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Re-circulating indoor vertical farm: Technicalities of an automated duckweed biomass production system and protein feed product quality evaluation

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ABSTRACT

Duckweeds are fast-growing and nutritious plants, which are gaining increased attention in different fields of application. Especially for animal nutrition, alternative protein sources are needed to substitute soybean meal. The current bottleneck is the standardized production of biomass, which yields stable quantities of a defined product quality. To solve this problem, an indoor vertical farm (IVF) for duckweed biomass production was developed. It consists of nine vertically stacked basins with a total production area of 25.5 m^2. The nutrient solution, a modified N-medium, re-circulated within the IVF with a maximum flow rate of 10 L min^{-1}. Nutrients were automatically added based on electrical conductivity. In contrast, ammonium was continuously supplied. A water temperature of 23 °C and a light intensity of 105 µmol m^{-2} s^{-1} with a photoperiod of 12:12 h were applied. During a 40-day production phase, a total of 35.6 kg of fresh duckweed biomass (equals 2.1 kg of dried product) was harvested from the IVF. On average, 0.9 kg day^{-1} of fresh biomass was produced. The dried product contained 32% crude protein (CP) and high levels of proteinogenic amino acids (e.g. lysine: 5.42 g, threonine: 3.85 g and leucine: 7.59 g/100 g CP). Biomass of this quality could be used as a protein feed alternative to soybean meal. The described IVF represents a modular model system for duckweed biomass production in a controlled environment and further innovations and upscaling processes.

1. Introduction

Soybean is one of the globally most important sources of protein (Jia et al., 2020). The main production areas are located in South and North America (Tallentire et al., 2018). However, soy production is related to deforestation (Henchion et al., 2017), environmental issues (de Visser et al., 2014) and transportation issues in order to meet the global demand (He et al., 2019). The increasing demand for animal protein drives soy production and therefore the environmental issues related to soy production (Henchion et al., 2017). In the EU in particular, soybean meal accounts for 48% of the protein-rich feed with a protein content above 30% crude protein (CP) (European Commission, 2021b). This leads to a protein deficit and dependence, especially in the feeding of monogastric animals (de Visser et al., 2014). However, the growth potential for common agricultural protein crops is limited, as the European Commission (2021a) expects a further decreasing availability of land for agricultural production.

In order to reduce these issues related to soy production, novel protein sources and efficient land-use strategies for crop production have to be considered. Duckweeds are gaining increasing attention in research and application due to their high growth rates and high protein content (Acosta et al., 2021). Therefore, duckweed must be taken into account as an alternative protein source. They are small floating freshwater plants, belonging to the family of *Lemnaceae* Martinov (1820). To

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date, 36 species are known (Bog et al., 2019). Duckweeds occur worldwide, except for the polar regions and other areas with extreme climatic conditions, such as deserts (Landolt, 1986). They are the fastest-growing angiosperms in the world (Ziegler et al., 2015). The species *Wolffiella hyalina* (clone 9525) reached relative growth rates (RGR) of up to 0.512 d^{-1} and can double its biomass every 32.2 h (Ziegler et al., 2015), while *Wolffia microscopica* (clone 2005) can double its biomass every 29.3 h (RGR: 0.559 d^{-1}) under axenic in vitro conditions (Sree et al., 2015).

Duckweed biomass is highly nutritious (Acosta et al., 2021), with protein contents of up to 45%, based on dry matter (DM) (Xu et al., 2021). Duckweed protein contains a high ratio of essential amino acids, making its protein composition valuable for animal nutrition (Chakrabarti et al., 2018) and human consumption (Appenroth et al., 2017). Several experiments were conducted with livestock to investigate the effects of duckweed biomass in the total diet. Broiler chickens reacted with increased growth to a fraction of up to 6% *Lemna minor* in complete feed as a substitute for sesame oil cake (Ahammad et al., 2003). Other trials showed that rates of up to 8% duckweed in all ages of chickens (Kabir et al., 2005) and up to 10% in finisher diets (Kusina et al., 1999) are possible. Also, a partial replacement of soybean meal by duckweed for feeding piglets and growing pigs has been realized (Moss, 1999). For laying hens, *Lemna gibba* could replace up to 10% soy bean or approximately 50% fishmeal without adverse effects on egg quality or laying performance (Zakaria and Shammout, 2018). A Leghorn hens diet, containing 25% *Lemna gibba*, resulted in a higher egg protein content and significantly increased yolk pigmentation compared to the control, which contained soybean meal and fishmeal as protein sources (Haustein et al., 1990). Increased egg yolk colour has also been confirmed by Anderson et al. (2011). Moreover, the nutritional value of duckweed has been shown for feeding ducks (Khanum et al., 2005), fish (Asimi et al., 2018), cattle (Huque et al., 1996) and sheep (Zetina-Córdoba et al., 2012). Generally, trial results varied based on the quality of the used duckweed and its composition.

Consequently, product quality has to be optimized and controlled to ensure appropriate and efficient animal nutrition. In duckweeds, growth rates and the nutritional composition can be influenced by cultivation conditions, such as light and temperature (Cui et al., 2011) as well as the composition of the nutrient medium (Petersen et al., 2021).

A novel strategy for duckweed cultivation, which has not been described in detail to date, can be the use of an indoor vertical farm (IVF). Generally, this strategy consists of several horizontal cultivation levels stacked above each other, usually operated with artificial lighting (Kozai et al., 2020). This way, the land utilization efficiency, meaning the cultivation area per ground area, can be increased and thus an extensive production with less available land is feasible (Coughlan et al., 2022). Soilless cultivation methods, e.g. hydroponics, can be integrated into IVFs and are already used for the production of different crops, such as tomatoes, cucumbers, peppers, strawberries as well as lettuce and other leafy greens (Sharma et al., 2018). Hydroponic IVFs operated in a controlled environment have the advantage that the grower can set all abiotic factors according to the plant demand for most efficient growth and nutrient accumulation. This includes, amongst others, nutrient concentration and composition, light source and settings, temperature and CO_2 levels (Benke and Tomkins, 2017). It allows for year-round production, independent of weather conditions and location. Standardizing the cultivation process and all relevant abiotic parameters aims at maximizing and stabilizing yields with a defined and high nutritive value at the same time. By re-circulating the nutrient solution in the hydroponic system (also known as "closed system"), the water and nutrient input can be reduced compared to "open systems". The water and fertilizer use efficiency for the cultivation of tomatoes was 22.7% higher for "closed systems" compared to "open systems" in both cases (de la Rosa-Rodríguez et al., 2020). Compared to conventional agriculture, up to 90% of irrigation water and 85% of fertilizers can be saved in a closed hydroponic system, while a productivity increase of up to 250% is possible (AlShrouf, 2017). Nutrient leaching into the environment can be largely avoided by using this cultivation method (Keuter et al., 2021).

For duckweed, IVFs have been described theoretically (Coughlan et al., 2022; Roman and Brennan, 2021), as a greenhouse-based continuous flow plant (Fujita et al., 1999) and as a small scale version on an experimental level (Petersen et al., 2022). A vertical farming system for duckweed cultivation has been described by Everett et al. (2012), while the Israel-based company Green-Onyx has a patented vertical farming module for *Wolffia* production. In all cases detailed information about the system, operational parameters as well as yielded biomass quantity and quality are scarce.

