



Amino acids profile of six freshwater Algae in the northern region of Bangladesh: A solution for the protein supplement of poultry feed

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ABSTRACT

Algae is an important natural source of protein, specifically amino acid source, which are the building blocks of enzymes, hormones, nucleic acids, etc. Herein, the amino acid composition was investigated in six commonly growing fresh-water algae. The number of amino acids in *Oscillatoria brevis*, *Spirulina platensis*, *Pithophora kewensis*, *Euglena viridis*, *Hydrodictyon reticulatum*, and *Aphanocapsa holsatica* were detected 15, 12, 17, 15, 14, and 15, respectively. Among studied algal species, the amino acids: proline ($29239.3 \pm 276.4 \mu\text{g/g}$), histidine ($10056.8 \pm 85.4 \mu\text{g/g}$), aspartic ($3030.6 \pm 48.6 \mu\text{g/g}$), threonine ($1492.3 \pm 31.8 \mu\text{g/g}$) were detected in the highest amount in *O. brevis*, *S. platensis*, *P. kewensis*, *A. holsatica*, respectively. It is reported that the range of the ratio between 50/50 to 60/40 of essential amino acid/(nonessential amino acid + conditional essential amino acid) promotes maximum growth performance and best energy utilization. The obtained results of this study suggested that a combination of *A. holsatica*, *H. reticulatum*, and *O. brevis* can provide a good ratio (48/52) of amino acids and the noble source of essential amino acids for feed or food supplements, especially for poultry's stamina and, consequently, welfare.

1. Introduction

Algae is a diverse group of thalloid, photosynthetic, chlorophyll-bearing, and microscopic to macroscopic organisms. They are mainly grown in aquatic environments. They also play an important role in the environmental food chain to maintain ecologic balance in aquatic systems [1]. There have been several reports on the utilization of algae in various applications, including cosmetics, auspicious feedstock, fatty acids, therapeutic agents, wastewater treatment, and biofuels [2–4]. There are several different types of algae grown in freshwater environments like *Oscillatoria brevis*, *Spirulina platensis*, *Pithophora kewensis*, *Euglena viridis*, *Hydrodictyon reticulatum*, *Aphanocapsa holsatica*, etc. The nutrient content of algae varies from one type to another, and their natural habitat also varies even within the same algal group [5].

Recently, enhanced awareness has grown worldwide about feed supplements to have antibiotics-free poultry meat. Although poultry is

fed with natural feed, some essential amino acids (lysine, methionine, threonine, and tryptophan) are often offered as manufactured complements [6]. To meet the production costs of essential amino acids and poultry feed, and to find alternative feed sources for corn, soybean feed, and animal protein, freshwater algae have attracted attention in recent years, revealing their nutrients and incorporating them into food products as supplements [7]. Globally, the market for algae-based dietary supplements and nutraceuticals reached 30 billion euros in 2020, and the growth rate of the market is assumed to be 50.6 %, and the trend is predicted to continue until 2026 [8]. They are enriched not only with amino acids, fatty acids, minerals, and vitamins but also with other health-promoting antioxidant substances like polyphenols and carotenoids [9,10]. Two well-known examples of algae that are commercially cultivated in North America and Europe are *Spirulina* and *Chlorella*, which are 'generally recognized as safe, and they are being used as a protein source in food products [11].

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In Bangladesh, algae can also be a potential source of nutrition and industrialization of algae-derived functional products. Several seaweeds from the Bay of Bengal have undergone research to reveal their nutritional properties [12–14]. However, many different types of freshwater algae have not been investigated yet, especially in the northern region of Bangladesh. The freshwater algae are grown abundantly in this area. Therefore, studying the amino acid content could be a great opportunity to pave the way to introduce the nutritional values of cost-effective freshwater algae and utilization of the algae as various food supplements for animals.

Considering algae as a highly attractive and sustainable source of feed supplements, it is important to find out more effective algal strains for the required nutrients. In the present study, for the first time, we analyzed the amino acid composition of the six commonly growing algae, *O. brevis*, *S. platensis*, *P. kewensis*, *E. viridis*, *H. reticulatum*, and *A. holsatica* by using High-Performance Liquid Chromatography (HPLC) to find out a natural, cost-effective amino acid enriched that is protein enriched locally available source for feed industry.

