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## Full Length Research Paper

# Evaluation of some biological activities of *Euglena gracilis* biomass produced by a fed-batch culture with some crop fertilizers

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The feasibility of growing *Euglena gracilis* in some crop fertilizers was investigated and some biological activities of the produced biomass were evaluated. A fed-batch mixotrophic culture was done in an airlift photobioreactor operated at an aeration rate of 0.2 vvm. A final biomass concentration of 2.6 gL<sup>-1</sup> obtained with NPK15:15:15 after 96 h of cultivation was significantly lower than the 5.7 gL<sup>-1</sup> obtained with BG11 medium under the same culture condition. There was no significant effect of supplementing conventional rat feeds with *Euglena* biomass or captopril (an antihypertensive drug) on the body weight of the rats. However, supplementation with *Euglena* biomass had significant ( $P < 0.05$ ) effects on some biological activities such as, inhibition of angiotensin converting enzyme (ACE), hypocholesterolaemic activity and increase in red blood count ( $P < 0.05$ ). Analysis of the dried algal biomass showed a protein content of 33.327%, fat content of 16.4%, poly unsaturated fatty acid (PUFA) content of 51.33 mgKOH/g, and vitamin E content of 171.3 mg/100 g.

**Key words:** Mixotrophic, angiotensin converting enzyme, hypocholesterolaemic activity, photobioreactor.

## INTRODUCTION

There is an increase in chronic–degenerative diseases all over the world. This has been attributed to environmental factors such as high caloric diet intake, lack of exercise, tobacco and alcohol consumption; and genetic predisposition (Torres-Duran et al., 2007). There is therefore, a growing interest in functional foods which

provide physiological benefits in addition to nutrients and energy (Lordan et al., 2011). Functional foods can be prepared by fortification of traditional foods with some ingredients which directly or indirectly have beneficial effects on human health. Although such ingredients can be synthetic or natural ingredients usually extracted from

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natural sources such as, plants, food by-products and microalgae which are preferred (Lordan et al., 2011; Dufosse et al., 2005).

Microalgae are good sources of antioxidants such as  $\alpha$ -tocopherol (Vitamin E), phytosterol and Carotenoids (Sivakurma et al., 2014; Luo et al., 2015). They are rich in a range of nutrients such as carbohydrates, proteins, essential amino acids, and vitamins, as well as some pharmaceuticals and bioactive molecules. Besides supplying amino acids and energy that are essential for growth and maintenance, food proteins of microalgae can act as an important source of biologically active peptides with antihypertensive, anti-amnesic, hypocholesterolemic, opioid, immunomodulatory, antioxidative, antimicrobial, antithrombotic and other activities (Irit et al., 2009; Lordan et al., 2011; Elena et al., 2015). It has been experimentally proven by *in vivo* and *in vitro* studies that *Spirulina platensis* is effective in treatment of certain allergies, anaemia, cancer, hepatotoxicity, viral and cardiovascular diseases (Bin-Meferij, 2008; Torres-Duran et al., 2007).

However, the type and level of such activities depend on species of microalgae, and even, to an extent, on the growth condition. For example, both chlorophyll and tocopherol contents of *Euglena gracilis* produced under heterotrophic conditions are different from those produced under photoautotrophic conditions (Ogbonna, 2009). Also, adjusting the nutrient, salinity and cultivation duration have been reported to enhance the accumulation of phytosterol in some microalgae spp. (Luo et al., 2015). The commercial production of these bioactive compounds in microalgae can be achieved by scaling up the production of microalgae biomass in photobioreactors. Sivakumar et al. (2014) have reported biomass and RRR -  $\alpha$  - tocopherol production in *Stichococcus bacillaris* strain siva2011 in a ballon bioreactor.

Various species of microalgae are currently produced as food and feed supplements. The choice of microalgae use in food and feed industries depend on their growth rates, nutrient composition and digestibility. Although *Euglena* species have many advantages such as high protein content and high digestibility for animal feed compared to *Chlorella* sp. (Chae et al., 2005), commercial cultivation of this species is faced with many problems. Their growth rates are relatively low, they are prone to contamination by fast growing heterotrophic species, and the current media used for their cultivation are very expensive. Thus, commercialization of *Euglena* culture requires both drastic reduction in the production costs, and production of high value products such as functional foods and pharmaceutical products such as  $\alpha$ -tocopherol and phytosterol (Luo et al., 2015).

