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# Comparing the chemical composition of lesser duckweed (*Lemna minor* L.) grown in natural and laboratory settings

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**ABSTRACT.** The aim of this study was to compare the chemical composition of lesser duckweed (*Lemna minor* L.) obtained from the natural environment and cultured under laboratory conditions. In the first variant, lesser duckweed was collected from a natural pond (LDN). Subsequently, the plants were cleaned of other aquatic flora and fauna, dried, and ground. In the second variant, lesser duckweed was cultured in an aquarium (LDC) illuminated from 04:00 to 24:00. The water temperature was 25 °C and the pH was maintained in the range of 6.5–7.3. The content of crude protein, fat, and fibre was similar in LDN and LDC, but the proportion of crude ash was higher in LDC. The content of total amino acids was 95.70 and 68.71 g/100 g crude protein in LDC and LDN, respectively. The concentrations of essential amino acids and nonessential amino acids were 43.75 and 51.96 g/100 g crude protein in LDC, respectively, and 32.10 and 36.61 g/100 g crude protein in LDN, respectively. Palmitic and stearic acids were found in higher quantities in LDN than LDC, whereas the oleic acid content was three-fold higher in LDN compared to LDC. Moreover, linoleic and α-linolenic acid concentrations were higher in LDN than LDC. Mineral analysis revealed elevated levels of Ca, Na, and Zn, while P and Mg levels were lower in LDC. Additionally, the levels of δ-, γ-tocopherols and α-tocotrienol were found to be higher in LDC, while δ- and γ-tocotrienols in LDC were below detectable limits. So, the significant influence of growth conditions on the nutrient composition of *L. minor* was shown. Optimizing growth conditions is pivotal for enhancing lesser duckweed production. It seems that *L. minor* can be a valuable source of essential nutrients and could serve as a supplementary food source for both domestic animals and humans. Nonetheless, anti-nutritional components, including toxic metals, should be monitored.

## Introduction

Insect larvae, algae, and duckweed are promising alternatives to traditional livestock feeds such as soybean and rapeseed meal, fish meal, or oilseeds due to their high protein or fat contents, along with a high abundance of vitamins, minerals and bioactive components (Xu et al., 2021; Boccardo et al., 2022; Zamri et al., 2023). Duckweeds (*Lemna* L., family *Lamnaceae*) are not only consumed by animals, but also by humans in Laos, Thailand, Myanmar and South-Asian countries like India, Pakistan, Bangladesh, where food is rich in carbohydrates but poor in protein (Appenroth et al., 2017). However, in the USA and Europe, duckweed is generally considered an aquatic weed. Nevertheless, its applications span diverse fields, including biological wastewater treatment (e.g. type of Lemna Co.), biogas and ethanol production, biotechnological production of bioactive compounds, ecotoxicology and lastly folk medicine, where it is valued for its diuretic, carminative, diaphoretic, and cholagogic properties

(Czerpak and Piotrowska, 2005; Verma and Suthar, 2014; Sońta et al. 2020). Numerous studies have explored the use of duckweed meal in livestock diets, particularly in poultry and growing pigs, with fewer studies focusing on ruminants (Hugue et al., 1996; Mwale and Gwaze, 2013; Gwaze and Mwale, 2015).

Duckweed species are small and floating aquatic plants that form dense mats on or just below the surface of water. These species have a cosmopolitan range and are adapted to a wide variety of geographic regions and climatic zones. The family *Lamnaceae* includes 4 genera: *Lemna*, *Spirodela*, *Wolffia*, and Wolfiella. The genus *Lemna* comprises 38 species, of which 5 occur in Poland: lesser duckweed syn. common duckweed (*L. minor* L.), least duckweed (*L. minuta* Kunth), gibbous duckweed (*L. gibba* L.), star duckweed (*L. trisulca* L.) and turion duckweed (*L. turionifera* Landolt). Duckweed species thrive in eutrophic waters, sheltered from wind and wave action, in temperatures ranging from 6 to 33 °C. Under optimal conditions of nutrient availability, water temperature, pH, and sunlight, duckweeds can double their mass within 16 h to 2 days. Consequently, their yield production can be very high, reaching 10–20 tons of dry matter (DM) per ha/year (Leng et al., 1995). According to Pagliuso et al. (2022) and Appenroth et al. (2017), duckweed species typically contain approximately (% DM) 20–35 protein, 4–7 fat, 44–54 fibre, and 14–20 ash. Duckweed species contain all nine essential amino acids (EAA) and are a rich source of many macro- and micronutrients, including Ca, P, Na, K, Mg, Fe, Mn, Cu, and Zn (Pagliuso et al. 2022). Appenroth et al. (2017) analysed the fatty acid profile of 6 duckweed species, revealing a high content of polyunsaturated fatty acids (PUFA) and lower levels of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). Duckweed also contains a diverse range of antioxidants, especially carotenoids and tocopherols, which are associated with reduced risk of many chronic diseases (Appenroth et al. 2017). On the other hand, duckweed can accumulate toxic metals, e.g. Cd, Cr, Pb, and others, as well as compounds such as oxalic acid or calcium oxalate crystals from the aquatic environment, potentially limiting its suitability as a food or feed ingredient. According to a report of the Food and Agriculture Organization of the United Nations (FAO, 1999), the levels of toxic metals in duckweed are generally low and pose no significant threat to human and animal health. However, it should be noted that this assessment depends largely on the concentration of these substances in the water.

The lesser duckweed is the most prevalent species in Poland, characterized by three or four combined, obovate, dark green fronds measuring 1.9–4.5 mm in length and 1.3–3.3 mm in width, each having three veins, and a 2-cm-long root (Ceschin et al., 2016).