Our team at the University of Applied Sciences Osnabrück, Germany developed a large-scale IVF, designed for duckweed biomass production. This is the first detailed report on an IVF system for duckweed cultivation. Aim of this research is the description of the construction, technicalities and operation of this automated and re-circulating IVF for the mass production of protein-rich *Lemna* biomass. The yielded qualities were evaluated for a possible use as a protein feedstuff.

2. Materials and methods

2.1. Experimental setting of the re-circulating indoor vertical farm during production

The IVF was operated for 40 consecutive days in order to produce protein-rich *L. minor* (clone 9441, Germany) biomass. The basins of the IVF were filled with local tap water (see Table S1) to a height of 5 cm and the reservoir to 25 cm, this resulted in a total volume of ca. 2000 L. A light intensity of 105 µmol m^{-2} s^{-1} and a photoperiod of 12:12 h light: dark cycle was used. The water temperature was set to 23 °C. The flow rate of the re-circulating water within the IVF was 10 L min^{-1}, which was treated with UV-C light to reduce the growth of unwanted ubiquitous organisms.

As nutrient solution, a modified N-medium with a NO_3^--N to NH_4^+-N ratio of 75%–25% was applied, which resulted in the highest RGR and protein yield of *L. minor* and *W. hyalina* (Petersen et al., 2021). The stock solutions were mainly prepared with commercially available fertilizers in order to reduce the operation cost. The EC value was set to 0.7 mS cm^{-1} to reach the target concentrations given in Table 1. No pesticides were applied throughout the whole production phase.

2.2. Sampling and analysis

Nutrient medium samples were taken at 5 time points during the production phase, while the ammonium concentration was measured at 14 time points. Ammonium and nitrate concentrations in nutrient media samples were determined with Reflectoquant® Ammonium and Reflectoquant® Nitrate Tests (0.2–7.0 mg l^{-1} NH_4^+, 5–225 mg l^{-1} NO_3^-; Merck KGaA, Darmstadt, Germany) and a RQflex®20 (Merck KGaA).

Table 1
Target nutrient concentrations (mmol l^{-1} and mg l^{-1}) in the modified N-medium.

Substance	Concentration (mmol l^{-1})	Concentration (mg l^{-1})
NO_3^--N	0.87	12.2
NH_4^+-N	0.25	3.5
PO_4^{3-}	0.1	9.5
K^+	0.98	38.4
Mg^{2+}	0.41	9.9
SO_4^{2-}	1.23	117.7
Ca^{2+}	1.34	53.5
Fe^{3+}	0.0028	0.15
B^{3+}	0.0024	0.025
Mn^{2+}	0.0013	0.07
Na^+	0.76	17.4
Zn^{2+}	0.0095	0.62
Cu^{2+}	0.0014	0.09

Other nutrients were analysed according to DIN EN ISO 11885:2009-09 (2009) with an ICP-OES (ICAP 7400, Thermo Fischer Scientific, Waltham, USA). Temperature, pH- and EC-values were logged via Pro Controller connect (Bluelab Corporation Ltd, Tauranga, New Zealand). Light intensities were measured for control with a Light Meter LI-250A (LI-COR Biosciences, Lincoln, USA).

Biomass harvesting was done six times during the 40-day production phase. The fresh biomass was subsequently oven dried at 65 °C for 72 h and stored for further use in animal feeding trials. The CP content of the dried biomass was determined using the Dumas method according to ISO 16634-1:2008-11 (2008). Acid detergent fibre and acid detergent lignin were analysed in accordance with DIN EN ISO 13906:2008-2011 (2008) and neutral detergent fibre was analysed according to ISO 16472:2006-04 (2006). The following substances were analysed by methods described by Commission Regulation (EC) No 152/2009, annex III: Tryptophan, method G; all other amino acids, method F; residual moisture and dry matter, method A; crude fibre, method I; crude fat, method H procedure B; crude ash, method M.

2.3. Calculations

In order to evaluate the protein quality of the dried biomass, the essential amino acid index (eAAI) and the amino acid ratio (AAR) were used. eAAI was calculated with an equation described by Oser and Albanese, 1959:

$$eAAI = \sqrt[n]{\frac{aa1}{AA1} * \frac{aa2}{AA2} * \ldots * \frac{aan}{AAn}} \quad (1)$$

whereas aa1, aa2, …aan are the amino acid contents in the CP of the tested sample and AA1, AA2, …AAn are the respective demands of broiler chickens (National Research Council, 1994; age of 3–5 weeks) or piglets (National Research Council, 1998; 10–20 kg body weight). The concept of ideal protein is widely used to assess the nutritive value of proteins (Santamaría-Fernández and Lübeck, 2020). For the calculations of amino acid ratios (AAR), amino acids concentrations are set in relation to the lysine content (Pastor, 2014):

$$AAR_{aa}(\%) = \frac{aa}{LYS} * 100 \quad (2)$$

whereas aa is the individual amino acid content and LYS is the lysine content in the CP of the tested sample. This ratio was calculated for every individual amino acid. This way, the amino acid ratio for feedstuffs was compared with the ideal amino acid ratio (IAAR) for broiler chickens (National Research Council, 1994) and piglets (National Research Council, 1998), matching the requirement of the respective species.

3. Results

3.1. Re-circulating indoor vertical farm

The presented IVF consists of nine rectangular production basins, positioned vertically above each other, and a reservoir at the bottom (Fig. 1A). Each basin is made of acrylic glass with a length of 195 cm, a width of 145 cm and in case of the production basins a height of 10 cm, while the reservoir has a height of 40 cm high. All production basins together amount to a total cultivation area of ca. 25.5 m^2. Acrylic glass was chosen because it is stable, break-proof, transparent and lighter than glass. All basins rest in an aluminium framework. Each basin rests on two permanently installed horizontal aluminium bars in the framework, the space between the basins is 16.7 cm. The aluminium bars are hollow to reduce the weight of the framework.