2. Materials and methods

2.1. Collection and processing of samples

O. brevis, *S. platensis*, *P. kewensis*, *E. viridis*, *H. reticulatum*, and *A. holsatica* species were collected from freshwater bodies of different locations in the Rajshahi division, Bangladesh. Initially, they were washed for a few minutes with running tap water to remove unwanted material from the surface and finally cleaned by washing with deionized water. They were cultured using Raj-media in the shed at room temperature. Individual samples were collected and dried at room temperature with continuous aeration using a ceiling fan. The dried sample was blended for fine powder and stored at 4 °C until further analysis.

2.2. Amino acid profiling

2.2.1. Sample preparation

The profile of amino acids was determined by following a slight modification of the process described in Olaofe et al., 2013 [15]. Dry algae powder (30 mg) from each species was weighed and taken into six different test tubes. The samples were hydrolyzed using seven ml of 6 N HCl and shaken smoothly for a few seconds. Mixtures were heated at 105 ± 5 °C for 24 h by keeping the test tubes in a beaker filled with sand and keeping the whole system in an incubator. The sand was used to reduce the possibility of concentration downfall of the samples. During the hydroxylation period, samples were sealed tightly with a cap and surrounded with aluminum foil paper. After 24 h, samples were taken out from the sand and allowed to stand until cooled down to room temperature. Then the mixture of HCl was diluted to 0.1 N, using deionized water to obtain the volume of 25 mL and filtered with a membrane filter (Pore size, 0.2 µm). The final solutions were poured into different vials to analyze with HPLC. Standards samples of amino acids were prepared with 0.1 N HCl.

2.2.2. HPLC equipment parameter and derivatization process

A Thermo Scientific™ Dionex™ Ultimate™ 3000 rapid separation system with a diode array detector (DAD) was used as HPLC equipment. The system was equipped with a C18 analytical column (Acclaim RSLC 120) having 2.2 µm particle size (100 mm × 2.1 mm) and 120 Å pore size. The run time was 15 min, flow rate and injection volumes were 0.722 mL/min and 1.00 µL, respectively, for each sample. The chromatographic signal was detected in 338 nm wavelength (λ) at 7.9 min for the first absorbance and at 7.1 min for the last absorbance in 262 nm when the sampler temperature was 22 °C and the column temperature was 40 °C. Mobile phase-A was prepared by dissolving sodium phosphate (1.78 g), sodium tetra borate (3.8137 g), sodium azide (0.0325 g), and the appropriate amount of 85 % phosphoric acid (to adjust pH at 7.8) in

HPLC grade acetonitrile to make final volume 1 L. Mobile Phase-B was prepared by mixing methanol, acetonitrile, and water in the amount of 450 mL, 450 mL, and 100 mL, respectively. Reagent for derivatization were prepared with 0.1 M Borate buffer at pH-9, 1 N HCl, OPA/MPA reagent in borate buffer solvent, 2.5 mg/mL FMOC solution in acetonitrile and injection diluent made by mixing 85 % H₃PO₄ and Mobile Phase-A.

2.2.3. Comparison of amino acid classes

Amount of different classes of amino acids were compared by the following formula:

$$\% \text{ of AA in a class of a species} = \frac{\text{Amount of amino acid in a class of a species} \times 100}{\text{Total amount of amino acid in the species}}$$

2.3. Statistical analysis

The data were generated with 3 times replications. They were statistically analyzed by SPSS software version 22. One-way ANOVA followed by the Duncane (DMRT) method of post hoc was carried out to find out the level of significance (p < 0.05).