We hypothesized that different growth conditions of the lesser duckweed (*Lemna minor* L.) could affect the chemical composition of its biomass.

The objective of this study was to compare the chemical composition, focusing on amino acid and long chain fatty acid profiles, as well as mineral elements, of the lesser duckweed cultured in both natural and laboratory environments.

## Material and methods

### Collection of lesser duckweed in the natural environment (LDN)

The lesser duckweed was collected from a pond located in the forest near Serock (Legionowo County, Masovian Voivodeship, Poland; 52.53751° N, 20.96155° E), away from traffic routes. The pond was surrounded by trees such as black alder (*Alnus glutinosa* L.), silver birch (*Betula pendula* Roth), red oak (*Quercus rubra* L.), alder buckthorn (*Frangula alnus* Mill.), and bird cherry (*Padus avium* Mill.). The following species were identified in the pond: mollusks such as great ramshorn snail (*Planorbarius corneus*), great pond snail (*Lymnaea stagnalis*), amphibians such as frogs (*Rana*) and newts (*Triturus*), as well as aquatic insects like water bugs (*Heteroptera*), beetles (*Coleoptera*), mayflies (Ephemeroptera), dragonflies (*Odonata*), caddisflies (*Trichoptera*). Representatives of the marsh and shoreline flora included the following genera: mint (*Mentha*), speedwell (*Veronica* L.), loosestrife (*Lysimachia* L.), rushes (*Juncus* L.), sedges (*Carex* L.), bulrush (*Schoenoplectus* Rchb.), willowherb (*Epilobium* L.), and yellow iris (*Iris pseudacorus* L.). The underwater flora was represented, among others, by the following genera: soakwort (*Elodea* Michx.), hornwort (*Ceratophyllum* L.), and water violet (*Hottonia palustris* L.). The lesser duckweed was collected manually using nets twice a month from June to mid-August (on the 1st and 15th of each month) and pre-cleaned of any remaining fauna and flora of the pond. The summer period was selected due to the most intensive growth of plants. During harvesting, the pH of the water was measured, and it ranged from 6.9 to 7.10 ± 0.15. Next, further cleaning was carried out on sieves under running water. After this procedure,

the duckweed was left to drain and transferred to the laboratory for drying at a temperature of 55–65 °C; subsequently, the drying coefficient was calculated. After drying, the duckweed was again cleaned, mainly from small shells and stems of other plants. After drying, the material was weighed, ground (ø – 1 mm), transferred to tightly sealed plastic bags, and stored at room temperature until further analysis.

## Collection of lesser duckweed in laboratory conditions (LDC)

The lesser duckweed for aquarium culture was harvested from the pond (52.53751° N, 20.96155° E) in July. Subsequently, it was transferred to the laboratory and thoroughly cleaned of any other fauna and flora. In the laboratory, the plants were acclimatized and propagated under aquarium culture conditions with bottom sediment substrate. The lesser duckweed was cultivated in an aquarium measuring 120 × 40 × 30 cm. The plants were illuminated from 04:00 to 24:00 using two fluorescent lamps: the first one (T8 AQUA GLO, HAGEN®, Germany) provided light intensity within the range of chlorophyll a and b activity, with a light intensity of 120 lx, and a colour temperature of 18 000 K; the second lamp (T8 SUN-GLO, HAGEN®, Germany) emitted warm white light similar to daylight, with a light intensity of 210 lx and a colour temperature of 4 200 K. Water in the aquarium was purified using an internal sponge filter with flow rate control (Pat-Mini Filter 107715, Aquael, Poland), allowing for gentle water movement. The filter was also equipped with an aeration tube connected to the outlet nozzle, allowing for effective and efficient oxygenation of the water in the tank. Once the culture conditions had stabilized, a chemical analysis of the water was carried out (Table 1). The water temperature was maintained at 25 °C, and the pH of the water kept within the range of 6.5–7.3 ± 0.20. The total hardness was 300 ± 60 mg/l $CaCO_3$. Twice a week, a multi-nutrient fertilizer, FLOURISH (SACHEM Laboratories INC, USA), was applied at a dose of 3 ml, following the manufacturer's recommendations (Table 2). The duckweed was harvested once a week, leaving a small amount of plants for further propagation. The collected plants were drained, dried, and ground, similar to the collection from the pond.

**Table 1.** Chemical analysis of water used in breeding lesser duckweed (*L. minor* L.) in laboratory conditions

| Item | Value | Reference value* |
|---|---|---|
| Ammonia, mg/l | <0.13 | ≤0.50 |
| Nitrates, mg/l | 3.3 ± 0.50 | ≤50.0 |
| Nitrites, mg/l | <0.066 | ≤0.50 |
| Aluminium, µg/l | <10.0 | ≤200 |
| Cadmium (µg/l) | <0.50 | ≤5.0 |
| Copper, mg/l | 0.024 ± 0.005 | ≤2.0 |
| Lead, µg/l | 1.4 ± 0.20 | ≤10.0 |
| Manganese, µg/l | 0.69 ± 0.14 | ≤50.0 |
| Mercury, µg/l | <0.10 | ≤1.0 |
| Sodium, mg/l | 27.0 ± 4.0 | ≤200 |

*The Minister of Health's regulation (Law Journal, 2017.2294)