Cost of media represents a significant percentage of microalgae production costs. There are many formulations of culture media for laboratory cultivation of microalgae but regardless of the complexity and

effectiveness of the media commonly used, production costs are often prohibitive for commercial cultivation (Pacheco-Ruiz et al., 2004). Thus, for large-scale cultivation of microalgae, it is necessary to use cheaper and simple sources of media to reduce the production cost (Nwuche et al., 2014; Simental and Sanchez-Saavedra, 2003). Another method of reducing the cost of production is to increase the productivity. Some species of microalgae can grow photoautotrophically (using inorganic carbon and light), heterotrophically (using organic carbon in dark) or mixotrophically (using both inorganic and organic carbon sources in light) (Ogbonna and Moheimani, 2015; Ogbonna and Mchenry, 2015; Ozioko et al., 2015). It has been shown that productivity of *Euglena* is highest under mixotrophic condition (Afiukwa and Ogbonna, 2007). In this study, the potential of using some plant crop fertilizers as alternative cheap media for cultivation of *Euglena* was investigated under mixotrophic condition, and some biological activities of the resulting biomass were evaluated with a final objective of developing a high value functional food and feed.

## MATERIALS AND METHODS

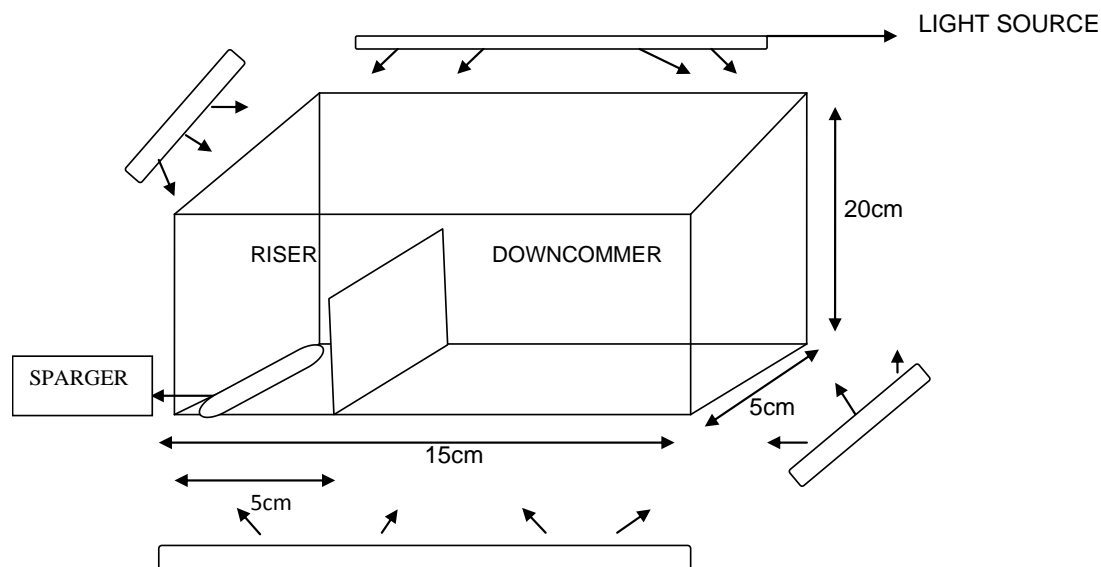
*Euglena gracilis* Z. was obtained from the Culture Collection Centre of the Institute of Applied Microbiology, University of Tokyo, Japan and sub cultured at OGB Biotechnology Research and Development Centre Enugu, Nigeria. Four types of crop fertilizers were investigated. They include NPK 15:15:15 (composed of 15% nitrogen, 15% phosphorus and 15% potassium), NPK 12:12:17 (composed of 12% nitrogen, 12% phosphorus and 17% potassium), NPK 20:10:10 (composed of 20% nitrogen, 10% phosphorus and 10% potassium), and boost extra liquid fertilizer from Candel (composed of 20% nitrogen, 20% potassium, 20% phosphorus, 1% manganese, 0.075% EDTA, 0.075% copper EDTA, 0.0012% cobalt EDTA, 0.075% zinc EDTA, 0.15% iron, 0.0315% boron, and 0.0012% molybdenum). A mineral salt medium (BG II) is composed of (in g/l)  $\text{KH}_2\text{PO}_4$ , 0.4;  $(\text{NH}_4)_2\text{HPO}_4$ , 0.2;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.5;  $\text{CaCO}_3$ , 0.2; Trace element solution, 1.0 ml/l and Iron (Fe) solution, 1.0 ml/l was used for the control. These elements are supplemented with ethanol as an organic carbon source, and peptone as an organic nitrogen source for mixotrophic cultures.