Each basin, except for the reservoir, has an outlet positioned in one

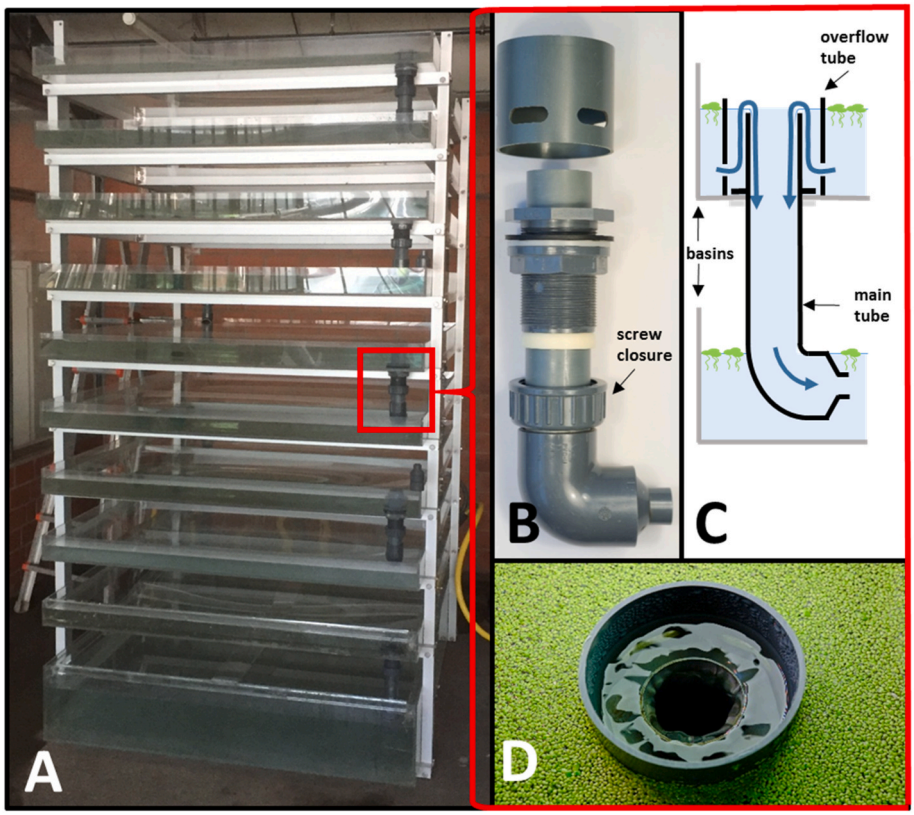

Fig. 1. Basic structure of the indoor vertical farm (IVF), consisting of the aluminium framework, nine acrylic glass production basins and one acrylic glass reservoir at the bottom (A). Close-up of the outlet, connecting the upper basin to the one below (B) and a schematic figure indicating the flow of water (C). Top view on the upper part of the outlet in an operational state of the IVF (D).

corner of the ground plate (Fig. 1B). These outlets consist of a main tube, at their bottom a 90° elbow-piece is connected. Inside rests a removable, conical reducing socket. This way, the speed of the outflow can be increased. A screw closure is implemented. In an open position, it is possible to move the main tube up and down according to the requirements. If closed, the tube rests firmly in its position. Over the top of the main tube, an overflow tube with a wider diameter is placed. This tube section has three oval shaped holes. By moving the main tube up or down, the height of the nutrient solution can be individually adjusted in each basin. If the main tube is positioned higher than the holes, nutrient solution will flow into the basin below while the floating duckweed is hindered to pass the barrier (Fig. 1C and D). This way, nutrient solution circulation throughout the whole system is achieved. The outlets are positioned on opposite sides of the basins, in order to create a circulatory nutrient solution flow within each basin.

A harvesting system is integrated in the IVF. Each basin has an outlet positioned at the shorter side wall. It consists of a T-piece. A manual gate valve is located in this structure. All T-pieces are connected to each other (Fig. 2) and this way form the complete harvesting system. All used tubes and pieces are made of polyethylene. When the gate valve is opened, the duckweed flows into the harvesting system together with the supernatant nutrient solution. At the bottom of the harvesting system, the duckweed is collected in a net. The nutrient solution flows into a container and can be pumped back into the IVF. The above-described IVF structure was completely provided by AquaLight GmbH (Bramsche, Germany).

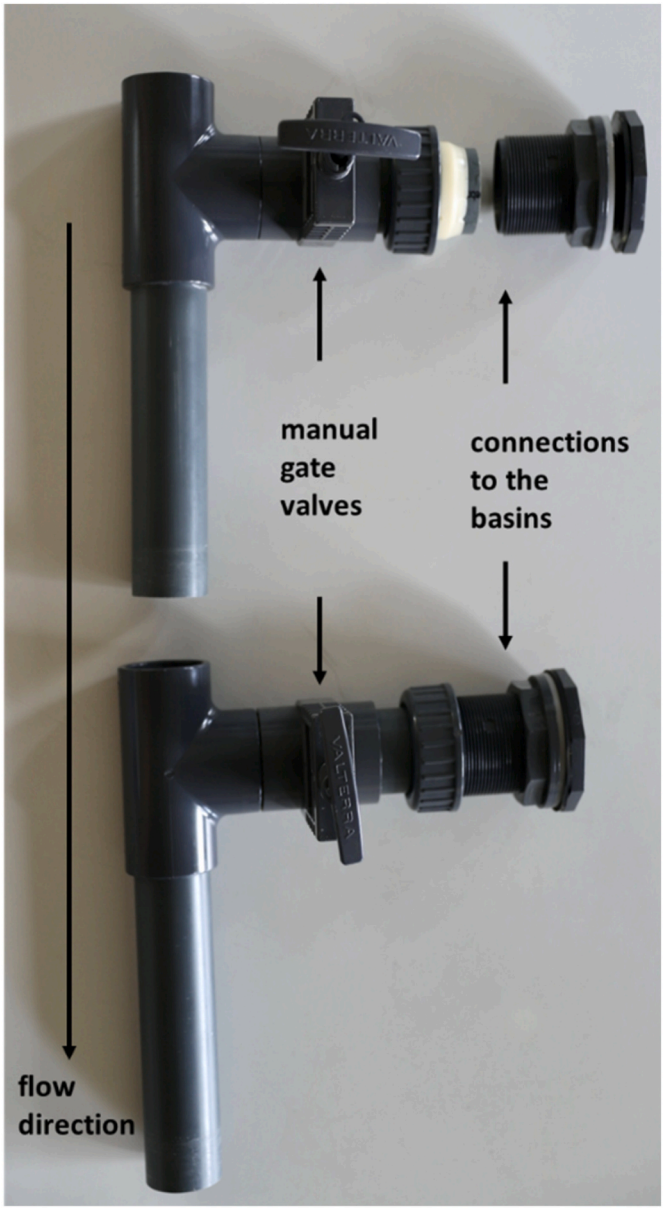

Fig. 2. Structure of two elements belonging to the harvesting system of the IVF. Such a structure is connected to each of the nine basins. By opening the manual gate valves, duckweed and supernatant nutrient solution flow out of the basins.

The described IVF is specially designed for the cultivation of duckweed. All relevant abiotic growth factors can be adjusted and partially controlled. A flow chart shows the structure and function of this IVF (Fig. 3). Local tap water and demineralized water are fed to the reservoir through flexible hoses. For both water sources, a pressure reducer valve is installed as well as an L-type ball valve to manually select between the water supply. When the IVF is firstly filled, tap water is used, while evaporation losses during the operation are replenished by demineralized water. On the inside wall of the reservoir, a mechanical float valve automatically regulates the water influx and compensates for evapotranspiration losses. By this measure, an uncontrolled water influx is prevented and the water level cannot rise above the top edge of the reservoir. The water circulation flow within the IVF is created by a submergible and adjustable feed pump (AquaForte DM-10000 Vario, SIBO BV, Veghel, The Netherlands) placed at the bottom of the reservoir. The water is pumped through a flexible hose into a UV-C clarifier (OSAGA UVC-55, Fischfarm Otto Schierhölter, Glandorf, Germany) to eliminate spores of ubiquitous algae and fungus as well as bacteria. In addition, a water smart flow meter (Gardena Deutschland GmbH, Ulm, Germany) to measure the flow rate and a gate valve to manually adjust the flow rate are installed. The water is flowing through a screen filter to avoid particles reaching the top basin. In order to reduce clogging of the filter, it is backwashed at frequent time intervals, regulated by a time switch. Magnetic valves redirect the flow direction, backwards through the screen filter for a few seconds and then into the drain. A drain valve ensures complete water outflow of the drain hose. Finally, a one-way check valve right before the top basin inlet avoids a gravity-driven backflow of water and duckweed into the UV-C clarifier when the feed pump is turned off.