**Table 2.** Composition of multicomponent fertilizer, %

| Ingredients | Contents |
|---|---|
| Total nitrogen | 0.07 |
| Available phosphate | 0.01 |
| Soluble potash | 0.037 |
| Calcium | 0.14 |
| Soluble magnesium | 0.10 |
| Combined sulphur | 0.27 |
| Boron | 0.008 |
| Cobalt | 0.0004 |
| Soluble copper | 0.0001 |
| Soluble iron | 0.32 |
| Soluble manganese | 0.0118 |
| Molybdenum | 0.0009 |
| Soluble zinc | 0.0007 |

sources of mineral ingredients: potassium chloride, calcium chloride, copper sulphate, magnesium chloride, ferrous gluconate, cobalt sulphate, magnesium sulphate, manganese sulphate, boric acid, sodium molybdate, zinc sulphate, yeast protein hydrolysate

## Chemical analysis of lesser duckweed

The concentrations of nutrients, fatty acids (FA), tocopherols and tocotrienols were analysed at The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences (Jabłonna, Poland). Amino acid concentrations were determined at The National Laboratory for Feedingstuffs in Lublin (Accreditation certificate of testing laboratory No. AB 856). The mineral content of duckweed and water used in laboratory conditions for duckweed culture were analysed by GBA POLAND Sp. z o.o., Łajski, a member of the GBA Group (Accreditation for testing laboratory No. AB 1095; Certificate of defence and security accreditation No. 37/MON/2021; Certificate AQAP 2110:2016; GMP+ Int. Registration No. GMP050609). The number of replications of the determinations was at least three. If the results of the repetitions of the determinations differed from each other above 10%, subsequent repetitions were made.

## Nutrient analysis

The contents of DM (934.01), total nitrogen (978.04), crude fat (CT) (930.09), crude ash (CA) (930.05), and crude fibre (978.10) in lesser duckweed were analysed according to AOAC International

(2011) standards. Neutral detergent fibre (NDF) in plants was assayed using a heat stable amylase and expressed excluding residual ash, following the method described by Mertens (2002). Acid detergent fibre (ADF) (973.18) in lesser duckweed was expressed excluding residual ash and acid detergent lignin (ADL) (973.18), analysed according to AOAC International (2011). The non-fibrous carbohydrate (NFC) content was calculated using the following formula (%):

NFC = 100 – (NDF + CP + CT + CA) (NRC, 2001).

Haemicellulose (HEM) and cellulose (CEL) contents were calculated as follows:

(%) NDF – ADF and ADF – ADL, respectively.

### Fatty acid analysis

FA present in dry duckweed samples were derivatized by the base- and acid-catalysed methylations, and subsequently quantified using capillary gas chromatography coupled with mass spectrometry (GC-MS) on a Shimadzu GC-MS-QP2010 Plus EI chromatograph (Tokyo, Japan) equipped with a BPX70 fused silica column (120 m × 0.25 mm id × 0.25 µm film thickness; Phenomenex, Torrance, CA, USA), a quadrupole mass selective detector (Model 5973N; Agilent Technologies, Santa Clara, CA, USA), and an injection port, with helium as the carrier gas, according to Białek et al. (2018). The total FA profile was determined as methyl esters (FAME) with nonadecanoic acid (C19:0) as the internal standard. FAME identification was validated based on electron impact ionization spectra of FAME and compared to authentic FAME standards (Sigma, St. Louis, MO, USA) and the NIST 2007 reference mass spectra library (National Institute of Standard and Technology, Gaithersburg, MD, USA). All FAME analyses were based on total ion current chromatograms and/or selected ion monitoring chromatograms.

### Tocopherol and tocotrienol analyses

Dry duckweed samples underwent alkaline saponification by adding ascorbic acid and a freshly prepared saponification reagent to the tube (Czauderna and Kowalczyk, 2007). The resulting mixture was flushed with a stream of argon, and subsequently placed in a shaking water bath at 80 °C. Afterward, the mixture was cooled to approximately 20 °C. Hexane and distilled water were added to the cooled mixture, followed by vortexing of the tube to ensure thorough mixing. The upper hexane layer was then collected to a vial. Organic solvents were then removed under a stream of argon gas. The vials containing the samples were stored at −20 °C until analysis (Czauderna et al., 2024).

Concentrations of α, δ, and γ forms of tocopherols and tocotrienols in duckweed samples were analyzed using reversed-phase (C18) liquid chromatography (UPLC) (SHIMADZU, Tokyo, Japan) (Czauderna et al., 2024). The chromatographic setup consisted of an ultra-fast liquid chromatograph (UFLC), with two LC-20ADXP pumps (UFLCXR), a SIL-20ACXR autosampler (LFLCXR), a CBM-20A communication bus module, a CTO-20A column oven, a DGU-20A5 degasser, a SPD photodiode array detector (DAD), and a fluorescence detector. Separation and quantification of all forms of tocopherols and tocotrienols in samples were achieved using a Kinetex C18 column (1.7 µm, 150 mm × 2.1 mm, id, 100 Å; Phenomenex, Torrance, CA, USA) fitted with a 4 mm × 2 mm id pre-column (Phenomenex). All separations were performed at a column temperature of 40 °C, while the ambient temperature was 21–24 °C.

### Amino acid and mineral analysis

The concentration of amino acids in dry duckweed samples was analyzed using ultra-performance liquid chromatography with spectrophotometric detection (UHPLC-UV), according to PB 59 KLP methods of January 14, 2014 (1st edition); tryptophan (TRP) was detected using high performance liquid chromatography with fluorescence detection (HPLC-FLD), as per 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 concentration of Ca, K, Mg, Na, Zn, Cu, and total P in dry duckweed samples were analyzed according to the PB-158/LF method of February 7, 2022 (7th edition). The PN-EN 15763:2010 method was used to determine the Cd and Pb contents in the samples. The concentration of minerals in duckweed samples was analyzed using atomic absorption spectroscopy, except for phosphorus, which was determined photometrically.

