



Partial replacement of fishmeal with *Spirulina platensis* and *Chlorella vulgaris* and its effect on growth and body composition of African catfish *Clarias gariepinus* (Burchell 1822)

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ABSTRACT

The study examined the effect of partial replacement of fishmeal with *Spirulina platensis* (SP) and *Chlorella vulgaris* (CL) powder in the diets of African catfish *Clarias gariepinus* (Burchell 1822) fingerlings on growth performance and body composition. Nine diets (45.14-48.45% crude protein) consisting SP or CL as fishmeal replacement at zero (control), 12.5, 25, 50 and 75%, were fed to triplicate groups of 10 fingerlings (7.82±0.08 g) each for 56 days. The results showed statistically higher weight gain, specific growth rate, protein efficiency ratio, relative growth rate, Fulton's condition factor (K) and a corresponding lower feed conversion ratio when the replacement of *C. vulgaris* was at 75%. Proximate analysis revealed a significantly greater carcass protein in the SP12.5% and the highest flesh lipid and gross energy in SP75% groups. Flesh lipid increased with increasing SP and CL levels. Polynomial curve estimation regression analysis revealed the optimum fishmeal replacement levels for best growth are at 68.5 and 69.4% for SP and CL, respectively. CL was found to be more efficient in terms of growth and effective feed utilisation than SP.

Keywords: Body composition, *Chlorella vulgaris*, Fishmeal, Growth, *Spirulina platensis*

Introduction

Increase in human population and overexploitation of natural water bodies have reduced the levels of fish captured from wild and led to increase in aquaculture production. Aquaculture is among the fastest growing food producing sectors globally which forms a potential solution to the reduction in global capture fisheries production (Lehane, 2013). The cost of feed in aquaculture production has led to reliance on feed ingredients, especially fishmeal (FM), due to its high profile of proteins and growth factors (NRC, 2011). The increased demand for fishmeal together with its static production necessitated the search for alternative, locally available protein sources as its replacement in fish feed.

Previously, researchers used plant protein as a replacement for the conventional fishmeal diet in feeding fish. These include various pulses and lupins in various fishes. However, soybean meal and related byproducts have been widely used in fish diet and are of prime importance in the tropical omnivorous fish feed (Goda *et al.*, 2007; Jimoh *et al.*, 2014). However, microalgae are mainly used in aquaculture nutrition as a sole component

or food additive, for colouring the flesh of salmonids and for inducing other biological activities (Richmond, 2004). Microalgae have high protein content and digestible amino acid profiles comparable with those of other reference food proteins (Becker, 2007; Reyes-Becerril *et al.*, 2013). They are also high in polyunsaturated fatty acids, β -carotene, antioxidants, sulphated polysaccharides and sterols (Otlis and Pire, 2001; Xue *et al.*, 2002). Numerous studies have been conducted on the usage of algae on different cultured fish as additives. Many of these studies demonstrated that algae as feed additives improved the growth and feed utilisation of the cultured fish because of effective absorption of dietary protein, enhancement of biological activities, stress reduction and disease resistance (Gatlin, 2007; Guroy *et al.*, 2011). *Chlorella* and *Spirulina* have great potential to be used in aquafeed due to their high protein content, indispensable amino and fatty acids, antioxidant pigments as well as immunostimulatory properties (Becker, 2007; Lordan *et al.*, 2011).

Chlorella vulgaris is a unicellular green alga that is very rich in chlorophyll, protein (51-58%, depending on

the species), crude lipid (14-15%) and essential nutrients like polyunsaturated fatty acid pigments, antioxidant vitamins such as B6, B12, β -carotene C, E and minerals (Tartiel, 2005; Becker, 2007; Badwy *et al.*, 2008). More importantly, amino acids such as aspartic acid, serine, alanine, leucine and glycine were found to contribute to 50% of dry matter of *Chlorella* (Badwy *et al.*, 2008). It also contains *Chlorella* growth factor (CGF) and β -1, 3-glucan, a strong immune stimulator, a free-radical scavenger as well as a reducer of blood fat (Spolaore *et al.*, 2006). Previously, researchers have investigated *Chlorella* as a feed additive or fishmeal replacement for growth enhancement and as an immune booster of different fish species such as Gibel carp (*Carassius auratus gibelio*) (Qihuan *et al.*, 2014; Xu *et al.*, 2014), rohu (*Labeo rohita*) (Pradhan and Das, 2015), *Chlorella* and *Scenedesmus* on Nile tilapia (*Oreochromis niloticus*) (Badwy *et al.*, 2008).

On the other hand, *Spirulina platensis* is a blue-green cyanobacterium that is very rich in protein, 55-70% depending on the species and the source (Phang *et al.*, 2000; Lordan *et al.*, 2011), essential fatty acids (GLA) and vitamins, essentially pro-vitamin A and B12 (Belay, 2002; Gouveia *et al.*, 2008; Dalle Zotte *et al.*, 2013). It is also rich in antioxidant pigments, especially phycocyanin and chlorophyll (Wang *et al.*, 2007). Previous researchers have examined the influence of *Spirulina* on the development, immune responses and nutrient utilisation of different fish species such as *Clarias gariepinus*, *Oplegnathus fasciatus* and *Cyprinus carpio* (Promya and Chitmanat, 2011; Abdulrahman and Ameen, 2013; Kim *et al.*, 2013; Abdulrahman and Ameen, 2014; Sayed and Fawzy, 2014).

