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Review Article

NUTRITIONAL ANALYSIS OF AZOLLA AND ITS POTENTIAL AS A PROTEIN SUPPLEMENT FOR HUMAN CONSUMPTION: A REVIEW

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ABSTRACT

Proteins are essential for numerous physiological processes. While traditional animal-derived proteins are nutritionally superior, their production has significant environmental burdens. Plant-based alternatives, though more sustainable, face challenges such as incomplete amino acid profiles, poor digestibility, and undesirable sensory attributes. Azolla, a fast-growing aquatic fern that is symbiotically associated with nitrogen-fixing cyanobacteria, has emerged as a potential sustainable protein source. This review explores the nutritional profile, digestibility, and amino acid composition of four Azolla species commonly found in India. Crude protein levels ranged from 19% to 42%, with promising essential amino acid content. The review also compares Azolla's potential with existing plant protein supplements such as soy, pea, chickpea, and potato, highlighting both benefits and limitations. Notably, Azolla has demonstrated efficacy as an economical feed supplement in poultry, livestock, and aquaculture sectors, improving growth performance and reducing production costs. Preliminary studies on Azolla protein hydrolysates suggest favourable digestibility and bioactivity, including antioxidant and antihypertensive effects, however its direct application in human nutrition remains underexplored.

Keywords: Azolla, Plant proteins, Protein energy malnutrition, Protein hydrolysates.

INTRODUCTION

Proteins are fundamental components of cells and are involved in diverse functions that are crucial for cellular processes (Fraga *et al*., 1995; Widłak, 2013). They act as catalysts (salivary amylase, DNA polymerase), receptors (GPCR's, TLR's), hormones (Insulin, oxytocin), carriers (haemoglobin, transferrin), membrane channels (Voltage-gated sodium channels, aquaporins), and structural elements (microfilaments, collagen), among many other roles (Fraga *et al*., 1995; Lane *et al*., 2007). Protein requirements for adults vary based on physical activity levels, sedentary individuals are recommended a daily dietary allowance of 0.85 g/kg of body weight and for adults with moderate or intense physical activity, 1.3g and 1.6g per kilogram body weight per day are recommended respectively (Campbell *et al*., 2008; *Recommended Dietary Allowances*, 1989; Tarnopolsky *et al*., 1992). Humans rely on consumption of plants and animals to meet their daily protein needs and recent studies indicate that protein consumption patterns vary globally, with animal-based protein intake generally higher in developed countries compared to developing countries (Henchion *et al*., 2021). Traditional animal-based proteins like meat and eggs are the gold standard due to their complete amino acid profiles, rapid post-prandial blood amino acid levels and superior digestibility. Economic development and urbanization have driven increased animal-based protein consumption worldwide, going from 61g to 80g per person per day between 1961 and 2011(Sans & Combris, 2016).

Developing nations like the Indian subcontinent largely depends on plant- and cereal- based sources to meet their daily protein needs due to socioeconomical, religious, and cultural reasons as a result of which a significant percentage of the population falls within the protein energy malnutrition (PEM) bracket (Zhang *et al*., 2022). Data from NHFS-5 2019-2020 showed 36% stunting, 32% underweight, and 19% wasting in children under the age of

