Journal of Animal and Feed Sciences, 33, 4, 2024, 542–546, https://doi.org/10.22358/jafs/193033/2024
The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna

Short communication

Evaluation of the biochemical composition of the greater duckweed *Spirodela polyrhiza* (L. Schleiden)

R. Miltko, M.P. Majewska, N. Frączek and B. Kowalik*

The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Department of Animal Nutrition, Instytucka 3, 05-110 Jabłonna, Poland

KEY WORDS: amino acid, chemical composition, minerals, *Spirodela polyrhiza*

Received: 27 August 2024
Revised: 5 September 2024
Accepted: 6 September 2024

* Corresponding author:
e-mail: b.kowalik@ifzz.pl

ABSTRACT. The objective of this study was to evaluate the nutritional value of *Spirodela polyrhiza* (L. Schleiden) (greater duckweed, GD) by analysing its chemical composition, mineral content and amino acid profile under laboratory conditions. GD was cultured in an aquarium illuminated from 04:00 to 24:00, maintaining a water temperature at 25 °C and a pH range of 6.5–7.3. The plants were collected, dried and ground for analysis. The content of crude protein, fat, ash, fibre and starch of GD was (% dry matter): 31.3, 5.7, 17.9, 10.5 and 7.8, respectively. The content of total amino acids, essential amino acids and nonessential amino acids was (g/100 g crude protein): 76.6, 36.5 and 40.1, respectively. The total content of minerals, macronutrients and trace elements in GD biomass was (g/100 g crude ash): 34.3, 34.2 and 0.1, respectively. In summary, our data indicate that *Spirodela polyrhiza* has a high content of crude protein and ash as well as significant amounts of amino acids and minerals. This suggests that GD could potentially be a valuable source of these nutrients for both animals and humans.

Introduction

Soybean oil, rapeseed meal, cereals and maize are among the most commonly utilised sources of protein, fat, and carbohydrates in animal rations. However, the use of these feed concentrates is often associated with high animal production costs as well as deforestation and long transport distances. Duckweed species are gaining increasing attention in livestock nutrition and are considered a potential sustainable source of protein and other essential nutrients. The aquatic family *Lemnaceae* (duckweeds) comprises small floating plants that are widely distributed around the world. Certain duckweed genera such as *Spirodela* sp., *Lemna* sp. and *Wolffia*, are rich in protein, lipids, starch, valuable minerals and other bioactive compounds (Xu et al., 2021). Duckweeds are known for their rapid reproduction in eutrophic waters. For example, in ponds, the production of *Spirodela polyrhiza* (L. Schleiden) can reach 24 t/ha/year on a dry matter (DM) basis (Sharma et al., 2019). Therefore, species of the duckweed genera can potentially be utilised as dietary supplements for humans and domestic animals (Xu et al., 2021). Moreover, duckweeds are used in biological wastewater treatment, biogas and ethanol production, and have prospective applications in the pharmaceutical and cosmetic industries (Hoang and Schubert, 2017; Sońta et al., 2019).

The genus *Spirodela* consists of two species: *S. polyrhiza* and *S. intermedia* (Sree et al., 2020). The greater duckweed (*S. polyrhiza,* GD) is the largest among the 34 described species within the family *Lemnaceae*. Individual fronds of *S. polyrhiza* can reach up to 15 mm in length and 5–10 mm in width, while fronds of the other genera, such as *Lemna, Wolffia, Landoltia* and *Wolffiella* are much smaller, ranging from 5 mm to less than 1 mm in length.

The upper surface of *S. polyrhiza* fronds is medium green, while the lower side is typically reddish-purple and has 5 to 11 veins. Additionally, *S. polyrhiza* develops between 7 and 21 roots (Said et al., 2022).

Previous studies have reported that GD has a high nutritional content, but its chemical composition can vary and the content of essential nutrients depends on the plant's growth conditions. For instance, the protein content (% DM) of this plant can range from 24.0 to 36.0, fat 1.7–7.2, crude fibre 8.8–14.5, crude ash 1.8–20.6, and starch 6.5–24.0 (Appenroth et al., 2017; Ma et al., 2017; Sharma et al., 2019; Xu et al., 2021; Said et al., 2023). *S. polyrhiza* contains all nine essential amino acids (EAA), ten non-essential acids (NEAA) and thirteen free amino acids (AA) (Sharma et al., 2019). According to Appenroth et al. (2017), the concentrations of critical AA in GD are within the recommended range of World Health Organization for human consumption. Moreover, *S. polyrhiza* is a rich source of calcium (Ca), magnesium (Mg), tin (Sn) and nickel (Ni) (Kumar et al., 2022). The reported findings on the chemical composition of *S. polyrhiza* are often inconsistent and incomplete.

