediately after capture (Pickering et al 1982). Blood samples (1 mL) were collected from the caudal vein of randomly selected fish, into three different tubes (EDTA, heparinized and serum). Blood samples from the anticoagulant tubes were used to determine the haematological parameters using XN-module automated haematology analyser (Sysmex, Germany). This analyser has a dedicated channel for nucleated RBC count, where the analyser makes use of side fluorescence to measure the nucleic acid content of the cell, while it measures the cell size with forward scatter (Da Rin et al 2017). Serum was obtained from the blood sample (serum tube) after centrifuging at 5000 g×rpm for ten minutes and used to determine biochemical parameters using Advia 2400 Chemistry System (Siemens Healthineers, Germany). Haematological and biochemical parameters were performed prior and after *A. hydrophila* infection.
Haematological parameters (RBC indices) were determined based on Seiverd (1983):

$$MCV\ (fl) = Hct/Hgb$$
$$MCH\ (pg.) = (Hgb \times 100)/RBC$$
$$MCHC\ (gdl^{-1}) = (Hgb/Hct) \times 100$$

Where:
MCV = Mean corpuscular volume
MCH = Mean corpuscular haemoglobin and
MCHC = Mean corpuscular haemoglobin concentration

$$\text{Serum globulin g } dL^{-1} = \text{Total protein} - \text{albumin}$$

Aeromonas hydrophila infection test. After feeding trial, 10 fish per replicate were infected with a virulent strain of *A. hydrophila* which was obtained from the University Putra Malaysia (UPM) microbial repository. Preliminary infection tests using similar groups of catfish fed commercial feed kept under same environmental conditions were performed to establish the LD_{50} of the bacterium. The original suspension of *A. hydrophila* was prepared by centrifuging an overnight culture (18 h) of the bacterium. Briefly, a loop full of the bacterium was inoculated into tryptic soy broth and incubated for 18 h at 30°C. The culture was centrifuged at 8000 rpm according to Marion (1998) for 10 min at 4°C and the supernatant was discarded. The cell pellet was washed twice in sterile phosphate buffer saline (PBS) solution (pH 7.4) and finally re-suspended in 25 mL fresh buffer. Varying concentrations (2.5×10^0–2.5×10^6 CFU mL^{-1}) of this suspension were then prepared using 10-fold serial dilutions.70 fish were divided into seven tanks comprising 10 fish each and then challenged with the various dilutions. They were monitored for mortalities within 96 h. The LD_{50} was found to be $2.5x10^6$ CFU mL^{-1} in this experiment and was subsequently used in the actual infection study.

After 16 weeks of feeding, the fish were injected intraperitoneally with 100 µL of the bacteria suspension. The fish were starved for 24 h before the infection and feeding was resumed following the challenge. Mortality was monitored for 18 days, and dead fish were removed and dissected aseptically to remove the intestine and liver which were then weighed and homogenized in sterile 10% physiological saline solution. The bacteria load from each organ was then determined using the drop-plate technique (Herigstad et al. 2001), following a series of serial dilutions. Butzler Camylobacter selective agar (BCSA) was used to recover the bacteria from the organs (Misra et al 1989).

Respiratory burst activity assay. Blood samples were collected from surviving fish (as described above) at days 10 and 18 post challenge. Leukocytes respiratory burst activity was determined as described by Kumari & Sahoo (2005). 100 µL each of blood sample and 100 µL of 0.2% nitro blue tetrazolium (Sigma, Aldrich Germany) were mixed, homogenized and then incubated at 25°C for half an hour. 50 µL of the homogenate was aliquoted into glass tubes and mixed with 1 mL of N, N-dimethylformamide (Sigma, Aldrich Germany). This was then centrifuged at 3000 rpm for 5 min. The optical density of the supernatant was measured at 540 nm with the aid of a microplate reader (Tecan, Switzerland).

Lysozyme activity. Serum lysozyme was determined turbidi-metrically as described by Zhou et al (2006). Lyophilised *Micrococcus lysodeikticus* (0.75 mg mL^{-1}) (Sigma, Aldrich, Germany) was suspended in 100 mL phosphate buffered saline (0.1m M, pH 6.4). 20 µL of the serum sample was added to 200 µL of the suspension in a 96-well plate which was first incubated at room temperature for 1 min and later for 10 min. The reduction in absorbance was measured at 570 nm in a plate reader (Tecan, Switzerland).
The lysozyme activity was calculated as follows:

$$\text{Units/mL enzyme} = (\Delta_{570/}\text{/min Sample-}\ \Delta_{570/}\text{/min})/\ (0.001)\ (0.02)$$

Where: dilution factor 0.001= $\Delta_{570\ as}$ per the unit definition.

Statistical analysis. All experimental data were pooled and analysed with one–way analysis of variance (ANOVA) using IBM SPSS Statistics for Windows, Version 21.0. Differences of means between individual treatments were determined using Duncan's multiple range test at 5% level of significance ($P<0.05$).

Results

Growth performance of C. gariepinus. The growth performances of the fish were monitored for a period of 16 weeks prior to bacterial challenge (Table 3). The fish in control treatment had the least growth performance in terms of weight, WG (225.82±0.19), specific growth rate, SGR (2.26±0.01) and relative growth rate, RGR (539.69±2.74), which are significantly lower ($P<0.0001$) compared to *S. platensis* and *C. vulgaris* treatments. However, there was no significant difference ($P>0.05$) between the algae treatments in relative to WG, SGR and RGR. There was no significant difference ($P>0.05$) in feed conversion ratio, though fish from the control had highest (0.91±0.01). Similarly, differences in the protein efficiency ratio among the algae treatments were negligible ($P>0.05$), while all the fish (100%) survived (Table 3).

Haematological profiling of C. gariepinus juvenile. Table 4 shows the haematological profile of *S. platensis* - *C. vulgaris* fed *C. gariepinus* infected with *A. hydrophilla*. The haemoglobin, haematocrit and red blood cells of algae fed fish are significantly higher ($P<0.05$) relative to the control-fed fish at both pre and post infection, although there was no significant difference ($P>0.05$) in these parameters among algae treatment at pre or post infection (Table 4). The mean corpuscular haemoglobin concentration, MCHC (25.88±0.05) of the control fish before infection is significantly lower ($P<0.0001$) compared to that of the algae treatment before inoculation. However, there was no significant difference ($P>0.05$) in the MCHC at post infection among all the treatments (Table 4).

The respiratory burst activity (RBA) for the post infection showed that differences between RBA at days 10 and 18 post infection (pi) were negligible ($P>0.05$), although the differences among each of the treatments were significant ($P<0.05$) (Table 4). Conversely, the lysozyme activity at day 18 pi is significantly higher ($P<0.0001$) than day 10 post infection, while the highest value of lysozyme was recorded at day 18 pi of CL75 fish (145±0.58 µ mL^{-1}) and the least is at day 10 post infection of control fish (113.00±0.58 µ mL^{-1}) (Table 4).