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5 in India (International Institute for Population Sciences, 2022). In a tribal area of Parol, Thane district, Maharashtra, a study of 225 children under the age of 5 reported 34.67% stunting, 38.67% underweight, and 36% wasting (Shingade, 2023). A study of 300 children from 6 urban slums in Behrampur city, Odisha, reported 55.3% underweight, 75 % wasting, and 42% stunting respectively (Sethy *et al*., 2017) A cross-sectional descriptive study, with a sample size of 1332 children aged 1-5 conducted at the Fatehpur Beri Urban Primary Health Centre (UPHC) South Delhi reported 27.2% stunting, 24.5% underweight, and 17.8% wasting (Goyal *et al*., 2023). A different cross-sectional study of 517 children between the ages 0-59 months from rural areas of Jabalpur district, Madya Pradesh, reported 35.8% underweight, 41.4% stunting, and 19.7% wasting (Toppo, 2018). Pulses account for 11-14% of the total protein intake, and although there is no specific data indicating the percentage of cereal protein consumption, this can be inferred from consumption patterns and the protein content of cereals. The Comprehensive National Nutrition Survey 2016-18 conducted by the Ministry of Health and Family Welfare (MoHFW), India, showed in most states, children between the ages 5-19 years consumed cereals on average >85% (Ministry of Health and Family Welfare (MoHFW), Government of India *et al*., 2019). (Deepak *et al*., 2025) examined the annual consumption pattern of cereals and found that rice consistently accounted for the highest proportion (108.44 kg/capita/year), followed by wheat (60.38 kg/capita/year), maize (7.63 kg/capita/year), and millets (6.61 kg/capita/year). Protein content of whole wheat flour was reported to be 12.84±0.02 g/100g of flour and 14.74±0.03 g/100g for 48h sprouted wheat flour (Perveen *et al*., 2024). A study on two different types of rice, Bapata (BPT, white rice) and Uma (coloured rice), commonly consumed in Tamil Nadu and Kerala showed that raw BPT contained 7.8±0.2 g of protein per 100g of rice and raw Uma contained 7.6±0.3 g of protein per 100g of rice (Shobana *et al*., 2011).

A study on raw sorghum (colloquially termed jowar) showed it contains 11.36±0.04 g of protein per 100g (Mohapatra *et al*., 2019). While animal-based proteins have their benefits, many studies have shown the negative effects of meat production on the environment, it utilizing 1/3rd of the global land area and generating 14% of all human-caused greenhouse gas emissions (Gerber & Food and Agriculture Organization of the United Nations., 2013; Opio *et al*., 2011; M. Swain *et al*., 2018).With the global population projected to reach 9-10 billion by the year 2050 roughly resulting in 60% increase in protein demand, it is bound to increase the logistical challenges of keeping up with the exponential protein needs (Andreoli *et al*., 2021; Baldi & Gottardo, 2017; United Nations Department For Economic And Social Affairs, 2025). In view of increasing health awareness and ethical concerns among consumers, preferences have shifted to vegetarianism and veganism with plant-based proteins receiving widespread acceptance. Several fern genera, including Diplazium, and Azollaceae are also being explored as potential alternatives due to their reported high protein content, ranging from 12.10% to 28.44% (Mohan Gupta *et al*., 2020; Deepak S. Patil, 2021).

Azolla is a genus of small floating aquatic ferns belonging to the family Salviniaceae, order Salviniales (Pereira, 2017). Taxonomically they are divided into two subgenera: Euazolla which includes *A.filiculoides*, *A. mexicana*, *A. caroliniana*, *A.microphylla*, and *A.ruba*; Rhizosperma which contains *A.pinnata*, *A. nilotica*, and *A. imbricata* (Korsa *et al*., 2024; Yang *et al*., 2022). Morphologically, they are 1-2cm in diameter with triangular or polygonal fronds, consists of a submerged lower ventral lobe that keeps them buoyant and an upper dorsal lobe that captures light, water, and CO_2 for photosynthesis. Azolla reproduces both sexually through specialized spores and asexually through fragmentation (Salazar *et al*., 2025). Due to its symbiotic relationship with nitrogen-fixing cyanobacteria *Anabaena azollae*, azolla exhibits rapid growth, capable of doubling its biomass in just 2-5 days. This review aims to explore the nutritional profile of *Azolla spp.* found in India, compare it with current plant-based protein supplements, highlight its successful application in the poultry industry, and discuss its potential and challenges as a protein supplement for human consumption.