3. Results

Six different freshwater algae were investigated to analyze the amino acid contents. Most of the amino acids were found in detectable amounts in all of the samples. About 15, 12, 17, 15, 14, and 15 amino acids were detected in *O. brevis*, *S. platensis*, *P. kewensis*, *E. viridis*, *H. reticulatum*, and *A. holsatica*, respectively. The percentages of each amino acid of investigated algal species were presented in Table 1 and the comparisons of different classes of amino acids from species to species were presented in Table 2 and Fig. 1. Threonine, histidine, methionine, valine, tryptophan, phenylalanine, isoleucine, leucine, and lysine were categorized as essential amino acids. Threonine was found prominently present (1492.3 ± 31.8 µg/g) in *A. holsatica* whereas this amino acid has not been detected in *S. platensis*. Histidine has been detected only in the case of *P. kewensis*, and *S. platensis* at the amount of 9.6 ± 2.9 and 10056.8 ± 85.4 µg/g, respectively. Similarly, isoleucine has been proven to be present only in *P. kewensis* at the amount of 0.12 ± 0.06 µg/g according to our study. Methionine was present at the quantity of 2.02 ± 0.12 µg/g, 0.77 ± 0.14 µg/g, 6.1 ± 0.5 µg/g, 19.17 ± 2.3 µg/g, 4.7 ± 0.3 µg/g, and 7.14 ± 0.24 µg/g in *P. kewensis*, *E. viridis*, *O. brevis*, *H. reticulatum*, *S. platensis*, and *A. holsatica*, respectively. Valine has not been detected in *S. platensis*.

Although tryptophan and phenylalanine have been detected in all studied species, the amount was not comparable to other amino acids. The maximum quantity of leucine and lysine have been quantified as 8055.3 ± 120.2 µg/g, and 7856.6 ± 0.2 µg/g, respectively in *A. holsatica*. Proline was the most prominent amino acid in all of the species except *H. reticulatum*. The highest amount of proline was found in *O. brevis* (29239.3 ± 276.4 µg/g) followed by 25097.6 ± 61.1 µg/g, 6578.0 ± 7.6 µg/g, 6522.7 ± 51.0 µg/g, and 3030.6 ± 48.6 µg/g in *S. platensis*, *E. viridis*, *A. holsatica*, and *P. kewensis*, respectively.

The percentage of essential, nonessential, and conditionally essential amino acids based on the total amount of amino acids present in the respective species of algae were presented in Table 2. The highest percentage of conditionally essential amino acid was 94.31 ± 0.91 which was found in *O. brevis*. Whereas, *P. kewensis*, *E. viridis*, and *S. platensis* were bound with 81.4 ± 2.52, 86.55 ± 1.36, and 70.7 ± 0.7% of the conditionally essential amino acid. The highest essential amino acid present in *A. holsatica* (72.1 %), whereas the conditionally essential amino acid present in that species, was about 26.78 %. Moreover, a sufficient amount of essential amino acid is present in *H. reticulatum* (65.31 ± 0.9896), and *S. platensis* (28.6997 ± 0.3075). The quantity of nonessential amino acid present in *H. reticulatum*, *P. kewensis*, *E. viridis*, *O. brevis*, *S. platensis*, and *A. holsatica* abound with 28.136 ± 1.16, 10.19 ± 0.26, 7.26 ± 0.23, 43 ± 0.03, 0.840 ± 0.0195, and 1.17 ± 0.026%,

Table 1Amino acid ($\mu\text{g/g}$) content of 6 species of freshwater algae.