Serum biochemistry of C. gariepinus juvenile. The serum biochemistry profile of *C. gariepinus* showed differences (P<0.05) prior and post *A. hydrophila* inoculation in all treatments (Table 5). The highest value of total protein (4.64±0.03 mg dL^{-1}), albumin (1.82±0.01 mg dL^{-1}) and globulin (2.82±0.02 mg dL^{-1}) were observed in pre-inoculated SP75 fish, while the least of these three parameters were recorded among the control after inoculation. However, there was no significant difference (P>0.05) among the algae treatment at either pre or post inoculation (Table 5). The total cholesterol is higher (P<0.0001) in the control before inoculation (99.81±0.03 mg dL^{-1}).

In addition, the alkaline phosphatase value was significantly higher (P<0.05) among algae group at post infection compared to the pre-infection and control fish (Figure 1). The aspartate aminotransferase (AST) value, 64.50±0.09 Iμ/L, of the control fish at post infection is significantly higher (P<0.0001) compared to other treatments. However, the AST values before infection are significantly higher (P<0.0001) than the AST values at post infection in all treatments (Figure 1).

The effects of *A. hydrophila* infection on the fish showed that CL50 fish survival rate of 73.34±3.33% is significantly higher (P>0.05) compared to other treatments and control fish at 20.33±0.88% (Table 6). The bacterial load at the control fish intestine ($1.29\times10^5\pm2.0\times10^3$ CFU mL^{-1}) and liver ($1.07\times10^5\pm1.42\times10^3$ CFU mL^{-1}) are significantly higher (P<0.0001) relative to other treatments, while there was no significant difference (P>0.05) in the intestine and liver bacteria load among SP75, CL50 and CL75 fish (Table 6).

Table 3

Growth performances of *Clarias gariepinus* fed different levels of *Spirulina platensis* and *Chlorella vulgaris* for 16 weeks prior to *Aeromonas hydrophilla* infection

Parameter	*Control*	*SP50%*	*SP75%*	*CL50%*	*CL75%*	*P*
IW (g)	41.84±0.22^a	41.87±0.03^a	41.85±0.16^a	41.88±0.04^a	41.84±0.02^a	0.99
FW (g)	267.67±0.31^a	275.54±0.06^b	271.78±0.06^b	275.59±0.05^b	272.20±0.06^b	0.0001
WG (g)	225.82±0.19^a	233.67±0.02^b	233.20±0.16^b	233.72±0.02^b	233.25±0.03^b	0.0001
SGR (% d^{-1})	2.26±0.01^a	2.28±0.01^b	2.29±0.01^b	2.29±001^b	2.28±002^b	0.0001
RGR (%)	539.69±2.74^a	558.11±0.39^b	557.31±2.52^b	558.15±0.57^b	557.53±0.10^b	0.0001
FC (g)	205.17±0.02 ^{a}	207.63±0.02 ^{a}	206.51±0.02^a	208.71±0.02^a	209.14±002^a	0.36
FCR	0.91±0.01^a	0.89±0.01^a	0.88±0.01^a	0.89±0.01^a	0.90±0.02^a	0.80
PI	46.37±4.58^b	41.85±1.28^{ab}	43.90±0.49^{ab}	40.24±0.49^a	43.19±0.32^{ab}	0.41
PER	4.96±0.02a	5.39±0.06ab	5.32±0.03ab	5.81±0.03b	5.40±0.02ab	0.75
K Factor	1.75±0.02a	1.88±0.02b	1.87±0.02b	1.89±0.02b	1.87±0.02b	0.001
Survival %	100	100	100	100	100	

FW: Final weight; IW: Initial weight; WG: Weight gain; FC: Feed consume; FCR: Feed conversion ratio; SGR: Specific growth rate; PER: Protein efficiency ratio; PI: Protein intake; RGR: Relative growth rate. Values represent mean±standard error, while row with different superscripts indicate significant difference.

Table 4

Haematological profile of *Clarias gariepinus* juveniles fed diets containing varying levels of *Chlorella vulgaris* and *Spirulina platensis* prior and post-exposure to *Aeromonas hydrophilla*

		CTRL	*SP50*	*SP75*	*CL50*	*CL75*	*P*
HGB (g/dL)	Pre	11.78±0.09^{c}	12.25±0.06^{d}	12.28±0.02^{d}	12.24±0.02^{d}	12.26±0.04^{d}	0.01
	post	10.70±0.02^{a}	11.45±0.06^{b}	11.50±0.06^{b}	11.52±0.06^{b}	11.50±0.04^{b}	0.01
HCT (%)	Pre	45.50±0.29^{c}	46.50±0.06^{d}	46.55±0.06^{d}	46.49±0.06^{d}	46.51±0.03^{d}	0.00
	Post	40.00±0.51^{a}	43.00±0.29^{b}	43.07±0.04^{b}	43.09±0.05^{b}	43.06±0.03^{b}	0.00
RBC (10^{6}cells/mm3)	Pre	3.89±0.06^{c}	4.02±0.04^{d}	4.05±0.03^{d}	4.02±0.02^{d}	4.04±0.03^{d}	0.02
	Post	3.08±0.04^{a}	3.40±0.03^{b}	3.44±0.04^{b}	3.50±0.06^{b}	3.43±0.02^{b}	0.02
MCV (fL)	Pre	117.00±0.99^{a}	115.60±0.99^{a}	115.05±0.76^{a}	115.75±0.62^{a}	115.42±0.66^{a}	0.00
	Post	129.78±2.51^{c}	126.62±1.19^{bc}	125.43±1.19^{b}	123.18±2.18^{b}	125.46±0.60^{b}	0.00
MCH (pg)	Pre	30.28±0.09^{a}	30.45±0.17^{a}	30.35±0.18^{a}	30.48±0.16^{a}	30.55±0.15^{a}	0.00
	Post	34.72±0.49^{c}	33.72±0.44^{bc}	33.49±0.36^{b}	32.92±0.59^{b}	33.41±0.32^{b}	0.00
MCHC (g/dL)	Pre	25.88±0.05^{a}	26.34±0.09^{b}	26.38±0.02^{bc}	26.33±0.01^{b}	26.33±0.05^{b}	0.00
	Post	26.76 ±0.02^{d}	26.63±0.04^{bd}	26.70±0.04^{bd}	26.73±0.02^{cd}	26.63±0.04^{bd}	0.00
WBC (10^{3}cells/mm3)	Pre	92.75±0.27^{a}	93.36±0.10^{c}	93.34±0.08^{c}	93.25±0.03^{bc}	93.21±0.03^{b}	0.00
	Post	99.00±0.25^{d}	96.87±0.04^{e}	96.56±0.04^{f}	96.00±0.38^{g}	96.59±0.02^{f}	0.00
RBA Post-challenge (A_{540})							
	D10	0.42±0.02^{a}	1.02±0.27^{de}	0.99±0.06^{cd}	1.53±0.39^{f}	0.83±0.01^{b}	0.05
	D18	0.43±0.01^{a}	1.10±0.26^{e}	1.00±0.02^{d}	1.55±0.39^{f}	0.88±0.08^{b}	0.05
Lysozyme activity post-challenge (U/mL)							
	D10	113.00±0.58^{a}	123.00±0.58^{b}	125.00±0.35^{d}	124.0±0.29^{c}	128.67±0.03^{e}	0.00
	D18	132.67±0.04^{f}	142.00±0.58^{g}	142.47±0.12^{g}	142.47±0.03^{g}	145.00±0.58^{h}	0.00

HGB: Haemoglobin; HCT: Haematocrit; RBC: Red blood cells; WBC: White blood cells; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; RBA: Respiratory burst activity; D: Day. Values represent mean ± standard error (N=9), while rows mean with different superscripts (a-h) indicate significant difference.