A gravity-driven flow from basin to basin is realized through the outlets installed in each basin. Before the nutrient solution reaches the reservoir, it passes a second UV-C clarifier. The reservoir contains an overflow, which is connected to the drain in order to prevent flooding of the IVF. Another technical device to ensure the safe operation of the IVF devices at all times is the use of a water level sensor (WPS 3000 plus, H-TRONIC, GmbH, Hirschau, Germany). When the water level reaches the lower sensor, the pumps, heating system and UV-C clarifiers will be automatically shut down to avoid damage by overheating or running dry. When the water level reaches the upper sensor, the same technical devices are automatically turned on again.

Liquid fertilizers from stock solutions are automatically added to the tap water in the IVF by an EC- based nutrient control and dosing system (Pro Controller connect and PeriPods, Bluelab Corporation Ltd, Tauranga, New Zealand). The Bluelab Pro Controller connect regularly measured and logged EC, pH and temperature data. The corresponding probes are measuring in the reservoir. As four dosing pumps were available, the six corresponding stock solutions were combined according to the following scheme: A - stock solution 1 & 4 (Ca^{2+}, Cl^{-}, K^{+}, NH_4^{+}, NO^{3-}); B - stock solution 3 & 5 (K^{+}, NH_4^{+}, PO_4^{3-}, SO_4^{2-}); C - stock solution 6 & 7 (Fe^{3+}, Mg^{2+}, SO_4^{2-}, trace elements); D - stock solution 3 (NH_4^{+}, SO_4^{2-}). The composition of the different stock solutions is given in Table S2. Dosing pumps A, B and C were used for an EC-based nutrient dosing into the reservoir to obtain the composition of the modified N-medium. Dosing occurred for 12 s when the EC-value was below 0.7 mS cm^{-1}. In order to avoid overconcentration, a lag-phase of 15 min for dosing of stock solutions A, B and C ensured enough time for a homogenous nutrient distribution within the IVF. Dosing pump D added

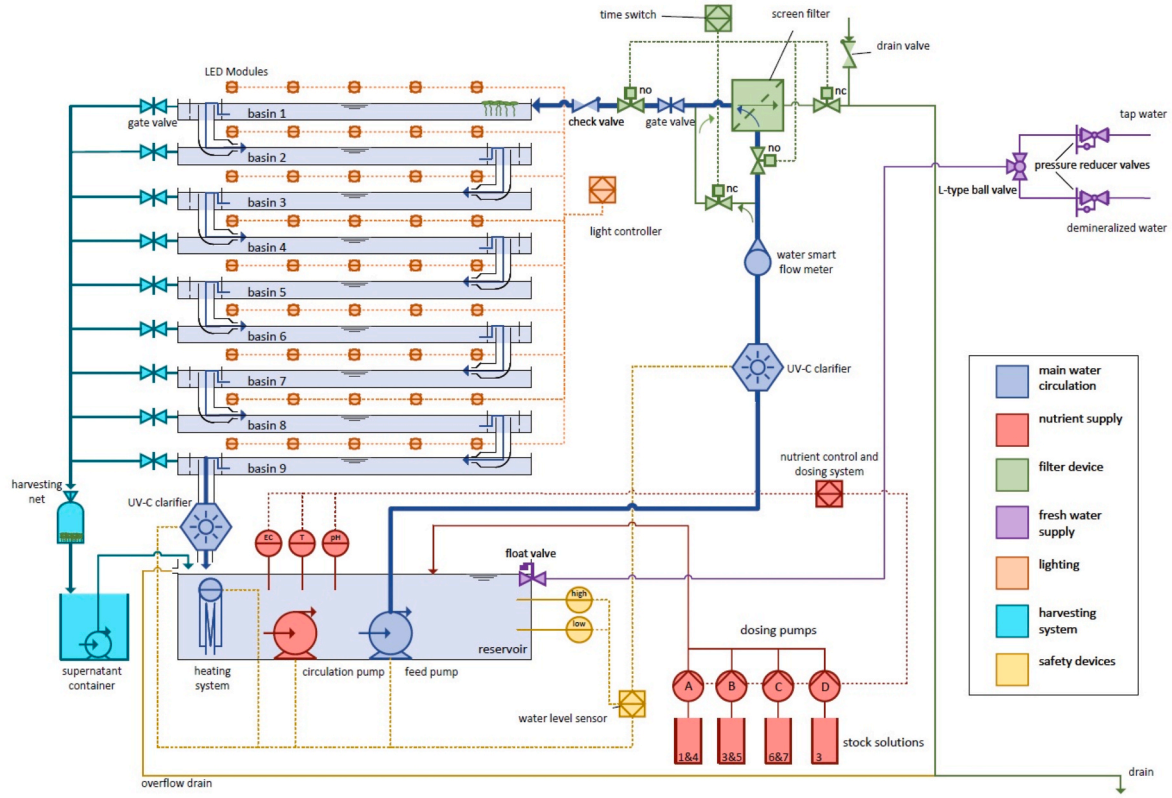

Fig. 3. Flow chart of the IVF. The continuous lines depict mass flow, while bold lines illustrate re-circulation in the IVF. Dotted lines indicate electricity or data flow.

stock solution 3 to the nutrient solution for 3 s every 40 min, independent of the actual EC-value. This continuous ammonium dosing was done to keep the NH_4^+-N concentration at a stable level.

The water temperature is adjusted and held constant by a heating system (Super Fish Smart Heater 500 W, Aquadistri BV, Klundert, The Netherlands) installed at the bottom of the reservoir. Due to the constant circulation of the water in the IVF, nutrients are equally distributed and a constant water temperature is expected throughout the whole system. A second submergible pump is placed at the bottom of the reservoir, which creates a continuous flow of the nutrient solution in the reservoir, in order to reach a fast homogenization of the added nutrients and to impede the adhesion of unwanted organisms to the reservoirs ground plate and walls. As an artificial light source, five LEDs (FLEX PRO 12 S4, SANLight GmbH, Bludenz, Austria) with a length of 1731 mm were installed 12.5 cm above the water surface in each basin. The LEDs are adjustable regarding their light intensity (0–250 μmol m^{-2} s^{-1}) and the daily operation duration (0–24 h). To adjust these parameters, a light controller with a Berryvine Farmee Client (Experior Micro Tech GmbH, Munich, Germany) is used. The LEDs are automatically turned on and off by this light controller on a daily basis. Five LED bars per layer are necessary to create an even illumination of the whole basin.