African catfish *Clarias gariepinus* (Burchell 1822) is regarded as one of the most important freshwater fish due to its fast growth, reproduction and adaptability in Africa, South-east Asia and other parts of the world (Fasakin *et al.*, 2003; Al-Dohail *et al.*, 2009). It has superior growth performance compared with other *Clarias* species. Hence, there is a need for a well-balanced diet to sustain and increase the commercial production of this important fish species at a reasonable cost to both farmers and consumers. Information available in literature regarding optimum dietary addition levels of *C. vulgaris* and *S. platensis* for the growth performance of most fish species are inconsistent. There is also scarce information on the effects of *C. vulgaris* on *C. gariepinus*. This may be partly attributed to the fact that *C. vulgaris* and *S. platensis* diets are yet to be commercially applied as a source of macronutrients in fish feed formulation (Teuling *et al.*, 2017). The high nutritional and health properties of the two algae and their usefulness to growth and body

composition of fish has attracted much research attention. But, no published information is available on the effect of *C. vulgaris* or *S. platensis* in African catfish. Thus, this study included *C. vulgaris* and *S. platensis* separately in pelleted diets, to ascertain the effects of their altered inclusion levels on growth enhancement and carcass composition of *C. gariepinus*. Additionally, the study sought to determine the comparative advantages of one alga over the other, as well to establish the optimal level that could positively replace fishmeal without adverse effect on *C. gariepinus* growth.

Materials and methods

Experimental diets

Spray-dried and thin cell *C. vulgaris* (cell-ruptured) and *S. platensis* powder used for this study were purchased from TST Biocuticals (Perak, Malaysia). The powder was used to formulate nine experimental diets based on the protein requirement of clariid fish fingerlings. The diets were prepared in sets of either *Chlorella* (CL) or *Spirulina* (SP) to replace fishmeal at various inclusion levels of 0, 12.5, 25, 50 and 75% resulting in diet levels of SP12.5% to SP75% or CL12.5% to CL75%. Fishmeal was replaced proportionally according to their inclusion level, while other feed ingredients were adjusted to attain isonitrogenous and caloric diets (Table 1). Diets were formulated using Pearson's square and Winfeed 2.8 software (Mirza, 2004). After milling essential feed components with a hammer mill (Disk Mill, FFC 454), vitamins, minerals and di-calcium phosphate (DCP) were carefully mixed using water, then pelleted into 1 mm diameter in a pelleting machine (model KCM, Y132M-4). The pelleted feed was oven dried at 70°C and preserved in a feed cold room (4°C) until use.

Experimental set-up

A total of 450 African catfish fingerlings were purchased from a native supplier in Balakong Hatchery, and acclimatised at the Institute of Biological Sciences Freshwater Aquarium Laboratory, University Malaya. Prior to stocking, the fingerlings (7.82 \pm 0.08 g) were starved for 24 h and 270 fingerlings were randomly stocked into 27 different tanks, with 10 fish per tank and 3 replicates per treatment. Experimental fish were fed twice daily between 9:00 and 16:00 hrs at 4% body weight for 56 days. Fish sampling and weighing were performed fortnightly. The tanks were monitored closely for mortality and dead fishes were removed and recorded for determination of survival rate. On the commencement of the feeding trials, 10 fish samples were homogenised,

Table 1. Ingredients and proximate composition (% as fed) of experimental diets

Ingredients (g kg ⁻¹)	Treatments (Replacement %)								
	Control	* <i>Spirulina platensis</i>				** <i>Chlorella vulgaris</i>			
		0%	12.5%	25%	50%	75%	12.5%	25%	50%
Fishmeal	250	218.8	187.5	125	62.5	218.8	187.5	125	62.5
<i>Spirulina</i>	0	31.2	62.5	125	187.5	0	0	0	0
<i>Chlorella</i>	0	0	0	0	0	31.2	62.5	125	187.5
Soya bean	503	494.5	501.9	500.3	501.9	512.3	521.7	540.5	559.2
Rice bran	139.3	150.9	143.9	148.5	143.9	133.6	127.9	116.5	105.1
Premix ^a	9	9	9	9	9	9	9	9	9
Cod liver oil	59.7	56.6	56.2	53.2	56.2	56.1	52.4	45.1	37.7
Supplements+binder ^b	39	39	39	39	39	39	39	39	39
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000
Nutrient levels (% Dry matter basis)									
Protein	47.84	48.45	46.74	45.58	45.59	45.14	45.53	46.35	46.03
Fat	9.27	10.62	9.91	8.87	8.92	10.48	8.43	8.07	7.37
Ash	8.87	9.28	8.49	8.09	8.27	8.98	8.53	8.00	7.30
Moisture	6.49	4.29	5.94	5.98	8.27	6.51	6.69	6.19	6.70
Crude fibre	2.02	3.19	1.76	2.29	1.29	2.89	1.99	2.37	2.12
NFE	25.51	22.17	27.16	29.19	27.66	26.00	30.83	29.02	30.48
Gross energy (KJ g ⁻¹)	19.24	19.53	19.51	19.17	18.94	19.17	18.98	19.05	18.99

^a Vitamin and Mineral premix supplied: vitamins A - 500IU; 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;

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 - .25 mg

^b Supplements plus binder: Lysine, Methionine, DCP and binder at 0.2; 0.7; 1.0; 20, respectively

**Spirulina* proximate composition: CP 60.71%; Crude lipid 8.68%; Energy 477.92 Kcal 100 g⁻¹

** *Chlorella* proximate composition: CP 58.0%; Crude lipid 9.59%; Energy 478.05 Kcal 100 g⁻¹

Fishmeal proximate composition: CP 66.67%; Crude lipid 9.55%; Energy 472.21 Kcal 100 g⁻¹

then frozen while 3 samples per replicate were euthanised with 10% clove oil, for subsequent carcass examination on termination of the experiments.

