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Nutritional Profile of *Spirulina platensis*, *Chlorella vulgaris* and *Azolla pinnata* to Novel Protein Source for Aquaculture Feed Formulation

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Abstract

The comparative study was conducted to determine the nutrition profile of the freshwater algae *Spirulina platensis*, *Chlorella vulgaris* and floating fern *Azolla pinnata* for the use of alternative protein source for aquaculture feed formulation. The freshwater algae and *A. pinnata* were cultured (30 days) in laboratory condition using standard culture methods. It was determined the growth rate, biochemical constituents (crude protein, carbohydrate, lipid ash and moisture), mineral contents, and profile of amino acid concentration of *S. platensis*, *C. vulgaris* and *A. pinnata*. The determined results were compared with fishmeal. The proximate composition, crude protein was significantly similar in *S. platensis* followed the *C. vulgaris* and *A. pinnata* showed lower then fishmeal. Carbohydrate content was significantly higher in *A. pinnata*, *C. vulgaris* and *S. platensis*. Also, the higher level of lipid was recorded in *C. vulgaris* than the fishmeal, *S. platensis* and *A. pinnata* show significantly low when compared with fishmeal. Ash content was significantly ($P < 0.05$) higher in *A. pinnata* followed by the *S. platensis* and *C. vulgaris* showed significantly similar with fishmeal. Moisture content was show significantly higher ($P < 0.05$) in fishmeal. Similarly the maximum mineral contents were significantly higher in *C. vulgaris* and *S. platensis*, *A. pinnata* show low level of mineral contents apart from phosphorus. As well as, the same trend was observed in profile of amino acids contents. The obtained results show required quantity of biochemical constituents for aquaculture feed formulation. It can be used least cost feed formulation for sustainable and environment safety aquaculture.

Keywords: *S. Platensis*; *C. Vulgaris*; *A. Pinnata*; Nutritional Profile; Amino Acid; Minerals

Introduction

Fisheries and aquaculture make crucial contributions to the world's well-being and prosperity. In the last five decades, world fish food supply has outpaced global population growth, and today fish constitutes an important source of nutritious food and animal protein for much of the world's population. In addition, the sector provides livelihoods and income, both directly and indirectly, for a significant share of the world's population [1]. Aquaculture's success and continuing growth have been more important for our world [2]. The world's population is projected to reach 9.3B in 2050 according to the medium variant of UN projections [5,6].

Fish is a key source of protein, essential amino-acids and minerals, especially in low-income, food-deficit countries [3-5]. Aquaculture growth has averaged 8% per year since the late 1970s (faster than human population growth), bringing fish production to a total of 142 Mt in 2008 [6]. About 115Mt are currently directed to human use, providing an estimated per capita supply of about 17 kg person-1yr -1, an all time high [7].

The aquaculture growth has relied heavily on fishmeal and fish oil. Fishmeal is an internationally traded, high protein powder, which results from the industrial processing of small pelagic fish (e.g. anchovy, sardine, capelin, and herring). It is a key component of the aqua feed of salmon, trout, shrimp and other farmed marine species [8], supplying essential amino acids, fatty acids and other micronutrients [9]. Due to these properties, FM has become one of the primary components of commercial feed formulations. The demand for FM in aquatic feeds has been estimated to account for 31% to 42.5% of total world FM production [10]. However, as a result of a decreasing supply of fishery byproducts and concerns over its quality, the aquaculture industry is now actively investigating alternatives nutrient sources [11]. In the last two decades, although worldwide FM production remained at a relatively stable level, it still could not match the rapid worldwide development of aquaculture [12]. The cost of FM increased constantly, which caused the price of commercial feed increase sharply. Thus, there is an urgent need to find alternative protein sources to make up for the shortage of FM and to secure a stable supply for commercial diets [13]. Now a day, the considerable interest and research have been focused on the developing unicellular organisms such as yeast, molds, bacteria, microalgae and fungi as additives to aquaculture feeds.

Algae are a diverse group of aquatic, photosynthetic organisms generally categorized as either macro algae (i.e. seaweed) or microalgae (unicellular). As aquatic relatives of plants, microalgae

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Table 1: Spirulina culture medium (Schlosser, 1994).

