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Mass production of *Arthrospira platensis* on the livestock manure for use as a protein source in animal feed

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ABSTRACT

The biotechnological applications of *Arthrospira platensis* require intensive cultivation for mass production, taking into consideration the economic perspectives. The livestock manure was tested for the cultivation of *A. platensis* EG5 for economic production of biomass and protein. The manure concentrations of 1.6, 3.2, 6.4 and 12.8 g L^{-1} significantly increased ($P \leq 0.5$) the biomass by 19.6, 44.9, 29.8, and 18.8 %, compared to the control Zarrouk's medium. Meanwhile, the control medium supported protein production more than manure media but with a nonsignificant difference ($P \leq 0.05$) with the manure concentrations of 3.2 and 6.4 g L^{-1}, where it reached 50±5.4 and 49.6±4.0 g $100g^{-1}$ dry biomass compared to 53.5±6.6 g $100g^{-1}$ for control medium. The metals; Fe, Zn, Cu, Mg and Mn were measured in *A. platensis* EG5 cultivated on different manure media and their concentration range was in good agreement with many animal feed requirements as reported by NRC. The biomass and protein productivity of *A. platensis* EG5 on a continuous open pond system using manure medium (3.2 g L^{-1}) was studied for three continuous culturing cycles and maintained mean biomass of 1.11, 1.42 and 1.35 kg m^{-3} and protein content of 48.5, 52 and 56 g $100g^{-1}$ for the 1st, 2nd, and 3rd harvested biomass. The biomass of *A. platensis* EG5 cultivated on manure medium was a good source of the essential amino acids; arginine, leucine, isoleucine, lysine, methionine, phenylalanine, threonine, and valine with concentrations comparable to the amino acids requirements of many animals as reported by the NRC. In conclusion, the cultivation of *A. platensis* EG5 on livestock manure for biomass and protein production maintained promising results. Therefore, we would recommend future detailed studies on using the manure commercial-scale cultivation of microalgae as a protein source to enhance the nutritional quality of fish and animal diets economically and sustainably.

INTRODUCTION

Undernutrition forms a public health dilemma, especially in developing countries, where, the growing population requires economic nutritional alternatives to meet human needs. Therefore, researchers and scientists have to develop novel renewable and economical solutions to prevent malnutrition; hence the attention has been turned to

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microalgae (**Khatoon and Pal, 2015**). Using of algae as a food for both humans and animals is one of these solutions because this valuable group of organisms contains a high percentage of proteins, carbohydrates, and lipids in addition to varieties of mineral and vitamins (**Renaud *et al.*, 1999; Pulz and Gross, 2004; Becker, 2007; Abdel-Hamid *et al.*, 2015a; Mofeed *et al.*, 2019**). Algae became a powerful raw material for many applications in our life not only as food and feed (**Becker, 2004; Pulz and Gross, 2004; Abdel-Hamid *et al.*, 2016**) but also in therapeutic pharmaceuticals (**Bansemir *et al.*, 2006; Madkour *et al***., **2012a, 2019; Abdel-Aal *et al.*, 2015; Deyab *et al.*, 2020**), nutritional and cosmetic industries (**Pulz and Gross, 2004**), in addition to its applications in the production of biofuel (**Abdel-Aal, 2013; Abdel-Aal and Mofeed, 2015; Abdel-Hamid *et al.*, 2015b; Abdel-Hamid *et al.*, 2019**), bioplastic and purification of waste or even as a biomarker for different types of pollutants in aquatic habitats (**Shaaban-Desouki *et al.*, 2004; Ward *et al.*, 2012; Mofeed and Mosleh, 2013; Abdel-Hamid *et al.*, 2014, 2017; Mofeed, 2017; Abdel Gawad and Abdel-Aal, 2018**).