Table 5

Serum biochemistry of *Clarias gariepinus* juveniles fed diets containing varying levels of *Chlorella vulgaris* and *Spirulina platensis* prior and post-exposure to *Aeromonas hydrophilla*

Parameters		*CTRL*	*SP50*	*SP75*	*CL50*	*CL75*	*P-value*
PG mg/dL	PRE	77.31±0.01^{i}	72.87±0.03^{d}	72.78±0.01^{c}	72.45±0.01^{b}	71.98±0.01^{a}	0.00
	POS	78.86±0.02^{j}	73.78±0.05^{h}	73.22±0.02^{g}	73.04±0.02^{f}	72.97±0.01^{e}	
TP g/dL	PRE	4.20±0.06^{d}	4.58±0.02^{e}	4.64±0.03^{e}	4.57±0.06^{de}	4.61±0.05^{e}	0.00
	POS	3.00±0.06^{a}	3.95±0.01^{b}	4.06±0.01^{bc}	4.13±0.03^{c}	4.05±0.01^{bc}	
Alb g/dL	PRE	1.75±0.01^{c}	1.80±0.06^{c}	1.82±0.01^{c}	1.79±0.06^{c}	1.80±0.06^{c}	0.00
	POS	1.30±0.01^{a}	1.59±0.01^{b}	1.62±0.01^{b}	1.64±0.01^{b}	1.61±0.01^{b}	
Glob g/dL	PRE	2.45±0.05^{c}	2.78±0.03^{d}	2.82±0.02^{d}	2.78±0.00^{d}	2.81±0.01^{d}	0.00
	POS	1.70±0.05^{a}	2.36±0.00^{b}	2.44±0.00^{bc}	2.49±0.03^{c}	2.44±0.00^{bc}	
A/GR%	PRE	0.71±0.01^{b}	0.65±0.03^{a}	0.65±0.00^{a}	0.64±0.02^{a}	0.64±0.02^{a}	0.02
	POS	0.76±0.02^{c}	0.67±0.00^{ab}	0.66±0.00^{a}	0.66±0.01^{a}	0.66±0.00^{a}	
Trig mg/dL	PRE	90.61±0.06^{g}	80.69±0.02^{f}	80.35±0.06^{e}	78.36±0.02^{c}	77.96±0.02^{b}	0.00
	POS	78.76±0.06^{d}	78.69±0.06^{d}	78.73±0.06^{d}	77.08±0.03^{a}	77.12±0.03^{a}	
TCL mg/dL	PRE	99.81±0.03^{g}	93.05±0.03^{e}	92.95±0.03^{d}	92.89±0.03^{d}	92.68±0.02^{c}	0.00
	POS	96.58±0.03^{f}	92.12±0.03^{ab}	92.19±0.03^{b}	92.05±0.03^{a}	92.08±0.03^{a}	
HDL mg/dL	PRE	59.86±0.02^{b}	66.93±0.04^{c}	70.66±0.02^{g}	69.18±0.03^{f}	69.21±0.06^{f}	0.00
	POS	54.44±0.02^{a}	66.87±0.02^{c}	67.06±0.02^{d}	67.69±0.03^{e}	67.04±0.05^{d}	
LDL mg/dL	PRE	59.11±0.03^{b}	58.70±0.03^{a}	58.68±0.03^{a}	58.66±0.03^{a}	58.65±0.02^{a}	0.00
	POS	70.76±0.03^{g}	62.46±0.03^{f}	62.35±0.02^{e}	61.97±0.03^{d}	61.77±0.06^{c}	

PG: Plasma glucose; TP: Total protein; Alb: Albumin; Glob: Globulin; A/GR: Albumin/globulin ratio; ALP: Alkaline phosphatase; AL: Alanine transaminase; AST: Aspartate transaminase; Crt: Creatinine; Trig: Triglyceride; TCL: Total cholesterol; HDL: High density lipoprotein and LDL: Low density lipoprotein cholesterol. Values represent mean±standard error (N=9), while rows mean with different superscripts (a-g) indicate significant differenc

Table 6

Relative percent survival (RPS) and bacteria loads in the intestine and liver of *Clarias gariepinus* fed with *Spirulina platensis* and *Chlorella vulgaris* at post *Aeromonas hydrophilla* infection

Diets	*RPS (%)*	*Intestine Cfu mL^{-1}*	*Liver Cfu mL^{-1}*
Control	20.33 ± 0.88^{a}	$1.29\times10^{5}\pm2000^{b}$	$1.07\times10^{5}\pm1420^{b}$
SP50%	46.67 ± 1.67^{b}	$1.09\times10^{4}\pm506^{a}$	$1.48\times10^{4}\pm197^{c}$
SP75%	60.00 ± 2.89^{c}	14.8 ± 0.20^{c}	417 ± 9.7^{d}
CL50%	73.34 ± 3.33^{d}	14.1 ± 0.19^{c}	589 ± 7.8^{d}
CL75%	53.31 ± 2.33^{bc}	420 ± 11.5^{c}	$1.02\times10^{4}\pm136^{a}$

Values are means of nine fish per treatments. Mean values on the same row (a-d) with different superscripts indicate significant difference.