S. No	Component	Stock solution	Quantity used	Concentration in final medium
1	**Solution 1**	500 ml		
	$NaHCO_3$		13.61g	$1.62x10^{-1}$
	Na_2CO3		4.03g	$3.80x10^{-2}$
	K_2HPO_4		0.50g	$2.87x10^{-3}$
2	**Solution 2**	500 ml		
	$NaNO_3$		2.5g	$2.94x10^{-2}$
	K_2SO_4		1.0g	$5.74x10^{-3}$
	NaCl		1.0g	$1.71x10^{-2}$
	$MgSO_4.7H_2O$		0.2g	$8.11x10^{-4}$
	$CaCl2.2H_2O$		0.04g	$2.72x10^{-4}$
	$FeSO_4.7H_2O$		0.01g	$3.60x10^{-5}$
	$Na_2EDTA.2H_2O$		0.08g	$2.15x10^{-4}$
3	**Trace metal solution (1ml)**	(gl^{-1} dH_2O)		
	$Na_2EDTA.2H_2O$		0.8g	$2.15x10^{-6}$
	$FeSO_4.7H_2O$		0.7g	$2.52x10^{-6}$
	$ZnSO_4.7H_2O$		1ml	$3.48x10^{-9}$
	$MnSO_4.7H_2O$		1ml	$8.97x10^{-9}$
	H_3BO_3		1ml	$1.62x10^{-7}$
	$Co\ (NO_3)_2.6H_2O$		1ml	$3.44x10^{-9}$
	$Na_2MoO_4.\ 2H_2O$		1ml	$4.13x10^{-9}$
	$CuSO_4.\ 5H_2O$		1ml	$2.00x10^{-11}$
4	**Vitamin solution** (1ml) Cyanocobalamine (vitamin B_{12})	(gl^{-1} dH_2O)	5mg	$3.69x10^{-9}$

Source: Andersen (2005).

thrive in aerated, liquid cultures where the cells have sufficient access to light, carbon dioxide and other nutrients [14]. Algae are primarily photo autotrophic and few species are heterotrophic in nature. Unlike terrestrial plants, which require fertile and or irrigation, microalgae can grow in a wide range of habitats [15]. Successful commercial utilization of microalgae has been established in the production of nutritional supplements, antioxidants, cosmetics, natural dyes and Poly Unsaturated Fatty Acids (PUFA) [16]. In order to be used in aquaculture, a micro algal strain has to meet various criteria, such as ease of culturing, lack of toxicity, high nutritional value with correct cell size and shape and a digestible cell wall to make nutrients available [15,17]. The main objective of the present study was conducted to analyze the nutrient profile such as proximate composition, mineral content and amino acid profile of two species of freshwater algae (*Spirulina platensis* and *Chlorella vulgaris*) and a floating fern *Azolla pinnata* for the purpose of using alternative protein source or additives for aquaculture feeds.

Materials and Methods

Culture of *Spirulina platensis*

Collection and cultivation of *spirulina platensis*: The pure *S. platensis* pure culture was collected from Spirulina production research and training center Kadachanendal, Madurai, Tamil Nadu, India.

Table 2: Bold's basal medium.

S. No	Component	Stock solution ($g\ L^{-1}$ dH_2O)	Quantity used	Concentration in final medium
1	**Macronutrients**			
	$NaNo_3$	25	10 ml	$2.94x10^{-3}$
	$CaCl_2.\ 2H_2O$	2.5	10 ml	$1.70x10^{-4}$
	$MgSO_4,\ 7H_2O$	7.5	10 ml	$3.04x10^{-4}$
	K_2HPO_4	7.5	10 ml	$4.31x10^{-4}$
	KH_2PO_4	17.5	10 ml	$1.29x10^{-3}$
	NaCl	2.5	10 ml	$4.28x10^{-4}$
2	**Alkaline EDTA solution**			
	EDTA	50	1ml	$1.71x10^{-4}$
	KOH	31		$5.53x10^{-4}$
3	**Acidified Iron solution**			
	$FeSO_4.\ 7H_2O$	4.98	1ml	$1.79x10^{-5}$
	H_2SO_4	-		
4	**Boran solution**			
	H_3BO_4	11.42	1ml	$1.85x10^{-4}$
5	**Trace metal solution**			
	$ZnSO_4.7H_20$	8.82		$3.07x10^{-5}$
	$MnCl.4H_2O$	1.44		$7.28x10^{-6}$
	MoO_3	0.71		$4.93x10^{-6}$
	$CuSO_4.5H_2O$	1.57		$6.29x10^{-6}$
	$Co\ (NO_3)_2.6H_2O$	0.49		$1.68x10^{-6}$

Source: Andersen (2005).

KBBM medium (BBM + 0.25% Sucrose +1.0% protease peptone) was developed for a Chlorella strain (Schuster et al., 1990).

Preparation of inoculums: The microalgae, *S. platensis*, was inoculated in Spirulina medium (Table 1) (Schlosser 1994) (100 ml mother culture + 900 ml basal medium) and the cultures were incubated for 15 days at 24±1°C in a thermo-statically controlled room and illuminated with cool inflorescence lamps (Phillips 40 W, cool daylight 6500 K) at an intensity of 2000 lux in a 12:12 h light dark regime.