### Effects of media concentration on the growth of *E. gracilis*

Erlenmeyer flasks (500 ml) containing 400 ml of varying concentrations of the crop fertilizers were prepared in four replicates and sterilized by autoclaving at 121°C for 15 min. Each flask was inoculated with approximately 10% of a pure culture of *E. gracilis* containing  $0.5 \times 10^7$  cells/ml and was incubated at room temperature ( $25^\circ\text{C} \pm 1$ ) indoors, between two parallel daylight fluorescent tubes at a light intensity of 2570 Lux. The culture flasks were manually shaken thrice daily and cell growth was monitored by taking samples and counting the cell number with microscope and Neubaur counting chamber.

### Fedbatch culture of the cells in an airlift photobioreactor

A pre-culture was prepared using 10% stock culture in 500 ml



**Figure 1.** A schematic diagram of an airlift Photobioreactor used for cultivation of *Euglena gracilis*.

Erlenmeyer flasks containing 300 ml of BG 11 or NPK NPK15:15:15 media. This was incubated under continuous light illumination (2570 lux) and was agitated manually (three times per day) for 10 days at room temperature. An airlift photobioreactor was constructed using plastic glass with thickness of 3 mm. The photobioreactor was 20 cm high, 5 cm wide and 15.0 cm long. The interior was divided into a riser and down comer columns with cross sectional areas of 25 and 50 cm<sup>2</sup>, respectively (Figure 1). The photobioreactor was filled with 70% ethanol and left to stand an overnight for sterilization. It was then rinsed with sterilized distilled water, followed by addition of 1.2 L of autoclaved 5.0 g/l of NPK15:15:15, supplemented with 0.5 g/l peptone or BG II. The photobioreactor was then inoculated with the pre-culture to give an initial cell concentration of  $5.5 \times 10^7$  cells/ml. Ordinary air was used for aeration (0.2 vvm) through a ring sparger. The bioreactor was surrounded by four daylight fluorescent tubes. Ethanol (2.0 ml) was added once per day to the culture. The light intensity was 2570 Lux on each of the four sides of the bioreactor. The cultivation was done at room temperature  $28^\circ\text{C} \pm 3^\circ\text{C}$ .

#### Biomass determination

Cell biomass was determined gravimetrically as dry cell weight per unit volume. A given volume of the culture was washed by diluting with distilled water and centrifuging at 3000 rpm for 15 min. The cell pellet was re-suspended in distilled water and then filtered through a pre-weighed Whatman No.1 filter paper, and dried in the oven at  $105^\circ\text{C}$  for 24 h. The filter paper containing the cell biomass was weighed and the weight of the biomass was obtained by subtracting the weight of the filter paper.

#### Feed preparation

Animal feed (Vital feed; Growers Grand Cereals Nig. Plc) (2.8 kg) bought at Nsukka local market was soaked in algal biomass culture containing  $4.2 \times 10^9$  cells (3.08 g biomass) and stirred very well to ensure proper mixing of the algal cells and feed particles. The wet feed containing algal biomass was dried under the sun with continual stirring to ensure even drying. The dried feed contained

an algal biomass of 1.1 g/kg which was stored in a clean dry container until it was time for use.

#### Biological activity of *Euglena* biomass in rat model

##### Experimental animals

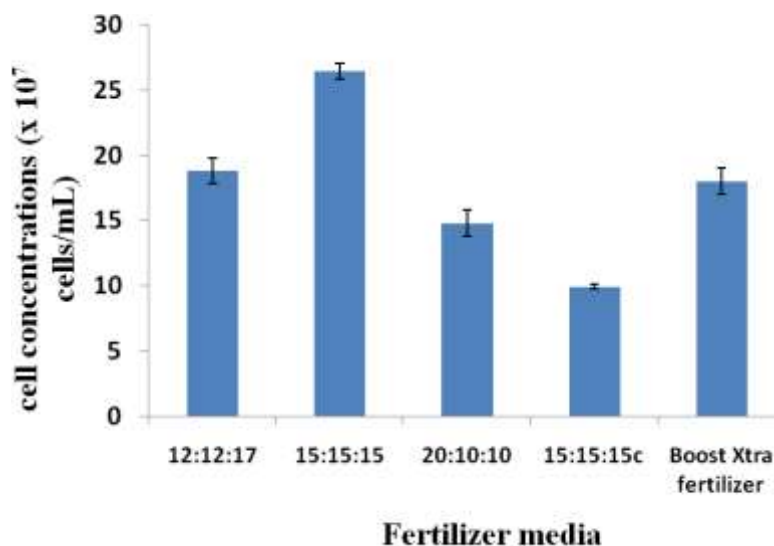
The animals used for the study were 15 male rats (*Rattus rattus*) each weighing between 100 and 150 g. They were purchased from the Animal House Unit of the Department of Veterinary Medicine, University of Nigeria, Nsukka. They were randomly separated into three groups (A, B and C), kept in three animal cages of five rats each and fed with the normal feed for one week of acclimatization. The animals in each cage were then fed 120 g of feed per day for a period of four weeks. Group A was fed with animal feed containing *Euglena* biomass (1.1 g/kg of the feed), and 100ml algal culture containing  $6.5 \times 10^7$  cells/ml (0.1 g) per cage in place of water every day. Thus, each rat in group A received 0.02 g *Euglena* biomass per day and group B received pure animal feed and 100 ml of water containing 0.25 mg/ml per cage of captopril (antihypertensive drug) every day. Thus each rat in group B received 5 mg per day of captopril. Group C was fed with pure animal feed and water only. The weight of the rats in each of the cages, were measured on daily basis using a weighing balance.