Proximate examination of trial diets and fish carcass

Proximate analysis (crude protein, dry matter, ash, crude fibre and crude lipid) was carried out on the ingredients, formulated diets and fish carcass as per AOAC (2000). Crude protein analysis was done *via* the Kjeldahl method (N×6.25) after acid digestion using Vapodest 50 (Gerhardt Germany). Dry matter and moisture were determined through oven drying at 105°C to a constant weight and ash *via* combustion in a muffle furnace (Memmert UFB500 and Carbolite Furnace Memmert CWF 11/13, Germany) at 600°C. Crude lipid was determined as per Bligh and Dyer (1959) with slight modification. This involved dissolving the sample in a mixture of 2:1 chloroform and methanol in Soxhlet apparatus (Gerhardt Soxtherm) for 8 h for SP and CL and petroleum ether extraction for other samples for 1.5 h. Fiber contents were determined by alkali and acid digestion of lipid residue. Nitrogen free extract (NFE) was calculated as 100 - (% crude lipid + % crude fibre + % crude protein

+ % crude ash) and gross energy (kJ g⁻¹) was calculated using the physiological values, CP × 23.9 + lipid × 39.8 + carbohydrates × 17.6 (Schulz *et al.*, 2005). The essential amino acid composition of freeze-dried algae meal was determined 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 and Meredith (1984). Meanwhile, the alkaline hydrolysis method by Nielsen and Hurrell (1985) was used to determine tryptophan. Fatty acid methyl esters were separated and measured following the method by Ichihara and Fukubayashi (2010) using Agilent 7820A gas chromatograph fitted with a capillary column (SLB-IL 100, 30 m × 0.25 mm × 0.20 μm, Supelco, USA) plus a flame ionisation detector (FID) using the temperatures of injector and detector at 250 and 260°C, respectively. The different FAMES were recognised by comparing their retention times with those of known standards from Sigma-Aldrich, USA, while fatty acids were quantified in milligram per gram of lipids, by addition of the internal standard C7:0 Sigma, USA (Vello *et al.*, 2014).

$$\text{Mean weight gain} = \frac{W_f - W_i}{n}$$

where, W_f = final weight; W_i = initial weight and n = number of fish.

$$\text{Relative growth rate (RGR)} = \frac{W_g}{W_i} \times 100$$

where, W_g = weight gain and W_i = initial weight

$$\text{Specific growth rate (FCR)} = \frac{(\log W_f - \log W_i)}{t} \times 100$$

where, $\log W_f$ = log of final weight, $\log W_i$ = log of initial weight, and t = time.

$$\text{Feed conversion ratio (FCR)} = \frac{F_i}{FW_g}$$

where, F_i = dry feed fed and FW_g = fish wet weight gain.

$$\text{Protein efficiency ratio (PER)} = \frac{MWG}{MPI}$$

where, MWG = mean weight gain and MPI = mean protein fed.

$$\text{Survival rate} = \frac{F_n}{I_n} \times 100$$

where, F_n = final quantity of fish at the end of experiment and I_n = initial number of fish at the beginning of experiment.

$$\text{Protein productive value (PPV)} = \frac{FPE}{FPB} \times 100$$

where, FPE = total fish protein at the end and FPB = total fish protein at the beginning of feeding experiment.

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

where, W = the weight of fish (g) and L = standard length (cm) (Htun-Han, 1978).

Growth performance indices

Fish weights and feed supplied were measured and recorded fortnightly to compute the growth indices following the method by Oliva-Teles and Goncalves (2001).

Statistical analysis

One-way analysis of variance (ANOVA) with SPSS version 21 statistical package (SPSS Inc., Chicago) was used to analyse the experimental data. Duncan's multiple range analysis (Duncan, 1955) was used to compare differences between treatments and considered to be significant when $p < 0.05$. Curve estimation (polynomial) regression analysis was performed with the aid of Wolfram Mathematical 11 version (11:0.1.0) student edition (Wolfram Research Inc., Champaign IL, USA), to estimate the optimum level of dietary replacement with

CL and SP respectively relative to the weight gain of *C. gariepinus* fingerlings.

Results

Proximate composition of *Spirulina platensis*, *Chlorella vulgaris* and experimental diets

Table 1 shows the formulations and proximate composition of the different diets used in the study. The crude protein of the experimental diets (%) ranged from 45.14 to 48.45, lipid 7.37 to 10.62, ash 7.30 to 9.28, moisture 4.29 to 8.27, fiber 1.29 to 3.19, NFE 22.18 to 30.84 and gross energy 18.94 to 19.53 KJ g⁻¹. This can be compared to the composition of *S. platensis* (CP 60.71%; crude lipid 8.68%; energy 20.00 KJ g⁻¹), *C. vulgaris* (CP 58.0%; crude lipid 9.59%; energy 20.00 KJ g⁻¹) and fishmeal (CP 66.67%; crude lipid 9.55%; energy 19.76 KJ g⁻¹) used for the diets (Table 1).

Significant differences ($p < 0.05$) were observed in total fatty acids, saturated fatty acids, total monounsaturated fatty acids, total n-6 and n-3 (18, PUFAs), n-3 and n-6 long chain (LC) PUFAs among dietary treatments (Table 2). The CL12.5% diet had significantly ($p < 0.05$) higher total fatty acids (TFA) than control and other treatments, while CL50% diet had significant higher ($p < 0.05$) linoleic acid, C18:2n6 (20.11%). Saturated fatty acids (SFA), the majority of which was made up of palmitic acid (C16:0) was significantly higher ($p < 0.05$) in the SP50% diet, whereas of the total monounsaturated fatty acids that predominated in SP12.5% (24.33%), a substantial amount was made up of oleic acid (18:1n9c) as found in SP25% (12.61%). Both linolenic and docosahexaenoic (18:3n3 and 22:6n3DPA) acids were higher in CL25%, while eicosapentaenoic acid (EPA), 20:5n3 was higher in the CL75% diet.

All the 10 essential amino acids were present in varying amounts in all the diets (Table 3). Among the diets, SP50% was considerably greater ($p < 0.05$) in histidine, arginine, threonine, valine, methionine, isoleucine, leucine and phenylalanine. Lysine and tryptophan were significantly ($p < 0.05$) higher in CL12.5% diet.

Growth performance

The growth performance of CL75% fish were significantly ($p < 0.05$) higher in terms of body weight gain (16.32±0.01 g), SGR (3.15±0.00%), PER (2.33±0.01), RGR (209.02±1.05%), K factor (1.89±0.02) and a corresponding lower FCR (0.93±0.01), while SP12.5% recorded highest ($p < 0.05$) PPV of 83.61±0.04 (Table 4). However, optimum level of replacement of fishmeal with CL relative to the growth of the fish was found to be 69.4%, while optimum replacement with SP was 68.5% (Fig. 1a and b).