Culture in plastic troughs: Culture troughs after the troughs were sun dried for 8 h. Later, the plastic troughs were filled with tap water up to 25 L and mixed well with the pure nutrient media (N-8) (Tables 2 & 3) [18]. 1 L of mother were cleaned well with bleach and were rinsed until bleach smell had totally gone off culture of *S. platensis* was inoculated in the plastic troughs. The plastic troughs were vigorously aerated to provide required quantity of oxygen and to keep cells and media in suspension. The required concentration of algae developed after 30 days of inoculation. The plastic troughs were kept in open under 100% outdoor light exposure. A constant temperature of 25-30°C was maintained throughout the growth period.

Culture of *Chlorella vulgaris*

Collection of pure mother culture of *C. vulgaris*: *C. vulgaris* mother culture was collected from Vivekananda Institute of Algal Technology (VIAT), R.K.M. Vivekananda College, Chennai, Tamil

Table 3: Composition of N-8 nutritive media (Vonshak, 1986).

S. No	Name of chemical used	Quantity/ml distilled H_2O	Required / l(Final Conc.)
1	Sodium nitrate	15 g/500 ml	10 ml
2	Potassium phosphate	0.4g/200 ml	5 ml
3	i. Iron sulfate	0.3 g/100 ml	0.1 ml
	ii. Citric acid	0.3 g/100 ml	
	iii. Boric acid	0.15 g/100 ml	
	iv. Manganese chloride	0.15 g/100 ml	
4	i. Zinc sulfate	0.022 g/100 ml	0.1 ml
	ii. Copper sulfate	0.079g/100 ml	
	iii. Ammonium molybdate	0.015g/100 ml	
	iv. Ammonium vanadate	0.023g/100 ml	
	v. EDTA	0.25 g/100 ml	
	vi. Cobalt chloride	0.012g/100 ml	
5	Vitamin B_{12}	0.007g/100 ml	20 ml
6	Na_2 EDTA	3 g/100 ml	20 ml

Source: Andersen (2005).

Nadu, and India.

Preparation of inoculums: The microalgae, *C. vulgaris*, was inoculated in Bold Basal medium (100 ml mother culture + 900 ml basal medium) (Table 2) [19,20] and the cultures were incubated for 15 days at 24 ± 1°C in a thermo-statically controlled room and illuminated with cool inflorescence lamps (Phillips 40 W, cool daylight 6500 K) at an intensity of 2000 lux in a 12: 12 h light dark regime.

Culture in glass tanks: Culture containers were well cleaned with bleach, rinsed and sun dried for 8 h. Then plastic troughs were filled with tap water up to 25 L and mixed well with the pure nutrient media (N-8 medium) [18]. 1 L of mother culture of *C. vulgaris* was inoculated in the glass tanks. The tanks were vigorously aerated to provide required quantity of oxygen and to keep cells and media in suspension. The required concentration of algae was developed after 30 days of inoculation. The tanks were kept open under 100% outdoor light exposures. A constant temperature of 25-30°C was maintained throughout the growth period.

Counting of algal cells and filtering method

Sampling was done once in five days basis using 10 ml capacity vials. *Chlorella* cells in each vial were preserved by adding 2-3 drops of formalin. One ml of sample was carefully filled in Neubauer Hemocyto meter groove [21] and covered with glass slide. The cells were enumerated under compound microscope. Hand tally counter was used for reliable counting. Algal cells were calculated by the following mathematical expression.

(Cells (ml^{-1}) = Total number of cells counted/$10\times4\times10^{-6}$)

Filtering method: Printing polyester/ Nylon fabric cloth with a mesh between 30-60 microns is using filter. After use, the filter should be carefully washed, as quickly as possible, and then dried away from direct sunlight.

Culture of *Azolla pinnata*

Materials: Wet clay soil, cow dung, urea, Azolla culture and plastic tubs.

Collection of pure Mother culture *Azolla pinnata*: The pure cultures of *A. pinnata* were collected from Azolla cultivation and Research Centre, Tamilnadu Agriculture University Coimbatore, Tamilnadu.

Culture of *A. pinnata* in troughs [22]

A trough of 24L was taken to which a sediment layer of 3cm clay was made. 20L of water was poured into the trough. 0.5% of urea was dissolved in the water along with 1 kg cow dung extract. This composition was allowed to stand for 2 days. The trough was kept out door in direct sunlight. To this medium, *A. pinnata* cultures were added. The *A. pinnata* growth was monitored daily. After thirty days the *A. pinnata* cultures were collected and dried in incubator. The *A. pinnata* were sun dried for three days until they become crispy while retaining their greenish coloration. The dried leaves were then milled using a hammer mill to produce leaf meal, which was then stored in containers until further use.