##### Collection of samples for analysis

At the end of the fourth week, blood samples were collected from each of the rats in EDTA bottles and test tubes. The thoracic cavity of each rat was opened, and the lungs were removed. The lungs were then washed with 60 ml of ice-cold 0.9% saline, and then kept in an ice-cold 0.02 M potassium phosphate buffer at pH 8.3. The lungs were homogenized in the ice-cold buffer and kept in the refrigerator for analysis.

##### Determination of the angiotensin converting enzyme (ACE) activity of *Euglena* biomass

The lungs of the animals were homogenized in ice-cold 0.02 M



**Figure 2.** Effects of crop fertilizer type on the growth of *E. gracilis* 12:12:17 = NPK 12:12:17 with ethanol; 20:10:10 = NPK 20:10:10 with ethanol; 15:15:15 = NPK 15:15:15 with ethanol; 15:15:15c = NPK 15:15:15 without ethanol; Boost xtra = Boost xtra fertilizer with ethanol. The volume of ethanol added was 1.0 ml/L of the medium, while the concentrations of the fertilizers were 5.0g/L, 0.5 g/L, 2.5 g/L and 5.0 mL for NPK15:15:15, NPK20:10:10, NPK12:12:17 and Boost Xtra fertilizer, respectively.

potassium phosphate buffer at pH 8.3 (30 ml of buffer per set of lungs). The ACE activity in these homogenized lungs was then assayed following the method of Cushman and Cheung (1971).

#### Determination of hypocholesterolemic effect of *Euglena* biomass

The serum cholesterol content of the blood collected from the rats was assayed, using Monoreagent Enzyme Cholesterol (an enzymatic colourimetric test kit, AXIOM diagnostic, Germany). Cholesterol esterase and cholesterol oxidase were added to the sample to release free cholesterol and hydrogen peroxide. The hydrogen peroxide was released from a red dyestuff with 4-Aminoantipyrine and phenol under the catalytic action of peroxidase. The intensity of the red colour formed is directly proportional to the concentration of cholesterol in the sample and was determined photometrically at 550 nm.

#### Effect of *Euglena* biomass on red blood cell

The blood of the rats collected in EDTA containers was diluted by adding 2  $\mu$ l of blood in 4.0 ml of diluents (a solution of formol – citrate) to give a final dilution of 1 in 201. The diluted sample was then mixed and loaded into haemocytometer. When the cells have settled out of suspension, the cells were counted and calculated thus:

$$\text{Red Cell Count/l} = \frac{N \times DF \times 10^6}{A \times D}$$

N = the number of cells counted; DF = dilution factor (201),  $10^6$  converts to cells per litre.

A = the area of chamber counted (0.2 mm<sup>2</sup>), D = the depth of chamber (0.1 mm).

#### Proximate analysis

The protein content of the microalgae was determined by Kjeldahl method, the crude fibre content was determined by the use of Wendee method while the fat content was determined using continuous solvent extraction method (Pearson, 1976). The ash content of the algae biomass was determined using the procedure of AOAC (1990).

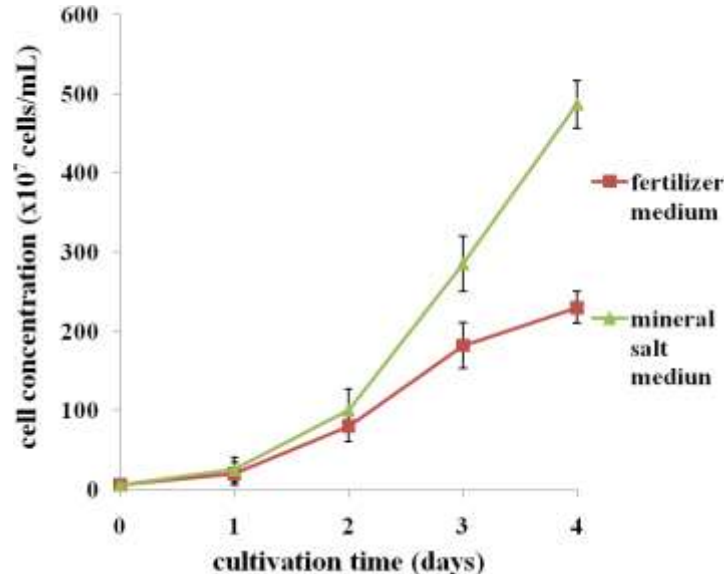
#### Statistical analysis

All the data were analyzed statistically using one way analysis of variance (ANOVA), and the means were separated using Least Significant Different methods.