Arthrospira platensis Gomont 1892 is the most applicable algae throughout the recent decades due to the fact that in terms of the chemical composition it is a very rich food source of both macro- and micronutrients including high-quality protein content up to 70% of the dry weight, essential fats (e.g., gamma-linolenic and oleic acids), essential amino acids, vitamins (e.g., B12), β-carotene, lipids and polysaccharides with a distinctive high content of calcium, iron, phosphorous, minerals, sulfated and phycocyanin, besides its antioxidant capacity (**Phang *et al***., **2000; Babadzhanov *et al.*, 2004; Abdel-Daim *et al*, 2015, 2020; Mofeed, 2019**). Moreover, *A. platensis* showed neither chronic nor any acute toxicity or even harmful effects, making it safe to use as part of diets for human food and as a dietary supplement of poultry and fish feed production as a non-conventional high protein source (**Habib *et al.*, 2008; El-Sheekh *et al.*, 2014**). *Arthrospira platensis* as many microalgae can be cultivated in both closed and open systems to get a commercial value. Cultivation of *A. platensis* needs basic elements such as phosphorus, nitrogen and carbon (**Davis, 1977**), with a significant high need to both bicarbonate and carbonate (**Binaghi *et al.*, 2003**). The cost of *A. platensis* biomass productivity can vary according to the used nutritional source, therefore, it was cultivated in the large farms using fertilizers as nutrient sources (**Kendirli, 2010; Madkour *et al***., **2012b**). Recently the agro-industrial wastes were used as raw materials in the economic production of *A. platensis* (**Markou *et al.*, 2018**). There are some pioneering attempts in this field, such as the use of sugarcane mills residues in the cultivation of *Arthrospira* to produce a protein (**Pelizer *et al.*, 2015**). Another way by utilization of the disposals of pig and chicken farms, as the source of nutrients, in the production of *Spirulina* (**Ungsethaphand *et al.*, 2007, 2009**). Furthermore, **Cheunbarn and Peerapornpisal (2010)** utilized wastewaters with its carbonized substances to produce algal biomass. The biomass which is obtained from the cultivation on animal farms wastes can be used in animal feed as being a valuable nutrient source (**Chaiklahan *et al.*, 2010**). The livestock manure characterized by the high organic and mineral load; represented mainly by nitrogen (N) and phosphorus (P) (**Petersen *et al***., **2007**), which are very important components for the cultivation of microalgae. There are few published studies about the cultivation of *A. platensis* on livestock manure as a source of nutrients (e.g. **Mitchell and Richmond, 1988**). Therefore, the present study aimed to examines the efficiency of using large animal manure for mass production of *A. platensis* vis the conventional cultivation process to reduce the cost of biomass and protein production.

MATERIALS AND METHODS

1. Preparation of livestock manure

The cattle manure was obtained from a livestock farm near Mansoura city (Egypt). The manure was dried in the open air for two days and then oven-dried at 100^{o}C until constant weigh. The dried manure wasground to a fine powder and stored in a refrigerator at -20^{o}C.

2. Preparation and analysis of manure stock solution

A manure stock solution was prepared at a concentration ratio of 1.0 kg manure powder: 5 L distilled water, autoclaved at 121^{o}C for 15 min, filtrated through cotton and Whatman No. 1 filter papers, re-autoclaved and then kept at 4^{o}C. The concentrations of some nutritionally important elements (Table 1) were determined in the prepared manure stock solution to evaluate its potential as cultivation medium for microalgae including; total inorganic nitrogen (TIN), dissolved phosphorus (DP), iron (Fe), magnesium (Mg), zinc (Zn), and copper (Cu). All analyses were carried out according to **APHA (2005).**

Table 1: Element constitutes of manure stock solution.

Parameters	Concentration
TIN, g L^{-1}	33.6
DP, g L^{-1}	6.30
Fe, g L^{-1}	1.38
Mg, g L^{-1}	3.98
Cu, mg L^{-1}	6.18
Zn, mg L^{-1}	6.76

3. Test microalga and growth conditions

The cyanobacterium strain *Arthrospira platensis* EG5 (NCBI: txid 2175805) was obtained from the Hydrobiology Laboratory, Freshwater and Lakes Division, National Institute of Oceanography and Fisheries, NIOF, Egypt. The strain was grown in liquid Zarrouk's medium (**Zarrouk, 1966**) with a continuous light intensity of 3.5 ± 0.2 Klux at 28 ± 2^{o}C.