## Results

The contents of main nutrients, CA, NFC, and CEL were very high, but NDF, ADL, and HEM levels were lower in LDC compared to LDN (Table 3). On the other hand, the crude protein, fat and fibre contents were similar in LDN and LDC.

The amino acids composition of crude protein is presented in Table 4. Generally, the content of the 18 amino acids tested was found to be higher in LDC in comparison to LDN. Specifically, the total

**Table 3.** Main composition of lesser duckweed (*Lemna minor* L.), % dry matter (DM)

| Component | LDN | LDC |
|---|---|---|
| DM, % fresh matter | 9.81 ± 0.14 | 7.92 ± 0.04 |
| Organic matter | 88.45 ± 0.77 | 85.93 ± 2.62 |
| Crude protein | 12.12 ± 1.05 | 13.00 ± 0.92 |
| Crude fat | 2.39 ± 0.49 | 2.87 ± 0.49 |
| Crude ash | 11.55 ± 0.77 | 14.07 ± 2.62 |
| Crude fibre | 13.50 ± 1.10 | 13.59 ± 0.25 |
| NDF | 61.74 ± 1.92 | 46.86 ± 0.59 |
| ADF | 23.11 ± 0.87 | 24.57 ± 0.86 |
| ADL | 14.18 ± 0.51 | 10.38 ± 2.10 |
| NFC | 23.12 ± 2.73 | 32.49 ± 5.35 |
| HEM | 38.64 ± 1.26 | 22.29 ± 1.06 |
| CEL | 8.92 ± 1.15 | 14.20 ± 1.60 |

NDF – neutral detergent fibre, ADF – acid detergent fibre, ADL – acid detergent lignin, NFC – non-fibrous carbohydrate, HEM – haemicellulose, CEL – cellulose; LDN – lesser duckweed in the natural environment, LDC – lesser duckweed in the laboratory conditions

**Table 4.** Amino acid composition of crude protein from lesser duckweed (*Lemna minor* L.), g/100 g crude protein

| Amino acids | LDN | LDC |
|---|---|---|
| Histidine* | 1.18 ± 0.19 | 1.69 ± 0.29 |
| Serine | 3.84 ± 0.61 | 5.17 ± 0.87 |
| Arginine* | 3.94 ± 0.62 | 5.04 ± 0.85 |
| Glycine | 3.90 ± 0.39 | 4.58 ± 0.48 |
| Aspartic acid | 7.90 ± 1.25 | 14.00 ± 2.37 |
| Glutamic acid | 9.41 ± 1.48 | 12.54 ± 2.12 |
| Threonine* | 3.42 ± 0.54 | 4.59 ± 0.78 |
| Alanine | 4.88 ± 0.83 | 6.67 ± 1.21 |
| Proline | 3.47 ± 0.38 | 4.83 ± 0.56 |
| Lysine* | 3.99 ± 0.43 | 5.59 ± 0.65 |
| Tyrosine | 2.09 ± 0.35 | 2.68 ± 0.49 |
| Valine* | 4.40 ± 0.59 | 6.20 ± 0.89 |
| Isoleucine* | 3.33 ± 0.48 | 4.53 ± 0.71 |
| Leucine* | 5.78 ± 0.77 | 7.72 ± 1.14 |
| Phenylalanine* | 3.34 ± 0.53 | 4.74 ± 0.80 |
| Cysteine | 1.12 ± 0.18 | 1.49 ± 0.25 |
| Methionine* | 1.37 ± 0.23 | 1.78 ± 0.32 |
| Tryptophan* | 1.35 ± 0.21 | 1.87 ± 0.32 |

*essential amino acids; LDN – lesser duckweed in the natural environment, LDC – lesser duckweed in the laboratory conditions

amino acid content was 95.70 and 68.71 g/100 g crude protein in LDC and LDN, respectively. The concentration of essential amino acids (EEA) and nonessential amino acids (NEAA) in LDC were 43.75 and 51.96 g/100 g crude protein, respectively. The EEA and NEAA contents in LDN were 32.10 and 36.61 g/100 g crude protein, respectively. The quantity of arginine, lysine (LYS), valine (VAL), leucine (LEU), serine, aspartic acid, glutamic acid, and alanine amino acids was higher in LDC.

The distribution of FA in CT in lesser duckweed is shown in Table 5. Palmitic (C16:0) and