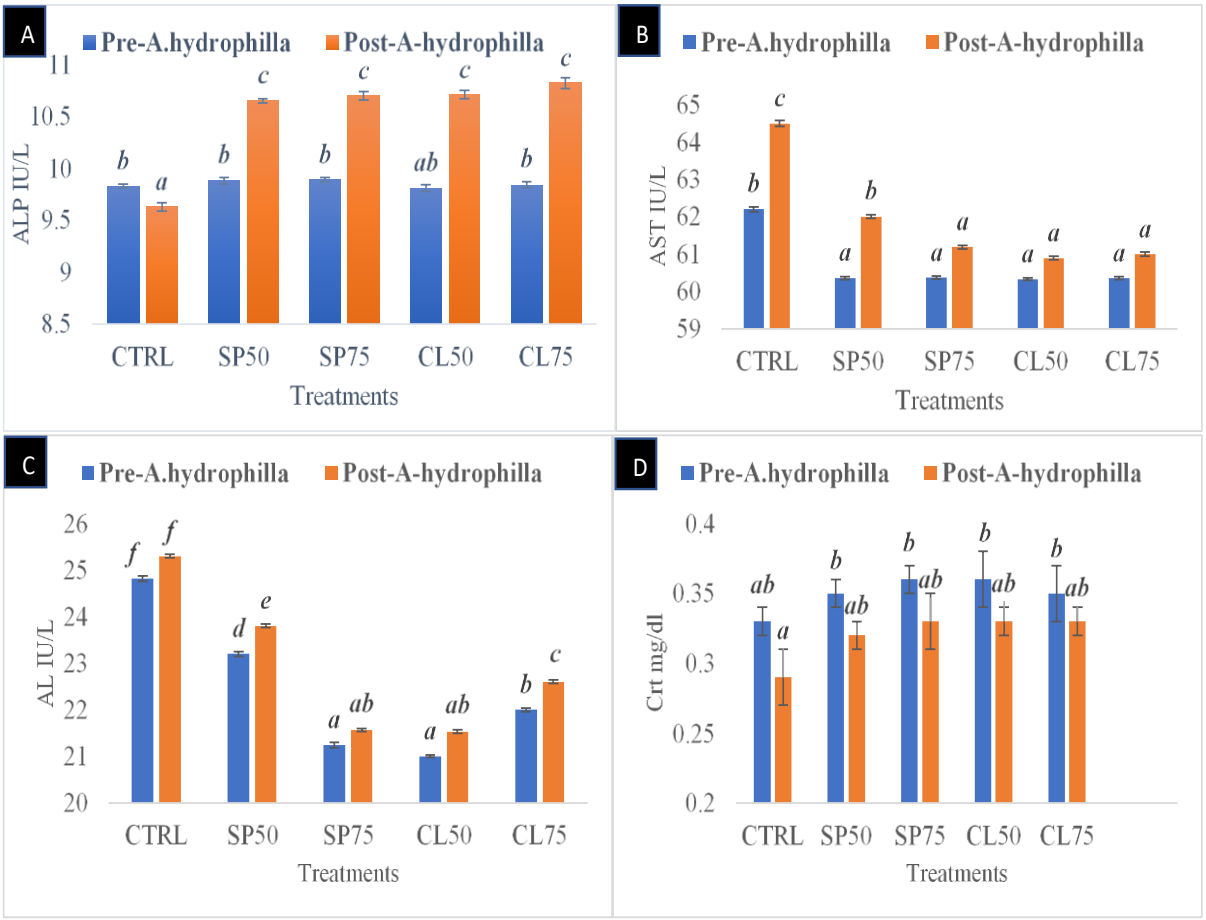

Figure 1. Changes in A: Alkaline phosphatase (ALP, IUL^{-1}); B: Aspartate aminotransferase (AST, IUL^{-1}); C: Alanine aminotransferase (AL, IUL^{-1}) and D: Creatinine (Crt, mg mL^{-1}) in response to *Aeromonas hydrophilla* infection. Values are means of nine fish per treatments and different letters (a-f) indicate significant difference at $P<0.05$.

Discussion. The current study evaluated the effects of partial inclusion of *S. platensis* and *C. vulgaris* diets on growth and innate immune response of *C. gariepinus* juveniles. Dietary *C. vulgaris* at 50% inclusion levels significantly enhanced the growth performance of African catfish in this study. However, there was no remarkable difference between CL50% and other algae treated groups in SGR, RGR and K-factor. The growth improvement of both *S. platensis* and *C. vulgaris* in this study may be attributed to their high digestibility (>80%) of nutrients, especially protein, lipid, amino acids and fatty acids (Sarker et al 2016; Teuling et al 2017). The inclusion of 3–5% supplementary *S. platensis* in fish diets has been observed to increase the ability of *C. gariepinus* to absorb

nutrients and hence, improve their growth (Promya & Chitmanat 2011; Sayed & Fawzy 2014). On the other hand, the growth stimulating effects of *C. vulgaris* in this study may be attributed to the influence of their thin and broken cell wall, which makes it highly digestible and together with the processing method (spray-drying by pressure release) that conserved most of the nutrients within the algae (Bengwayan et al 2010). Dietary *C. vulgaris* has also been found to promote the activity of digestive enzymes in the hepato-pancreas of *Carassius auratus gibelio*, leading to increasing diet utilization and growth (Xu et al 2014).

Growth performance such as SGR observed in the *S. platensis* diet (2.28%) is similar to the reports of Promya & Chitmanat (2011) and Teuling et al (2017) with SGR ranges of 2.14–2.98%, though SGR in the present study is higher than that reported by Sayed & Fawzy (2014) of 0.58–0.9%. In addition, the growth performance shown by *C. vulgaris* diet in this study is similar to the report of 2.95% SGR and 0.77 FCR by catfish fed 30% *C. vulgaris* diet (Teuling et al 2017).

In this study, all the pre-inoculated Hgb, Hct and RBCs were high and fell within the range of 10.34-12.41 g dL^{-1} reported for Hgb by Okorie-Kanu & Unakalamba (2015), Hct 40.02-46.60% and RBC 3.051-8.64×10^6 mm^{-3} (Akinrotimi et al 2011) for *C. gariepinus*. Thus, it can be suggested that all the experimental diets do not have negative consequence on the percentage of the total erythrocytes, Hct, Hgb levels as well as the oxygen carrying capacity of the blood. *S. platensis* and *C. vulgaris* have been reported to be rich in iron (Yeganeh et al 2015) and folic acid (Khani et al 2017), which was found to have a substantial effect on erythropoiesis through the increase of RBCs and haemoglobin counts (Kapoor & Mehta 1998). In addition, *S. platensis* and *C. vulgaris* are rich sources of bioactive and natural antioxidants like vitamins C and E, carotenoids and phycocyanin, which help in RBC production (Radhakrishnan et al 2014; Yeganeh et al 2015). The algae treated groups were, however, better than the control as they exhibited significantly higher values in RBCs, Hgb and Hct both at pre- and post-infections.

Furthermore, fish biochemistry profiles are valuable and reliable indicators of the health status of fish (Dawood et al 2016). In this study, all pre-inoculated biochemical parameters were within the range reported for *C. gariepinus* as reported by Myburgh et al (2008). Blood glucose of algae fed fish were persistently lower compared to the control fish in this study. It has been suggested that high concentration of glucose, cholesterol and triglycerides in juvenile tilapia blood, is partially due to the hydrolysis of feed into smaller particles such as peptide and amino acids which are readily assimilated by the fish (Montoya-Mejía et al 2017). Blood glucose levels also have been suggested to be relative to the amount of carbohydrate digested (Hemre et al 1990). Hence, the concentrations of blood glucose in the algae-fed fish can be attributed to high digestibility and energetic status of the algae diets. However, there is need for further study on the consequences of persistence high blood glucose especially during fish infections. This become imperative as persistent high plasma glucose concentration maintained through glycogenesis during stressful condition has been suggested as a good stress indicator in fish over adrenaline or cortisol (David et al 2005). Therefore, the substantial variation in the pre- and post-infection plasma glucose may be ascribed to the injection of *A. hydrophila*, which triggered a remarkable upsurge in plasma glucose, partly due to increased energy requirement to neutralize the toxin secreted by the bacteria.