The whole IVF (Fig. 4) was housed in a mosquito net to reduce the risk of an infestation with insect-transmitted pathogens, as in the summer of 2020 an infection of the duckweed with the fungus *Pythium myrothulium* occurred, a soil-borne pathogen (Brand et al., 2021). It is assumed that insects acted as vectors and transferred the pathogen into the aquatic system. Another possible path of infection is through working staff. However, because the path of the infection could not be reconstructed clearly, the mosquito net was installed as a countermeasure. This way, insects are hindered to reach the IVF. Additionally, a disinfection procedure was obligatory for all people working at the IVF. During the infection, it was recognised that the fungal spots primarily occurred in the corners of the basins. In order to reduce dead zones

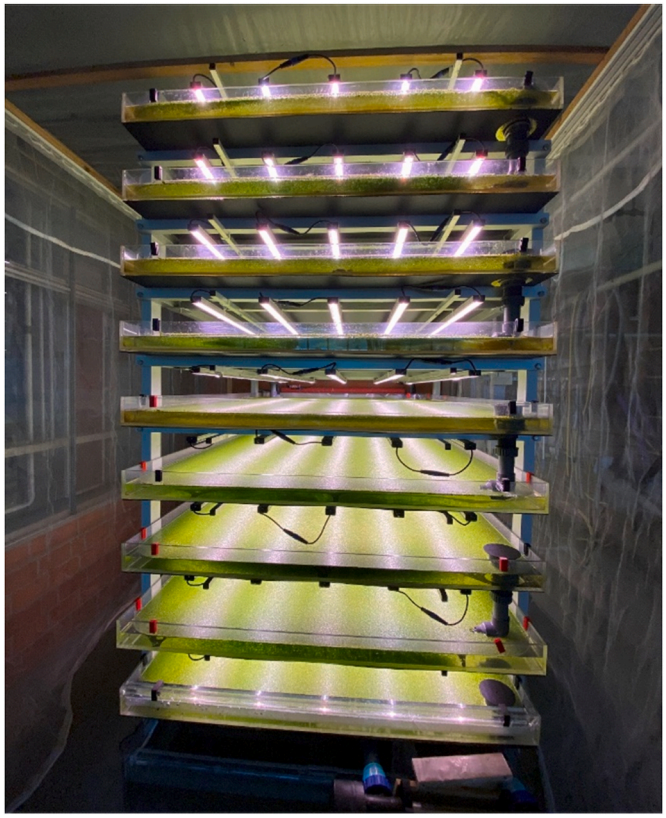

Fig. 4. Fully planted indoor vertical farm for duckweed production in operation.

regarding water and duckweed movement, the edges were rounded by installing plastic shields. This way, the nutrient solution can circulate well within each basin. After the mosquito net was installed, no infection occurred anymore.

3.2. Nutrient solution

The cumulative volume of the EC-based stock solution dosing during the 40-day production phase was 326 ml for stock solutions 1 & 4, 336 ml for stock solutions 3 & 5 and 328 ml for stock solutions 6 & 7. For the continuous ammonium dosing, a total of 722 ml of stock solution 3 was added to the IVF. The average pH during the production phase was 6.1 ± 1.1. Average measured concentrations of nutrients in the solution as well as the variation coefficient are presented in Table 2.

The concentrations of the individual nutrients were not completely constant during the production phase. The variation coefficients differed, depending on the substance. A variation coefficient of 3% for calcium and 4% for sulphate was calculated, indicating a stable nutrient concentration. In contrast, high variation coefficients of 61% for ammonium-N, 86% for zinc and 94% for manganese indicate severe fluctuations in nutrient concentrations during the production phase. On average, nitrate-N and ammonium-N were present in the nutrient solution in a ratio of 4.3:1, while the calcium to magnesium ratio was 5.5:1.

3.3. Biomass yield and quality

Over the whole 40-day production phase a total of 35.6 kg duckweed biomass (FW) was harvested from the IVF with a yield of 6 ± 1 kg FW per harvest and a harvest interval of 6.7 ± 1.4 days. On average, 0.9 ± 0.15 kg day^{-1} (FW) were yielded. One kg of fresh biomass resulted in 59 ± 2 g of dried product after oven drying. The corresponding yield for the dried product in the whole IVF was 2.1 kg in total or 53 ± 10 g day^{-1} on average. These results would extrapolate to ca. 6.3 kg of FW or 370 g dried product per week. The total dried biomass of the duckweed production in the IVF had a residual moisture of 7.8% and a protein content of 32% in DM. The biomass composition is shown in Table 3.

The CP consisted mainly of Aspartic and Glutamic acid with contents of 11.9 and 10.5 g/100 g CP, respectively, but also essential amino acids such as lysine (5.42 g/100 g CP), threonine (3.85 g/100 g CP) and leucine (7.59 g/100 g CP) are present. Proteinogenic amino acids account for 88.1% of the crude protein. The complete amino acid profile of the harvested *L. minor* biomass is shown in Fig. 5.

Carbohydrates were analysed using both the usual Weender analysis with a separation into crude fibre and N-free extracts and the more detailed detergent analysis. Crude fibre accounts for 24.9% of carbohydrates. However, considering the neutral detergent fibre (NDF), fibrous compounds are contained more extensively at a level of 66.2%. Sugar content was below the detection limit of the method (<1%) and the starch concentration was 1.08% in DM. The complete carbohydrate composition is shown in Fig. 6.

Table 2
Nutrient solution concentrations (mg l^{-1}) and variation coefficients (%) for a duckweed production phase of 40 days in the re-circulating indoor vertical farm. Number of measurements n = 5, except for ammonium (n = 14).

Substance	average ± standard deviation (mg l^{-1})	variation coefficient (%)
NO_3^--N	11.5 ± 2.3	20
NH_4^+-N	2.7 ± 1.7	61
PO_4^{3-}	6.2 ± 2.3	37
K^+	18.2 ± 5.8	32
Mg^{2+}	11 ± 1.3	12
SO_4^{2-}	160.8 ± 6.4	04
Ca^{2+}	61 ± 1.6	03
Fe^{3+}	0.008 ± 0.003	45
B^{3+}	0.01 ± 0.007	68
Mn^{2+}	0.12 ± 0.11	94
Na^+	19.1 ± 1.5	08
Zn^{2+}	0.09 ± 0.08	86
Cu^{2+}	0.013 ± 0.009	69

Table 3
Average dry matter composition of the dried *Lemna* biomass (n = 2, except where stated).

Analytical substance	Concentration in dry matter
Crude protein	32.0 ± 0.7
Crude fat (n = 1)	4.8
Crude fibre	10.7 ± 0.1
Crude ash (n = 1)	20.3

4. Discussion

4.1. Re-circulating indoor vertical farm

Cultivation in IVFs is already established for certain agricultural crops. Depending on the plant cultivated, the IVF structure has to be adapted to the crop requirements. Plant morphology and abiotic requirements have to be considered during the design and construction process of an IVF. Today's cultivation systems are adapted to plants with roots, which are traditionally grown in soil, such as tomatoes, peppers or leafy greens. Nutrient film technique (NFT), ebb and flow, drip and aeroponic are typical hydroponic production systems (Sharma et al., 2018). For duckweed, these systems are not applicable, because its morphology as an aquatic plant is not comparable to the above-mentioned crops. Duckweeds usually require a water body to float on, which can be most likely compared to a deep water culture. In order to keep the water and nutrient input to a minimum, it was decided to re-circulate the nutrient solution within the IVF instead of a batch production. To create a space efficient production a vertical structure with nine basins was built. Per square meter of ground floor 9 m^2 of production area are available. Coughlan et al. (2022) described 15 m^2 per square meter of ground floor in a theoretical approach under different constructional conditions. In general, many features and aspects of the presented IVF were also described by Coughlan et al. (2022), but our construction is the first practical IVF application for duckweed biomass production.