**Table 5.** The fatty acid composition of lesser duckweed (*Lemna minor* L.), g FAME/100 g crude fat*

| Fatty cids | LDN | LDC |
|---|---|---|
| SFA | | |
| C8:0 | 0.136 ± 0.017 | 0.027 ± 0.004 |
| C9:0 | nd | 0.044 ± 0.025 |
| C10:0 | 0.266 ± 0.024 | 0.062 ± 0.008 |
| C12:0 | 0.210 ± 0.016 | 0.183 ± 0.011 |
| C14:0 | 1.343 ± 0.042 | 0.854 ± 0.001 |
| C15:0 | 0.087 ± 0.029 | 0.277 ± 0.006 |
| C16:0 (PL) | 12.244 ± 0.546 | 4.800 ± 0.018 |
| C17:0 | 0.029 ± 0.007 | 0.064 ± 0.007 |
| C18:0 | 4.741 ± 0.036 | 2.970 ± 0.025 |
| C20:0 | 0.044 ± 0.010 | 0.085 ± 0.015 |
| C22:0 | nd | 0.028 ± 0.007 |
| C23:0 | nd | 0.063 ± 0.004 |
| C24:0 | 0.065 ± 0.017 | 0.110 ± 0.002 |
| ΣSFA | 19.165 ± 0.544 | 9.567 ± 0.027 |
| MUFA | | |
| C14:1 c7 | 0.084 ± 0.014 | 0.245 ± 0.012 |
| C14:1 c9 | nd | 0.208 ± 0.001 |
| C15:1 c7 | nd | 0.054 ± 0.008 |
| C15:1 c10 | 0.079 ± 0.027 | 0.084 ± 0.010 |
| C16:1 c7 | 0.172 ± 0.028 | 0.049 ± 0.009 |
| C16:1 c9 | 0.170 ± 0.009 | 0.079 ± 0.002 |
| C16:1 c10 | 0.565 ± 0.005 | 0.134 ± 0.007 |
| C18:1 c9 (OL) | 5.792 ± 0.040 | 1.755 ± 0.002 |
| C18:1 c11 | 0.178 ± 0.013 | 0.080 ± 0.011 |
| ΣMUFA | 7.039 ± 0.033 | 2.688 ± 0.015 |
| PUFA | | |
| C16:2 c9,c12 | 1.203 ± 0.042 | 1.479 ± 0.067 |
| C18:2 c9,c12 (LA) | 11.463 ± 0.234 | 2.983 ± 0.100 |
| C18:3 c9,c12,c15 (LNA) | 15.031 ± 0.052 | 1.576 ± 0.002 |
| C20:2 c11,c14 | 0.019 ± 0.006 | 0.046 ± 0.003 |
| ΣPUFA | 27.716 ± 0.180 | 6.084 ± 0.142 |
| ΣPUFA n-6 | 12.684 ± 0.232 | 4.508 ± 0.143 |
| ΣPUFA n-3 | 15.031 ± 0.052 | 1.576 ± 0.002 |
| n-6/n-3 | 0.844 ± 0.018 | 2.861 ± 0.094 |
| ΣPUFA/ΣSFA | 1.447 ± 0.032 | 0.636 ± 0.017 |

FAME – fatty acids as corresponding fatty acid methyl esters; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids, ΣPUFA n-6 – C16:2 c9,c12; C18:2 c9,c12; C20:2 c11,c14; ΣPUFA n-3 – C18:3 c9,c12,c15; 3 n-6/n-3 – the ratio of n-6 PUFA to n-3 PUFA; LDN – lesser duckweed in the natural environment, LDC – lesser duckweed in the laboratory conditions; nd – not detectable

stearic acids (C18:0) were the most abundant SFA, with significantly higher levels observed in LDN compared to LDC. The total SFA content was two-fold higher in LDN compared to LDC. Oleic acid (C18:1 c9) dominated monounsaturated fatty acids (MUFA), with its concentration being three-fold higher in LDN than in LDC.

The total MUFA content was also higher in LDN compared to LDC. Linoleic acid (C18:2 c9, c12) and α-linolenic acid (C18:3 c9, c12, c15) were the main PUFA, with higher concentrations observed in LDN compared to LDC. As a consequence, the n-6/n-3 ratio of fatty acids was 3.4-fold higher in LDC than in LDN.

Among macroelements, very high levels of Ca and Na, and low of P and Mg were detected in lesser duckweed cultured under laboratory conditions (Table 6). Moreover, CA from LDC contained 3.3 times more Zn compared to LDN. Additionally, in terms of toxic heavy metals, Cd levels were higher, while that of Pb were lower in LDC compared to LDN.

**Table 6.** Macro and trace elements (g/100 g crude ash), and heavy metals (mg/100 g crude ash) in lesser duckweed (*Lemna minor* L.)

| Item | LDN | LDC |
|---|---|---|
| Macro elements | | |
| calcium | 14.84 ± 0.213 | 22.64 ± 0.134 |
| potassium | 8.66 ± 0.169 | 7.11 ± 0.192 |
| magnesium | 5.12 ± 0.173 | 3.24 ± 0.236 |
| sodium | 2.25 ± 0.092 | 4.32 ± 0.195 |
| phosphorus | 6.48 ± 0.083 | 1.47 ± 0.108 |
| Trace elements | | |
| copper | 0.001 ± 0.000 | 0.013 ± 0.004 |
| zinc | 0.029 ± 0.009 | 0.097 ± 0.011 |
| Total mineral elements | 37.378 ± 0.098 | 38.895 ± 0.189 |
| Heavy metals | | |
| cadmium | 0.029 ± 0.002 | 0.603 ± 0.010 |
| lead | 0.339 ± 0.021 | 0.188 ± 0.003 |
| Total heavy metals | 0.368 ± 0.017 | 0.791 ± 0.014 |

LDN – lesser duckweed in the natural environment, LDC – lesser duckweed in the laboratory conditions

In this study, the concentrations of δ- and γ-tocopherols, and α-tocotrienol were higher in the LDC lesser duckweed than LDN (Table 7). The δ- and γ-tocotrienols content of the LDN duckweed reached 0.550 and 1.874 µg/100 g DM, respectively. Unfortunately, δ- and γ-tocotrienols concentrations in LDC were below the detection limit.