Amino acid	Freshwater Algae (Present study)						Other sources of amino acid in poultry feed	
	<i>P. kewensis</i>	<i>E. viridis</i>	<i>O. brevis</i>	<i>H. reticulatum</i>	<i>S. platensis</i>	<i>A. holsatica</i>	Soya-cake [31]	Rapeseed cake [32]
Aspartic acid	360.1 \pm 54.3 ^b	514.7 \pm 4.4 ^a	77.4 \pm 3.3 ^c	101.1 \pm 18.8 ^c	91.42 \pm 19.6 ^c	85.5 \pm 12.14 ^c	32.9 \pm 0.36 ^a	0.02 \pm 0.00 ^b
Glutamic acid	2.02 \pm 0.36 ^d	2.857 \pm 0.3 ^d	219.2 \pm 3.2 ^a	209.8 \pm 7.2 ^b	200.1 \pm 6.61 ^b	185.6 \pm 6.7 ^c	47.1 \pm 0.66 ^a	0.05 \pm 0.01 ^b
Asparagine	4.8 \pm 0.3 ^b	0.36 \pm 0.06 ^c	7.8 \pm 0.4 ^a	0.3 \pm 0.06 ^c	ND	ND	ND	ND
Serine	0.12 \pm 0.04 ^d	8.9 \pm 0.2 ^c	25.4 \pm 1.1 ^b	31.01 \pm 2.4 ^a	ND	10.18 \pm 0.3 ^c	13.9 \pm 0.58 ^a	0.01 \pm 0.00 ^b
Glutamine	0.12 \pm 0.06 ^d	8.8 \pm 2.4 ^c	25.4 \pm 1.1 ^b	31.01 \pm 2.4 ^a	ND	10.18 \pm 0.3 ^c	ND	0.05 \pm 0.01 ^b
Threonine	2.5 \pm 0.18 ^d	4.7 \pm 0.3 ^c	728.9 \pm 48.6 ^b	439.2 \pm 9.2 ^a	ND	1492.3 \pm 31.8 ^c	11.68 \pm 0.52 ^c	0.01 \pm 0.00 ^b
Histidine	9.6 \pm 2.9 ^b	ND	ND	ND	10056.8 \pm 85.4 ^a	ND	7.36 \pm 0.68 ^b	0.01 \pm 0.00 ^b
Arginine	9.05 \pm 0.24 ^c	3.63 \pm 0.2 ^c	155.3 \pm 6.1 ^a	155.1 \pm 10.5 ^a	143.1 \pm 26.9 ^{ab}	120.2 \pm 17.1 ^b	18.4 \pm 0.04 ^a	0.02 \pm 0.00 ^b
Alanine	24.7 \pm 0.71 ^a	19.1 \pm 1.3 ^b	23.6 \pm 2.1 ^d	0.42 \pm 0.06 ^e	3.04 \pm 0.24 ^d	7.2 \pm 0.18 ^c	11.8 \pm 0.48 ^b	0.01 \pm 0.00 ^a
Tyrosine	ND	ND	37.7 \pm 2.4 ^b	40.4 \pm 4.17 ^a	2.5 \pm 0.3 ^d	32.02 \pm 1.4 ^c	8.9 \pm 0.66 ^a	ND
Methionine	2.02 \pm 0.12 ^d	0.77 \pm 0.14 ^d	6.1 \pm 0.5 ^{bc}	19.17 \pm 2.3 ^a	4.8 \pm 0.3 ^c	7.14 \pm 0.24 ^b	3.18 \pm 0.05	0.006
Valine	13.51 \pm 1.67 ^a	7.14 \pm 0.6 ^c	1.3 \pm 0.4 ^d	10.12 \pm 0.18 ^b	ND	1.37 \pm 0.18 ^d	13.8 \pm 0.46	0.016
Tryptophan	0.71 \pm 0.12 ^{de}	21.5 \pm 0.5 ^a	10.2 \pm 0.2 ^b	7.26 \pm 0.18 ^c	1.19 \pm 0.12 ^d	0.36 \pm 0.06 ^e	19.76 \pm 0.66	ND
Phenylalanine	25.8 \pm 10.24 ^e	7.4 \pm 0.2 ^f	155.6 \pm 14.3 ^b	179.16 \pm 0.54 ^a	138.9 \pm 2.56 ^c	127.14 \pm 0.24 ^d	14.0 \pm 0.34 ^b	0.01 \pm 0.00 ^a
Isoleucine	0.12 \pm 0.06	ND	ND	ND	ND	ND	21.1 \pm 0.66 ^a	0.01 \pm 0.45 ^b
Leucine	138.2 \pm 0.9 ^b	154.3 \pm 0.5 ^b	ND	2.44 \pm 0.18 ^c	0.714 \pm 0.42 ^c	8055.3 \pm 120.2 ^a	13.6 \pm 0.46 ^c	0.02 \pm 0.00 ^b
Lysine	20.53 \pm 1.55 ^a	6.7 \pm 0.3 ^c	0.83 \pm 0.18 ^{ce}	ND	3.39 \pm 0.36 ^d	7856.6 \pm 0.2 ^b	13.6 \pm 0.46 ^b	0.02 \pm 0.1 ^a
Proline	3030.6 \pm 48.6 ^d	6578.0 \pm 7.6 ^c	29239.3 \pm 276.4 ^a	ND	25097.6 \pm 61.1 ^b	6522.7 \pm 50.1 ^c	13.98 \pm 0.54 ^f	0.02 \pm 0.00 ^e

ND: Not detected.