4. Experiment Design

Six treats of manure media were prepared at the concentrations illustrated in Table 2, to evaluate the mass production potentiality of the animal manure. The Zarrouk's medium was used as a control medium for growth of *A. platensis* EG5. The pH of the prepared media was adjusted to 9.5±0.5 by $NaHCO_3$ and the salinity to 1.0 g L^{-1} by NaCl. The nitrogen concentration in the prepared manure media ranged from 67.2 to 2150 mg L^{-1}, while that of Zarrouk's medium was 400 mg L^{-1}. Meanwhile, the phosphorus concentration in the prepared manure media ranged from 12.6 to 403 mg L^{-1} and in Zarrouk's medium was 88.8 mg L^{-1}. The prepared media were inoculated by a one-weekold culture of *A. platensis* EG5 to obtain initial biomass concentration of 0.064± 0.015 g L^{-1} dry weight biomass (DWB); dried at 60°C). The culture flasks were incubated at 28 ± 2°C under a continuous light intensity of 3.5 ± 0.2 Klux. The growth was determined as means of DWB (g L^{-1}) every two days for ten days incubation period.

Table 2: The text treats of manure media.

Manure media treats	Manure sock solution (ml L^{-1})	Manure powder (g L^{-1})	Nitrogen (mg L^{-1})	Phosphorus (mg L^{-1})
Treat I	2.0	0.4	67.2	12.6
Treat II	4.0	0.8	134.4	25.2
Treat III	8.0	1.6	268.8	50.2
Treat IV	16	3.2	537.6	100.8
Treat V	32	6.4	1075.2	201.6
Treat VI	64	12.8	2150.4	203.2

5. Analysis of microalga biomass

The protein content was estimated in *A. platensis* EG5 biomass cultivated on Zarrouk's and manure treats media at days 2, 6 and 10 of growth using Lowry's method (**Lowry *et al.*, 1951**). Also, the concentrations of the metals; iron (Fe), magnesium (Mg), zinc (Zn), copper (Cu) and manganese (Mn) were measured in *A. platensis* EG5 biomass at the end of incubation time. The metals were measured according to **AOAC (2000)** official method 985.01 using atomic absorption spectrophotometry (Shimadzu AA-6200).

6. Verification experiment for large scale production of *A. platensis* EG5

The manure medium Treat IV, with the concentration of 3.2 g L^{-1}, maintained the highest biomass and protein content, so this medium treat was used for the large scale production of *A. platensis* EG5. A fibreglass open pond with dimensions of 2.5 m × 1.5 m and culture capacity of 1.0 m^3 (Figure 1) was used for the large scale mass production. The open pond supported with a constant stirring system consists of paddle wheels and air pump, to ensure the continuous mixing, aeration and recirculation of the culture. For mass production, the open pond was filled with 500 litres manure medium (490 litres of pre-sterilized tap water and eight litres of the manure stock solution). The prepared medium was inoculated by a fresh culture of *A. platensis* EG5 cultivated on manure medium (Figure 1). The culture was incubated for two weeks and then refreshed by 500 litres of manure medium to obtain 1.0 m^3 culture volume. After another two weeks, about 80% of the *A. platensis* EG5 culture was harvested by filtration through frame lined with plankton net fabric (3µm Mesh) (Figure 1) and the culture was refreshed by ½ strength manure medium. The mass production process was continued for two months during the summer season, and the biomass productivity was estimated every two weeks. The air temperature during the cultivation period fluctuated between 32 and 42°C and the culture temperature fluctuated between 28 and 36°C. Meanwhile, the light intensity fluctuated between 102.45 and 113.24 Klux. The protein content in the harvested biomass was estimated using Lowry's method (**Lowry *et al.*, 1951**) and the amino acid profiles of *A. platensis* EG5 cultivated on Zarrouk's and manure media were determined according to AOAC Method No.982.30E (**AOAC, 2011**).

Figure 1: Large scale mass production of *Arthrospira platensis* EG5 on manure medium. A) Prepared inoculum of *A. platensis* EG5 cultivated in glass tanks with 20 Littre capacity, B) Cultivation in the fibreglass open pond, C) Harvesting process by plankton net fabric (3μm Mesh) and D) Dried biomass of *A. platensis* EG5.