Alterations in enzyme profiles remain important toxicity biomarkers that have been widely used in evaluating the biochemical and physiological function of vital organs in fish. ALT and AST activities are important pointers of the liver and kidney function in fish (Coz-Rakovac et al 2005). Increased serum ALT and AST have been shown to be associated with cellular damage, increased porous membrane and inter-cellular activity, as a result of morphological damage to the liver (Pan et al 2003). This study shows that AST and AL increased after *A. hydrophila* inoculation. This increase can be associated with the increase in the generation of reactive oxygen species (ROS) in response to *A. hydrophila* bacteria (Banaee et al 2017). However, their levels were shown to be decreased in the treatment groups relative to the control, irrespective of pre- or post-inoculation status. This is in conformity with the findings of Vasudeva Rao et al (2006) in *Labeo rohita* fed a diet containing herb, *Achyranthes aspera* after *A. hydrophila* exposure

and in Argulus infested goldfish treated with azadirachtin (Kumar et al 2013). ALP is an enzyme that transports glycogen, synthesizes protein and certain enzymes and regulates secretary activities (Pradhan & Das 2015). Reduction in serum/tissues ALP and acid phosphatase activities in carp due to stress induced by the sublethal effect of nitrite was reported by (Das et al 2004). Increased ALP activity was recorded in *L. rohita* fed with *C. vulgaris* from 60-100 days and turmeric for 60 days, after *A. hydrophila* infection (Pradhan & Das 2015; Sahu et al 2008). This is in consonance with the observation of this study as the ALP (except for the control) increased significantly post *A. hydrophila* infection especially in *C. vulgaris* (CL75%) treated group. Intensification of phosphatase signifies a higher breakdown of energy reserves that is normally used for fish growth and survival (Pradhan & Das 2015).

Fish are more exposed to bacteria and viruses due to their aquatic habitat. There was no mortality throughout the 16-week experiment prior to *A. hydrophila* infection. However, after 24 h post *A. hydrophila* infection, all fish developed clinical signs like greyish-white lesion on the surface of the body, caudal fin, loss of balance and redness of the fin bases and loss of appetite, followed by mortality as early as 48 h post inoculation. This signifies that *A. hydrophila* is pathogenic to *C. gariepinus* as previously reported by Taufek et al (2018). The lower mortality and quick wound recovery observed in both algae treated group as compared with the control group, suggested some level of non-specific immunity. This assertion on non-specific immune boosting of both *S. platensis* and *C. vulgaris* has been reported by some authors (Abdel-Tawwab & Ahmad 2009; Andrews et al 2011; Kim et al 2013; Pradhan & Das 2015; Promya & Chitmanat 2011; Qihuan et al 2014).

Furthermore, fish serum lysozyme activity is understood to emanate from leukocytic origins (Cecchini et al 2000; Lie et al 1989). Significantly higher lysosome activities at days 10 and 18 were noticed for both *C. vulgaris* and *S. platensis* supplementation levels and more importantly, highest in CL75%. This suggests that *C. vulgaris* may contain some bioactive ingredients that are involved in stimulating the immune response of the fish. This corroborates with the observations in Atlantic salmon and *C. carpio* (Møyner et al 1993; Siwicki & Studnicka 1987), which exhibited higher serum lysozyme activities at post infection with *Aeromonas salmonicida* and *Aeromonas punctata*, respectively. A report by Zhang et al (2014) showed that dietary *C. vulgaris* can increase fish lysozyme and SOD, and contains bioactive materials that can regulate fish innate and adaptive immunity.

Conclusions. This study assessed the effects of diets (comprising of 50% and 75% *S. platensis* and *C. vulgaris* replacement of fishmeal protein) on the haematological and immunostimulatory responses of *C. gariepinus*. The findings of this study therefore suggested that substituting fishmeal with both 50% *C. vulgaris* and *S. platensis* (12.5% dietary inclusion) improved the fish growth. Replacing fishmeal with 75% of both *S. platensis* and *C. vulgaris* (18.75% dietary inclusion) was able to enhance the immune function by improving the haematological parameters, decreasing the TCL and elevation of HDL indicating the potentials of both algae to control of atherosclerosis. However, for optimization of the benefits of *S. platensis* and *C. vulgaris* in intensive aquaculture, dosages, duration of study, specific species (*S. platensis* and *C. vulgaris*), administration methods, fish physiological conditions as well as species-specific responsiveness must be taken into consideration.

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References

Abdel-Latif H. M., Khalil R. H., 2014 Evaluation of two phytobiotics, *Spirulina platensis* and *Origanum vulgare* extract on growth, serum antioxidant activities and resistance of nile tilapia (*Oreochromis niloticus*) to pathogenic *Vibrio alginolyticus*. International Journal of Fisheries and Aquatic Studies 1:250-255.

Abdel-Tawwab M., Ahmad M. H., 2009 Live spirulina (*Arthrospira platensis*) as a growth and immunity promoter for Nile tilapia, *Oreochromis niloticus* (L.), challenged with pathogenic *Aeromonas hydrophila*. Aquaculture Research 40(9):1037-1046.

Ahamad B., Punniamurthy D., Kumar N., Malmarugan V., Suresh R., Ranganathan V., 2013 Outbreak of bacterial haemorrhagic septicaemia in freshwater carps in Thanjavur region of Tamil Nadu. Advanced Biotechnology 12(7):12-15.

Akinrotimi O., Bekibele D., Orokotan O., 2011 Select hematological values of the african catfish (*Clarias gariepinus*) raised in a water recirculating aquaculture system. International Journal of Recirculating Aquaculture 12(1):1-12.

Alain K., 2009 Isolation of *Aeromonas hydrophila* from naturally diseased thai pangas *Pangasius hypophthalmus* (MSc Thesis). Bangladesh Agricultural University, Mymensingh, Bangladesh.

Andrade L., Andrade C., Dias M., Nascimento C., Mendes M., 2018 Chlorella and spirulina microalgae as sources of sunctional foods. Nutraceuticals, and Food Supplements pp. 45-58.

Andrews S. R., Sahu N., Pal A., Mukherjee S., Kumar S., 2011 Yeast extract, brewer's yeast and spirulina in diets for *Labeo rohita* fingerlings affect haemato-immunological responses and survival following *Aeromonas hydrophila* challenge. Research in Veterinary Science 91(1):103-109.