Table 2. Fatty acid composition (mg g⁻¹) of diets containing various levels of *S. platensis* and *C. vulgaris*

Fatty acid	Control	SP12.5%	SP25%	SP50%	SP75%	CL12.5%	CL25%	CL50%	CL75%
ΣFAS	42.62±0.01	26.47±0.01	48.81±0.02	17.31±0.01	28.46±0.02	52.48±0.01	5.70±0.01	13.39±0.00	17.29±0.01
¹ ΣSFA	46.08±0.01 ^b	47.59±0.02 ^c	47.83±0.01 ^d	54.28±0.01 ⁱ	51.15±0.02 ^h	47.91±0.01 ^e	40.49±0.01 ^a	50.11±0.00 ^g	48.38±0.01 ^f
² ΣMUFA	23.05±0.02 ^g	24.33±0.01 ⁱ	23.16±0.02 ^h	21.00±0.02 ^e	20.12±0.02 ^d	22.47±0.0 ^f	15.46±0.01 ^a	19.71±0.01 ^c	18.94±0.01 ^b
³ ΣPUFA	30.89±0.02 ^g	28.12±0.21 ^b	29.03±0.01 ^d	24.73±0.02 ^a	28.72±0.01 ^c	29.64±0.01 ^e	44.06±0.02 ⁱ	30.17±0.01 ^f	32.70±0.01 ^h
C16:0	27.90±0.01 ^b	31.77±0.02 ^g	29.75±0.02 ^d	35.90±0.02 ⁱ	31.72±0.01 ^f	30.63±0.01 ^e	22.72±0.01 ^a	32.43±0.02 ^h	26.86±0.02 ^c
C18:0	9.50±0.01 ^h	8.80±0.01 ^c	8.89±0.01 ^d	9.43±0.01 ^g	10.25±0.01 ⁱ	9.28±0.01 ^f	6.31±0.01 ^b	9.26±0.01 ^e	2.39±0.02 ^a
C18:1n9c	11.59±0.02 ^d	12.43±0.01 ^g	12.61±0.01 ⁱ	11.54±0.01 ^c	11.82±0.01 ^f	12.57±0.01 ^h	7.38±0.01 ^b	11.63±0.02 ^e	2.52±0.02 ^a
⁴ Σ1n9	18.97±0.02 ^g	20.23±0.01 ⁱ	19.75±0.01 ^h	16.99±0.01 ^c	16.33±0.01 ^d	18.84±0.01 ^f	12.48±0.00 ^b	15.75±0.01 ^e	11.62±0.02 ^a
C18:2n6	13.22±0.02 ^d	12.51±0.02 ^c	13.80±0.02 ^e	14.00±0.02 ^f	15.24±0.01 ^h	14.75±0.01 ^g	9.63±0.01 ^b	20.11±0.01 ⁱ	5.85±0.01 ^a
⁵ Σn-6PUFA	13.45±0.01 ^d	12.61±0.01 ^c	13.95±0.01 ^e	14.09±0.01 ^f	15.50±0.01 ^h	14.94±0.00 ^g	10.35±0.00 ^b	20.27±0.01 ⁱ	6.40±0.01 ^a
⁶ Σn-3 PUFA	14.85±0.01 ^g	13.60±0.01 ^f	12.90±0.01 ^e	8.24±0.01 ^b	8.81±0.00 ^c	12.33±0.00 ^d	32.61±0.01 ⁱ	7.95±0.01 ^a	18.98±0.01 ^h
C20:5n3	5.93±0.01 ^h	5.69±0.01 ^g	5.27±0.00 ^f	3.60±0.01 ^c	3.32±0.00 ^a	5.24±0.00 ^e	3.40±0.00 ^b	4.35±0.00 ^d	7.84±0.00 ⁱ
C22:6n3	5.46±0.00 ^g	5.21±0.00 ^f	4.72±0.00 ^e	2.74±0.01 ^b	2.39±0.01 ^a	4.23±0.01 ^d	27.72±0.00 ⁱ	6.00±0.01 ^h	3.75±0.01 ^c
n-3/n-6PUFA	1.1	1.08	0.92	0.58	0.57	0.83	3.15	0.39	2.97

Values are means of triplicate of nine different feed samples. Values on the same row with different superscripts are significantly different (p<0.05)
¹Saturated fatty acids consist of: 4:0, 6:0, 8:0, 10:0, 11:0, 12:0, 13:0, 14:0, 15:0, 16:0, 18:0, 20:0 and 24:0
²Monounsaturated fatty acids comprise: 14:1, 15:1, 16:1, 18:1n9c, 18:1n7, 20:1n9, C22:1n9 and 24:1n9
³Polyunsaturated fatty acids consist of: 16:2n4, 16:3n4, 16:4n1, 18:2n6t, 18:2n6c, 18:3n4, 18:3n6, 18:3n3, 18:4n3, 20:2, 20:3n6, 20:3n3, 20:5n3, C22:2 and 22:6n3
n-3 fatty acids were: 18:3n3, 18:4n3; n-6 fatty acids: 18:2n6t, 18:2n6c, 18:3n6; n-3 Long chain polyunsaturated fatty acids found were: 20:3n3, 20:5n3 and 22:6n3; n-6 Long chain polyunsaturated fatty acids include: 20:3n6
⁴Consisted 18:1n9c, 20:1n9, C22:1n9 and 24:1n9
⁵Comprised: 18:2n6t, 18:2n6c, 18:3n6 and 20:3n6
⁶Included: 18:3n3, 18:4n3, 20:3n3, 20:5n3 and 22:6n3

Table 3. Essential amino acids (g 100 g crude protein⁻¹) of the study diets containing graded levels of *S. platensis* and *C. vulgaris*