## RESULTS

#### Effects of fertilizers on the growth of *E. gracilis*

*E. gracilis* grew in all the fertilizers tested but their growth rates were different for each fertilizer. The optima concentrations of the fertilizers were 5.0 g/L, 0.5 g/L, 2.5 g/L and 5.0 ml for NPK15:15:15, NPK20:10:10, NPK12:12:17 and Boost Xtra fertilizer, respectively (data not shown). A comparison of the final cell concentrations obtained with the optima concentrations of the different fertilizers is shown in Figure 2. NPK 15:15:15 gave the highest cell concentration of  $26.0 \pm 1.07 \times 10^7$  cell/ml ( $P < 0.05$ ). Statistically, the effectiveness of the various optima concentrations of fertilizers in supporting the growth of *E. gracilis* was ranked as NPK 15:15:15 > (NPK12:12:17 = Boost Xtra) > NPK 20:10:10.



**Figure 3.** A comparison of the growth of *E. gracilis* in BG II and NPK15:15:15 media at 2570 Lux using airlift bioreactor Fertilizer medium = NPK 15:15:15 medium; Mineral salt medium = BG II medium.

### Production of *E. gracilis* in an air-lift bioreactor

The time courses of *E. gracilis* growth in BG II and NPK15:15:15 media using the air-lift Photobioreactor are shown in Figure 3. Cell concentrations of  $2.30 \times 10^9$  cell/ml (2.65 g dry weight/L) and  $4.87 \times 10^9$  cell/ml (5.76g dry weight/L) were produced with NPK15:15:15 and BG II, respectively. In comparison with the flask cultures, the final cell concentrations in the airlift bioreactor were 8.8 and 18.7 times higher for NPK and BG II media respectively. Furthermore, the maximum cell concentration obtained with NPK15:15:15 in photobioreactor was 7.84 times higher than the value obtained in the 500 mL Erlenmeyer (Figure 4).

### Growth of experimental rats during the feeding experiment

The result of the average weight of the rats in the cages (Data not shown) illustrated that, the weights were increasing per day for all the groups of animals. However, the average weight gain of the group fed, with the animal feed supplemented with microalgae biomass was not significantly higher than the groups fed with feed supplemented with Captopril, and the group fed with the animal feed only (Figure 5).

### Angiotensin Converting Enzyme (ACE) inhibitory activity

The effect of feed supplementation on the antiostensin

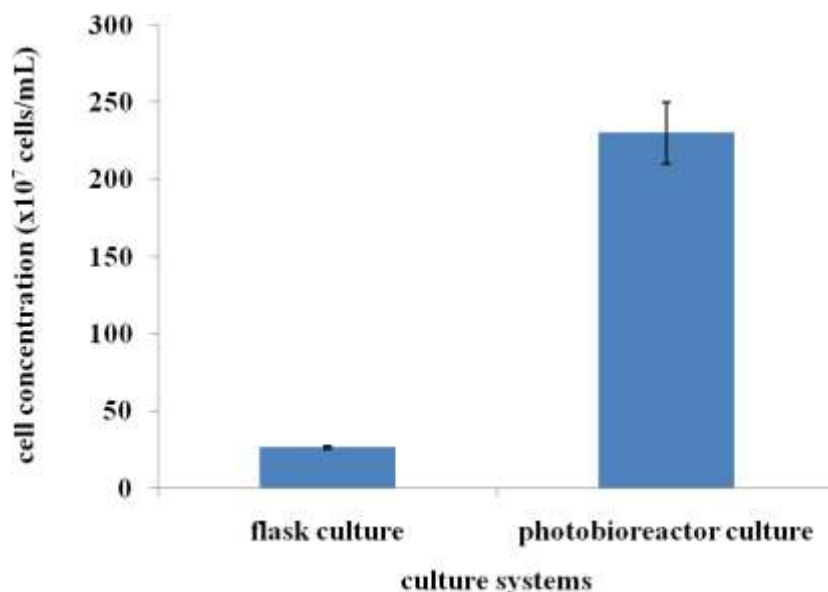
converting enzyme activity is shown in Figure 6. The angiotensin converting enzyme (ACE) activity was highest in group C rats (those fed with animal feed only). The ACE activities were significantly lower ( $p < 0.05$ ) in group A (rats fed with algal biomass and animal feed) and group B (rats fed with captopril and animal feed) than in group C rats. The enzyme activities were in the order of Group C > Group A > Group B.

