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Quantitative and Qualitative Traits of Duckweed (*Lemna minor*) Produced on Growth Media with Pig Slurry

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Abstract: Duckweed is a plant with high phytoremediation abilities, which is why it is used in the process of cleaning the aquatic environment. The present study aimed to determine the effect of various concentrations of pig slurry added to the growth media used to produce duckweed (*Lemna minor*) (laboratory Warsaw University of Life Sciences—SGGW) (experimental groups 1–9, pig slurry concentration (%): 1—2.00, 2—1.50, 3—1.00, 4—0.75, 5—0.50, 6—0.25, 7—0.12, 8—0.06, 9—0.03, control group 0—0.00). The contents of nutrients in the growth media could be classified as high (gr. 1–3), optimal (gr. 4–6), and deficient (gr. 7–9). Analyses were conducted for duckweed yield and growth medium parameters (pig slurry concentration, pH, salinity, temperature, TDS, and EC) on days 0, 10, 20, and 30 of the experiment. No growth or poor growth of duckweed were noted in groups 1, 6–9, and 0. In turn, satisfactory yields of duckweed green mass were recorded in groups 3–5, which allowed choosing them for further observations and analyses, including proximate composition (including protein content); contents of Ca, Mg, K, Na, Zn, Cu, Cd, Pb, Al, Cr, and α-tocopherol; and carotenoids—β-carotene, α-carotene, violaxanthin, zeaxanthin, lutein, amino acids, fatty acids as well as N-NH_4 and N-NO_3. The plant material had an acceptable proximate composition and nutritionally safe analyzed component contents. Appropriate, stable growth medium conditions allowed the production of satisfactory duckweed yields. The study results allowed us to conclude that it is feasible to obtain feed material meeting basic quality standards by maintaining a closed circuit of duckweed culture, and use in the agricultural environment is possible through harnessing pig slurry for its production and ensuring its optimal growth conditions.

Keywords: duckweed; pig slurry; growth; chemical composition; nutritional value; alternative protein source

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1. Introduction

Meeting nutritional demands of a continuously growing human population while maintaining a human-friendly environment poses a global problem today [1]. The growing demand for protein derived from animal products—meat, milk, eggs, has contributed to the intensification of animal production across the world [2,3]. The livestock population has increased, and 90% of these animals are reared in the industrial farming system and fed with standardized feedstuffs [4]. It is therefore necessary to ensure greater availability of plant-derived feed materials capable of covering energy and protein demands for the production of feed mixtures used in animal feeding. Among the many components commonly used in animal feed mixtures, soybean meal serves as the most valuable source of protein [5]. Other meals, like those made of rapeseed, sunflower, or legume seeds, are used less frequently and rather as additives to soybean meal [6]. The storage and disposal of animal waste, i.e., manure, dung, and slurry, is a serious issue for environmental protection strategies [7].

The European Union (EU) promotes an initiative to minimize waste and greenhouse gas emissions by converting waste into energy and other re-usable sources [8]. Therefore, the possibility of combining individual components of animal production into one closed circuit, namely using farm animal excreta to produce feed mixtures that can further be used in animal feeding/fattening [9], is currently extensively sought.

As part of the recovery of fertilizing substrates from various growth media, aquatic plants can be used for the treatment of various types of wastewater [10–12]. Duckweed has been found to be important in sustainable production and has spurred social interest due to its unique morphological characteristics and phytoremediating potential. It is a small plant composed of several leaves that is free-floating—it floats on the surface, quickly multiplies, and easily adapts to different environments [13,14]. Duckweed is commonly used for wastewater treatment at Lemna-type treatment plants [15]. Its application for water purification has been driven by its morphological features: a well-developed root system and rapid vegetative growth as well as ease of harvesting and further management [16]. The growth rate of duckweed in various growth media is determined by the media's abundance in nutrients (nutrient concentrations) and environmental conditions (temperature and pH of the medium, insolation, day length, and wind speed) [17,18]. Under optimal growth conditions, duckweed produces large amounts of biomass rich in protein and nutrients and can therefore be used in commercial livestock and aquaculture feeding [19]. Large-scale production of duckweed takes place in tropical countries, where natural conditions are appropriate for its rapid growth and high biomass production within a relatively short time span [20]. In animal feeding, it can be used as a meal (dried) or in a natural form (fresh green biomass). Duckweed production can provide 4–5 times more protein per hectare than soybean cultivation [21]. Other advantages of this aquatic plant include: (1) it is not genetically modified; (2) it does not contain gluten; and (3) it does not require arable land or the use of mineral (artificial) fertilizers. In contrast, potential threats include heavy metals, dioxins, phenols, pesticides, and pathogens it can accumulate [22].