Current Landscape of Plant-Based Protein Supplements

Proteins from pea, soy, hemp, potato, rice, chickpea, faba beans, lentils, and quinoa are gaining popularity as they offer comparable nutritional quality to animal proteins. Although plant-based proteins are associated with lower cardiovascular, lower inflammatory diseases and diabetes risks, they generally have poor digestibility and lower essential amino acid content than animal proteins (Gorissen & Witard, 2018; Mariotti, 2019). The presence of anti-nutrients such as glucosinolates, phenolic compounds, and phytic acid, along with digestive enzyme inhibitors induce undesirable flavours, adding complexity to the isolation process. Though recent advances in food technology have enabled the modification of these proteins to overcome digestibility issues—through processes such as isolation, concentration, hydrolysis, and fermentation—these techniques add to the production costs which is ultimately transferred to consumers (Ahnen *et al*., 2019; Kostrakiewicz-Gierałt, 2024).

Soy Proteins

Protein digestibility-corrected amino acid score (PDCAAS) is a method used for the measurement of protein quality in human nutrition. It is calculated by comparing the concentration of the first limiting essential amino acid in the test protein with the concentration of the same amino acid in a reference protein and correcting it by multiplying with the true digestibility of the protein. A PDCAAS value of 1.00 would indicate that the protein provides necessary amounts of essential amino acids (Schaafsma, 2000). With a PDCAAS value of 0.92 – 1.00, soy protein represents one of the most nutritionally complete plant-based protein sources and demonstrates high digestibility and nutritional quality comparable to animal proteins (Hughes *et al*., 2011). Its consumption has been associated with improved cardiovascular health by lowering total cholesterol, LDL cholesterol, and triglycerides as well as metabolic health particularly in insulin sensitivity (Azadbakht *et al*., 2007; Faria *et al*., 2018; Hoie *et al*., 2007). The most notable concern is allergenicity, with soy being recognized as a major food allergen that limits its use in many food applications (Kutzli *et al*., 2021). Soy proteins are particularly affected by "beany" and "grassy" odors, which result from a combination of over 20 volatile compounds produced during soybean growth and processing (Sakai *et al*., 2022). The beany flavor is also linked to derivatives of secondary lipid oxidation and naturally occurring bitter-astringent tastes from saponins and isoflavones.

Pea Proteins

Pea protein concentrates, with a PDCAAS value of 0.89 (Rutherfurd *et al*., 2015), has shown satiety-increasing properties, blood pressure lowering-effects, potential to lower postprandial TAG levels, and a possibility to mediate the glycemic response (Braden *et al*., 2023; Li *et al*., 2011; Marinangeli *et al*., 2009; Marinangeli & Jones, 2011). Pea proteins face similar taste challenges, with products often associated with distinct off-flavors described as "beany" or "vegetal" caused by aldehyde molecules. Other volatile molecules related to off-flavours including alcohols, ketones, and pyrazines, increase significantly as pea protein concentrations increases in food formulations. Sensory evaluation confirms that pea protein addition results in bitter and metallic tastes, as well as astringent aftertastes, likely resulting from isoflavones, phenolic acids, and saponins (Flores *et al*., 2023; McCarron *et al*., 2024).

Potato Proteins

Potato protein with a PDCAAS of 0.56 (after boiling) is lower than other plant proteins like wheat and soy but contains all nine essential amino acids required by the human body (Bailey *et al*., 2023). Research has shown that potato proteins can effectively stimulate muscle protein synthesis, can improve cholesterol homeostasis, has anti-hypertensive properties, has anti-obesity properties, anti-inflammatory/ antioxidant properties (Kaspar *et al*., 2011; Liu *et al*., 2022; Liyanage *et al*., 2009; Oikawa *et al*., 2020; Pihlanto *et al*., 2008; Robert *et al*., 2006). Beyond the core health benefits, potato protein demonstrates versatility as a functional ingredient such as being an effective carrier of vitamin B_{12} and other hydrophobic bioactive compounds (Mendes *et al*., 2020). Lysine plays a crucial role in protein synthesis to maintain positive nitrogen balance, cross-linkage in type 1 collagen, is a precursor in carnitine synthesis and glutamate synthesis in mammalian brains (Papes *et al*., 2001; Tanphaichitr *et al*., 1971; Yamauchi & Shiiba, 2008; Zello *et al*., 1993). Given its high lysine content, it can be consumed by persons relying on cereals for proteins as lysine is a limiting essential amino acid in cereals.