Anyanwu M. U., Chah K. F., Shoyinka V. S., 2015 Evaluation of pathogenicity of motile *Aeromonas species* in African catfish. International Journal of Fishries and Aquatic Studies 2(3):93-98.

Badwy T. M., Ibrahim E., Zeinhom M., 2008 Partial replacement of fishmeal with dried microalga (*Chlorella* spp. and *Scenedesmus* spp.) in Nile tilapia (*Oreochromis niloticus*) diets. Paper presented at the 8th International Symposium on Tilapia in Aquaculture. Central Laboratory for Aquaculture Research, Egypt, Cairo, 12-15 October 2008.

Banaee M., Soleimany V., Nematdoost Haghi B., 2017 Therapeutic effects of marshmallow (*Althaea officinalis L.*) extract on plasma biochemical parameters of common carp infected with *Aeromonas hydrophila*. Veterinary Research Forum 8(2):145-153.

Becker E. W., 2004 Microalgae for aquaculture. In Richmond A. (ed), Handbook of microalgal culture: biotechnology applied phycology. Blackwell Publishing Ltd., pp. 380-391.

Becker E. W., 2007 Micro-algae as a source of protein. Biotechnology advances 25(2):207-210.

Bengwayan P. T., Laygo J. C., Pacio A. E., Poyaoan J. L. Z., Rebugio J. F., Yuson A. L. L., 2010 A comparative study on the antioxidant property of Chlorella (*Chlorella* sp.) tablet and glutathione tablet. E-International Scientific Research Journal 2(1):25-35.

Bligh E. G., Dyer J. W., 1959 A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37(8):911–917.

Cecchini S., Terova G., Caricato G., Saroglia M., 2000 Lysozyme activity in embryos and larvae of sea bass (*Dicentrarchus labrax* L.), spawned by broodstock fed with vitamin c enriched diets. Bulletin-european association of fish pathologists 20(3):120-124.

Choudhury D., Pal A., Sahu N., Kumar S., Das S., Mukherjee S., 2005 Dietary yeast RNA supplementation reduces mortality by *Aeromonas hydrophila* in rohu (*Labeo rohita* L.) juveniles. Fish and shellfish immunology 19(3):281-291.

Coz-Rakovac R., Strunjak-Perovic I., Hacmanjek M., Lipej Z., Sostaric B., 2005 Blood chemistry and histological properties of wild and cultured sea bass (*Dicentrarchus*

labrax) in the North Adriatic Sea. Veterinary research communications 29(8):677-687.
Da Rin G., Vidali M., Balboni F., Benegiamo A., Borin M., Ciardelli M. L., Dima F., Di Fabio A., Fanelli A., Fiorini F., Francione S., Germagnoli L., Gioia M., Lari T., Lorubbio M., Marini A., Papa A., Seghezzi M., Solarino L., Pipitone S., Tilocca E., Francione S., 2017 Performance evaluation of the automated nucleated red blood cell count of five commercial hematological analyzers. International Journal of Laboratory Hematology 39(6):663-670.
Das P. C., Ayyappan S., Das B. K., Jena J. K., 2004 Nitrite toxicity in Indian major carps: sublethal effect on selected enzymes in fingerlings of *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 138(1):3-10.
David M., Kumar R., Mushigeri S., Kuri R., 2005 Blood glucose and glycogen levels as indicators of stress in the freshwater fish, *Labeo rohita* under fenvalerate intoxication. Journal of Ecotoxicology & Environmental Monitoring 15(1):1-5.
Dawood M. A. O., Koshio S., Ishikawa M., Yokoyama S., El Basuini M. F., Hossain M. S., Nhu T. H., Dossou S., Moss A. S., 2016 Effects of dietary supplementation of *Lactobacillus rhamnosus* or/and *Lactococcus lactis* on the growth, gut microbiota and immune responses of red sea bream, *Pagrus major*. Fish and Shellfish Immunology 49:275-285.
Enyidi U., 2017 Chlorella vulgaris as protein source in the diets of african catfish *Clarias gariepinus*. Fishes 2(4):17.
Han J., Kang G., Kim J. K., Kim S. H., 2002 The present status and future of Chlorella. Food Science and Industry 6:64-69.
Harikrishnan R., Rani M. N., Balasundaram C., 2003 Hematological and biochemical parameters in common carp, *Cyprinus carpio*, following herbal treatment for *Aeromonas hydrophila* infection. Aquaculture 221(1-4):41-50.
Heinrikson R. L., Meredith S. C., 1984 Amino acid analysis by reverse-phase high-performance liquid chromatography: precolumn derivatization with phenylisothiocyanate. Analytical Biochemistry 136(1):65-74.
Hemre G. I., Lie Ø., Lambertsen G., 1990 Digestibility of different carbohydrate sources in cod (*Gadus morhua*), and its relation to glucose content in blood and urine. Fiskeridirektorates Skrifter Serie Ernaering 3(1):3-9.
Herigstad B., Hamilton M., Heersink J., 2001 How to optimize the drop plate method for enumerating bacteria. Journal of Microbiological Methods 44(2):121-129.
Hudaidah S., Putri B., Samara S., Adiputra Y., 2019 Effect of partial replacement of fish meal with *Spirulina platensis* meal in practical diets and culture location on growth, survival, and color enhancement of percula clownfish *Amphiprion percula.* Paper presented at the IOP Conference Series: Earth and Environmental Science. Indonesia, East Java, 1 March 2019.
Jian-Feng N., Guang-Ce W., Lin X. Z., Zhou B. C., 2007 Large-scale recovery of C-phycocyanin from *Spirulina platensis* using expanded bed adsorption chromatography. Journal of Chromatography B 850(1-2):267-276.
Kang M. S. Chae H., Sim S., 2004 Chlorella as a functional biomaterial. Korean Journal of Biotechnology and Bioengineering 19(1):1-11.
Kapoor R., Mehta U., 1998 Supplementary effect of spirulina on hematological status of rats during pregnancy and lactation. Plant Foods for Human Nutrition 52(4):315-324.
Khani M., Soltani M., Mehrjan S. M., Foroudi F., Ghaeni M., 2017 The effects of *Chlorella vulgaris* supplementation on growth performance, blood characteristics, and digestive enzymes in Koi (*Cyprinus carpio*). Iranian Journal of Fisheries Sciences 16(2):832-843.
Khanzadeh M., Fereidouni A. E., Berenjestanaki S. S., 2016 Effects of partial replacement of fish meal with *Spirulina platensis* meal in practical diets on growth, survival, body composition, and reproductive performance of three-spot gourami (*Trichopodus trichopterus* Pallas, 1770). Aquaculture International 24(1):69-84.