Natural fertilizers are very valuable nutrient-rich by-products of animal production and are useful for crop fertilization. The content of nutrients in fertilizers varies and depends on many factors, including animal species and breed, feed mixture type, and bedding in terms of both its type and use. In order to preserve the highest fertilizing value, certain methods are recommended regarding the collection, storage, and use of natural fertilizers [23]. Slurry is a mixture of feces and urine with an admixture of water and is generated during pig and cattle rearing. The problem faced by producers of these animals is to provide appropriate tanks for its storage and sufficiently large areas of arable land for its agricultural management. Excess volumes of slurry applied to arable land lead to excessive accumulation of nitrogen and phosphorus in the soil, which leach into surface and underground waters [24]. Incorrect use of slurry can also lead to its direct runoff into water bodies and their local contamination [25]. Algae bloom (proliferation of blue-green algae—cyanobacteria) and the toxic effects of ammonia and nitrites cause the death of aquatic plant and animal species [26,27]. Additionally, slurry is a source of greenhouse gases, such as nitrous oxide and methane [28].

The aim of this research was to determine the effect of different concentrations of pig slurry added to the growth media used to produce duckweed as well as to determine the chemical composition and nutritional value of duckweed.

2. Materials and Methods

2.1. Course of the Experiment

The experiment was carried out in a laboratory (Warsaw University of Life Sciences—SGGW) with temperature maintained at 23 ± 1 °C, a light:dark cycle of 16:8 h, and a light intensity of 7000 lux. Thirty plastic vessels with a capacity of 14 L each were placed in the room, and each was filled with 12 L of clean water (municipal water/tap water). The liquid fraction of pig slurry from a farm where pigs were fed with a cereal feed mixture with the

addition of protein (soybean meal and rapeseed meal) and a mineral-vitamin premix, was used as a medium for duckweed growth.

Table 1 presents the chemical composition of the slurry used in the study. The determinations were made according to the procedures in force at the Regional Chemical and Agricultural Station in Warsaw/Poland. Slurry was mixed with water to obtain growth media with the following concentrations: group 1—2.0%, group 2—1.5%, group 3—1.0%, group 4—0.75%, group 5—0.50%, group 6—0.25%, group 7—0.12%, group 8—0.06%, and group 9—0.03%; and the control medium (group 0) without the liquid slurry—0%. Three replicates (media preparation and duckweed growth) were performed for each group. The content of nutrients in the prepared media varied and could be categorized as: very high (groups 1–3), optimal (groups 4–6), and deficient (groups 7–9).

Table 1. Chemical composition and pH of pig slurry used in the study (mean values and standard deviations).

Specification	Pig Slurry
pH	7.52
Dry matter (%)	4.21 ± 0.21
Nitrogen total (% fresh weight)	0.58 ± 0.06
N-NH_4 (% fresh weight)	0.390 ± 0.078
P total (% fresh weight)	0.11 ± 0.02
P_2O_5 (% fresh weight)	0.25 ± 0.04
K (% fresh weight)	0.30 ± 0.06
K_2O (% fresh weight)	0.36 ± 0.07
Mg (% fresh weight)	<0.10
MgO (% fresh weight)	<0.16
Ca (% fresh weight)	0.13 ± 0.03
CaO (% fresh weight)	0.18 ± 0.4
Pb (mg/kg d.m.)	<10.2
Cd (mg/kg d.m.)	0.31 ± 0.07
Ni (mg/kg d.m.)	<5.1
Cr (mg/kg d.m.)	6.3 ± 1.6
Cu (mg/kg d.m.)	243 ± 49
Zn (mg/kg d.m.)	1083 ± 217
Hg (mg/kg d.m.)	<0.010
Fe (mg/kg d.m.)	1601 ± 320
Mn (mg/kg d.m.)	717 ± 143
B (mg/kg d.m.)	44.0 ± 8.8
Mo (mg/kg d.m.)	11.9 ± 2.4
Na (% fresh weight)	0.05 ± 0.01

d.m.—dry matter.

Duckweed used in the experiment was obtained from an aquarium shop that professionally cultures it. About 200 plants (*Lemna minor*, LM) were placed in each vessel. During the 30-day pot test, the dynamics of duckweed growth were observed by counting the number of plants every 5 days. All duckweed plants were collected from the vessels with the highest duckweed yield (groups 2, 3, 4, 5) and subjected to chemical analyses. The following parameters were measured throughout the experiment (days: 0, 10, 20, and 30) using a Combo 5IN1 m model EZ-9909SP: pH, total content of dissolved solids (TDS), electrical conductivity of liquid (EC), salination, and temperature of the growth media. The results obtained represent the mean value from the three vessels for each replication.