### Plasma cholesterol level

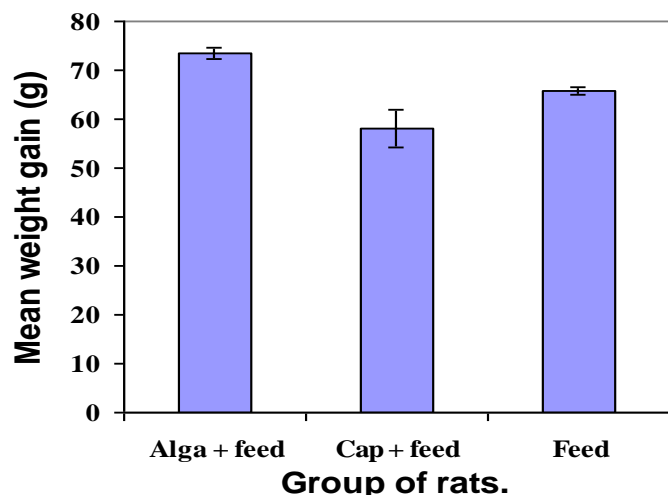
The results of the effect of *Euglena* biomass on plasma cholesterol level is shown in Figure 7. The plasma cholesterol level is highest in group C (rats fed with animal feed and water only) with an average concentration of  $189.5 \pm 7.9$  mg/L. The cholesterol level was significantly ( $p < 0.05$ ) reduced in the rats fed with feed supplemented with *Euglena* biomass. Supplementation of feed with captopril also reduced the cholesterol level in the rats but was not as effective as *Euglena* biomass.

### Effect of feed supplementation with *E. gracilis* biomass on their red blood cell count

The effects of *Euglena* biomass on red blood cells in the rats are shown in Figure 8. Supplementation of the feed with *E. gracilis* biomass significantly increased the red blood counts in the rats ( $p < 0.05$ ). The red blood counts of the rats fed with feed supplemented with *E. gracilis*



**Figure 4.** A comparison of the maximum cell concentrations of *E. gracilis* obtained in airlift bioreactor and 500 ml Erlenmeyer flask NPK15:15:15 medium was used and the light intensity was 2570 Lux in both reactors.



**Figure 5.** The mean weight gain of rats fed with various feeds after one month of feeding Alga+feed = Group fed with animal feed supplemented with microalgae biomass (group A). Cap+feed = Group fed with animal feed supplemented with captopril (group B). Feed = Group fed with animal feed only (group C).

biomass, feed supplemented with captopril or fed alone were  $8.9 \pm 0.02 \times 10^{11}$  RBC/L,  $6.3 \pm 0.02 \times 10^{11}$  RBC/L, or  $7.8 \pm 0.04 \times 10^{11}$  RBC/L, respectively, showing that captopril reduced red blood cells in rats.

#### Proximate composition of *E. gracilis* biomass

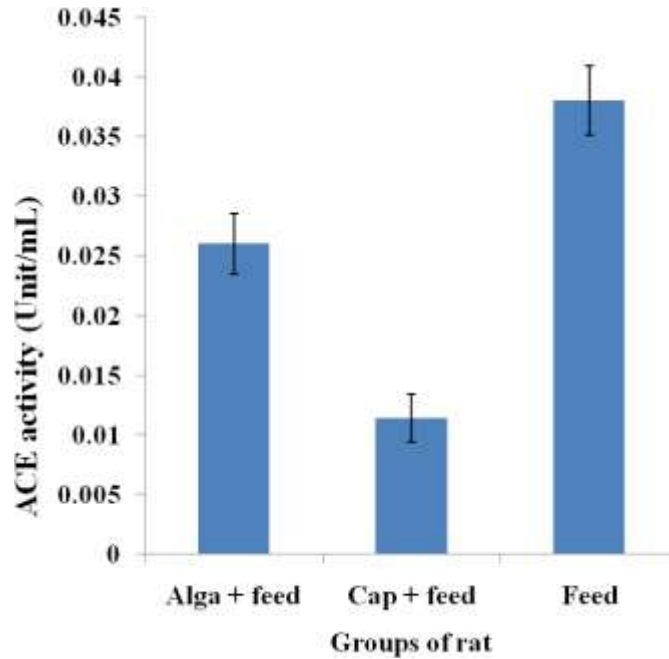
The result of analysis for some of the intracellular

contents of *E. gracilis* biomass is illustrated in Table 1. *E. gracilis* has a high protein content of 33.327%, Vitamin E content of 171.3 mg/100g, and Polyunsaturated Fatty acid (PUFA) content of 51.33 mg/KOH.