An IVF for duckweed production, intended for pharmaceutical use, is described by Everett et al. (2012). The former company Biolex Therapeutics Inc. (USA) chose the approach of a sterile production process using single-use seed bags, production bags and harvest bags in an IVF consisting of 8 vertical shelves. Lighting, ambient air supply, media composition and temperature were considered, but no process parameters or settings were mentioned. As the presented study aimed to produce duckweed biomass for feed purposes, a sterile production process, especially regarding the size of the IVF, seems unfeasible. Therefore, the way of duckweed production in our IVF can hardly be compared to the approach described by Everett et al. (2012).

4.2. Operation and nutrient management

A light intensity of approximately 100 μmol m^{-2} s^{-1} was chosen because it yielded good RGR results for *L. minor* (Petersen et al., 2022) and is close to the recommended optimum regarding energy input and biomass output (Yin et al., 2015). An increase in photoperiod can increase duckweed growth rate and biomass yield (Yin et al., 2015), but the growth of unwanted biotic parameters, such as ubiquitous algae and biofilm formation, has to be considered in such a non-sterile production system. The uncontrolled growth of algae can result in reduced duckweed growth (Roijackers et al., 2004). As countermeasures, the target temperature, flow rate, light intensity, photoperiod and nutrient concentration were adapted based on prior experiences (Brand et al., 2021; Petersen et al., 2021, 2022).

Nutrient management is critical to successfully cultivate duckweed

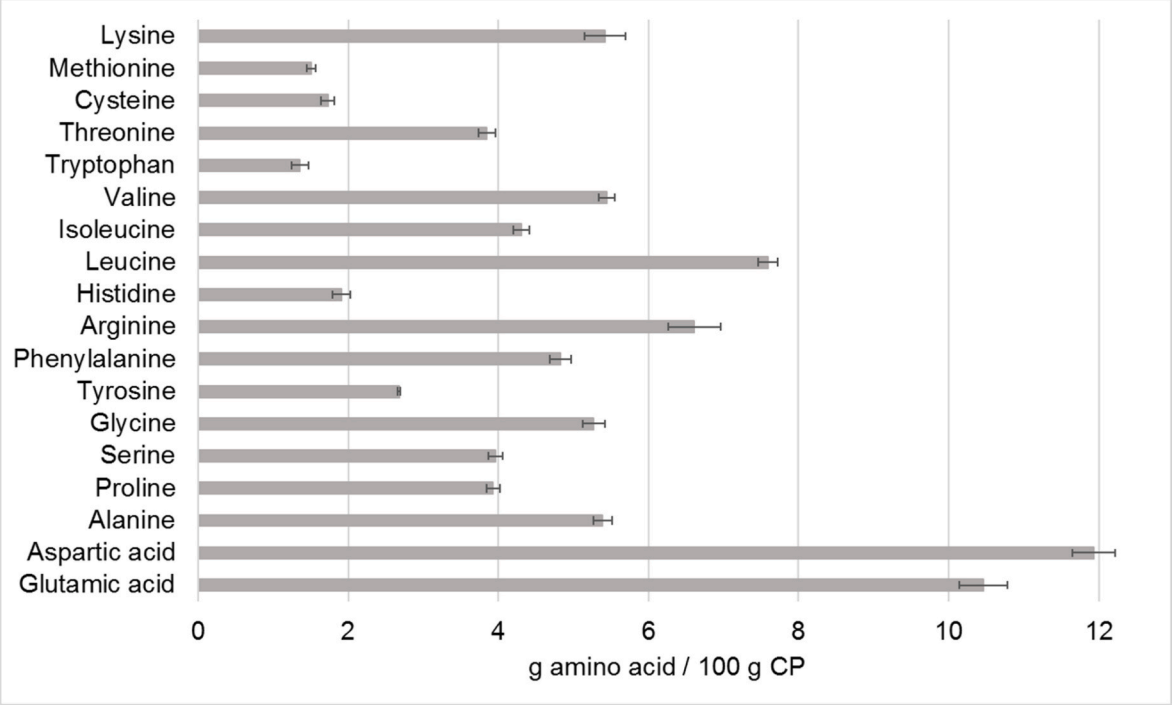

Fig. 5. Average amino acid composition of the crude protein in the total harvested *L. minor* biomass (n = 2). Error bars indicate standard deviation.

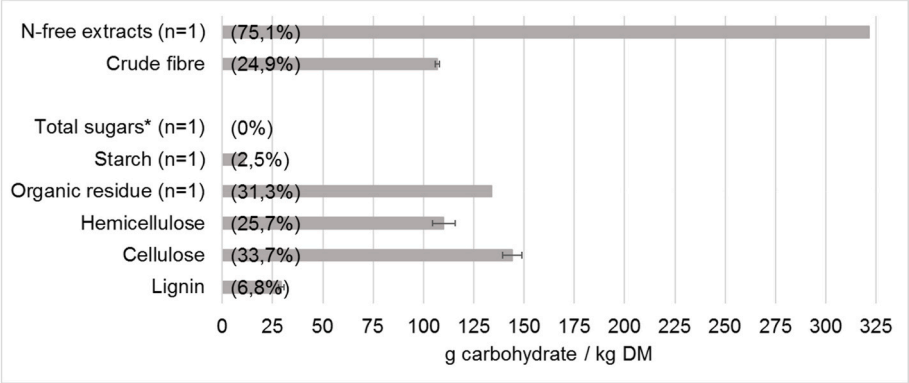

Fig. 6. Content of carbohydrates in the dry matter (n = 2, except where stated). Values in brackets indicated the share of individual compounds or fractions of total carbohydrates. Error bars indicate standard deviation.

biomass over a long time period. Different nutrient media are described and optimized for duckweed cultivation (Appenroth, 2015). However, in a continuous cultivation process, it is critical to keep the nutrient concentrations and ratios as stable as possible to maintain maximum growth rate at all times. Duckweeds quickly and preferentially take up ammonium over nitrate (Zhou et al., 2021), but keeping the concentration and ratio stable is important for high growth rates and crude protein contents (Petersen et al., 2021). Therefore, a continuous ammonium supply in intervals was installed.

The average concentration of nitrate-N in the liquid medium in the IVF was 0.7 mg l^{-1} below the target value (12.2 mg l^{-1}) and ammonium-N was 0.8 mg l^{-1} below target value (3.5 mg l^{-1}). Except for magnesium, sulphate, calcium, manganese and sodium (which were all present in high concentrations in the local tap water), all other nutrients were below the target value. In case of potassium, on average only half of the intended concentration was present in the solution, while iron was detected in a concentration more than ten times lower compared to the target value.