Amino acid	Control	SP12.5%	SP25%	SP50%	SP75%	CL12.5%	CL25%	CL50%	CL75%
Histidine*	2.02±0.06 ^b	2.07±0.02 ^{bc}	2.11±0.06 ^{bc}	2.38±0.02 ^d	1.85±0.00 ^a	2.01±0.01 ^b	2.17±0.00 ^c	1.87±0.00 ^a	2.00±0.00 ^b
Arginine*	6.47±0.27 ^{bc}	6.57±0.06 ^c	6.49±0.14 ^c	7.47±0.09 ^d	6.29±0.04 ^{bc}	6.10±0.01 ^{ab}	6.30±0.01 ^{bc}	5.76±0.01 ^a	6.08±0.04 ^{ab}
Threonine*	3.68±0.05 ^{ab}	3.78±0.05 ^{bc}	3.79±0.10 ^{bc}	4.43±0.06 ^d	3.75±0.03 ^b	3.82±0.02 ^{bc}	3.92±0.00 ^c	3.57±0.00 ^a	3.77±0.01 ^b
Valine*	3.64±0.05 ^b	3.70±0.05 ^b	3.97±0.09 ^c	4.14±0.04 ^c	3.62±0.01 ^b	4.09±0.20 ^c	3.23±0.00 ^a	3.62±0.00 ^b	3.57±0.00 ^b
Methionine A*	2.38±0.02 ^{bc}	2.40±0.04 ^c	2.68±0.05 ^c	2.91±0.02 ^f	2.32±0.02 ^b	2.64±0.01 ^{de}	2.57±0.01 ^d	2.11±0.00 ^a	2.13±0.00 ^a
Lysine*	5.40±0.14 ^c	5.09±0.08 ^b	6.12±0.14 ^c	4.87±0.09 ^f	3.87±0.02 ^a	6.12±0.08 ^c	3.64±0.01 ^a	5.71±0.01 ^d	4.85±0.00 ^b
Isoleucine*	3.24±0.03 ^c	3.35±0.05 ^d	3.53±0.07 ^c	3.80±0.02 ^f	3.36±0.01 ^d	3.49±0.01 ^e	2.83±0.00 ^a	3.11±0.00 ^b	3.09±0.00 ^b
Leucine*	6.43±0.09 ^b	6.44±0.10 ^b	6.94±0.15 ^c	7.18±0.04 ^d	6.28±0.02 ^b	6.78±0.00 ^c	5.62±0.02 ^a	6.42±0.01 ^b	6.35±0.00 ^b
Phenylalanine/TAA*	3.87±0.07 ^{ab}	4.04±0.06 ^c	4.07±0.09 ^c	4.80±0.00 ^e	3.95±0.01 ^{bc}	3.97±0.01 ^{bc}	4.28±0.01 ^d	3.76±0.01 ^a	4.07±0.00 ^c
Tryptophan*	0.83±0.01 ^d	0.79±0.00 ^{cd}	0.79±0.00 ^{cd}	0.78±0.00 ^{cd}	0.70±0.00 ^b	0.76±0.00 ^c	0.72±0.01 ^{bc}	0.68±0.00 ^b	0.59±0.00 ^a

Values are means of triplicates of nine different feed samples. Mean values on the same row with different superscripts are significantly different (p<0.05). *Amino acid requirement of *C. gariepinus*: Histidine - 1.2; Arginine - 3.6; Threonine - 2.8; Valine - 2.4; Methionine A - 2.3; Lysine - 4.8; Isoleucine - 2.0; Leucine - 3.5; Phenylalanine/TAA - 4.0; Tryptophan - 0.5, [Source: Uys (1989); Unprasert. (1984)]. TAA: Total amino acid

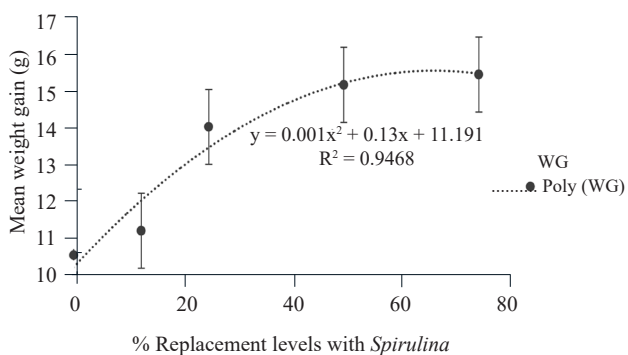


Fig. 1a. Mean weight gain of *C. gariepinus* fingerlings fed graded levels of *Spirulina* for 56 days. The optimum level is at 68.5%.

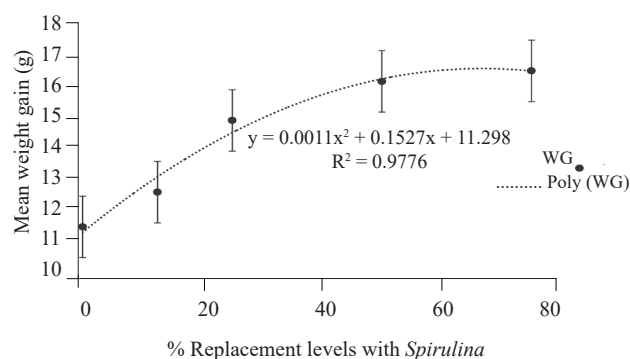


Fig. 1b. Mean weight gain of *C. gariepinus* fingerlings fed graded levels of *Chlorella* for 56 days. The optimum level is at 69.4%.

Table 4. Growth parameters of *C. gariepinus* fingerlings fed different levels of *S. platensis* or *C. vulgaris* diets for 8 weeks