The content of each amino acid is presented as mean \pm SD (n=3). The mean amount is significantly different ($p < 0.05$) which was analyzed by one-way ANOVA. The data were analyzed by the Duncan method of post hoc test that indicates that the values containing the same letter as superscription in a row are not different.

Table 2

The percentage of essential, conditional essential, and non-essential amino acids against total amino acids.

Sample	Conditional amino acid	Essential amino acid	Nonessential amino acid
<i>P. kewensis</i>	81.4 \pm 2.5 ^c	5.9 \pm 0.46 ^d	10.19 \pm 0.2 ^b
<i>E. viridis</i>	86.6 \pm 1.3 ^b	2.59 \pm 0.2 ^e	7.26 \pm 0.2 ^c
<i>O. brevis</i>	94.3 \pm 0.9 ^a	3.64 \pm 0.1 ^e	1.1 \pm 0.03 ^d
<i>H. reticulatum</i>	4.7 \pm 0.4 ^f	65.3 \pm 0.1 ^b	28.1 \pm 1.1 ^a
<i>S. platensis</i>	70.7 \pm 0.7 ^d	28.7 \pm 0.3 ^c	0.8 \pm 0.02 ^d
<i>A. holsatica</i>	26.7 \pm 0.001 ^e	72.1 \pm 0.05 ^a	1.17 \pm 0.03 ^d

The amount of amino acid is presented as mean \pm SD (n=3). The mean amount of amino acid is significantly different according to one-way ANOVA ($p < 0.05$). The data were analyzed by the Duncan method of post hoc, which indicates that the value containing different letter as subscript in a single column is significantly different from each other.

respectively (Table 2 and Fig. 1). From the above results, it can be concluded that freshwater algae have the potential to utilize as a protein source i.e. amino acid source.

4. Discussions

As a potential source of amino acids, six different types of freshwater algae that are available in the Rajshahi region of Bangladesh were considered for analyzing the amino acid profile in this study. Although the amino acid content of freshwater algae was studied previously [7, 16], the amino acid profiles of *O. brevis* was described for the first time in this study. There were statistically significant differences between the groupings of amino acids for all algal species. Aspartic acid which plays a role in the inter-reconversion of carbohydrates and protein through the tri-carboxylic acid cycle and is involved in the development of the nervous system and enhances adult neurological activities of rats and chickens [17,18], was highest in *E. viridis*. Valine, which improves insulin sensitivity [19], and the amino acid alanine, a key component of the glucose-alanine cycle in mammals [20,21] were found highest in *P. kewensis* (24.701 $\mu\text{g/g}$). Arginine, leucine, glutamine, and methionine are also present in all of the species that can promote cell signaling transduction during protein synthesis [22]. The highest amount of leucine was present in *A. holsatica*. Moreover, *P. kewensis* and *E. viridis* also contained a promising amount of leucine. Phenylalanine is another essential amino acid that is present in all of the respective species of

algae according to our study. The phenylalanine is present in *H. reticulatum* in the highest amount followed by *O. brevis*, *S. platensis*, *A. holsatica*, *P. kewensis*, and *E. viridis* and they are in different subsets at 5 % statistically significant differences (Table 2). In this study, it was observed that among the studied algal species, *O. brevis* contains a higher amount of proline (~29239.3 $\mu\text{g/g}$) which could help to maintain the collagen integrity of the vascular wall, especially in case of skin burning for cellular regeneration [23]. Hence, like other microalgal species, *O. brevis* can be a promising source of proline for human supplements as well as poultry feed [24]. However, proline has not been detected in *H. reticulatum*.