The presented data show that nutrient concentrations in our IVF were not stable at all times. The fluctuations differed in intensity for different nutrients. They were more intense for nutrients, such as ammonium, boron, manganese, iron and zinc, and less intense in the case of magnesium, sulphate and sodium. For calcium and sulphate, the variation coefficient is below 5%, while for manganese it is above 90%.

The ratio between nitrate-N and ammonium-N was 4.3:1 on average during the whole production phase. This is very close to the target ratio of 4:1, which achieved high RGRs, protein contents and protein yields in *L. minor* and *W. hyalina* (Petersen et al., 2021). Hecht and Mohr (1990) and Mehrer and Mohr (1989) explained that ammonium accumulation is not well regulated by plants, thus a higher ammonium concentration can have detrimental effects on plants. They called it ammonium toxicity syndrome. It has been reported that also the ratio of calcium to magnesium influences *L. minor* growth. The obtained ratio of 5.5:1 is in-between the reported ratios of 3:1 and 6.1:1, which resulted in RGRs of 0.164 d^{-1} and 0.148 d^{-1}, respectively (Walsh et al., 2020).

The continuous ammonium dosing stabilized the EC-value, meaning it decreased only slowly. As a consequence, stock solutions A, B and C were seldom dosed, which lead to a decreasing concentration of certain

nutrients over time. Furthermore, the amount of added ammonium was not adapted to the varying quantity of duckweed in the IVF at different points of time, which could explain the fluctuations in ammonium-N concentrations. These examples show that the use of EC-based dosing systems is inaccurate and can cause a diminishment or enrichment in certain substances in a re-circulating system, when there is an imbalance between the stock solution composition and the actual plant requirement. This imbalance will increase the longer a re-circulating system is in operation. A nutrient deficiency or overconcentration can cause reduced plant growth and quality and in severe cases even death of the cultivated plants. Therefore, new approaches should be tested, such as stationary ion-selective (Richa et al., 2021), ion-sensitive field-effect transistors (Bamsey et al., 2012) or mid-infrared sensors (Fan et al., 2012) coupled with a dosing system. When such a system will work reliably with mineral fertilizers in the future, the use of other promising nutrient sources, such as swine wastewater (Zhou et al., 2019) or anaerobically digested swine wastewater (Hu et al., 2019), can be tested and evaluated in an IVF.

4.3. Biomass quantity and harvesting process

The presented IVF for an automated duckweed biomass production continuously yielded fresh duckweed biomass. An average of 0.9 kg FW/53 g dried biomass was harvested per day with the above-described settings, which extrapolates to 620 kg month^{-1} ha^{-1} or 7.54 t year^{-1} ha^{-1} DM. This is comparable to the *L. minor* harvest of 702.5 kg month^{-1} ha^{-1} DM with an average protein content of 27% reported by Chakrabarti et al. (2018), when grown on inorganic fertilizers. Devlamynck et al. (2021) reported a yield of 8.1 t year^{-1} ha^{-1}, based on a 175-day growing season, when cultivating *L. minor* on a synthetic N-medium. Other studies report potential productivities of up to 68 t year^{-1} ha^{-1} (Mohedano et al., 2012), 70 t year^{-1} ha^{-1} (Calicioglu et al., 2021) or even 105 t year^{-1} ha^{-1} (Zhou and Borisjuk, 2019). These studies indicate that yields can be increased, however, growth conditions need to be considered when comparing these productivities. None of the other data sets used for these projections were obtained from a cultivation in a comparable environment. The applied conditions are optimized for a continuous production in the presented IVF.

The harvesting process in the presented IVF was executed by visual judgment at irregular time intervals. When the fronds overlap in several layers within the basins, a variable quantity of *L. minor* was removed via the installed harvesting system from the continuous production process in the IVF. After harvesting, a duckweed surface coverage of ca. 80% was always left in the basins to obtain reproducible results. However, this resulted in a slightly varying quantity of biomass left in the basins to continue growing. In order to quantify the duckweed biomass per basin at any time and thereby determine the optimal moment for harvest, in a first step the capacity limit for optimal duckweed growth in the IVF must be identified. In a second step the duckweed density in each basin must be automatically determined (e.g. by optical sensors coupled with an image processing software (Coughlan et al., 2022), in order to automatically initiate and stop the harvesting process at defined duckweed densities. In any case, a more frequent harvesting regime can be recommended. This favours nutrient recovery and biomass production (Xu and Shen, 2011). Calicioglu et al. (2021) suggested a harvest frequency of 1 day and a harvest ratio of 0.35 g g^{-1}. These suggestions, however, have to be adapted to the growth rates in the duckweed IVF.

4.4. Biomass quality

The achieved CP content of 32% (DM) has been reported previously by Kabir et al. (2005). However, widely varying compositions for *L. minor* have been investigated with CP contents ranging from 18.4 (Yilmaz et al., 2004) to 40.2% CP in DM (Khanum et al., 2005). Common protein sources for feed production are soybean meal and rapeseed meal. Those CP levels range from 38.8 to 52.1% (DM) and 30.3–37.5% (DM), respectively (Durst et al., 2021). Novel protein sources, such as insects or microalgae *Chlorella* contain CP at levels of 45.3% and 39.5%, respectively (Durst et al., 2021). The aim of our study was to produce duckweed as a protein-rich input for feed production. Therefore, strategies to increase CP content and thus nutritional value are required as part of product optimization.

Plant composition and thus CP content is influenced by cultivation conditions and, in particular, cultivation medium (Gwaze and Mwale, 2015). Comparable CP contents have been achieved using the same nutrient solution in a small re-circulating IVF for a cultivation phase of one week (Petersen et al., 2022). For the mass production of *L. minor*, cultivated on inorganic fertilizers, a CP content of 27% was reported by Chakrabarti et al. (2018), but compared to our cultivation conditions, their nitrate concentration was higher (15.3 mg l^{-1}) and no ammonium concentrations were reported. *Lemna minor* grown in a system with a constant supply of nutrients and average NO_3–N and NH_4–N concentrations of 6.3 and 0.3 mg l^{-1}, respectively, reached a CP content of 21.9%. This increased up to 39.4%, when the ammonium-N concentration increased to 39.1 mg l^{-1} (Iatrou et al., 2019). On the other hand, a decreasing ammonium-N content resulted in a decreased protein content (Hu et al., 2019). A synthetic N-medium with a concentration of 122 mg l^{-1} NO_3^--N and 0.71 mg l^{-1} NH_4^+-N yielded a CP content of 35% (Devlamynck et al., 2021). Khanum et al. (2005) reported 40.2% CP with a nutrient solution containing 26.6mg l^{-1} NH_4^+. The reported data indicate an impact of ammonium and nitrate concentrations on CP content. In order to increase the biomass CP levels, the nutrient control and dosing system can be optimized to continuously reach stable target values for ammonium and nitrate (see 4.2). Beyond that, increasing the target NH_4^+-level in the nutrient medium is a possible strategy to improve the CP content. However, a solely increased NH_4^+-content has been associated with a decreased plant productivity (Petersen et al., 2021) and NO_3^--N contents must be adapted as well.