2.2. Data Analyses

The harvested fresh duckweed was divided into two portions. The first was determined for dry matter content and after drying (temperature 60 °C, time 12 h) for contents of total protein, crude ash, crude fat, crude fiber, and minerals. The second portion was frozen. After thawing and freeze-drying, it was analyzed for the contents of carotenoids, fatty acids, nitrates and nitrites, as well as for amino acid composition. The chemical

composition of duckweed was determined according to the AOAC standard methods [29]. Dry duckweed samples were homogenized and mineralized (HNO_3, H_2O_2, and HCl) using a Model DK 20 device (VELP Scientifica, Usmate, Italy). The phosphorus content of duckweed was determined according to a method using vanadomolybdo-phosphoric acid using a Genesys 10 UV-VIS spectrophotometer (ultraviolet and visible light range) (Thermo Electron Corporation, Madison, WI, USA). The contents of Ca, Mg, K, Na, Zn, Cu, Cd, Pb, Al, and Cr in duckweed were determined using a SOLAAR atomic absorption spectrometer (AAS) (Thermo Elemental, Cambridge, UK). Carotenoids were separated, and their content was determined using an HPLC system (Dionex, Sunnyvale, CA, USA) equipped with a CoulArray electrochemical detector (ESA Inc., Chelmsford, MA, USA). Fatty acid methylation was carried out via transesterification [30]. Fatty acid concentrations were determined using an Agilent 7890 GC gas chromatograph (Agilent Technologies, Waldbronn, Germany), a flame ionization detector, and a Varian Select FAME column (Varian, Agilent Technologies, Waldbronn, Germany) according to the methodology described by Puppel et al. [31]. The amino acid composition was determined according to the methodology described by Appenroth et al. [32], and the level of nitrates and nitrites were determined according to the methodology described by Mir [33].

2.3. Statistical Analysis

The results were statistically elaborated using the IBM SPSS Statistics 28 package. The Shapiro–Wilk test was used to check the normal distribution. Differences between the groups were tested with the Kruskal–Wallis test. Boferroni correction for multiple comparisons was used. The tables present mean result and standard error. The differences were determined to be statistically significant at $p \leq 0.05$ or $p \leq 0.01$.

3. Results

3.1. Duckweed Growth

Figure 1 presents the mean yield of duckweed in groups 1–9 after the 30-day experiment.

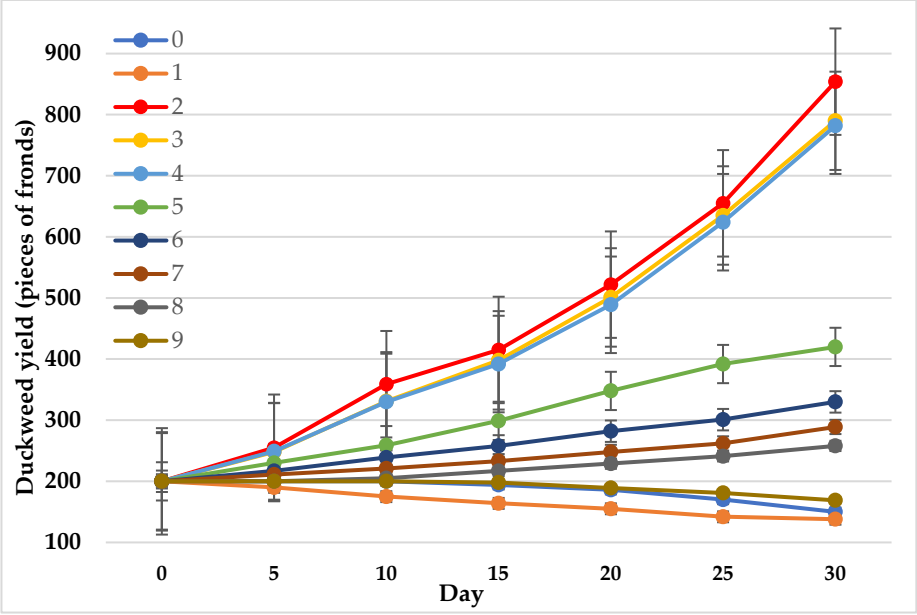

Figure 1. Yield of duckweed produced in growth media with various concentrations of pig slurry. Legend: slurry concentration in the growth medium: group 0—0.00%, group 1—2.00%, group 2—1.50%, group 3—1.00%, group 4—0.75%, group 5—0.50%, group 6—0.25%, group 7—0.12%, group 8—0.06%, and group 9—0.03%.