Fishmeal (FM)

The processed feed grade fishmeal (trash fish) was purchased from Rosen fisheries, Marathakkara, Thrissur, and Kerala.

Analysis of the proximate composition: Analysis of crude protein, moisture, lipid and ash ingredients (*S. platensis, C. vulgaris, A. pinnata* and fishmeal) were performed according to standard [1,5] procedures. Dry matter was determined by drying at 105°C until a constant weight was obtained. Ash content was determined by burning in a muffle furnace at 525°C for 12h. Crude protein (N*6.25) was analyzed by the Kjeldahl method after acid digestion. Crude lipid was analyzed by Soxhlet method. Ingredients gross energy was determined by using the Oxygen Bomb Calorimeter (230 VAC; Sl. No. 26036; Advance Research Instrument Company, New Delhi, India).

Analyses of Amino acid Profile: The profile of amino acids was performed by high performance thin layer chromatographic (HPTLC) method [23]. The peak area of the samples were compared with standard amino acids and quantified. All the twenty standard amino acids were classified into following four groups based on their Rfvalues to avoid merging of individual amino acids while elution. The Group-1 contained: asparagine, glutamine, serine, proline and metheonine; Group-2: aspartic acid, glutamic acid, alanine, valine and phenyl alanine; Group-3: lysine, glycine, threonine, tyrosine and isoleucine; Group-4: histidine, argentine, cystine, tryphtophan and leucine. Each group consisted of 1 mg of each 5 amino acids dissolved with 5 ml distilled water.

Content of Minerals and electrolytes analysis: The mineral elements such as Ca^{2+}, Zn^{2+}, Mg^{2+}, Mn^{2+}, and Cu^{2+} in the respective samples were analyzed using the Atomic Absorption Spectrophotometer (AAS) of Perkin-Elmer, Model 2380 in air acetylene flame. The representative dried samples for trace element analysis were prepared following the triple acid digestion method [24]. The phosphorous content in the diets and animal tissues were analyzed using the spectrophotometric method (Vanadomolybdate

Table 4: Growth rate of *S. platensis, C. vulgaris and A. pinnata.*

Days	*S. platensis* (cells/ml $^{-1}$)	*C. vulgaris* (cells/ml $^{-1}$)	*A. pinnata* (g)
Initial	80.50 ± 0.50^{g}	72.16 ± 5.79^{f}	300.00 ± 10.00^{g}
5th Day	93.16 ± 6.60^{f}	89.83 ± 9.00^{e}	425.00 ± 25.00^{f}
10th Day	120.66 ± 8.25^{e}	102.33 ± 5.53^{d}	560.00 ± 32.78^{e}
15th Day	141.00 ± 4.76^{d}	111.00 ± 4.50^{d}	675.00 ± 25.00^{d}
20th Day	162.66 ± 8.94^{c}	122.66 ± 2.75^{c}	811.66 ± 34.03^{c}
25th Day	177.00 ± 3.50^{b}	142.5 ± 5.56^{b}	966.66 ± 32.53^{b}
30th Day	198.00 ± 6.72^{a}	167.00 ± 5.07^{a}	1175.00 ± 25.00^{a}
F value	145.89	93.84	382.76

Each value is a mean ± SD of three replicate analysis, within each column means with different superscripts letters are statistically significant P<0.05 (one way ANOVA and subsequently post hoc multiple comparison with DMRT).

method) using a spectrophotometer (Elico SL150, UV visible spectrophotometer) at 470 nm. The Na^{+} and K^{+} present in HCl digested sample were estimated following the simple flame photometric method of [25]. Using a simple flame photometer (Elico flame photometer, model CL 220). NaCl and KCl were used as standards.

Statistical analysis

The paired sample t-test and DMRT (SPSS, 16.0-version, IBM software) were performed to determine whether significant variation between the treatments existed. All these tests used a significance level of 5% (P<0.05). Data's are reported as Mean ± standard deviation.

Results

Growth rate of *S. platensis, C. vulgaris* and *A. pinnata*

The laboratory cultured *S. platensis; C. vulgaris* and *A. pinnata* growth rate are proved in (Table 4). The 30 day of *S. platensis*, *C. vulgaris* and *A. pinnata* growth rate was statistically significant when compared with initial day. Initial day of *S. platensis* was 80.50 ± 0.50 (cells/ml-1), it is improved in thirtieth day in 198.00 ± 6.72 (cells/ml -1), the initial day of *C. vulgaris* was 72.16 ± 5.79 (cells/ml -1), it was improved in thirtieth day in 167.00 ± 5.07 (ml-1) and also, the initial day of *A. pinnata* was 300.00 ± 10.00 (g), it is improved in thirtieth days in 1175.00 ± 25.00 (g). *S. platensis*, *C. vulgaris* and *A. pinnata* growth rate was statistically significant at P< 0.05.