Parameter	Control	SP12.5%	SP25 %	SP50%	SP75%	CL12.5 %	CL25%	CL50%	CL75%
IW (g)	7.82±0.06 ^a	7.88±0.05 ^a	7.88±0.01 ^a	7.87±0.03 ^a	7.85±0.03 ^a	7.87±0.04 ^a	7.82±0.00 ^a	7.89±0.05 ^a	7.81 ±0.02 ^a
FW (g)	19.29±0.03 ^a	19.91±0.03 ^b	22.33±0.01 ^d	23.30±0.01 ^f	23.52±0.03 ^g	20.42±0.04 ^c	22.60±0.01 ^e	23.88±0.02 ^h	24.14±0.01 ⁱ
WG (g)	11.47±0.03 ^a	12.04±0.09 ^b	14.45±0.01 ^d	15.43±0.02 ^f	15.67±0.01 ^g	12.55±0.01 ^c	14.78±0.01 ^e	15.99±0.05 ^h	16.32±0.01 ⁱ
RGR %	146.63±1.63 ^a	152.90±2.03 ^b	183.53±0.14 ^d	196.07±1.05 ^f	199.69±0.81 ^g	159.50±0.89 ^c	189.01±0.08 ^e	202.78±1.84 ^g	209.02±0.61 ^h
SGR (% day ⁻¹)	2.92±0.00 ^a	2.95±0.00 ^b	3.07±0.00 ^d	3.11±0.00 ^g	3.12±0.00 ^f	2.98±0.00 ^c	3.08±0.00 ^e	3.14±0.00 ^h	3.15±0.00 ⁱ
FI (g)	17.75±0.21 ^b	16.89±0.08 ^{ab}	16.96±0.02 ^{ab}	16.17±0.01 ^a	17.92±0.04 ^b	16.84±0.02 ^{ab}	16.40±0.01 ^a	16.20±0.02 ^a	16.26±0.03 ^a
FCR	1.55±0.01 ⁱ	1.40±0.02 ^h	1.17±0.01 ^f	1.05±0.01 ^c	1.14±0.01 ^e	1.34±0.01 ^g	1.11±0.13 ^d	0.95±0.01 ^b	0.93±0.01 ^a
PI	8.49±0.01 ^g	8.52±0.00 ^g	7.93±0.00 ^c	7.37±0.00 ^c	8.17±0.02 ^f	7.60±0.01 ^d	7.14±0.01 ^b	7.04±0.03 ^a	7.03±0.00 ^a
PER	1.35±0.01 ^a	1.41±0.02 ^b	1.82±0.01 ^d	2.09±0.01 ^g	1.92±0.01 ^e	1.65±0.01 ^c	2.07±0.01 ^f	2.27±0.01 ^h	2.33±0.01 ⁱ
PPV	58.19±0.09 ^d	83.61±0.04 ⁱ	78.84±0.10 ^h	46.80±0.01 ^c	36.24±0.07 ^a	60.39±0.06 ^c	39.73±0.03 ^b	75.55±0.33 ^g	72.31±0.12 ^f
K Factor	1.39±0.00 ^a	1.50±0.03 ^b	1.66±0.03 ^d	1.69±0.02 ^{de}	1.73±0.02 ^e	1.58±0.01 ^g	1.67±0.01 ^f	1.84±0.01 ^g	1.89±0.01 ^h
Survival (%)	86.67±8.82 ^a	96.67±3.33 ^a	96.67±3.33 ^a	93.33±3.33 ^a	100.00±0.0 ^a	96.67±3.33 ^a	93.33±3.33 ^a	96.67±3.33 ^a	96.67±3.3 ^a

Values are means of a triplicate of ten fish. Mean values on the same row with different superscripts are significantly different ($p < 0.05$). SGR: Specific growth Rate; FCR: Feed conversion ratio; RGR: Relative growth rate; PI: Protein intake; PER: Protein efficiency ratio; K: Fulton's condition factor; PPV: Protein productive value.

Table 5 clearly shows significant ($p < 0.05$) differences in the experimental fish carcass composition compared to the control group. The mean protein value (61.97%±0.10) recorded for SP12.5% was significantly ($p < 0.05$) higher than all other experimental fish groups except for SP25%. Crude lipid (14.81%±0.08) and gross energy (20.81 KJ g⁻¹±1.65) were significantly ($p < 0.05$) higher in SP75% than other diets, whereas ash, moisture and NFE were significantly ($p < 0.05$) lower in SP75%, control and SP25% respectively.

Discussion

This study investigated the effect of *S. platensis* and *C. vulgaris* as partial replacement for fishmeal protein on growth and body compositions of African catfish (*C. gariepinus*) fingerlings. The basal diet was replaced with 0 to 75% *S. platensis* and *C. vulgaris*. The findings from this study showed that experimental diets contained similar fatty acids, essential amino acids and gross energy, relative to the control diet, although the two microalgae varied in their chemical composition of essential amino acid and

fatty acid profiles. The crude protein and lipid values of the experimental diets fall within the range recommended for optimal growth of catfish fingerlings (FAO, 2009). The experimental diets contained high quantity of amino acids which are greater than values reported by Jimoh *et al.* (2014) for sesame seed meal fed to African catfish and slightly higher than values recommended for African catfish by Unprasert (1994), except for the values obtained for methionine in CL75%, lysine in SP75 and CL25%. Cysteine was however not determined, as Lovell (1989) reported that non-EAAs like cysteine and tyrosine could replace about 60 and 50% catfish requirements for methionine and phenylalanine, respectively.

Fatty acids (FAs) are imperative constituents of biomembranes of fish besides providing a premise of vitality (Parrish *et al.*, 2012). In many fish species, eicosapentaenoic acid (EPA) and arachidonic acid (ARA) are essential for vital metabolic functions (Tocher, 2015). Microalgae are the principal producers of FAs. Fish, for the most part, require linolenic acid (18:3n-3) for optimal

Table 5. Proximate composition (% dry weight) of the whole body of *C. gariepinus* fed *S. platensis* and *C. vulgaris* diets