**Table 7.** Content of tocopherols and tocotrienols in the lesser duckweed (*Lemna minor* L.), µg/100 g dry matter

| Specification | LDN | LDC |
|---|---|---|
| α-tocopherol | 2.019 ± 0.403 | 1.958 ± 0.112 |
| δ-tocopherol | 0.094 ± 0.000 | 2.935 ± 0.077 |
| γ-tocopherol | 1.586 ± 0.102 | 3.761 ± 0.157 |
| α-tocotrienol | 0.207 ± 0.005 | 0.628 ± 0.023 |
| δ-tocotrienol | 0.550 ± 0.066 | nd |
| γ-tocotrienol | 1.874 ± 0.658 | nd |

LDN – lesser duckweed in the natural environment, LDC – lesser duckweed in the laboratory conditions; nd – not detectable

# Discussion

## Protein and amino acids

Proteins, along with fats and carbohydrates, constitute the basic components of both human and animal diets. Supplying proteins to the body in adequate quantity and quality is crucial for health and performance. Ge et al. (2012) and Mwale and Gwaze (2013) reported that duckweed had a high protein content of 9–20% DM in nutrient-poor media, with this proportion reaching even 24–41% DM in nutrient-rich media. In our experiment, the percentage of protein in lesser duckweed ranged from 12.1 to 13.0%. This finding suggests that the *L. minor* was grown in nutrient-poor water, probably with low nitrogen concentration. (Mohedano et al., 2012). On the other hand, Hanczakowski et al. (1995) have shown that the protein content is influenced by the anatomical structure of lesser duckweed and the significant contribution of fronds to its biomass, as well as the abundance of nutrients in the water. According to Appenroth et al. (2017), the protein content in the biomass depends on culture conditions and duckweed species. Xu et al. (2011) observed a reduction in protein content and a concurrent rise in starch content in duckweed biomass when plants were transferred from a culture pond enriched with diluted pig effluent to clean water or salty water. Zhao et al. (2015) have found that the proportion of protein in different duckweed species can range from 6.8 to 44%, depending on the intensity of light and nitrogen concentration in the water. The average protein percentage in the species *L. punctata, L. minor,* and *L. gibba* has been reported to be 16.3, 17.5, and 21.5%, respectively (Dewanji, 1993; Chen et al., 2012; Aguilera-Morales et al., 2018). However, the CP content in lesser duckweed observed in the present experiment was lower than that reported by other authors (Falaye et al., 2022; Said et al., 2022). Considering the results of our study regarding protein content, it is unlikely that duckweed could replace high-protein feedstuffs in the nutrition of domestic animals and serve as a primary source of protein for humans. According to Bhanthumnavin et al. (1971), *Wolffia arriza* produces more DM than the traditional crops when it comes to annual yield. It seems that the protein yield of duckweed is several times greater than that of traditional crops. Sońta et al. (2020) showed that the protein content in *L. minuta* is approximately 354–361 g/kg DM. It is possible to obtain 2–4 t protein/ha/year from ponds, which is comparable to the protein yield from alfalfa cultivation.

The content and profile of specific amino acids in feedstuff and food products are important factors in assessing their nutritional quality. Protein from various duckweed species can provide the full spectrum of amino acids required for animal and human nutrition. However, Ifie et al. (2021) suggested that the geographical distribution of duckweed harvest may influence the amino acid profile. The lesser duckweed contains all ten EAA and eight different NEAA. According to our results, the concentration of EAA and NEAA in LDC was higher compared to LDN, indicating that culture conditions affect the content and profile of amino acids in *L. minor*. The concentrations of EAA (isoleucine (ILEU), LEU, LYS, threonine (THR), VAL) in LDC were shown to be higher than the corresponding values observed in *L. minor* grown under laboratory conditions (Appenroth et al., 2017). The levels of EAA in LDN, with the exception of tryptophan, were demonstrated to be lower than in the lesser duckweed collected from the water surface in a fish pond (Falaye et al., 2022).

Soybean and rapeseed meals are among the most widely utilized protein components in rations for domestic animals, serving as the main source of amino acids for livestock. However, soybean and rapeseed protein prices are rising. In comparison, both LDN and LDC exhibited higher concentrations of EAA than soybean and rapeseed meals (Lagos and Stein, 2017; Zhang et al., 2020; Kaiser et al., 2022). Nonetheless, the concentrations of histidine (HIS) and TRP were lower in LDN compared to soybean and rapeseed meals. Certain duckweed species offer a promising alternative for meeting the EAA requirements outlined by WHO (2007), and can support the growth and development of animals and humans. Our research findings revealed that HIS, LEU, LYS, methionine, and THR levels in lab-cultured duckweed were higher than those recommended by WHO (2007), whereas the contents of these EAA in lesser duckweed harvested from the pond were lower.

## Fat and fatty acids

Feeding fat to humans and domestic animals is mainly used to increase the energy density of the diet. In our study, the CT proportion in lesser duckweed ranged from 2.39 to 2.87%, with a slight dependence on the growth conditions of the plants. Appenroth et al. (2017) showed that the CT content was higher when six duckweed species were cultured in laboratory conditions, ranging from approximately 4.0 to 6.5%. The latter authors observed an increase in the fat content in *W. hyaline* (6.0%), while duckweed species contain lower fat proportions: *L. punctata* – 4.0%, *L. minor, L. gibba,* and *S. polyrhiza* – 4.5%. Thus, it appears that CT content may depend on duckweed species. Our data are inconsistent with findings of Fiordelmondo et al. (2022), who observed an increase in the CT proportion to 5.10% in lesser duckweed biomass harvested from fish farm ponds. Overall, the CT content of LDN and LDC was generally lower than that reported for *L. minor* in other studies.