Proximate composition

The proximate composition of FM, *S. platensis*, *C. vulgaris* and *A. pinnata* are proved in (Table 5*)*. The protein content was significantly higher (P<0.05) in *S. platensis* and Fish meal, followed by the *C. vulgaris* and *A. pinnata*. The carbohydrate level showed significantly higher (P<0.05) in *A. pinnata* followed by the *C. vulgaris* and *S. platensis* when comparison with FM. The lipid content showed significantly higher level in *C. vulgaris* followed by the *S. platensis* and fishmeal. *A. pinnata* showed significantly lower level of lipid. The ash content was significantly higher in *A. pinnata*, but *S. platensis* and *C. vulgaris* showed slightly lower than FM. The moisture content was higher in FM. Also, the gross energy was higher in FM followed by the *S. platensis*, *C. vulgaris* and *A. pinnata*.

Profile of Amino acids

The profiles of amino acids were detected through HPTLC analyses from the *S. platensis, C. vulgaris*, *A. pinnata* and fishmeal are presented in (Table 6). Totally fourteen amino acids were detected, among these nine are EAA (arginine, histidine, isoleucine, leucine, lysine, threonine, methionine, phenyl alanine and valine), remaining five are non essential amino acids (alanine, glycine, proline, glutamic acid and serine). The EAA such as, arginine, histidine, isoleucine, leucine, methionine and phenyl alanine contents were significantly higher (P<0.05) in *C. vulgaris* and *S. platensis*, but *A. pinnata* had not significant difference when compared with FM. Other EAA, lysine, threonine and valine contents had not significant difference in *C. vulgaris* and *S. platensis*, but *A. pinnata* have significantly lower (P<0.05) when compared with FM. The non essential amino acids such as, alanine, glycine and serine was observed had no significant difference in *C. vulgaris* and *S. platensis*, but *A. pinnata* have significantly lower level when compared with fishmeal. Other non essential amino acids proline and glutamic acid have significantly higher values in *C. vulgaris* and *S. platensis* when compared with FM.

Content of minerals and electrolytes

The minerals such as, Ca, K, Cu, Zn, Mg and Mn were significantly

Table 5: Proximate composition of fishmeal, cultured *S. platensis, C. vulgaris and A. Pinnata.*

Proximate composition	Ingredients				F Value
	Fishmeal	*S. platensis*	*C. vulgaris*	*A. pinnata*	
Protein (%)	60.4 ± 1.18^{a}	61.74 ± 1.06^{a}	55.7 ± 2.10^{b}	28.01± 1.15^{c}	365.39
		-19.341 (0.003)	8.849 (0.013)	1.870 (0.000)	
Carbohydrate (%)	4.78 ± 0.45^{d}	10.93 ± 0.31^{c}	15.28 ± 0.39^{b}	30.07 ± 2.42^{a}	220.65
		-76.087 (0.000)	-303.109 (0.000)	-22.235 (0.002)	
Lipid (%)	7.5 ± 0.25^{b}	5.09 ± 0.17^{c}	10.65 ± 0.87^{a}	3.18 ± 0.49^{d}	114.62
		52.178 (0.000)	-8.800 (0.013)	31.177 (0.001)	
Ash (%)	9.50 ± 0.55^{b}	9.00 ± 0.29^{b}	9.00 ± 0.54^{b}	13.5 ± 1.13^{a}	29.155
		3.331 (0.080)	86.603 (0.000)	-11.945 (0.007)	
Moisture (%)	10.00 ± 0.65^{a}	5.60 ± 0.45^{b}	6.30 ± 0.50^{b}	4.00 ± 0.35^{c}	77.584
		38.105 (0.001)	42.724 (0.001)	34.641 (0.001)	
Gross energy k.cal/g	2.85	2.781	2.747	2.713	

Each value is a mean ± SD of three replicate analysis, within each row means with different superscripts letters are statistically significant P<0.05 (one way ANOVA and subsequently post hoc multiple comparison with DMRT, paired sample 't' test also applied).

Table 6: Amino acids contents in Fish meal, cultured *S. platensis, C. vulgaris and A. pinnata* (g/100g dry weight).