We hypothesised that GD could be a potential rich source of nutrients for humans and domestic animals. The aim of the present study was to assess the nutritional value of *S. polyrhiza* by examining its basic chemical composition, mineral content and amino acid profile under controlled laboratory conditions.

Material and methods

The GD used in this study was collected in June 2024 year from the Komornicki Canal (Legionowo Country, Masovian Voivodeship, Poland; 52.47927° N, 20.91978° E). Subsequently, the plants were transferred to the laboratory and thoroughly cleaned of any aquatic fauna, such as snails and insects as well as aquatic flora like hornwort (*Ceratophyllum*) and lesser duckweed (*Lemna minor* L.). The pure *S. polyrhiza* was then transferred to an aquarium. The culture conditions followed the method described by Miltko et al. (2024). The duckweed was harvested once or twice a week, leaving a small number of plants for further propagation. The harvested plants were drained, dried and ground as described by Miltko et al. (2024).

Nutrient concentrations were analysed at The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences (Jabłonna, Poland). The AA profile was determined at The National Laboratory for Feeding stuffs in Lublin (Accreditation Certificate No. AB 856). The mineral contents of GD and water used under laboratory conditions for plant culture were determined by GBA POLAND Sp. z o.o., Łajski (Accreditation Certificate No. AB 1095).

The contents of DM, total nitrogen, crude fat, crude ash, starch, crude fibre, acid detergent fibre (ADF), acid detergent lignin (ADL), neutral detergent fibre (NDF) in GD were determined according to AOAC International (2011).

AA concentration in dry GD samples was analysed using the PB 59 KLP methods as per January 14, 2014 (1st edition); tryptophan concentration was determined according to the Commission Regulation (EC) No. 152/2009 of January 27, 2009, laying down the methods of sampling and analysis for the official control of feed. The profile of AA was performed using ultra-performance liquid chromatography with spectrophotometric detection (UHPLC-UV); high performance liquid chromatography with fluorescence detection (HPLC-FLD) was used to determine the concentration of tryptophan.

Additionally, the concentrations of Ca, K, Mg, Na, Zn, Cu, and total P in dry *S. polyrhiza* were determined using the PB-158/LF method of February 7, 2022 (7th edition). The amount of minerals in GD was analysed through using atomic absorption spectroscopy (ASA), except for P, which was performed photometrically.

Results and discussion

Protein and amino acids

In the present study, the crude protein (CP) content of GD was determined at 31.3% DM (Table 1). This value was similar to the previous report on the same species, which indicated a protein

Table 1. Main composition of greater duckweed (*Spirodela polyrhiza*), % dry matter

Component	Greater duckweed
Dry matter, % FM	6.6
Organic matter	82.1
Crude protein	31.3
Crude fat	5.7
Crude ash	17.9
Crude fibre	10.5
NDF	52.9
ADF	23.1
ADL	8.0
Starch	7.8

FM – fresh matter, NDF – neutral detergent fibre, ADF – acid detergent fibre, ADL – acid detergent lignin content of approximately 30.5% for GD harvested from a pond treated with inorganic fertilizers (Sharma et al., 2019). In contrast, Appenroth et al. (2017) found a lower protein content of approx. 25% in GD cultivated in laboratory settings. It should be stressed that the variability in GD protein content may depend on the cultivation conditions of the plant, including nutrient availability, particularly nitrogen and phosphorus as well as light intensity (Smith, 2014). Appenroth et al. (2017) observed that high light intensity and elevated nitrate levels affected the protein content of different duckweed species, which ranged from 6.8 to 45.0% DM. For instance, the protein content in lesser duckweed (*Lemna minor* L.) and GD collected from outdoor tanks with organic manures, varied between 36.9 to 36.4% DM (Kumar et al., 2022). Conversely, our previous study showed that the protein content of lesser duckweed harvested from natural ponds and laboratory cultivation was significantly lower, reaching 12.1 and 13.0% DM, respectively (Miltko et al., 2024). In addition, Yu et al. (2011) demonstrated that the protein content of GD extracted from fresh, frozen and ambient temperaturedried samples, was 52.1, 44.3 and 45.6% DM, respectively. This suggests that the proportion of protein in GD tissues not only depends on cultivation conditions but also on the form of biological material used for protein extraction.