7. Statistical analysis

All the experiments were run in triplicate. The collected data were statistically analyzed by Tukey test to compare means using Statistical Package (Statistix 8.1). The level of significant difference was set at $P \leq 0.05$.

RESULTS

1. Cultivation on manure media

The growth curves of *Arthrospira platensis* EG5 cultivated on different concentrations of manure media vis the standard control Zarrouk's medium are shown in Figure 2. As seen from these curves, Treats III, IV, V and VI enhanced the growth of *A. platensis* over that recorded with Zarrouk's medium (0.54 ± 0.15 g L^{-1}) during the entire period of the experiment, giving the maximum value of 0.78 ± 0.12 g L^{-1}, for Treat IV (3.2 g manure L^{-1}) followed by Treat V (0.696 ± 0.06 g L^{-1}). Meanwhile, both of Treats I and II suppressed the growth. Protein content revealed that Zarrouk's medium supported higher production of protein (53.5 ± 6.6 g 100 g^{-1} dry biomass) than those produced by all Treats (except Treats I and II) with nonsignificant difference ($P > 0.05$), especially for Treats IV and V, where it reached 50 ± 5.4 and 49.6 ± 4.0 g 100 g^{-1} dry biomass, respectively (Figure 3).

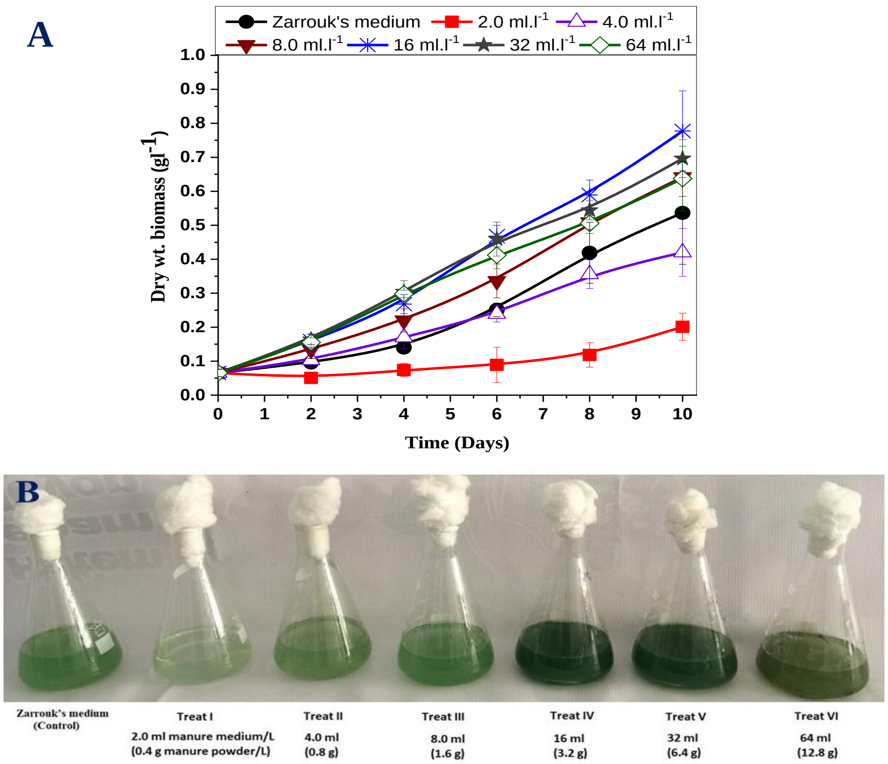

Figure 2: Changed in growth of *Arthrospira platensis* EG5 grown on Zarrouk's and different treats of manure media (A) and *A. platensis* EG5 growth at day ten (B).