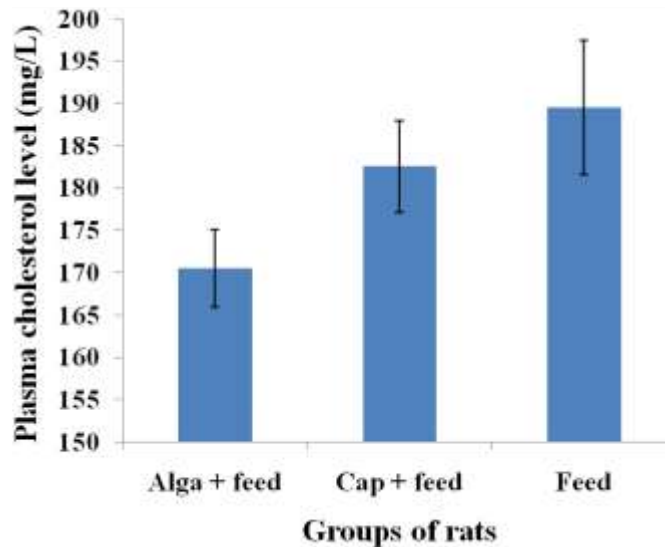
#### DISCUSSION

This study revealed that NPK (nitrogen, phosphorus, and potassium.) 15:15:15 fertilizer can support the growth of *E. gracilis* and the optimum concentration of the fertilizer for cell growth was 5 g/L. NPK fertilizer was recognized as important requirement for induced growth of phytoplanktons (Davies et al., 2006). Tavares et al. (2015) reported on the cultivation of *Ankistrodesmus gracilis* and *Haematococcus pluvialis* in NPK medium with productivities comparable with cultures in commercial medium (WC or CHU<sub>12</sub>). The reason for the differences in the effectiveness of the various crop fertilizers tested is not clear.

However, Korbitz et al. (2011), in their work on effects of different fertilizer on algal development, reported that fertilizers with high levels of phosphate and nitrate had the most algal growth. In addition, the differences in the turbidity of the individual fertilizer culture medium affected light penetration into the cultures, which in turn would affect photoautotrophic growth. Poor distribution of light inside the culture medium is known as a strong limiting factor to the growth of microalgae. Although growth of other species of microalgae on NPK fertilizer had been reported, to the best of our knowledge, this is the first report on the cultivation of *E. gracilis* on different crop fertilizers. The mineral salt medium (BG II) supported



**Figure 6.** Effects of supplementing feeds with *E. gracilis* biomass and captopril on the angiotensin converting enzyme activities in rats Alga+feed = Group fed with animal feed supplemented with algae biomass (group A). Cap+feed = Group fed with animal feed supplemented with captopril (group B). Feed = Group fed with animal feed and water only (group C).

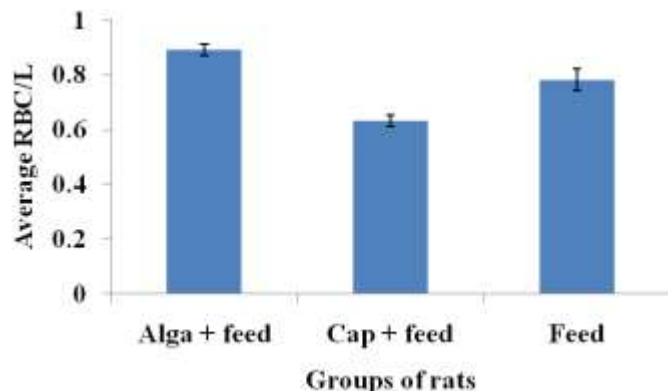


**Figure 7.** Effect of feed supplementation with *E. gracilis* biomass on plasma cholesterol level in rat. Alga+feed = Group fed with animal feed supplemented with *Euglena* biomass (group A). Cap+feed = Group fed with animal feed supplemented with captopril (group B). Feed = Group fed with animal feed and water only (group C).

better growth of *E. gracilis* than NPK15:15:15 probably because of the presence of some components, contained

in the synthetic media. Hence, there is need to supplement NPK 15:15:15 with other components





**Figure 8.** Effect of feed supplementation with *E. gracilis* biomass on their red blood cell count. Alga+feed = Group fed with animal feed supplemented with *Euglena* biomass (group A). Cap+feed = Group fed with animal feed supplemented with captopril (group B). Feed = Group fed with animal feed and water only (group C).