The AA composition of crude protein in GD is displayed in Table 2. The analysis identified eighteen AA, including ten EAA and eight NEAA. The total AA content in GD was 76.6 g/100 g CP of which: EAA accounted for 36.5 g/100 g CP, while NEAA 40.1 g/100 g CP. The AA profile observed in GD was generally consistent with a previous report on GD grown under laboratory conditions, except for aspartic acid (ASP), valine (VAL), and cysteine (CYS), which were found to be higher in our study, while threonine (THR) and tyrosine (TYR) levels were lower compared to those reported by Appenroth et al. (2017). In contrast, AA levels in GD biomass, determined in the present study, were significantly higher than those recorded for GD cultured with organic manure in tanks (Sharma et al., 2019). According to Pagliuso et al. (2022), the diversity and quantity of GD AA are related to the ecotype of the species. For example, the concentrations of EAA (histidine (HIS), valine (VAL), phenylalanine (PHE), isoleucine (ILE) and lysine (LYS)) were higher in *S. polyrhiza* 7498 compared to *S. polyrhiza* 9509 and 9264. In addition, NEAA (aspartic acid (ASP), glutamic acid (GLU), glycine (GLY) and serine (SER)) levels were found to be higher in *S. polyrhiza* 9346 and 9509 compared to 9264 (Pagliuso et al., 2022).

Table 2. Amino acid composition of crude protein from greater duckweed (*Spirodela polyrhiza*), g/100 g crude protein

Essential amino acids (EAA)		Non-essential acids (NEAA)	
Histidine (HIS)	1.4 ± 0.15	Serine (SER)	4.1 ± 0.71
Arginine (ARG)	4.6 ± 0.65	Glycine (GLY)	4.2 ± 0.30
Threonine (THR)	3.7 ± 0.55	Aspartic acid (ASP)	9.8 ± 1.51
Lysine (LYS)	4.4 ± 0.52	Glutamic acid (GLU)	9.5 ± 1.42
Valine (VAL)	5.7 ± 1.01	Alanine (ALA)	5.2 ± 0.92
Isoleucine (ILE)	3.8 ± 0.54	Proline (PRO)	3.8 ± 0.21
Leucine (LEU)	6.5 ± 0.51	Tyrosine (TYR)	2.4 ± 0.19
Phenylalanine (PHE)	3.9 ± 0.42	Cysteine (CYS)	1.11 ± 0.12
Methionine (MET)	1.4 ± 0.21		
Tryptophan (TRP)	1.1 ± 0.11		
Total EAA	36.5 ± 0.47	Total NEAA	40.1 ± 0.67

Fat

The crude fat content in GD was determined at 5.7% DM (Table 1). Pagliuso et al. (2022) reported that the fat content in *Spirodela* varied widely when cultured under laboratory conditions, ranging from 0.2 to 8% DM. The lipid content of *S. polyrhiza* was 2.0% lower in the present study compared to a previous study by Kumar et al. (2022). However, it should be noted that GD in the latter study was cultured in outdoor tanks with organic fertilisation. Other research has documented varying crude fat contents for different duckweed species: 1.05% DM for *Lemna punctata* and *S. polyrhiza*, 6.5% DM for *Wolffiella hyalina*, and up to 14.0% DM for *Wolffia arrhizal* 8853 and *Wolffia borealis* 9144 (Tang et al., 2015; Appenroth et al., 2017). Chakrabarti et al. (2018) have suggested that differences in fat concentrations between individual duckweed species, including GD, are influenced by growing and climatic conditions as well as nutrient availability.

Ash and minerals

The crude ash (CA) content of GD in the present study was 17.9% DM (Table 1). This result was consistent with previous work, which reported ash levels in *S. polyrhiza* collected from ponds ranging from 18.5 to 20.6% DM (Sharma et al, 2019). GD contains higher ash content compared to other duckweed species, such as *L. minor* (11.5 to 14.1% DM) (Miltko et al., 2024) and *L. obscura* (5.6% DM) (Demann et al., 2023). This elevated ash content makes GD a more valuable source of nutrients and a potentially valuable feed ingredient for livestock.