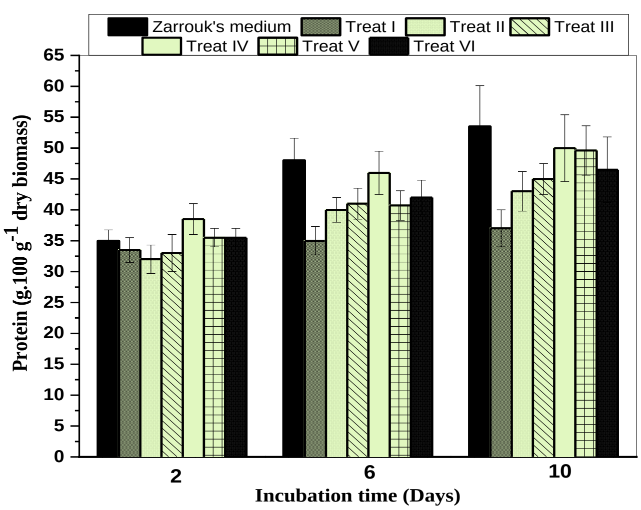

Figure 3: Protein content of *Arthrospira platensis* EG5 grown on Zarrouk's and different treats of manure media.

Table (3) shows the effect of manure media concentration on the accumulation of heavy metals; Fe, Zn, Cu, Mg and Mn in *A. platensis* EG5 biomass. Treat I (0.4 g L^{-1}) maintained the lowest concentrations of heavy metals which gradually increased by increasing manure concentration in the medium until the concentration of 1.6 g L^{-1} (Treat

III). It is noticeable that Fe, Zn and Mn obtained their maximum concentration (124.75 ± 3.75, 0.885 ± 0.105 and 2.91 ± 0.82 mg 100 g^{-1}, respectively) with Treat III, and then decreased with the higher concentrations of manure. While Cu gave its maximum (0.60 ± 0.08 mg 100 g^{-1}) with Treat IV and Mg with Treat V (692.5 ± 50.5 mg 100 g^{-1}). It is of interest to mention that *A. platensis* EG5 biomass cultivated on 3.2 g manure L^{-1} (Treat IV), which gave the highest biomass production for all Treats, accumulate higher concentrations of heavy metals than those recorded for Zarrouk's medium, especially for Mg (668.5 ± 66.5 mg 100g^{-1}) compared to 277.5 ± 30.5 mg 100g^{-1} with Zarrouk's medium (Table 3).

Table 3: Heavy metals concentrations in *Arthrospira platensis* EG5 cultivated on Zarrouk's and different treats of manure media.

Media	Concentration (mg 100 g^{-1})				
	Fe	Zn	Cu	Mg	Mn
Zarrouk's medium	93.25 ± 6.95	1.05 ± 0.215	0.42 ± 0.105	277.5 ± 30.5	2.76 ± 0.78
Treat I	41.65 ± 5.75	0.740 ± 0.151	0.485 ± 0.075	260.5 ± 27.5	2.34 ± 0.71
Treat II	106.3 ± 9.4	0.810 ± 0.140	0.56 ± 0.07	336.5 ± 27.5	2.60 ± 0.78
Treat III	124.75 ± 3.75	0.885 ± 0.105	0.58 ± 0.07	559.5 ± 10.5	2.91 ± 0.82
Treat IV	116.5 ± 7.5	0.865 ± 0.045	0.60 ± 0.08	668.5 ± 66.5	2.87 ± 0.67
Treat V	86.6 ± 9.8	0.535 ± 0.065	0.58 ± 0.04	692.5 ± 50.5	2.69 ± 0.75

2. Large scale production of *Arthrospira platensis* EG5.

The productivity of *A. platensis* EG5 on Treat IV manure medium for continuous large scale biomass production was studied for three continuous culturing cycles. The mean biomass production was 1.11, 1.42 and 1.35 kg m^{-3} for the 1st, 2nd and 3rd harvested biomass, respectively. Also, the harvested biomass maintained a high protein content of 48.5, 52 and 56 g 100 g^{-1} for the 1st, 2nd and 3rd harvested biomass, respectively (Fig. 4).