**Table 1.** Proximate composition of *E. gracilis* biomass .

Intracellular contents	Percentage (%) weight
Protein	33.3
Ash	4.8
Fat	16.4
Fibre content	17.2
Moisture content	7.8
Vitamin E	171.3mg/100g
PUFA	51.33mgKOH/g

provided its cost is effective. It is also noteworthy that, the simple airlift photobioreactor used in this study gave significantly higher cell concentration of *E. gracilis* ( $p < 0.01$ ) than the value obtained in flask cultures, indicating the high potential of scaling up *E. gracilis* cultivation with NPK15:15:15 for reduced cost of biomass production.

There was no significant difference ( $p > 0.05$ ) in the average weight gain of rats fed with various feeds. In other words, although *E. gracilis* resulted in higher weight gain, the difference was not statistically significant. On the other hand, Dvir et al. (2000) reported that, supplementation of feed with *Porphyridium* sp biomass resulted in a significant weight gain in rats. The difference in the results may be due to the difference in the species microalgae used, the concentration of biomass, the length of feeding and the type the animal feed. The rats fed with *E. gracilis* biomass- supplemented feed reduced level of ACE activity in their lungs compared with the control. This may be probably as a result of peptic digestion of the algal biomass in the animals' intestine to release some biologically active tetrapeptides such as Ala-Lys-Tyr-Lys, Tyr-Lys-Tyr-Tyr, Lys-Phe-Tyr-Gly, and

Tyr-Asn-Lys-Leu. These have been shown to have ACE inhibitory activity *in vitro* and blood pressure lowering *in vivo* (Suetsuna and Nakano, 2000; Jao et al., 2012; Jiang et al., 2006; Darewicz et al., 2014). However, the extent of reduction of ACE activity by algae biomass supplementation was not as high as that of captopril, which is a known drug, used to lower ACE activity. It is possible that, further reduction in the ACE activity can be achieved by increasing the amount of algae biomass in the feed.

It is also interesting to note that a hypocholesterolaemic effect was observed in the rats fed with a feed supplemented with *E. gracilis* biomass. Abd El Baky and El-Barty (2013) and Bansal and Jaswal (2009) also reported that there was reduction in plasma cholesterol concentration in animals fed with *Dunaliella salina* and *Spirulina* biomass. This attributed to a fact that, *D. salina* biomass contains carotenoid which inhibit the cholesterol synthesis through the inhibition of  $\beta$ - hydroxyl  $\beta$ -methylglutaryl CoA (HMG- CoA) synthesis; an enzyme that is involved in cholesterol biosynthesis. Some authors also reported that, significant hypocholesterolaemic effect was observed following administration of diets containing *Porphyridium* sp in comparison with animals fed with the control diet (Dvir et al., 2009). They attributed the hypocholesterolaemic effect to steroid excretion, increased fecal bile acid and increased intestinal viscosity. In addition, in rat, mice and human studies, *Spirulina* has been reported to show positive effect with respect to serum cholesterol reduction and elevation of HDL – cholesterol level and HDL to LDL ratio (Abd El Baky and El-Barty, 2013; Fong et al., 2000). However, to the best of our knowledge, this is the first report showing that *E. gracilis* is capable of reducing serum cholesterol in rats.

Supplementation of feed with *E. gracilis* biomass also resulted in a significant increase in the total RBC count when compared with the controls. This could probably be due to other nutritional values of the microalgae. *E. gracilis* contains a lot of important nutrients such as protein, vitamins, and PUFA. Venkataraman et al. (1980) also reported similar result on the profile of RBC of rats fed with microalgae. In their work, the RBC of rats fed with 24% and 36% *Scenedesmus acutus*, gave a higher RBC than the rats fed with 12 % casein. However, Stewart et al. (2008) reported that there was no significant difference in RBC of the rats fed with astaxanthin-rich microalgae biomass (*Haematococcus pluvialis*) and the control. Thus, the effect of microalgae on RBC depends on the species of microalgae used. To the best of our knowledge, the present study is the first report on the effect of *E. gracilis* on red blood cell.

The significant reduction in total RBC count of rats fed with captopril is an apparent side effect of this drug and indicates the need to take multivitamins and other nutrient supplements while taking captopril. This also highlights the significance of using microalgae and other

natural products to treat some of these diseases and health problems.

## Conclusions

NPK15:15:15 can serve as a cheap basal medium for the production of *E. gracilis* biomass. There is however, a need to supplement other components. *E. gracilis* biomass could serve as a rich source of biologically active compounds which can alleviate some physiological problems in animals such as hypercholesterol, Anaemia and hypertension.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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