Chickpea protein

Chickpea protein is a highly nutritious, cost-effective protein source with a PDCAAS between 0.75 – 0.84 (Nosworthy *et al*., 2020). The health benefits of chickpea protein include short term increased insulin sensitivity, various bioactive properties including angiotensin-converting enzyme inhibition after alcalase treatment, antioxidant activity, anti-inflammatory effects, hypoglycemic properties, and anticarcinogenic activity (Chino *et al*., 2017; Ercan & El, 2016; Johnson *et al*., 2005; Magee *et al*., 2012; Nestel *et al*., 2004; Torres-Fuentes *et al*., 2015; Yust *et al*., 2003). The removal of off-flavors remains a major challenge across all pulse proteins, with few mitigation methods proving successful. Chickpea and other pulse proteins share a persistent beany flavour which is a limiting factor for major consumer acceptance.

Nutritional Profile of *Azolla*

The mineral content and amino acid profiles of commonly found Azolla species in India is summarized in Table 1 and Table 2 respectively.

Table 1. Amino acid profiles of Azolla species found in India.

Essential Amino acid (EAA)	***Azolla caroliniana*** [a] (Anokhi Chandrababu *et al*., 2024)	***Azolla pinnata*** [a] (Anokhi Chandrababu *et al*., 2024)	***Azolla filiculoides*** [b] (Leterme *et al*., 2009)	***Azolla microphylla*** [a] (Sanginga & Van Hove, 1989)
Arginine	5.66±0.30	5.97±0.28	0.61	1.90
Histidine	2.55±0.10	2.59±0.27	0.26	0.47
Isoleucine	5.19±0.28	5.51±0.21	0.48	1.07
Leucine	9.30±0.19	9.76±0.15	0.94	2.29
Lysine	4.39±0.09	4.28±0.04	0.53	1.62
Methionine	1.29±0.21	1.41±0.05	0.17	0.43
Phenylalanine	6.06±0.50	6.06±0.5	0.55	2.17
Threonine	5.29±0.14	5.35±0.29	0.53	1.13
Tryptophan	1.36±0.04	1.41±0.03	0.15	-
Valine	6.73±0.19	6.54±0.18	0.62	1.07

Non-essential amino

acids (NEAA)				
Alanine	7.34±0.15	7.27±0.11	0.75	1.45
Aspartic acid	10.99±0.41	10.50±0.80	1.05	2.33
Cysteine	0.16±0.02	0.09±0.05	0.23	0.43
Glutamic Acid	14.46±0.38	13.75±0.25	1.4	4.04
Glycine	5.84±0.06	5.47±0.22	0.68	1.26
Proline	4.20±0.13	4.43±0.08	0.85	-
Serine	4.20±0.13	5.34±0.1	0.56	1.07
Tyrosine	4.02±0.11	4.28±0.22	0.37	2.17

[a] expressed as g/100g dry weight, [b] expressed as g/100g of protein

Table 2. Mineral composition of Azolla species.

Minerals	***A.caroliniana*** [a] (Anokhi Chandrababu *et al*., 2024)	***A.pinnata*** [a] (Anokhi Chandrababu *et al*., 2024)	***A.filiculoides*** [b] (Leterme *et al*., 2009)	***A.microphylla*** [c] (Seephueak *et al*., 2025)
S	-	-	0.39	-
Ca	0.5684	0.594	0.93	1.1
K	1.2670	1.447	2.25	1.8
P	0.2628	0.252	0.346	0.5
Na	0.1533	0.150	0.277	0.3
Zn	0.0054	0.006	0.0029	-
Al	0.0462	0.499	-	-
Mn	0.0482	0.038	0.142	-
Fe	0.3857	0.322	0.172	80
Cu	0.0038	0.005	0.013	-

[a] mineral content expressed as ppm, [b] mineral content expressed as g/kg DM, [c] expressed as g/100g DM

Proximate composition

Crude protein

Crude protein in *A.pinnata* on average was 26.5±0.08% on DM basis (Bhatt *et al*., 2020; Said *et al*., 2023). *A.caroliniana* was found to contain 19.0% ± 0.5% of which 14.91 g per 100g is digestible protein (Winstead *et al*., 2024) and *A filiculoides* contained 19.5±1.8% protein on DM basis. (Brouwer *et al*., 2018). *A.microphylla* showed to contain 36% to 42% protein when quail manure was used during cultivation (Latif *et al*., 2023). Another study reported *A.filiculoides* to contain 23.2% protein on DM basis. (Leterme *et al*., 2009).