The FA profile of fat is crucial for assessing the nutritional value of plants from the family *Lamnaceae* for both human and animal consumption. The content of total FA (SFA, MUFA, and PUFA) was higher in LDN compared to LDC, amounting to 53.90 and 18.4 g FAME/100 g CT, respectively. However, the levels of major SFA (i.e., C16:0 and C18:0), were lower in LDC compared to LDN. Appenroth et al. (2017) observed in a laboratory study that the sum of SFA and C16:0 content was lower in *L. minor* than in *L. punctata*. A higher SFA content may adversely affect the incidence of civilization-related diseases in humans and increase the SFA content in animal products. On the other hand, lesser duckweed harvested from a pond is a very good source of PUFA, with C18:2 and C18:3 being the two predominant acids. PUFA play a crucial role in various physiological processes in the body. The proportions of C16:0, C18:2, and C18:3 in LDN and LDC accounted for approx. 72.0 and 51.0% of total FA, respectively. Li et al. (2008) suggested that this difference might be due to growth conditions, as the low concentration of nitrogen and exogenous glucose in the aquatic environment could influence the accumulation of lipids in duckweed fronds. Our results are consistent with those of Tang et al. (2015), who showed that C16:0, C18:2, and C18:3 constituted approximately 80% of total FA in *L. aequinoctialis, S. polyrhiza,* and *W. globosa* collected from Lake Chao.

Interestingly, C9:0, C22:0, C23:0, C14:1 c9, and C15:1 c7 were not identified in the LDN biomass in the latter study. Tang et al. (2015) also failed to identify C22:0, as well as C16:1, and C20:0, in four duckweed species. Moreover, Yean et al. (2013) did not detect C18:3 and C18:4 in *L. minor*, *L. minuta*, and *L. aequinoctialis*. These findings suggest that the fatty acid profile may vary significantly depending on the duckweed species.

The n-6/n-3 ratio is a crucial parameter used to evaluate the nutritional value of fat for consumption. Our study showed that the n-6/n-3 ratio was strongly dependent on the FA profile of lesser duckweed. According to FAO (1999), the n-6/n-3 ratio should

ideally fall within the range between 1 and 4. The n-6/n-3 ratio in LDN and LDC was 0.844 and 2.861, respectively, thus our results fulfilled this requirement. CT from LDN was rich in linolenic acid (15.03 g FAME/100 g CT), resulting in a lower n-6/n-3 ratio compared to LDC. In this study, the n6/n3 ratio in LDC closely resembled that of rapeseed oil (2.2) (Jahreis and Schaefer, 2011). It should be noted that a high n6/n3 ratio in plants grown for food, such as olive and sunflower oils (15 and 150, respectively), or green pea and chickpea (5.7 and 21.8, respectively) (Jahreis and Schaefer, 2011; Jahreis et al., 2016), have been linked to the pathogenesis of many civilization diseases. Appenroth et al. (2017) reported the highest n:6/n:3 ratio for *W. microscopica* (0.61), the lowest for *S. polyrhiza* (0.25), while *L. minor* exhibited an intermediate value of 0.45. Our results demonstrated a higher ratio than those reported by Appenroth et al. (2017), indicating potential variations depending on growth conditions and species-specific factors.

## Ash and mineral composition

CA represents the inorganic fraction of organic matter of food products and forage. It encompasses the total mineral content and serves as a significant indicator for assessing feed quality. *Lemna* typically exhibits a CA percentage ranging from 7 to 36%, *Spirodela* biomass contains from 1 to 16% CA, and *Wolffia* from 10 to 23% (Pagliuso et al., 2022). The CA proportion in the biomass of lesser duckweed investigated in this experiment varied from 11.5% in LDN to 14.1% in LDC. Sońta et al. (2020) obtained a higher ash proportion in the biomass of *L. minuta* (approx. 22%) cultured in a plastic container with growth medium. In addition, Appenroth et al. (2018) reported a CA percentage of approximately 18% in the genus *Wolffia* when cultivated on plastic trays with growth medium. Comparatively, the ash content of lesser duckweed exceeds those of various forage ingredients such as lucerne hay (10%), corn stover (5–7%), wheat, oats, barley straw (4–6%), or grain (2–3%) (Table of chemical composition and nutritional value of domestic feed, 2015).

Duckweed species are capable of absorbing high quantities of macro- and micronutrients, as well as heavy metals, from water owing to their phytoremediation abilities (Appenroth et al., 2018). Absorption of mineral elements depends on their chemical form and the life stage of duckweed species, whether free-floating, suspended on the water surface, well-rooted, or rootless. Our study observed higher concentrations of Ca, Na, and Zn in LDC compared to LDN. The higher Ca concentration in LDC corresponded to increases in $CaCO_3$ (300 mg/l) levels in the water. Moreover, the elevated macro- and micronutrient contents in LDC result from the fertilization of lesser duckweed. It seems that the mineral composition of lesser duckweed is influenced by their concentration in the water. Interestingly, Mazen et al. (2003) reported that calcium oxalate raphide crystals, located in the vacuoles, played a role in the regulation of calcium levels in the fronds of *L. minor*. Additionally, Appenroth et al. (2017) suggested that changing the mineral composition of the medium can modulate the concentration of macro- and micronutrients in duckweed plants. Moreover, Appenroth et al. (2018) showed that different clones of the genus *Wolffia* exhibited varying mineral concentrations even when cultivated under the same conditions. These authors reported that the concentrations of Ca, Na, and Zn were in the range of 19.2–25.7, 0.18–0.38, and 22.5–50.0 mg/kg freeze-dry weight respectively in individual *W. arrhiza* clones. In the present study, the amount of P was four times higher in LDN compared to LDC. Counterintuitively, excess phosphorus is completely neutral for aquatic plants; however, an excessively high amount in relation to other minerals may lead to a lower concentration of minerals such as zinc or copper, corroborating our findings. Spiegel et al. (2013) suggested that duckweed could serve as a mineral source and could be combined with other ingredients, such as cereals, to provide a balanced diet.