In groups 0, 1, and 9, duckweed either did not proliferate or its green mass remained unchanged or decreased. In turn, the greatest increase in duckweed yield was noted in groups 2, 3, and 4. After 30 days of observation, a two-fold increase in duckweed green mass was noted in group 5, and small increases were noted in groups 6, 7, and 8, which generally showed a descending trend in duckweed yield. The results achieved for duckweed green mass growth allowed the selection of groups 2–5 for further observations and analyses.

3.2. Growth Medium Analyses

Figures 2–6 present the results of determinations of five quality parameters of the growth media performed for groups 2–5 (acidity—pH, total content of dissolved solids—TDS, electrical conductance of liquid—EC, salinity, and temperature) on days 0, 10, 20, and 30 of the experiment.

Figure 2 presents changes in the pH values of the growth media over 30 days of observations. On day "0", the pH values of media used in groups 2–5 ranged from 7.82 to 7.92 and on days 0–10 and 20–30 showed an ascending tendency, whereas between days 10 and 20, they were observed to fluctuate. Over the 30-day experimental period, the pH value showed a stable progression only in Group 5. On day 30, the highest pH was noted for Group 5—8.39 (0.50% slurry concentration in the medium), and the lowest was noted for group 4—8.06 (0.75% slurry concentration in the medium). The differences noted in pH values between groups 2 and 5 on day "0" and 30 were 0.12 and 0.33, respectively.

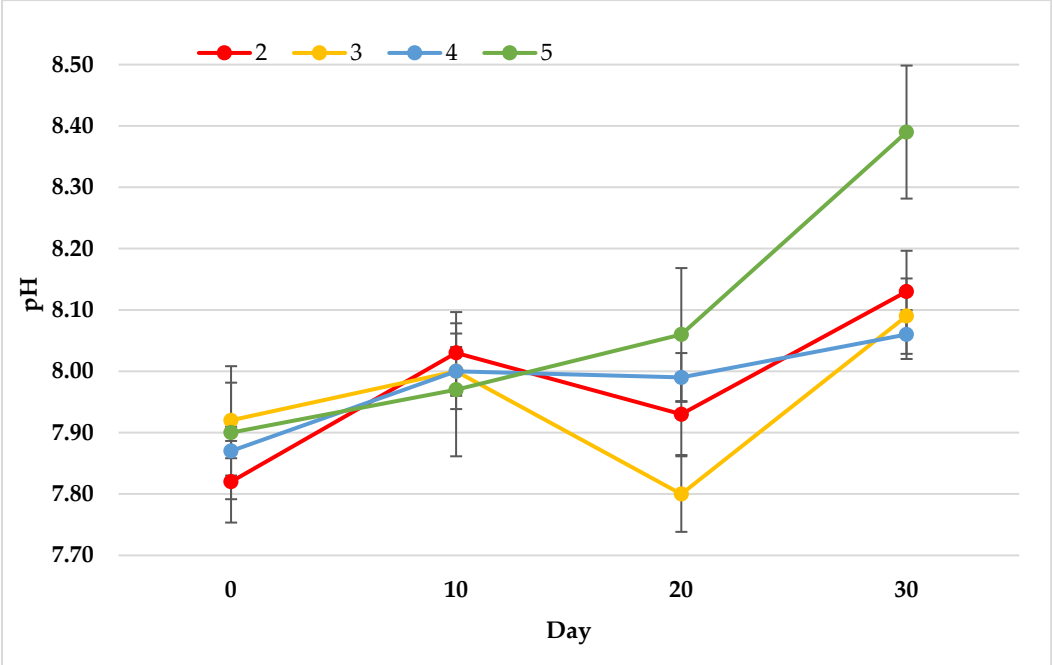

Figure 2. Changes in growth medium pH over time (day 0, 10, 20, and 30) in Groups 2–5. Legend: slurry concentration in the growth medium: Group 2—1.50%, Group 3—1.00%, Group 4—0.75%, and Group 5—0.50%.

Figure 3 depicts changes in the total content of all mobile ions of aqueous solutions of pig slurry (growth medium). From day "0" until day 20, the TDS contents were stable, and differences noted between groups were small. In the third stage of observations (days 20–30), the TDS contents increased significantly in all analyzed groups. A stable increase in TDS value over time (days 0–30) was noted only for Group 5. At the onset (day "0") and end (day 30) of the experiment, the TDS values ranged from 546 to 677 ppm in Group 2

and from 680 to 792 ppm in Group 5. The differences between these groups noted on the aforementioned days reached 131 and 112 ppm, respectively.