Moisture

Moisture content was significantly high in *A. microphylla* 93-95% and *A. caroliniana* was shown to have a mean moisture content of 91.81±0.03% (Anokhi Chandrababu *et al*., 2024). *A.pinnata* had an average moisture content of 90.87% (Said *et al*., 2023) which is significantly higher than 67±1.44% reported by another study (Das *et al*., 2018).

Ash Content

One study reported total ash content of *A.pinnata* to be 20.3±0.28% (Bhatt *et al*., 2020), which is less when compared to 31.2% reported by another study (Said *et al*., 2023). *A.caroliniana* was reported to contain 12% total ash (Azhar *et al*., 2018) while a different study reported 19.6±1.47% (Kösesakal & Yıldız, 2019). *A.microphylla* was shown to have a total ash content of 19.23%. (Latif *et al*., 2023). *A. filiculoides* had 19.9±0.07% (Hamidan *et al*., 2024) which is higher than previously reported 11.2% by a separate study (Leterme *et al*., 2009)

Dietary Fiber

A.pinnata was reported with NDF 44.28±0.18% ADF 39.4±0.06% (Bhatt *et al*., 2020) which is significantly higher compared to 11.15%. reported by another study (Said *et al*., 2023). *A.microphylla* was shown to contain fiber at 7% (Latif *et al*., 2023) which is significantly less compared to the 18.07% reported by another study (Azhar *et al*., 2018). *A. caroliniana* was shown to have 13.44% total fibre (Azhar *et al*., 2018). A study in *A.filiculoides* reported NDF and ADF values 52.3% and 33.3% respectively (Leterme *et al*., 2009).

Azolla protein hydrolysates

Protein hydrolysates show several advantages over whole proteins in terms of digestibility, absorption, and biological activity. Research has shown that hydrolysates are absorbed more swiftly and efficiently than whole proteins leading to greater plasma amino acid availability. They also possess various bioactive properties, including antioxidant, antihypertensive, and hypoglycemic activities (Nasri, 2017). A study analysed the effect of *A.pinnata* protein hydrolysates (AFPH) as an O/W emulsion system on the human gut microbiota and were assessed for cytotoxic effects (Qoms *et al*., 2024). AFPH were prepared by hydrolysing Azolla fern protein concentrates (AFPC) with Alcalase in sodium phosphate buffer (pH 8.5, 50mM), at a 3:100 w/w ratio for 6h at 55 °C. The study reported no cytotoxic effects on colon cells, with a survival rate of >95%. Additionally, it showed increased populations of *Bifidobacteria* and *Lactobacilli*, and great antihypertensive, antidiabetic, and antioxidant activities, under different conditions. Despite studies reporting high protein content in azolla, very few reports have explored the safety and efficacy of AFP (azolla fern protein) or AFPH.

Azolla in the Poultry Industry

With an excellent protein content and essential amino acid content and ease of bio-production of Azolla makes it one of the cheapest, economic, potential, and maintainable feed alternatives for livestock and poultry