The amount of conditional essential amino acid is remarkable in the case of *E. viridis*, *P. kewensis*, and *S. platensis* and there are statistically significant differences in all of the species. The percentage of essential amino acids is highest in *A. holsatica* which is followed by *H. reticulatum* in satisfactory amounts. The ratio of essential amino acid/(nonessential amino acid + conditional essential amino acid) is reported to have an important effect on growth performance, feed utilization (feed efficiency, protein efficiency ratio, and N retention) as well as on the body chemical composition in any organism [25]. Several research groups reported a ratio in the range between 50/50 to 60/40 promotes maximum growth performance and assures the best energy utilization [26–28]. In the present investigation, the ratio for *A. holsatica*, and *H. reticulatum* were found 72.07/27.9 and 65.6/32.8, respectively. Therefore, these two species can be used as standard raw materials for food or feed supplements as well as essential amino acid recompense. In *S. platensis* the ratio was 29/71 which can be mixed in the desired ratio with the previously mentioned two species to obtain a composition having a referenced ratio. Although the ratio of essential amino acid/(nonessential amino acid + conditional essential amino acid), for *O. brevis* was smallest (4/96) it contains the highest (29239 $\mu\text{g/g}$) amount of proline in the investigated samples which acts as a precursor molecule for the synthesis of excitatory neurotransmitter [29,30]. Ensuring a good combination of all the amino acids can be benevolent for high quality feed production. Hence, the obtained results of this study suggested that a combination of *A. holsatica*, *H. reticulatum*, and *O. brevis* can provide a good ratio (48/52) of all different categories i.e. essential, conditional, and nonessential amino acid sources. Soya cake and rapeseed cake have been used for poultry feed in this area as a protein source for the preparation of poultry feed. Upon comparison with the amino acid contents of soya cake and rapeseed cake that are reported in the previous studies, it was found that at least 3 to 10-fold