Kim S. S., Rahimnejad S., Kim K. W., Lee B. J., Lee K. J., 2013 Effects of dietary supplementation of Spirulina and Quercetin on growth, innate immune responses, disease resistance against *Edwardsiella tarda*, and dietary antioxidant capacity in the juvenile olive flounder *Paralichthys olivaceus*. Fisheries and Aquatic Sciences 16(1):7-14.
Kopp R., Brabec T., Mareš J., Maršálková E., Maršálek B., 2019 The utilization of algae with the aim to increase the fatty acid content in muscle of common Carp (*Cyprinus carpio* L.). Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis 67(1):91-99.
Kumar S., Raman R., Kumar K., Pandey P., Kumar N., Mallesh B., Mohanty S., Kumar A., 2013 Effect of azadirachtin on haematological and biochemical parameters of Argulus-infested goldfish *Carassius auratus* (Linn. 1758). Fish Physiology & Biochemistry 39(4):733-747.
Kumari J., Sahoo P., 2005 Effects of cyclophosphamide on the immune system and disease resistance of Asian catfish *Clarias batrachus*. Fish and Shellfish Immunology 19(4):307-316.
Lie Ø., Evensen Ø., Sorensen A., Froysadal E., 1989 Study on lysozyme activity in some fish species. Diseases of Aquatic Organisms 6:1-5.
Lu H. K., Hsieh C. C., Hsu J. J., Yang Y. K., Chou H. N., 2006 Preventive effects of *Spirulina platensis* on skeletal muscle damage under exercise-induced oxidative stress. European Journal of Applied Physiology 98(2):220.
Marion J. E., 1998 Water quality for pond aquaculture. Retrieved from International Center for Aquaculture and Aquatic Environment, Alabama Agricultural Experiment Station, Auburn University, Alabama.
Metsoviti M., Lachanidou G., Manolios A., Berillis P., Katsoulas N., Papapolymerou G., Karapanagiotidis I., 2018 The effect of fishmeal and fish oil replacement by dietary microalgae species on liver histology in gilthead seabream (*Sparus aurata*). Paper presented at the 3rd International Congress on Applied Ichthyology & Aquatic Environment, Greece, Volos, 8-11 November 2018.
Misra S. K., Bhadra R. K., Pal S., Nair G. B., 1989 Growth of *Aeromonas* spp. on Butzler Campylobacter selective agar and evaluation of the agar for the primary isolation of *Aeromonas* spp. from clinical specimens. Journal of Clinical Microbiology 27(2):346-347.
Montoya-Mejía M., García-Ulloa M., Hernández-Llamas A., Nolasco-Soria H., Rodríguez-González H., 2017 Digestibility, growth, blood chemistry, and enzyme activity of juvenile *Oreochromis niloticus* fed isocaloric diets containing animal and plant byproducts. Revista Brasileira de Zootecnia 46(12):873-882.
Møyner K., Røed K. H., Sevatdal S., Heum M., 1993 Changes in non-specific immune parameters in Atlantic salmon, *Salmo salar* L., induced by *Aeromonas salmonicida* infection. Fish and Shellfish Immunology 3(4):253-265.
Myburgh J. G., Botha C. J., Booyse D. G., Reyers F., 2008 Provisional clinical chemistry parameters in the African sharptooth catfish (*Clarias gariepinus*). Journal of the South African Veterinary Association 79(4):156-160.
Okorie-Kanu C. O., Unakalamba N. J., 2015 Normal haematological and blood biochemistry values of cultured Heteroclarias hybrid in South East Nigeria. Comparative Clinical Pathology 24(5):1015-1020.
Pan C. H., Chien Y. H., Hunter B., 2003 The resistance to ammonia stress of *Penaeus monodon* Fabricius juvenile fed diets supplemented with astaxanthin. Journal of Experimental Marine Biology and Ecology 297(1):107-118.
Perez-Velazquez M., Gatlin III D. M., González-Félix M. L., García-Ortega A., de Cruz C. R., Juárez-Gómez M. L., Chen K., 2019 Effect of fishmeal and fish oil replacement by algal meals on biological performance and fatty acid profile of hybrid striped bass (*Morone crhysops*♀ × *M. saxatilis*♂). Aquaculture 507:83-90.
Pickering A., Pottinger T., Christie P., 1982 Recovery of the brown trout, *Salmo trutta* L., from acute handling stress: a time-course study. Journal of Fish Biology 20(2):229-244.

Pradhan J., Das B. K., 2015 Effect of dietary *Chlorella vulgaris* on liver enzymatic profiles of rohu *Labeo rohita* (Hamilton, 1822). Indian Journal of Fisheries 62(2):132-136.
Priya K., Mukherjee S., Pal A., Sahu N., 2004 Effect of dietary lipids on histological changes in hepatic tissues of *Catla catla* fingerlings. Indian Journal of Veterinary Pathology 28(2):121-124.
Promya J., Chitmanat C., 2011 The effects of *Spirulina platensis* and *Cladophora algae* on the growth performance, meat quality and immunity stimulating capacity of the African sharptooth catfish (*Clarias gariepinus*). International Journal of Agriculture and Biology 13(1):77-82.
Qihuan Z., Ming Q., Wei X., Zhen G., Rong S., Zhitao Q., 2014 Effects of dietary administration of Chlorella on the immune status of gibel carp, *Carassius auratus gibelio*. Italian Journal of Animal Science 13(3):653-656.
Raji A. A., Alaba P. A., Yusuf H., Bakar N. H. A., Taufek N. M., Muin H., Alias Z., Milow P., Razak S. A., 2018 Fishmeal replacement with *Spirulina platensis* and *Chlorella vulgaris* in African catfish (*Clarias gariepinus*) diet: Effect on antioxidant enzyme activities and haematological parameters. Research in Veterinary Science 119:67-75.
Radhakrishnan S., Bhavan P. S., Seenivasan C., Shanthi R., Muralisankar T., 2014 Replacement of fishmeal with *Spirulina platensis*, *Chlorella vulgaris* and *Azolla pinnata* on non-enzymatic and enzymatic antioxidant activities of *Macrobrachium rosenbergii*. The Journal of Basic and Applied Zoology 67(2):25-33.
Reda R. M., El-Hady M., Selim K. M., El-Sayed H. M., 2018 Comparative study of three predominant gut *Bacillus strains* and a commercial *B. amyloliquefaciens* as probiotics on the performance of *Clarias gariepinus*. Fish & Shellfish Immunology 80:416-425.
Romano N., 2018 Alternative and new sources of feedstuffs. In: Enzymes in human and animal nutrition: Princples and perspectives. Nunes C., Kumar V. (eds), pp. 381-401, Academic Press, London, UK.
Sabur M., 2006 Studies on the ecology of the pathogenic bacteria Aeromonas hydrophila in indigenous and exotic carps under polyculture condition (PhD Dissertation), Bangladesh Agricultural University, Mymensingh, Bangladesh.
Şahan A., Özütok S., Kurutaş E. B., 2016 Determination of some hematological parameters and antioxidant capacity in Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) Fed Ginger (*Zingiber officinale* Roscoe) to *Aeromonas hydrophila*. Turkish Journal of Fisheries and Aquatic Sciences 16(1):197-204.
Sahu S., Das B. K., Mishra B. K., Pradhan J., Samal S. K., Sarangi N., 2008 Effect of dietary *Curcuma longa* on enzymatic and immunological profiles of rohu, *Labeo rohita* (Ham.), infected with *Aeromonas hydrophila*. Aquaculture Research 39(16):1720-1730.
Sakai M., 1999 Current research status of fish immunostimulants. Aquaculture 172(1-2):63-92.
Salnur S., Gultepe N., Hossu B., 2009 Replacement of fish meal by yeast (*Saccharomyces cerevisiae*): effects on digestibility and blood parameters for gilthead sea bream (*Sparus aurata*). Journal of Animal and Veterinary Advances 8(12):2557-2561.
Sarker P., Gamble M., Kelson S., Kapuscinski A., 2016 Nile tilapia (*Oreochromis niloticus*) show high digestibility of lipid and fatty acids from marine *Schizochytrium* sp. and of protein and essential amino acids from freshwater *Spirulina sp.* feed ingredients. Aquaculture Nutrition 22(1):109-119.
Sayed A. E. D. H., Fawzy M. A., 2014 Effect of dietary supplementation of *Spirulina platensis* on the growth and haematology of the catfish *Clarias gariepinus*. Journal of Advances in Biology 5(2):625-635.
Schulz C., Knaus U., Wirth M., Rennert B., 2005 Effects of varying dietary fatty acid profile on growth performance, fatty acid, body and tissue composition of juvenile pike perch (*Sander lucioperca*). Aquaculture Nutrition 11(6):403-413.
Seiverd C. E., 1983 Hematology for medical technologists. Lea & Febiger, Philadelphia, 946 p.