Poultry birds

Research shows that Azolla meal can be successfully incorporated into broiler chicken diets at varying levels with positive results in FCR, egg production, meat tenderness and body weight gain. Inclusion levels of 5% as a feed ingredient to replace sesame meal improved growth rate, FCR, and energy efficiency without negative effects on mortalities (Namra *et al*., 2010; Pramanik, 2002). One study showed no adverse effects in broilers with a maximum inclusion level of 7.5% of feed with Azolla (S. P. Sahu *et al*., 2021). Another study found that a 15% inclusion led to the highest body weight gain with no adverse effects on growth performance (Samad *et al*., 2020). Studies have also shown that egg production in chicken increased from 47 to 53.2 per bird when fed 100g fresh azolla per bird per day (Bidya Shankar Sinha *et al*., 2018). Chickens fed 200 g/kg Azolla instead of sesame oil had good egg mass and feed conversion efficiency as reported by another study (Khatun *et al*., 1999). A study on Pekin laying ducks reported an increase in the same parameters (B. Swain *et al*., 2018). From the above studies, inclusion levels of 5-7% were found to be appropriate for safe and profitable broiler production due to reduced FCR, mortalities, and production costs (Islam & Nishibori, 2016).

Livestock and cattle

Due to climate changes, there are reported shortages of green fodder for dairy animals and Azolla with its low lignin content, great protein concentrations and enhanced yield is an excellent alternative to green fodder and dairy feed (G. Kumar & Chander, 2017). In cross breed dairy cows supplemented with 1.5-2 kg of Azolla along with concentrate feed an increased milk yield of 11% was reported (A. S. Kumar *et al*., 2020). A study on lactating buffaloes reported that after 60 days of feeding 1.5 kg of fresh Azolla per day with conventional feed cottonseed cake, the milk yield increased by 1.30L/day on average, 9.30 L/day from 8.0 L/day (Meena *et al*., 2017). A report by (Sihag *et al*., 2018) showed that 15% of sundried Azolla can replace concentrated mixes of goat diets without any adverse effects, reducing feed costs for goat farmers. Replacing 25% linseed cake by 6% Azolla in the diets of Corriedale sheep increased body weight, DM digestibility and FCR (El Naggar & El-Mesery, 2022). A study on *A.microphylla* demonstrated that it can replace 50% of soybean meal in small scale farms in Thailand reducing the production cost for them (Seephueak *et al*., 2025).

Aquaculture

Fishmeal is the primary source of proteins used in aquatic feed and is prepared from wild fish causing their populations to decrease. This overreliance on wild fishes is driving up production costs and has restrained the growth of aquaculture. Azolla offers a viable solution and can be used as potential protein alternative with research on aquatic species including common carp, redbelly tilapia, catfish, fringed-lipped carp, calbasu, thai silver barb, tilapia, rohu and freshwater prawn showing improved growth and health (Yohana *et al*., 2023). A study showed that incorporating 40% of azolla in the diet of *L.fimbriatus* during fry-to-fingerling does not affect fish growth and survival but is 24.48% cheaper than the control diet used (Gangadhar *et al*., 2015). A report on *A. pinnata* demonstrated that 25% of commercial fish feed (CFF) can be substituted with *A.pinnata* reducing the overreliance on CFF and production costs (Das *et al*., 2018). (Lumsangkul *et al*., 2022) reported enhanced serum and skin mucus immunities against *S. agalactiae* in Nile tilapia when fed 100g kg^{-1} under bio-floc conditions, demonstrating immune enhancing capabilities of Azolla. (Mosha *et al*., 2020) reported significant average daily gain and specific growth rate, higher total serum proteins, respiratory burst activity, lysozymes/myeloperoxidase activity in GIFT tilapia at 15% inclusion level of azolla in feed.

Azolla as a Suitable Alternative for Human Consumption

Although current literature illustrates the potential and usage of Azolla in animal feed applications, much less is known about direct human consumption. Given its rapid biomass doubling and minimal cultivation requirements, Azolla is a promising candidate that offers a climate-resilient, scalable food alternative in regions of food insecurity and environmental stress. To make this transition from animal feed to human superfood, critical research gaps like toxicology, digestibility, protein bioavailability, sensory acceptability and allergenicity of these protein concentrates/hydrolysates must be addressed. Further research should also explore ways to genetically improve, boosting essential amino acid content and reducing anti-nutritional factors. By integrating Azolla into sustainable food systems, we can address global food insecurities and be used as an efficient protein source in interstellar space travel.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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