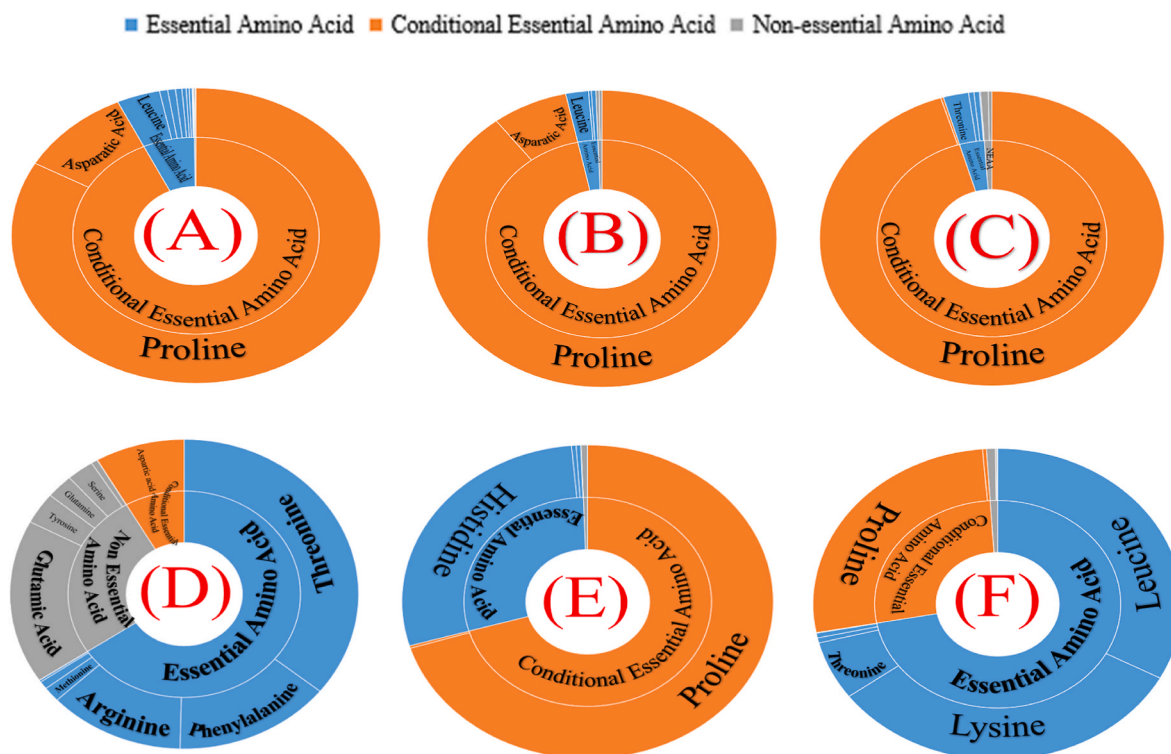


Fig. 1. Sunburst diagram of a variation of amino acids in (A) *P. kewensis*, (B) *E. viridis*, (C) *O. brevis*, (D) *H. reticulatum*, (E) *S. platensis*, (F) *A. holsatica*, (G) Soya-cake, and (H) Rape-seed cake (i.e.:Essential Amino Acid, Conditional Essential Amino Acids, Non-essential Amino Acid).

greater content of several essential amino acids like threonine, methionine, valine, phenylalanine, and lysine in *A. holsatica*, *O. brevis*, *S. platensis*, and *H. reticulatum*. Apart from this, some conditional amino acids especially, proline were found more than 200 fold in all targeted algae except *H. reticulatum* than the traditionally used soya cake and rapeseed cake. Therefore, it can be stated that freshwater algae enriched with conditional essential amino acids, especially a mixed combination of *A. holsatica*, *O. brevis*, and *H. reticulatum* can be utilized in case of threatening physiological conditions as food/feed supplements.

Although a huge amount of amino acids was found in the investigated freshwater algae, mineral contents, heavy metals, and some other nutritional parameters could not be evaluated in this study. We have only focused on the amino acid contents for finding a protein-enriched alternative for poultry feed and food supplements. Further, future investigation for the other parameters is required. However, this study recommends that algae in different combinations may be an optimistic source of protein that can be utilized for the preparation of food and feed, especially poultry feed.

5. Conclusion

To use freshwater algae as a sustainable protein source in animal feed or a dietary supplement, identification of the most appropriate algae (species) is crucial in terms of their amino acid content and composition. In this work, the amino acid composition of six locally available freshwater algae in Rajshahi, Bangladesh, were analyzed for a better combination of required protein sources. It was observed that *A. holsatica*, *O. brevis*, and *H. reticulatum* contain the highest amount of essential amino acid ($72.1 \pm 0.05\%$), Conditional essential amino acid ($94.3 \pm 0.9\%$) and nonessential amino acid ($28.1 \pm 1.1\%$), respectively. Therefore, a mixed combination of *A. holsatica*, *O. brevis*, and *H. reticulatum* could be suggested as a noble source of amino acids for feed or food supplements as well and individuals can be used to fulfill the shortage of particular amino acids.

Author's statement

No informed consent or human or animal rights apply to this study.

Credit authorship contribution statement

Md. Moniruzzaman: Conceptualization, Supervision, Writing–Original Draft, Review & Editing, Funding acquisition, Sample collection and characterization, Project administration; Dr. Md. Nurul Huda Bhuiyan: Supervision, Writing–Original Draft, Review & Editing; Dr. Md. Mahmudul Hassan Mondol: Writing–Review & Editing; Nazim Uddin Ahmed: Supervision, Statistical analysis of amino acid data; Mst. Munni Akter: Sample collection and characterization, Investigation; Mahci Al Basher: Formal data analysis; Farhana Jahan: Formal data analysis; Dr. Arfatun Nahar Chowdhury: Formal data analysis; Iftekhar Md. Noor: Formal data analysis; Dr. Sabrina Naz: Supervision, Writing–Review & Editing.

Declaration of competing interest

All authors declared that they have no known competing financial interests or personal relationships for publishing the submitted manuscript with title “Amino acids profile of six freshwater Algae in the northern region of Bangladesh: A solution for the protein supplement of poultry feed”.

Data availability

Data will be made available on request.

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