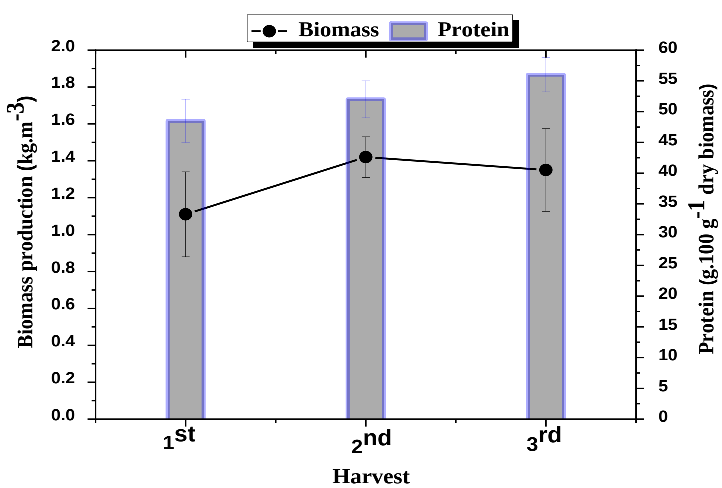

Figure 4: Biomass productivity and protein content of *Arthrospira platensis* EG5 cultivated on manure medium under field conditions.

3. Amino acids profile

It is well known that the protein quality depends on its essential amino acid content. Amino acid profiles of *A. platensis* EG5 grown on Zarrouk's and manure media are given in Table 4. The essential amino acids (EAA) of *A. platensis* EG5 biomass cultivated on Zarrouk's media comprises 33.22 ± 6.32 g 100 g^{-1} (51.5% of the total amino acids) and 28.93 ± 6.4 g 100 g^{-1} (51.2%) of that grown on manure medium (Table 4). Out of the identified EAA, leucine, isoleucine, lysine, phenylalanine, threonine and valine were detected in high concentrations. The EAA content of *A. platensis* grown on Zarrouk's medium was dominated by leucine (6.86 ± 0.9 g 100 g^{-1} DWB) followed by threonine (4.58 ± 0.74 g 100 g^{-1}), lysine (4.03 ± 0.72 g 100 g^{-1}), isoleucine (3.59 ± 0.89 g 100 g^{-1}) and the lowest value of 1.53 ± 0.63 g 100 g^{-1} was detected for tryptophan (Table 4). Meanwhile, the highest amounts of EAA in *A. platensis* grown on manure medium was as follow; leucine (4.31 ± 0.72 g 100 g^{-1}), valine (4.14 ± 0.74 g 100 g^{-1}), isoleucine (3.94 ± 0.34 g 100 g^{-1}), threonine (3.81 ± 0.94 g 100 g^{-1}) and the lowest value of 1.09 ± 0.75 g 100 g^{-1} was detected for histidine (Table 4). Glutamic acid, Aspartic acid, Alanine, and glycine were the highest detected non-essential amino acids (NEAA) (Table 4). Glutamic acid was the major amino acid in *A. platensis* EG5 grown on Zarrouk's and manure media with amounts of 7.97 ± 0.46 g 100 g^{-1} and 7.03 ± 0.69 g 100 g^{-1} followed by aspartic acid 6.98 ± 0.94 g 100 g^{-1} and 5.74 ± 0.55 g 100 g^{-1} and alanine 4.22 ± 1.45 g 100 g^{-1} and 4.16 ± 0.82 g 100 g^{-1}, respectively. Whereas, the lowest content of 1.06 ± 0.22 g 100 g^{-1} and 0.68 ± 0.16 g 100 g^{-1} were detected for cysteine, respectively (Table 4).

Table 4: Amino acid composition of *Arthrospira platensis* EG5 cultivated on Zarrouk's and manure media. Data are the mean of 2 determinations of 2 different harvest ± SE.

Amino Acid	Zarrouk's Medium		Manure medium	
	% of DWB[1]	% of total AA[2]	% of DWB	% of total AA
Essential amino acids (EAA):				
Arginine	3.44 ± 1.33	5.26	2.46 ±1.01	4.43
Histidine	-	-	1.09 ± 0.75	1.96
Isoleucine	3.59 ± 0.89	5.49	3.94 ± 0.34	7.1
Leucine	6.86 ± 0.91	10.5	4.31 ± 0.72	7.77
Lysine	4.03 ± 0.72	6.17	3.63 ± 1.27	6.54
Methionine	2.17 ± 0.68	3.32	1.47 ± 0.57	2.65
Phenylalanine	3.78 ± 1.23	5.78	3.54 ± 1.07	6.38
Threonine	4.58 ± 0.74	7.01	3.81 ± 0.94	6.87
Tryptophan	1.53 ± 0.63	2.34	-	-
Valine	3.24 ± 0.52	4.96	4.14 ± 0.74	7.46
Nonessential amino acids (NEAA):				
Alanine	4.22 ± 1.45	6.46	4.16 ± 0.82	7.5
Aspartic acid	6.98 ± 0.94	12.2	5.74 ± 0.55	10.3
Cysteine	1.06 ± 0.22	1.62	0.68 ± 0.16	1.23