Amino acids	Ingredients				F Value
	Fish meal	*S. platensis*	*C. vulgaris*	*A. pinnata*	
Arginine*	0.85±0.07^{b}	0.96 ± 0.11^{b}	1.28 ± 0.14^{a}	0.87 ± 0.09^{b}	10.649
		-4.763 (0.041)	-10.640 (0.009)	-1.732 (0.225)	
Histidine*	1.37±0.18^{b}	1.56 ± 0.11^{ab}	1.75 ± 0.12^{a}	0.97 ± 0.15^{c}	16.377
		-10.970 (0.008)	-4.701(0.042)	23.094 (0.002)	
Isoleucine*	2.50±0.20^{b}	2.76 ± 0.11^{a}	2.84 ± 0.13^{b}	1.42 ± 0.15^{c}	56.481
		-8.413 (0.014)	-5.004 (0.038)	37.412 (0.001)	
Leucine*	6.67±0.12^{c}	8.20 ± 0.11^{b}	9.10 ± 0.21^{a}	6.49 ± 0.12^{c}	221.46
		-46.765 (0.000)	-265.004 (0.000)	(*)	
Lysine *	1.27±0.15^{a}	1.38 ± 0.07^{a}	1.52 ± 0.15^{a}	0.77±0.17^{b}	16.228
		-2.382 (0.140)	-36.500 (0.001)	43.301 (0.001)	
Threonine*	1.54±0.11^{a}	1.37 ± 0.13^{a}	1.39 ± 0.10^{a}	0.99 ± 0.05^{b}	15.872
		25.981 (0.001)	14.722 (0.005)	15.877 (0.004)	
Methionine*	1.35±0.16^{c}	1.89 ± 0.08^{ab}	1.74 ± 0.05^{a}	1.61 ± 0.14^{b}	11.628
		-6.141 (0.026)	-11.691 (0.007)	-22.517 (0.002)	
Phenyl alanine*	1.35±0.14^{c}	2.05 ± 0.11^{ab}	1.87 ± 0.17^{a}	1.63 ± 0.10^{b}	15.654
		-30.022 (0.001)	-40.415 (0.001)	-12.124 (0.007)	
Valine*	0.60±0.11^{a}	0.65 ± 0.10^{a}	0.65 ± 0.15^{a}	0.58±0.09^{a}	0.288
		-2.165 (0.163)	-8.660 (0.013)	1.732 (0.225)	
Alanine**	1.32±0.05^{a}	1.18 ± 0.10^{a}	1.20 ± 0.08^{a}	0.84 ± 0.10^{b}	17.647
		6.928 (0.020)	4.850 (0.040)	16.628 (0.004)	
Glycine**	1.25±0.19^{a}	1.18 ± 0.17^{a}	1.30 ± 0.20^{a}	0.94 ± 0.20^{a}	2.104
		-8.660 (0.013)	6.062 (0.026)	53.694 (0.000)	
Proline **	1.85±0.11^{b}	2.04 ± 0.07^{a}	2.12 ± 0.10^{a}	1.06 ± 0.09^{c}	80.442
		-46.765 (0.000)	-8.227 (0.014)	68.416 (0.000)	
Glutamic acid**	0.65±0.14^{c}	0.79 ± 0.07^{c}	1.73 ± 0.13^{a}	1.27 ± 0.10^{b}	56.342
		-187.06 (0.000)	-3.464 (0.074)	-26.847 (0.001)	
Serine **	1.63±0.20^{a}	1.54 ± 0.05^{a}	1.71 ± 0.11^{a}	1.23 ± 0.10^{b}	8.203
		-1.540 (0.264)	1.039 (0.408)	6.928 (0.020)	

Each value is a mean ± SD of three replicate analysis, within each row means with different superscripts letters are statistically significant P<0.05 (one way ANOVA and subsequently post hoc multiple comparison with DMRT, paired sample 't' test also applied). (*), the correlation and t cannot be computed because the SE of the difference is '0'. *Essential amino acids, **Non essential amino acids.

higher (P<0.05) in *C. vulgaris* followed by the *S. platensis* and *A. pinnata* when compared with FM. The concentration of Na and Fe were significantly higher in *S. platensis* followed by the *C. vulgaris* and *A. pinnata* when compared to the FM. The phosphorus content was significantly higher in *A. pinnata* when compared with FM.