Treatments	Composition					
	Crude protein	Crude lipid	Ash	Moisture	NFE	Gross energy
Initial	54.85±0.09 ^a	7.16±0.00 ^a	20.95±0.04 ^h	5.00±0.03 ^c	12.06±0.08 ^d	17.81±0.20 ^a
Control	59.79±0.00 ^d	10.21±0.03 ^e	17.71±0.11 ^d	2.83±0.01 ^a	9.48±0.08 ^c	19.75±0.56 ^d
SP12.5%	61.97±0.10 ^e	7.53±0.01 ^b	18.39±0.10 ^f	4.04±0.00 ^d	8.07±0.01 ^b	18.98±0.49 ^b
SP25%	61.10±0.40 ^e	10.16±0.00 ^e	18.82±0.00 ^g	3.99±0.01 ^d	5.95±0.38 ^a	19.45±0.72 ^c
SP50%	58.30±0.25 ^c	12.14±0.03 ^d	17.62±0.01 ^d	3.92±0.01 ^d	8.04±0.22 ^b	19.92±0.26 ^c
SP75%	57.81±0.40 ^b	14.81±0.08 ^g	16.08±0.05 ^a	3.61±0.19 ^{bc}	7.71±0.34 ^b	20.81±1.65 ^g
CL12.5%	59.44±1.07 ^{cd}	13.30±0.15 ^f	16.78±0.06 ^{bc}	3.47±0.00 ^b	7.03±0.98 ^{ab}	20.50±0.96 ^f
CL25%	57.68±0.17 ^b	12.55±0.11 ^e	18.04±0.00 ^c	3.63±0.03 ^{bc}	8.11±0.03 ^b	19.95±0.20 ^c
CL50%	60.17±0.24 ^{dc}	7.16±0.00 ^a	16.93±0.06 ^c	4.07±0.03 ^d	11.69±0.14 ^d	19.01±0.75 ^b
CL75%	59.93±0.57 ^d	10.13±0.03 ^e	16.54±0.01 ^b	3.71±0.03 ^c	9.71±0.50 ^c	19.79±0.93 ^d

Values are means of triplicate groups of 3 fish per replicate. Mean values in the same column with different superscripts are significantly different ($p < 0.05$).

growth. African catfish has the ability to change dietary linolenic acid to EPA (20:5n-3) which is sufficient to meet its metabolic demands (Tucker and Hargreaves, 2004). The n-3 EFA prerequisite could be met by either 1 to 2% linolenic acid or 0.5 to 0.75% n-3 HUFAs (Satoh *et al.*, 1989), which can be supplied by supplementing marine fish oil, for example, menhaden oil in the diet (Morris *et al.*, 1995; Manning *et al.*, 2007). This can be likened to the palm oil supplement in the experimental diets. However, fatty acids play vital roles in the development of catfish. The EFA requirements of *C. gariepinus* according to Uys and Hecht (1985) are linoleic acid (18:2n6), arachidonic (20:4n-6), linolenic (18:3n3), eicosapentaenoic (20:5n3) and docosahexaenoic (22:6n3) acids in the ratio 1:1. The values for linoleic, linolenic, eicosapentaenoic and docosahexaenoic acids obtained in the present study are higher than the values obtained for African catfish by Li *et al.* (2009) from dried *Schizochytrium* sp. diets.

The outcome of this study shows that *C. gariepinus* fingerlings readily accepted the experimental diets as they exhibited good growth. This study further reveals that replacing fishmeal with *C. vulgaris*, positively correlated to the growth of the fingerlings resulting in higher weight gain and SGR. This might be as a result of growth factor in *Chlorella* sp. (Bengwayan *et al.*, 2010), while higher PER may be due to high protein digestibility. *C. vulgaris* diets (50 and 75%) have higher CP compared to other CL treatments and this high protein could induce the fish to use protein as energy source resulting in significant weight gain. Optimum weight gain was achieved at 69.4% replacement of FM with *C. vulgaris* and higher substitution beyond this could not provide a further increase in weight gain. Increasing the replacement level of fishmeal with *Chlorella* sp. above 50% did not improve the growth performance of Nile tilapia (Badwy *et al.*, 2008). Conversely, Khani *et al.* (2017) showed that optimum growth performance of Koi carp is best attained at 5% *C. vulgaris* replacement of fishmeal.

In addition, previous studies have attributed the comparable growth performance associated with *Chlorella* diets, to growth promoters such as sufficient amounts of macro nutrients and *Chlorella* growth factors, present in *C. vulgaris* (Yamaguchi, 1996; Badwy *et al.*, 2008). More so, the growth performance associated with dietary *Chlorella* could be attributed to high digestibility of microalgae and possession of significant concentration of polysaccharides, lipids, minerals and other bioactive compounds involved in physiological activities (Xu *et al.*, 2014; Khani *et al.*, 2017). Khani *et al.* (2017) suggested that *Chlorella* diets increase the digestive enzymes in the pancreas and intestine of Koi carp, thereby enhancing the diet utilisation rate through increase in the activity of

digestive enzymes. Another study by the author on nutrient digestibility of dietary *Chlorella* showed that it was better digested and assimilated than the commercial diet.

The present study also showed that *S. platensis* diets improve growth performance of *C. gariepinus* fingerlings as indicated by the weight gain, FCR, PER and SGR. As the *Spirulina* inclusion level increased, the body weight gain of the fingerlings also increased, although this increase showed an indirect relationship with the CP of *Spirulina* diets. The findings here show that dietary *Spirulina* at the same inclusion level (50:50) with FM gives better PER, which suggests that the diet is well digested and assimilated. It has been suggested that *S. platensis* improves the intestinal flora in fish, thereby allowing a breakdown of ingestible feed components to extract more nutrients and stimulates the production of enzymes that transport fats for metabolisms instead of storage within the fish (James *et al.*, 2006). Abdel-Tawwab and Ahmad (2009) stated that the role *Spirulina* plays in the digestibility of nutrients and its numerous vitamins and minerals is responsible for its positive effects on fish development. The present study agrees with the works of Nandeeshia *et al.* (1998) and Palmegiano *et al.* (2005) who successfully replaced FM with up to 60 and 100% dietary FM of *C. carpio* and Siberian sturgeon (*Acipenser baeri*) respectively, with no negative consequence.

Although some studies had recommended partial (0.5-5%) replacement of fishmeal with *S. platensis* for optimal growth performance (Abdel-Tawwab and Ahmad, 2009; Promya and Chitmanat, 2011), a total replacement (100%) was reported to show improved growth performance. These studies also suggested that major inclusion (25-100%) of dietary *Spirulina* significantly increased the digestive enzymes activity of common carp, rohu and catla (Nandeeshia *et al.*, 1994; Umesh *et al.*, 1994; Nandeeshia *et al.*, 1998). More importantly, the cellular structure of *Spirulina* lacks cellulose and is thus, easily digestible (Promya and Chitmanat, 2011). Similarly, Teuling *et al.* (2017) reported that protein apparent digestibility coefficient of *C. vulgaris* in *C. gariepinus* is about 80% and nutrient digestibility of the algae does not relate to its mechanical cell wall hardness.