Glutamic acid	7.97 ± 0.46	12.2	7.03 ± 0.69	12.7
Glycine	3.12 ± 0.21	4.77	2.66 ± 0.23	4.79
Proline	2.45 ± 0.21	3.75	2.72 ± 0.21	4.9
Serine	3.07 ± 0.38	4.7	2.86 ± 0.58	5.16
Tyrosine	2.27 ± 0.22	3.47	1.24 ± 0.18	2.24
Total amino acid (TAA)	**64.36 ±0.74**		**55.48±10.83**	
EAA	**33.22 ±6.32**		**28.39 ± 6.4**	
NEAA	**31.14 ±4.42**		**27.09 ± 4.43**	

[1] Percent of amino acid (AA) in dry weight biomass (DWB), [2] Percent of AA to total amino acid (TAA).

DISCUSSION

The growing population is always accompanied by a terrible increase in the need for economical renewable alternative food resources. The present study aims to obtain an animal feed with high protein nutritional value and economic cost. Animal wastes had been known since ancient times as a rich and costless source of organic fertilizers that can be used for mass production of algae which used as animal and fish feed (**Zhou *et al.*, 2012**). The obtained results clarified that varying the animal manure concentration in *A. platensis* EG5 culture medium had a significant influence on biomass production, where, the manure medium with the concentrations of 1.6, 3.2, 6.4 and even 12.8 g L^{-1}, stimulated the biomass production by 19.6, 44.9, 29.8, 18.8 % respectively, compared to Zarrouk's medium (Figure 2). **Costa *et al.* (2004)** in his trial to increase biomass by addition of urea reported that high nitrogen content stimulates growth and biomass productivity of algae cultures. This hypothesis is consistent with the obtained results; as the analysis of animal wastes showed significant high nitrogen content (168 mg g^{-1} manure powder). **Costa *et al.* (2001)** and **Madkour *et al.* (2012b)** also noted that different nitrogen sources or/and concentrations affected the development of *A. platensis*. Beside of that, the obtained results clarified the efficiency of animal manure media for biomass production in large-scale during three continuous culturing cycles and under field conditions (Figure 4) indicating that animal manure was the factor with the greatest influence on biomass production. Anent the importance of protein as an essential component of the biomass, Treat IV gave the highest protein content (38.5 ± 2.2 % of DWB) after two days (Figure 3), however after 6 and 10 days Zarrouk's medium supported higher protein content (53.5 ± 6.6 % of DWB), but with nonsignificant differences ($P > 0.05$) compared to 50 ± 5.4 % of DWB at Treat IV manure medium. In this context, **Koru and Cirik (2003)** reported that the variation in protein content referred to the differences in the nutrition media, especially the concentration of nitrogen (**Ungsethaphand, *et al.*, 2007, 2009**). These findings reflect the economic advantage of using animal manure as an alternative source of nutrients in the cultivation media without causing a significant decrease in protein content and these results were ensured during large scale production of *A. platensis* G5 on manure medium (Figure 4).