To obtain a high protein feedstuff, the duckweed biomass can be processed by protein extraction and protein isolation. This has been investigated with various techniques and plant protein sources e.g. alfalfa, clover, grass or macroalgae (Santamaría-Fernández and Lübeck, 2020). These processes mainly consist of three steps: First, plant material is chopped and the “green juice” is pressed out (Hojilla-Evangelista et al., 2017). In the next step, protein is precipitated with different techniques, such as coagulation or acidification. In the last step, protein is concentrated by separation and drying (Santamaría-Fernández and Lübeck, 2020). Rojas et al. (2014) observed a high digestibility in piglets and a high amino acid content for a *Lemna* protein concentrate with 68% CP indicating an improved product quality after processing. However, protein yields from leaves are mostly below 50% with an exception for alfalfa (Santamaría-Fernández and Lübeck, 2020).

In conclusion, future trials can assess, whether adaptions of cultivation conditions or further processing steps are more effective regarding protein enrichment. Therefore, plant productivity but also general efficiency of the processing steps and cultivation as well as the respective effect on nutritional value have to be weighed.

It has been stated for duckweed that the amino acid profile is stable for individual species (Appenroth et al., 2017). The findings of Amado et al. (1980) indicate that amino acid contents of different *L. minor* clones are comparable only to a limited extent. As clone 9441 (*L. minor*) was used in this study and the study of Appenroth et al. (2017), the amino acid profiles of these studies can be compared. Deviations in amino acid content are generally below 1 g amino acid per 100 g CP. However, Arginine is an exception with a difference of 1.9 g Arg/100 g CP. This can be supported by the findings of Devlamynck et al. (2021), who detected a significant influence of cultivation conditions on the Arginine content.

For all livestock species, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine are essential amino acids. Moreover, arginine is essential for poultry. Cysteine and tyrosine are semi-essential amino acids because they can

only be produced from methionine and phenylalanine, respectively (Fuller, 2004). Two methods (amino acid index and ideal amino acid ratio) were used to assess the amino acid profile of the harvested biomass. Considering the amino acid index (AAI), it appears that the nutritional value of *L. minor* protein is comparable to soybean meal and rapeseed meal, but also to other novel protein sources like algae (*A. platensis*) and insect meal of black soldierfly (BSF) larvae meal (Table 4).

The AAI neglects the fact that essential amino acids are needed in defined proportions and deficiencies of individual amino acids cannot be compensated by a surplus of other amino acids. Deficiency in one essential amino acid leads to loss of appetite and consequently to undernutrition (Santamaría-Fernández and Lübeck, 2020). In order to match the requirements of the livestock species, all essential amino acids must be supplied in a defined proportion (Kamphues et al., 2014). Therefore, the concept of ideal protein can be considered, where the ratio for individual amino acids is expressed relative to lysine (100) (Pastor, 2014). The amino acid ratios of the mentioned feedstuffs and the requirements for the ideal protein of piglets and broiler chicks can be evaluated. The respective values are shown in Table 4.

In pigs, lysine is the first amino acid to be deficient when the CP content of the diet is reduced. In poultry, this first limiting amino acid is methionine (Díaz-Gaona et al., 2021). The lysine content of the yielded biomass is comparable to rapeseed meal (5,3 g/100 g CP), but especially soybean meal (6.1 g/100 g CP (Sauvant et al., 2004)) has a higher lysine content than the *L. minor* biomass (5.4 g/100 g CP). The meal of BSF larvae has also a higher lysine content (6,6 g/100 g CP, Makkar and Ankers, 2014). With regard to the amino acid ratio, duckweed contains more sulphur-containing amino acids (SAA, Met + Cys) than soybean meal and BSF larvae meal. Moreover, *L. minor* protein is rich in tryptophan, arginine, valine, leucine and arginine, and matches the requirements of pigs and poultry with an exception for the sulphur containing amino acids. This deficiency in SAAs has previously been confirmed for *L. minor* by Devlamynck et al. (2021) and also Appenroth et al. (2017). As the described parameters only consider amino acid contents and do not regard availability, future studies might investigate the amino acid digestibility for livestock, such as broiler chickens.

5. Conclusion and further perspectives

This is the first detailed report on the construction, technicalities and operation as well as biomass yield and quality of an IVF for duckweed biomass production. An average daily FW yield of 0.9 kg with a CP content of 32% in the DM over a 40-day production phase is a first accomplishment in this field. The investigated parameters indicate that the produced duckweed biomass can be used as a soybean meal replacement in monogastric animal nutrition. However, in order to maximize the nutritional value of the duckweed biomass, CP contents should be increased.

The optimization of the presented IVF is still in progress, aiming at a maximum level of automation, biomass production and product quality with a minimum resource input. Therefore, in the future, the energy, water and nutrient input will be recorded automatically. This way, it is possible to determine the input in relation to the yield. New approaches in nutrient supply will be tested and evaluated, as well as new technologies to optimize the harvesting process.

This IVF can be used as a model system for the conduction of scientific experiments or duckweed biomass production in a controlled environment as well as for further innovations and upscaling processes.

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CRediT authorship contribution statement

Finn Petersen: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft, Writing – review & editing, Visualization. **Johannes Demann:** Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft, Writing – review & editing, Visualization. **Jannis von Salzen:** Investigation, Data curation, Visualization. **Hans-Werner Olfs:** Writing – review & editing, Supervision, Funding acquisition. **Heiner Westendarp:** Supervision, Project administration, Funding acquisition. **Petra Wolf:** Writing – review & editing, Supervision. **Klaus-Jürgen Appenroth:** Writing – review & editing, Supervision. **Andreas Ulbrich:** Conceptualization, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Table 4
Amino acid ratios (%) of various protein sources compared to the ideal amino acid ratio (IAAR) of broiler chickens and piglets.

	Lemna minor clone 9441						IAAR	
	This study	Appenroth et al. (2017)	A. platensis[a]	BSF larvae[b]	Soybean meal[c]	Rapeseed meal[c]	broiler chicks[d]	piglets[e]
Lysine	100	100	100	100	100	100	100	100
Methionine	28	32	59	32	23	38	38	26
Met + Cys	60	54	72	33	47	84	72	57
Threonine	71	82	72	56	64	81	74	64
Tryptophan	25	–	43	8	21	23	15	18
Valine	100	92	87	124	78	94	82	69
Isoleucine	79	74	83	77	75	76	73	55
Leucine	140	146	181	120	120	126	109	97
Histidine	35	30	61	45	43	49	32	31
Arginine	122	94	107	85	121	113	110	40
Phenylalanine	89	90	89	79	82	73	63	59
Phe + Tyr	138	152	162	183	136	127	122	92
AAI broiler chickens	1.20	–	1.23	1.25	1.24	1.22		
AAI piglets	1.34	–	1.38	1.40	1.39	1.37		

[a] Safi et al. (2013).
[b] black soldierfly, Makkar et al. (2014).
[c] Sauvant et al. (2004).
[d] 3–5 weeks, National Research Council (1994).
[e] 10–20 kg body weight, National Research Council (1998); AAI: amino acid index.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2022.134894.

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