Table 3. Macro- and micronutrients (g/100 g crude ash) in greater duckweed (*Spirodela polyrhiza*)

Macronutrients		Micronutrients	
Calcium	16.3 ± 0.20	Copper	0.02 ± 0.01
Potassium	5.6 ± 0.20	Zinc	0.08 ± 0.01
Magnesium	3.8 ± 0.36		
Sodium	4.9 ± 0.23		
Phosphorus	3.6 ± 0.07		
Total macronutrients	34.2 ± 0.21	Total micronutrients	0.10 ± 0.01

The mineral composition of CA in GD is detailed in Table 3. The analysis identified seven minerals, including five macronutrients and two trace elements. Macro- and micronutrients concentrations were 34.2 and 0.1 g/100 g CA, respectively. Noteworthy is the high GD content of Ca K, Na and Mg, amounting to 16.3, 5.6, 4.9 and 3.8 g/100 g CA, respectively. Meanwhile, Kumar et al. (2022) showed that the highest levels of these macronutrients were most abundant in *Pistia stratiotes, Wolffia globsa* and *S. polyrhiza*. Duckweed species, including *S. polyrhiza*, utilise their natural ability as aquatic macrophytes to absorb nutrients and minerals from environment, including wastewater, acting as phytoremediators. This process converts these nutrients into biomass that can serve as an alternative animal feed source. Similarly, Appenroth et al. (2017) observed that the mineral profile of aquatic plants was affected by the culture medium.

Carbohydrates

Spirodela polyrhiza, along with other duckweed species, contain a variety of carbohydrates such as starch, crude fibre and its detergent fractions. In the present study, the starch content was determined at 7.8% DM (Table 1). This observation is in line with the results of Appenroth et al. (2017), who reported a similar starch content of approximately 7.0% DM. On the other hand, higher levels of starch have been recorded by Ma et al. (2017) and Xu et al. (2021), with values reaching 11.0 and 23.3% DM, respectively. According to Appenroth et al. (2017), the starch content is significantly lower than the protein content in duckweeds grown under optimal conditions, which is consistent with our results. Moreover, the starch content in duckweed species tend to increase when phosphate or nitrate levels are low, as well as in the presence of certain heavy metals (Zhao et al., 2015). In contrast, Said et al. (2022) estimated that the percentage of nitrogen free extract (NFE), including starch, in *S. polyrhiza* was 58.0% DM. According to these authors, duckweed with a high number of roots often has a higher NFE level. GD, being the duckweed with the highest number of roots and largest fronds, is capable of storing substantial amounts of nutrients, including carbohydrates. On the other hand, reduced nutrient availability and low light intensity, along with increased GD population density, may lead to smaller leaf size and fewer roots.

In the present study, the crude fibre content in GD amounted to 10.5% DM (Table 1), which was consistent with findings from Demann et al. (2023). On the contrary, higher crude fibre contents were previously observed in *S. polyrhiza* and 11 other species of the genus *Wolffia* (14.5 and 26.2% DM, respectively) (Appenroth et al., 2018; Said et al., 2022). According to the latter authors, the high dietary fibre content of duckweed can improve Western diets by incorporating low-energy food ingredients, which may contribute to the prevention of lifestyle diseases. Duckweed grown under optimal conditions and harvested regularly have a crude fibre content in the range of 5–15% DM, depending on the species.

Conclusions

In summary, our data show that *Spirodela polyrhiza* contains a high content of crude protein and ash. Greater duckweed appears to be a good source of amino acids, as its composition of essential amino acids surpasses most plant proteins and is more similar to animal protein profile. The contents of macronutrients, especially calcium, potassium and sodium, is comparable to that of cereal grains. Consequently, *S. polyrhiza* shows potential as an alternative natural feed source for aquaculture and livestock and may also be considered for use as a food product for human consumption.

Conflict of interest

The Authors declare that there is no conflict of interest.

References

AOAC International, 2011. Official methods of analysis association of AOAC International. 18th edition. Arlington, VA (USA)

Appenroth K-J., Sowjanya Sree K., Böhmc V., Hammann S., Vetterd W., Leiterere M., Jahreis G., 2017. Nutritional value of duckweeds (Lemnaceae) as human food. Food Chem. 217, 266–273, https://doi.org/10.1016/j.foodchem.2016.08.116

Appenroth K.-J., Sree K.S., Bog M. et al., 2018. Nutritional value of the duckweed species of the genus *Wolffia* (Lemnaceae) as human. Food. Front. Chem. 6, 483, https://doi.org/10.3389/fchem.2018.00483

Chakrabarti R., Clark W.D., Sharma J.G., Goswami R.K., Shrivastav A.K., Tocher D.R., 2018. Mass production of *Lemna minor* and its amino acid and fatty acid profiles. Front. Chem. 6, 479, https://doi.org/10.3389/fchem.2018.00479

Demann J., Petersen F., Dusel G., Bog M., Devlamynck R., Ulbrich A., Olfs H.-W., Westendarp H., 2023. Nutritional value of duckweed as protein feed for broiler chickens – digestibility of crude protein, amino acids and phosphorus. Animals. 13, 130, https://doi.org/10.3390/ani13010130