With regards to concentration of metals in produced *A. platensis* EG5, the concentrations of Fe and Mg in *A. platensis* cultivated on Treat IV (1165 and 6685 mg/kg DWB, respectively) were higher than the requirement of many animals (30 - 50 and 1200 – 1800 mg/kg DM, respectively) and the maximum tolerable level (500 and 6000 mg/kg DM, respectively) (**NRC, 1985, 2005, 2007; McDowell, 2003**). However, the high level of Fe in *A. platensis* EG5 does not pose a harm for animal feed where high concentration of Fe was reported to reduce the uptake of Cu, P, Zn, and Mn (**McDowell, 2003; Suttle, 2010**). In contradiction, Zinc in *A. platensis* EG5 cultivated on Treat IV was lower (8.65 mg/kg DWB) than the sheep requirements (20 - 33 mg/kg DM) (**NRC, 1985, 2007; McDowell, 2003**). The mean concentration of Cu in *A. platensis* EG5 biomass (6.0 mg/kg DM) was close to sheep requirements (7 - 15 mg/kg DM) according to the reports of **NRC (1985, 2007)** and **McDowell (2003)**. The range of Mn concentration in *A. platensis* EG5 biomass (22 and 35.4 mg/kg DWB) was compatible with the required range of sheep (20 - 40 mg/kg DM) (**NRC, 1985, 2007; McDowell, 2003; McDowell, 2003; Suttle, 2010**). In general, the measured metals concentrations in *A. platensis* EG5 cultivated on Treat IV were in good agreement with the range of animal feed requirements reported by **NRC (1985, 2007)** and **McDowell (2003).**

The nutritional quality of protein depends mainly on its essential amino acid composition (**Becker, 2007; WHO, 2007**). The microalgae protein contains all essential amino acids and some species can be compared with soy and egg protein (**Galland-Irmouli *et al.*, 1999**). It is obvious from the results of amino acid profile that *A. platensis* EG5 cultivated on manure medium are a good source of the essential amino acids; arginine, leucine, isoleucine, lysine, methionine, phenylalanine, threonine and valine and their levels are in agreement with that reported by **Habib *et al.* (2008)**. In addition, the percent of the amino acids (AA) to the total amino acids (TAA) and to the dry weight biomass (DWB) of *A. platensis* EG5 grown on manure medium (Table 4) exceeded the amino acids concentrations required by different animals reported by the **National Research Council (1977, 2011)** (Table 5).

Table 5: Amino Acid Requirements of Seven Animals (Adapted from the National Research Council, 1977)

Amino acid	Eel fingerling	Carp fry	Channel catfish	Chinook salmon fingerling	Chick	Young Pig	Rat
Arginine	3.9[1] (1.7)[2]	4.3 (1.65)		6.0 (2.4)	6.1 (1.1)	1.5 (0.2)	1.0 (0.2)
Histidine	1.9 (0.8)			1.8 (0.7)	1.7 (0.3)	1.5 (0.2)	2.1 (0.4)
Isoleucine	3.6 (1.5)	2.6 (1.0)		2.2 (0.9)	4.4 (0.8)	4.6 (0.6)	3.9 (0.5)
Leucine	4.1 (1.7)	3.9 (1.5)		3.9 (1.6)	6.7 (1.2)	4.6 (0.6)	4.5 (0.9)
Lysine	4.8 (2.0)		5.1 (1.23)	5.0 (2.0)	6.1 (1.1)	4.7 (0.65)	5.4 (1.0)
Methionine[3]	4.5 (2.1)[3]	3.1 (1.2)	2.3 (0.56)	4.0 (1.6)[4]	4.4 (0.8)	3.0 (0.6)	3.0 (0.6)
Phenylalanine[5]				5.1 (2.1)[6]	7.2 (1.3)	3.6 (0.45)	5.3 (0.9)
Threonine	3.6 (1.5)			2.2 (0.9)	3.3 (0.6)	3.0 (0.4)	3.1 (0.2)
Tryptophan	1.0 (0.4)			0.5 (0.2)	1.1 (0.2)	0.8 (0.2)	1.0 (0.2)
Valine	3.6 (1.5)			3.2 (1.3)	4.4 (0.8)	3.1 (0.4)	3.1 (0.4)
% of total protein in the diet	42	38.5	24	40 - 41	18	13 - 20	13 - 20

[1] Percent of AA to TAA, [2] Percent of AA in dry diet, [3] In the absence of cysteine, [4] Methionine plus cysteine, [5] In the absence of tyrosine, [6] Phenylalanine plus tyrosine.

CONCLUSION

The biotechnological applications of *A. platensis* require intensive cultivation for mass production, taking into consideration the economic factors. Based on the promising results of economic mass production of *A. platensis* EG5 on livestock manure, we would recommend future detailed studies on using the livestock manure for commercial-scale cultivation of microalgae as a protein source to enhance the nutritional quality of fish and animals diets economically and sustainably.

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