Sheikhlar A., Goh Y. M., Ebrahimi M., Romano N., Webster C. D., Alimon A. R., Daud H., Javanmard A., 2018 Replacement of dietary fishmeal for fenugreek seed meal on the growth, body composition, innate immunological responses and gene expression of hepatic insulin-like growth factors in African catfish (*Clarias gariepinus*). Aquaculture Nutrition 24(6):1718-1728.

Sheikhlar A., Meng G. Y., Alimon R., Romano N., Ebrahimi M., 2017 Dietary Euphorbia hirta extract improved the resistance of sharptooth catfish *Clarias gariepinus* to *Aeromonas hydrophila*. Journal of Aquatic Animal Health 29(4):225-235.

Shi X., Chen F., Chen G. H., Pan Y. X., Zhu X. M., Liu X., Luo Z., 2017 Fishmeal can be totally replaced by a mixture of rapeseed meal and Chlorella meal in diets for crucian carp (*Carassius auratus gibelio*). Aquaculture Research 48(11):5481-5489.

Siwicki A., Studnicka M., 1987 The phagocytic ability of neutrophils and serum lysozyme activity in experimentally infected carp, *Cyprinus carpio* L. Journal of Fish Biology 31:57-60.

Supamattaya K., Kiriratnikom S., Boonyaratpalin M., Borowitzka L., 2005 Effect of a Dunaliella extract on growth performance, health condition, immune response and disease resistance in black tiger shrimp (*Penaeus monodon*). Aquaculture 248(1-4):207-216.

Taufek N. M., Aspani F., Muin H., Raji A. A., Razak S. A., Alias Z., 2016 The effect of dietary cricket meal (*Gryllus bimaculatus*) on growth performance, antioxidant enzyme activities, and haematological response of African catfish (*Clarias gariepinus*). Fish Physiolology and Biochemistry 42(4):1143-1155.

Taufek N. M., Simarani K., Muin H., Aspani F., Raji A. A., Alias Z., Razak S. A., 2018 Inclusion of cricket (*Gryllus bimaculatus*) meal in African catfish (*Clarias gariepinus*) feed influences disease resistance. Journal of Fisheries 6(2):623-631.

Teuling E., Schrama J. W., Gruppen H., Wierenga P. A., 2017 Effect of cell wall characteristics on algae nutrient digestibility in Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*). Aquaculture 479:490-500.

Thanigaivel S., Chandrasekaran N., Mukherjee A., Thomas J., 2016 Seaweeds as an alternative therapeutic source for aquatic disease management. Aquaculture 464:529-536.

Toranzo A. E., Magarinos B., Romalde J. L., 2005 A review of the main bacterial fish diseases in mariculture systems. Aquaculture 246(1-4):37-61.

Vasudeva Rao Y., Das B. K., Jyotyrmayee P., Chakrabarti R., 2006 Effect of *Achyranthes aspera* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. Fish and Shellfish Immunology 20(3):263-273.

Watanuki H., Ota K., Tassakka A. C. M. A. R., Kato T., Sakai M., 2006 Immunostimulant effects of dietary *Spirulina platensis* on carp, *Cyprinus carpio*. Aquaculture 258(1-4):157-163.

Wise D. J., Camus A. C., Schwedler T. E., Terhune J. S., 2004 Health management. In: Biology and culture of channel catfish. Developments in Aquaculture and Fisheries Science. Tucker C. S., Hargreaves J. A. (eds), pp. 444-503, Elsevier.

Xu W., Gao Z., Qi Z., Qiu M., Peng J. Q., Shao R., 2014 Effect of dietary Chlorella on the growth performance and physiological parameters of gibel carp, *Carassius auratus gibelio*. Turkish Journal of Fisheries and Aquatic Sciences 14(1):53-57.

Yeganeh S., Teimouri M., Amirkolaie A. K., 2015 Dietary effects of *Spirulina platensis* on hematological and serum biochemical parameters of rainbow trout (*Oncorhynchus mykiss*). Research in Veterinary Science 101:84-88.

Zhang Q., Qiu M., Xu W., Gao Z., Shao R., Qi Z., 2014 Effects of dietary administration of Chlorella on the immune status of gibel carp, *Carassius auratus gibelio*. Italian Journal of Animal Science 13(3):3168.

Zhou J., Song X. L., Huang J., Wang X. H., 2006 Effects of dietary supplementation of A3α-peptidoglycan on innate immune responses and defense activity of Japanese flounder (*Paralichthys olivaceus*). Aquaculture 251(2-4):172-181.

*** AOAC, 2005 Official methods of analysis of AOAC international. In: Horwitz W., Latimer J. W. (eds), Current through Revision 1, 2006. Washington DC, 18th Edition. AOAC International press Gaithersberg, MD, USA.

*** FAO, 2018 The State of world fisheries and aquaculture-meeting the sustainable development goals. Retrieved from Fisheries and Aquaculture Department, Food and Agriculture Organization of the United Nations, Rome, 210 p.
*** Mirza Y., 2004 Winfeed 2.8 Software. Cambridge: Winfeed (UK) LTD.

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