Duckweed species have the capacity to accumulate many heavy metals, posing a potential threat to the normal development and health of humans and animals. These heavy metals can infiltrate different parts of the soil-water-plant-animal-human food chain (Ociepa-Kubicka et al., 2012). In our study, the concentration of Cd was higher, while that of Pb was lower in the LDC lesser duckweed compared to LDN. It should be noted that the content of Pb and Cd in the water used for the *L. minor* culture did not exceed reference values ($\leq 5$ and $\leq 10$ µg/l, respectively). Unfortunately, the concentration of Pb and Cd in the pond water was not analyzed. Sońta et al. (2020) showed that the levels of Pb, Cr, and especially Cd and Al in *L. minuta* depended on the culture medium. The utilization of natural water resources does not pose a significant risk of heavy metal absorption for duckweed species because the concentration of these metals in water is generally low (Sońta et al. 2019). According to FAO (1999), duckweed species can be a good source of trace elements for feeds dedicated to domestic animals.

Appenroth et al. (2018) investigated the levels of heavy metals in 11 species of the genus *Wolffia*. These authors demonstrated that the Pb content ranged from 13 to 1020 μg/kg freeze-dry weight, while the Cd content varied from 9 to 590 μg/kg freeze-dry weight.

### Tocopherols and tocotrienols

Several researchers worldwide have shown that the plants from the family *Lamnaceae* contain bioactive compounds, including tocopherols, tocotrienols, carotenoids, and phytosterols(Appenroth et al., 2017; 2018; Sońta et al., 2020). Compounds, such as α-, β-, δ-, and γ-tocopherols, as well as α-, β-, δ-, and γ-tocotrienols are essential components of vitamin E. Tocopherols play diverse roles in the body, e.g., as antioxidants (protecting PUFA in cell membranes from oxidation), regulators of the activity of multiple enzymes, as well as stabilizers and modulators of functional properties of cell membranes (Finno and Valberg, 2012). Tocotrienols have been shown to inhibit HMG-CoA reductase, the main enzyme involved in cholesterol biosynthesis. These compounds also demonstrate anticancer activity by inducing apoptosis, enhancing the immune system, and exhibit cardioprotective and antiosteoporotic effects (Xiong et al., 2023). The present study found that the content of tocopherols and tocotrienols varied and depended on the growth condition of *L. minor*. Interestingly, LDC contained higher levels of δ- and γtocopherols, as well as α-tocotrienol compared to LDN. Conversely, α-tocopherol concentration was slightly elevated in LDN compared to LDC, while δ- and γ-tocotrienols were not detected in LDC. Stewart et al. (2021) found that a wide range of light intensities could affect carotenoid and α-tocopherol levels in *L. gibba* biomass. Therefore, a mixed lighting approach, predominantly with low light intensities, supplemented by brief exposures to high light, can increase carotenoid and tocopherol concentrations in plants. It is plausible that the increase in α-tocopherol and δ-, γ-tocotrienol levels in lesser duckweed biomass (LDN) was associated with exposure to sunlight in natural settings. Moreover, Polutchko et al. (2022) observed a rise in α-tocopherol content in *L. minor* grown in a local pond exposed to natural sunlight. On the other hand, the culture of lesser duckweed under laboratory condition (LDC), with permanent lighting and 4h dark phase, may have led to increased levels of other tocopherols in biomass. Stewart et al. (2021) demonstrated increased amounts of zeaxanthin and anthraxanthin in *L. gibba* biomass after exposure to constant high-intensity lighting. It should be noted that (13Z)-β-carotene was not detected in a previous study on *W. cylindracea* and *W. globosa* 9498 grown under laboratory conditions (Appenroth et al., 2018). The latter authors also found that the αtocopherol content in *W. arrhiza* and *W. microscopica* was 12.8 and 0.5 mg/100 g freeze-dry weight, respectively. Therefore, it cannot be ruled out that duckweed species and the lighting intensity affect the concentration of tocopherols and other bioactive components. It is interesting to note that the concentration of tocopherols in forage can fluctuate depending on factors such as growth phase, exposure to solar radiation, conservation method, and grass species. Shingfield et al. (2005) noted that drying grass instead of ensiling resulted in lower α-tocopherol concentrations in dry grass. Moreover, Weiss (1998) reported that the amount of α-tocopherol lost during ensiling or drying of grass could vary from 20 to 80%. Lynch et al. (2001) observed that the α-tocopherol content in various fresh forages followed this decreasing order: pasture grass>meadow grass>white clover>red clover (Lynch et al., 2001). Therefore, the inclusion of fresh or dried duckweed biomass in diets could enhance the quality of feedstuffs by increasing the content of bioactive compounds, such as tocopherols, tocotrienols and carotenoids.

## Conclusions

Our results demonstrate the significance of growth conditions on the nutrient content in *Lemna minor* L. Optimizing factors, such as temperature, light intensity, and nutrient availability, are key to enhancing duckweed production. While lower levels of crude protein, fat, and ash were recorded in the present study compared to other researches, we found higher concentrations of essential amino acids in laboratory-grown *L. minor* compared to pond-harvested plants. The lesser duckweed is a good source of polyunsaturated fatty acids, especially when cultured in the pond. In terms of macro- and micronutrients, Ca, Na, and Zn were the main components found in crude ash of duckweed biomass collected under laboratory conditions. Moreover, our data have demonstrated that *L. minor* contains significant amounts of vitamin E.

Overall, *L. minor* shows promise as a valuable source of nutrients that could supplement the diets of both domestic animals and humans, especially because, unlike the corn or soybean, is not a genetically modified plant. However, the concentration of antinutrient components, especially heavy metals, should be under constant monitoring in duckweed cultures.

Rapidly-growing plants, including duckweed species, also produce the highest yields of protein, fat, minerals, and other nutrients.

## Conflict of interest

The Authors declare that there is no conflict of interest.

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