The optimum replacement of fishmeal with SP and CL was 68.5 and 69.4%, respectively. However, any further increase in the inclusion level of these algae beyond the optimum will not improve the growth of *C. gariepinus* fingerlings further and this has been previously reported for catla (*Catla catla*) by Nandeeshia *et al.* (2001). These variabilities obtained in growth response of fishes to dietary *Spirulina* and other algal species in the different studies visibly revealed that the growth response to algae might be species-specific. Other

possible factors are variability in the nutrient composition, inclusion level of *Spirulina* or other algae as well as the nutritional contents of the test diets (Nandeeshia *et al.*, 1998; Olvera-Novoa *et al.*, 1998; Nandeeshia *et al.*, 2001; Takeuchi *et al.*, 2002). Olvera-Novoa *et al.* (1998) suggested that the harmful effects of inclusion of high levels of *Spirulina* in the diet on fish growth are the decline in phosphorous accessibility and reduction in feed palatability. This call for further study on the prolonged effect of extended feeding of *C. gariepinus* with high inclusion levels of algae.

Fulton's (K) factor provides reliable information on the actual state of health, growth and well-being of fish (Araneda *et al.*, 2008). The present study reveals that both *C. vulgaris* and *S. platensis* diets showed better Fulton's condition factor and survival rate when compared to the fish fed control diet. This further corroborates the high growth performance exhibited by fish fed algal diets and hence, it can be suggested that together these factors signify the good health condition of the fish.

The results obtained in this study showed that there is a positive correlation between the dietary protein and the fish muscle CP. The whole body composition of *C. gariepinus* fed dietary *Chlorella* and *Spirulina*, shows higher CP than the control. This could be due to the higher protein productive values observed with the algal diets, which signify good assimilation of the protein. It has been suggested that fish do not have a specific protein requirement, but a definite requirement for essential amino acids (EAA) content of the protein (Miles and Chapman, 2007). This is due to the fact that the dietary proteins will be digested and broken down into amino acids, which can be used efficiently for maintenance, health and synthesis of worn out tissues and thus, result in maximum feed efficiency and growth (Miles and Chapman, 2007). This assertion is supported by the profiles of EAA exhibited by the algal diets as observed in the present study.

In this work, protein levels of fish carcass decreased as the inclusion of *S. platensis* increased while fat increased in a dose dependent manner. The high fat and energy levels recorded in some of the carcasses might be because of varied inclusion levels of the palm oil which was mixed with the fish oil. This corroborates the findings of researchers who opined that when plant proteins are included in the diets of fish, there is potential increase in fish lipid (Yildirim-Aksoy *et al.*, 2007; Bake *et al.*, 2016). The findings in this study are consistent with other studies, which found *Spirulina* to increase fat deposition (Atack *et al.*, 1979; Watanabe, 1990; Nandeeshia *et al.*, 1998; 2001). However, it differs from that of *C. carpio* where a significant decrease occurred in body lipid composition by similar *Spirulina* administration. This is due to the effects of different *Spirulina* species on fat

deposition (Nandeeshia *et al.*, 1998; Nandeeshia *et al.*, 2001). Abdel-Tawwab and Ahmad (2009) stated that the influence of *Spirulina* on body lipid as well as protein are linked with their production plus build-up level, as well as the growth rate of the animal. *Chlorella* fed fish shows decrease in fat deposit in flesh with increase in inclusion levels in the diets. This could be due to the high quantity of polyunsaturated fatty acids in *Chlorella* in this study or due to the presence β -1, 3-glucan, a strong immune booster, free-radical forager and reducer of blood fat. *Chlorella* had superior activity in hindering lipid peroxidation compared to glutathione and has antioxidant properties (Bengwayan *et al.*, 2010).

There was a positive correlation between energy in the carcass and dietary lipid. Lipids are important sources of energy (Webster and Lim, 2002) as they have more energy per unit weight than any other organic compound; one gram of fat comprises about two folds as much aggregate energy as a single gram of protein or carbohydrate (Guillaume *et al.*, 2001). Previous researchers have shown that considerable use of vegetable oil as an energy source in fish diets produced growth response in fish (Babalola and Adebayo, 2007; Aderolu and Akinremi, 2009). Fish oil and palm oil at ratio of 1:3 were used in this study and the results obtained have demonstrated that they are good energy source and had no palatability problem. Previously, the inclusion of vegetable oils in the diet of seabream was found to promote fat build up in the liver (Caballero *et al.*, 2004). However, there seems to be no direct relationship between energy in the carcass and diets. This implies that energy in the carcass might have been derived from different components of the diets, mostly lipids.

This study determined the effect of partial substitution of fishmeal by *S. platensis* and *C. vulgaris* to assess the practicability of their use in *C. gariepinus* feed. Findings from this study revealed that SP and CL can optimally replace fishmeal up to 68%. Essential nutrients such as amino acids and fatty acids found in fishmeal are also abundantly present in SP and CL diets, while the carcass composition of fish fed SP and CL diets showed higher crude protein and energy compared to fishmeal fed carcass. Thus, findings from this study suggest that both *S. platensis* and *C. vulgaris* could substitute fishmeal in *C. gariepinus* diets. Nonetheless, further studies are still required to evaluate the long term effects of feeding *C. gariepinus* with dietary *S. platensis* and *C. vulgaris* from fingerlings to adult. The ability of both algae to thrive in different growth media would reduce feed cost involved in aquaculture production. Globally, fishmeal and oil production are declining and hence, *Chlorella* and *Spirulina* could play an important role to supply not only protein but EAAs and EFAs to *C. gariepinus* as they are sustainable sources of these products. However, large scale

production of these algae through advanced technology is recommended. This would serve as a sustainable source of protein for aquaculture and would help to curtail the over dependence and competition between the aquaculture and other livestock sectors on fishmeal.

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