Discussion

Proximate composition and profile of amino acid in *S. platensis and C. vulgaris*

Microalgal biomass is a rich source of nutrients, such as n-3 and n-6 fatty acids, proteins, minerals, and other essential nutrients [26]. Early interest of Spirulina focused mainly on its potential source of protein and vitamins. In the present study, laboratory culture of *S. platensis* showed protein 61.74%, carbohydrate 10.93%, lipid 5.09%, ash 9.00% and moisture 11.6%. Similarly, [27] and [28] reported that the composition of commercial Spirulina powder is 60% protein, 20% Carbohydrate, 5% fat, 7% minerals and 3-6% moisture making it a low fat, low calorie and cholesterol free source of protein has a balanced composition of amino acids with concentrations of methionine, tryptophan, and other amino acids almost similar to those of casein although, this depends upon the culture media used [29] reported that the Schlosser medium inoculated *S. platensis* grow with various level of temperature (30-40°C) which showed protein decreased from 64% to 59%. More than 40 different species of microalgae are cultured intensively for direct or indirect feeding through production of zooplanktons and Artemianauplii.

The most common algal species cultured are diatoms, Skeletonema costatum, Thalassiosira psuedonanna, Chaetoceros gracilis, Chaetoceros calcitrans, the flagellates Isochrysis galbana, Tetraselmis suecica, Monochrysis lutheri, Chlorococcalean and *Chlorella*. Among all, *Chlorella* has become an important source of food for rotifers due to its nutritional value and physical compatibility [30]. *Chlorella* was

Table 7: Concentration of minerals in fishmeal, cultured *S. platensis, C. vulgaris and A. pinnata* (mg/100g).

Minerals	Ingredients				F value
	Fishmeal	*S. platensis*	*C. vulgaris*	*A. pinnata*	
Ca	0.0034 ± 0.001^{c}	0.0029 ± 0.001^{b}	0.0039 ± 0.0004^{a}	0.0044 ± 0.0002^{bc}-2.165 (0.163)	9.759
		(*)	-9.238 (0.012)		
Na	0.149 ± 0.012^{d}	0.512 ± 0.025^{a}	0.354 ± 0.013^{b}	0.298 ± 0.015^{c}	232.064
		-48.364 (0.000)	-355.070 (0.000)	-86.025 (0.000)	
K	0.124 ± 0.013^{d}	0.322 ± 0.015^{b}	0.373 ± 0.017^{a}	0.155 ± 0.01^{c}	230.115
		-171.473 (0.000)	-107.820 (0.000)	-17.898 (0.003)	
P	13.15 ± 0.37^{d}	15.2 ± 0.23^{c}	17.2 ± 0.21^{b}	19.6 ± 0.12^{a}	367.81
		-25.362 (0.002)	-43.843 (0.001)	-44.687 (0.001)	
Cu	0.0068 ± 0.002^{b}	0.0025 ± 0.0002^{c}	0.0152 ± 0.003^{a}	0.0042 ± 0.001^{bc}	27.136
		4.138 (0.054)	-14.549 (0.005)	4.503 (0.046)	
Zn	0.138 ± 0.015^{d}	0.0412 ± 0.002^{b}	0.0623 ± 0.025^{a}	0.0233 ± 0.017^{c}	479.43
		-36.506 (0.001)	-84.004 (0.000)	-82.272 (0.000)	
Fe	0.515 ± 0.014^{bc}	0.135 ± 0.012^{a}	0.226 ± 0.024^{b}	0.101 ± 0.011^{c}	36.624
		-17.321 (0.003)	-19.226 (0.003)	8.083 (0.015)	
Mg	0.461 ± 0.34^{c}	0.811 ± 0.042^{b}	5.24 ± 0.12^{a}	0.501 ± 0.021^{d}	343.264
		-2.034 (0.179)	-21.879 (0.002)	-10.642 (0.009)	
Mn	0.0259 ± 0.004^{b}	0.048 ± 0.002^{b}	0.0634 ± 0.0022^{a}	0.0097 ± 0.0012^{c}	197.619
		-2.252 (0.153)	-36.084 (0.001)	3.835 (0.062)	

Each value is a mean ± SD of three replicate analysis, within each row means with different superscripts letters are statistically significant P<0.05 (one way ANOVA and subsequently post hoc multiple comparison with DMRT, paired sample 't' test also applied). (*), the correlation and t cannot be computed because the SE of the difference is '0'. Ca, Calcium; Na, Sodium; K, Potassium; P, Phosphorus; Cu, Copper; Zn, Zinc; Fe, Iron; Mg, Magnesium; Mn, Manganese.

first studied as a possible food source in Japan, United States and Germany after World War II [31]. In the present study, laboratory culture of *C. vulgaris* contained 55.7% of CP, 15.28% carbohydrate, 10.65% of lipid, 9.00% ash, 9.30% of moisture and 2.747 k.cal/g of energy. The present results correlated with spray dried *C. vulgaris* contained crude protein 52.8%, fat 8.1%, fiber 20.8%, carbohydrates 5.6%, energy 1291 kJ, moisture 3.6% and ash 9.13% in 100 g [32,33] reported that the *Chlorella* sp. cultivated by pig, poultry and cow dung showed chemical composition 32 to 39.91% protein, 5.50 to 7.30% fat, 4.64 to 5.91% fiber, 9.09 to 10.90% ash and NFE 37.08 to 47.04%.