EC (European Commission), 2009. Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed, http://data.europa.eu/eli/reg/2009/152/oj

Hoang P.T.N., Schubert I., 2017. Reconstruction of chromosome rearrangements between the two most ancestral duckweed species *Spirodela polyrhiza* and *S. intermedia.* Chromosoma 126, 729–739, https://doi.org/10.1007/s00412-017-0636-7

Kumar G., Sharma J.G., Goswami R.K., Shrivastav A.K.., Tocher D.R., Kumar N., Chakrabarti R., 2022. Freshwater macrophytes: a potential source of minerals and fatty acids for fish, poultry, and livestock. Front. Nutr. 9, 869425, https://doi.org/10.3389/fnut.2022.869425

Ma Y.B., Zhu M., Yu C.J., Wang Y., Liu Y., Li M.L., Sun Y.D., Zhao J.S., Zhou G.K., 2017. Large-scale screening and characterisation of *Lemna aequinoctialis* and *Spirodela polyrhiza* strains for starch production. Plant Biol. 20, 357–364, https://doi.org/10.1111/plb.12679

Miltko R., Majewska M.P., Wojtak W., Białek M., Kowalik B., Czauderna M., 2024. Comparing the chemical composition of lesser duckweed (*Lemna minor* L.) grown in natural and laboratory settings. J. Anim. Feed Sci. 33, 357–367, https://doi.org/10.22358/jafs/189963/2024

Pagliuso D., Grandis A., Fortirer J.S., Camargo P., Floh E.I.S., Buckeridge M.S., 2022. Duckweeds as promising food feedstocks globally. Agronomy, 12, 796, https://doi.org/10.3390/agronomy12040796

Said D.S., Chrismadha T., Mayasari N., Widiyanto T., Ramandita A., 2022. Nutritional content and growth ability of duckweed *Spirodela polyrhiza* on various culture media. IOP Conf. Ser.: Earth Environ. Sci. 1062, 012009, https://doi.org/10.1088/1755-1315/1062/1/012009

Sharma JaiGopal, Clarkb W.D., Kumar S.A., Kumar G.R., Tocherb D.R., Chakrabarti R., 2019. Production potential of greater duckweed *Spirodela polyrhiza* (L. Schleiden) and its biochemical composition evaluation. Aquaculture 513, 734419, https://doi.org/10.1016/j.aquaculture.2019.734419

Smith S.D.P., 2014. The roles of nitrogen and phosphorus in regulating the dominance of floating and submerged aquatic plants in a field mesocosm experiment. Aquat. Bot. 112, 1–9, https://doi.org/10.1016/j.aquabot.2013.07.001

Sońta M., Rekiel M., Batorska M., 2019. Use of duckweed (*Lemna* L.) in sustainable livestock production and aquaculture – a review. Ann. Anim. Sci. 19, 257–271, https://doi.org/10.2478/aoas-2018-0048

Sree K.S., Appenroth K.J., 2020. Worldwide Genetic Resources of Duckweed: Stock Collections. In: The Duckweed Genomes. Compendium of Plant Genomes. Cao X., Fourounjian P., Wang W., Editors; Springer: Cham; Switzerland AG; pp. 39–46, https://doi.org/10.1007/978-3-030-11045-1_3

Tang J., Li Y., Ma J., Cheng J.J., 2015. Survey of duckweed diversity in Lake Chaoand total fatty acid, triacylglycerol, profiles of representative strains. Plant Biol. 17, 1066–1072, https://doi.org/10.1111/plb.12345

Xu J., Shen Y., Zheng Y., Smith G., Sun X.S., Wang D., Zhao Y., Zhang W., Li Y. 2021. Duckweed (*Lemnaceae*) for potentially nutritious human food: A review. Food Rev. Int. 39, 3620–3634, https://doi.org/10.1080/87559129.2021.2012800

Yu G., Liu H., Venkateshan K., Yan S., Cheng J., Sun X.S., Wang D., 2011. Functional, physiochemical, and rheological properties of duckweed (*Spirodela polyrhiza*) protein. Transactions of the ASABE. 54, 555–561, https://doi.org/10.13031/2013.36459

Zhao Z., Shi H.J., Wang M.L., Cui L., Zhao H., Yun Z., 2015. Effect of nitrogen and phosphorus deficiency on transcriptional regulation of genes encoding key enzymes of starch metabolism in duckweed (*Landoltia punctata*). Plant Physiol. Biochem. 86, 72–81, https://doi.org/10.1016/j.plaphy.2014.11.007