In the present study, fourteen amino acids were detected in cultured *S. platensis*, and C. vulgaris, among these nine are EAA (arginine, histidine, isoleucine, leucine, lysine, threonine, methionine, phenyl alanine and valine), remaining five are non essential amino acids (alanine, glycine, proline, glutamic acid and serine). Similarly, [34] has reported the presence of these amino acids in different temperature treated *S. platensis*. Also, [35] reported the presence of these amino acids in commercially cultivated *S. platensis* [36]. Reported that presence of these amino acids in novel processing method treated *C. vulgaris* [37]. Reported that, five amino acids (aspartic acid, serine, alanine, leucine and glycine) were collectively responsible for 50% or more of the total dry matter content of *Chlorella* and Scenedesmus.

In the present study, minerals and trace elements such as, Ca, K, P, Cu, Zn, Mg, Mn, Na and Fe contents were detected in *S. platensis* and *C. vulgaris*. Similarly, [26] reported that Spirulina contain Na, K, Ca, Mg, Fe, Cr, Cu, Zn, Mn, Se and P; Also, *Chlorella* was rich in P (1761.5 mg), Na (1346.4 mg), K (749.9 mg), Ca (593.7 mg), Mg (344.3 mg), and Fe (259.1 mg); other mineral contents included Mn (2.09 mg), Zn (1.19 mg), Se (0.07 mg), Cu (0.06 mg), and Cr (0.02mg) respectively (Table 7). Reported that Spirulina is a rich source of minerals and it contains 6.82-9.60% of total minerals. Spirulina is considered to be a rich source of protein, vitamins, minerals, EAA and fatty acids Gamma-Linolenic Acid (GLA) and antioxidant pigments, such as carotenoids [38]. In addition, it is effective as an immune modulator [39], and using dried Spirulina as a feed supplement [40].

Proximate composition and amino acid profile of *A. pinnata*

The greens (green plants) have long been recognized as the cheapest and most abundant potential source of proteins because of their ability to synthesize amino acids from a wide range of virtually unlimited and readily available primary materials [41].

In the present study, the laboratory cultured *A. pinnata* showed proximate composition such as CP 28.01%, carbohydrate 30.07%, lipid 3.18%, ash 13.5%, moisture 8%, and a gross energy value 2.713 kcal/ kg [42]. Studied the chemical analysis in Azolla meal (AZM) contained of dry matter (DM) basis of 21.4% CP, 12.7% crude fiber, 2.7% ether extract, 16.2% ash and 47% carbohydrate. The proximate composition of *A. pinnata* is mentioned on dry matter basis: CP 21.6%; ash 15.4% crude fiber 16.6%; dry matter 6.6%; crude fat 3.8%; with a caloric value of 4.2 kcal/g and *invitro* digestibility of around 78%. The composition of Azolla appears to be rich because of the 21.6% crude protein with EAA, including a rich source of lysine, along with arginine and methionine. Azolla is a lysine rich protein

it is useful as a fresh food to animals including fish [43]. The present results are similarly finding with some previewers proximate analyses in AZM using for alternative protein source for broilers [44] and Nile tilapia [45]. In the present study, nonessential amino acids (arginine, histidine, isoleucine, leucine, lysine, threonine, methionine and phenyl alanine), and EAA (valine, alanine, glycine, proline, glutamic acid and serine) were detected in Azolla. Similarly [46,47], reported that the presences of these similar amino acids are rich in AZM.

In the present study, such minerals and electrolytes (Ca, Co, Cu, K, Mn, Mg, Zn, Fe, Na, and P) are identified in Azolla meal. Similarly, reported by [47-51], in his compiled study of various Azolla species. Azolla is also able to store phosphorus and potassium from water. It is also rich in Fe (1000-8600 ppm), Cu (3-210 ppm) and Mn (120-2700 ppm) [48,52-55].

Conclusion

In the present study, the laboratory cultured *S. platensis*; *C. vulgaris* and *A. pinnata* contain accepted level of biochemical constituents, essential amino acids and minerals for the feed formulation to aquaculture organisms. Based on these results and previous findings, it is decided to laboratory trial level on replace the fishmeal with these ingredients individually in the feed formulations for aquaculture organism. Also, these ingredients used for further study to feed formulation and feeding experiment conducting on freshwater prawn species at laboratory level and lab to land program.

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