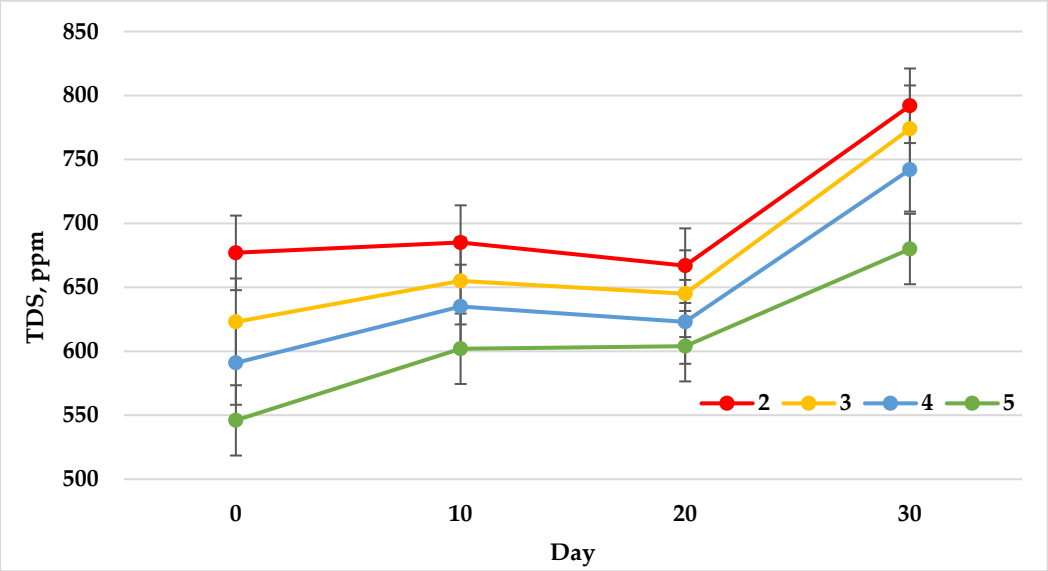

Figure 3. Changes in TDS content of the growth medium (total content of solids dissolved in water) over time (days 0, 10, 20, and 30) in Groups 2–5, ppm. Legend: slurry concentration in the growth medium: Group 2—1.50%, Group 3—1.00%, Group 4—0.75%, and Group 5—0.50%.

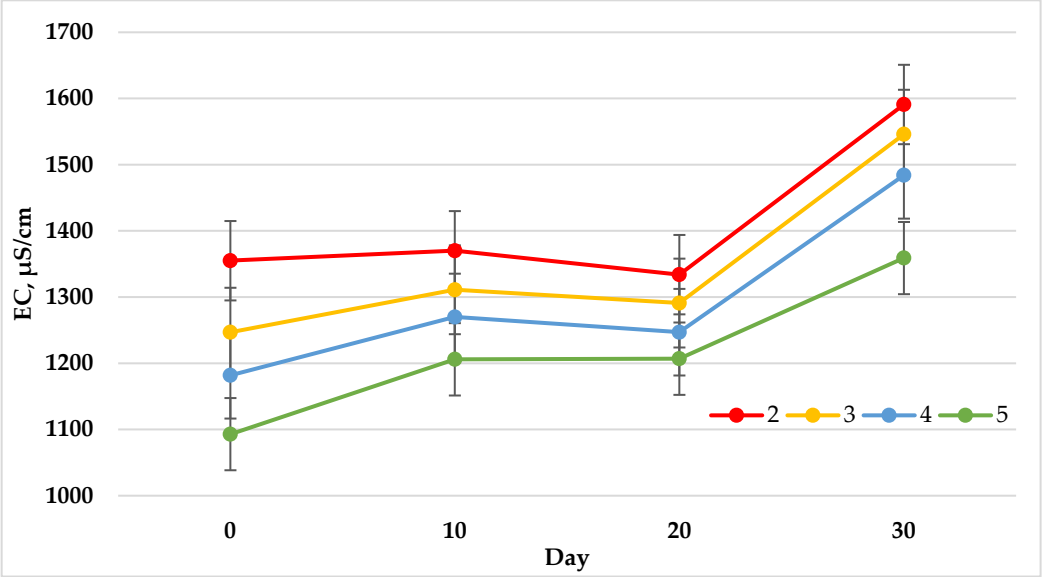

Figure 4. Changes in EC (electrical conductance of liquid) of the growth medium over time (days 0, 10, 20, and 30) in Groups 2–5, µS/cm. Legend: slurry concentration in the growth medium: Group 2—1.50%, Group 3—1.00%, Group 4—0.75%, and Group 5—0.50%.

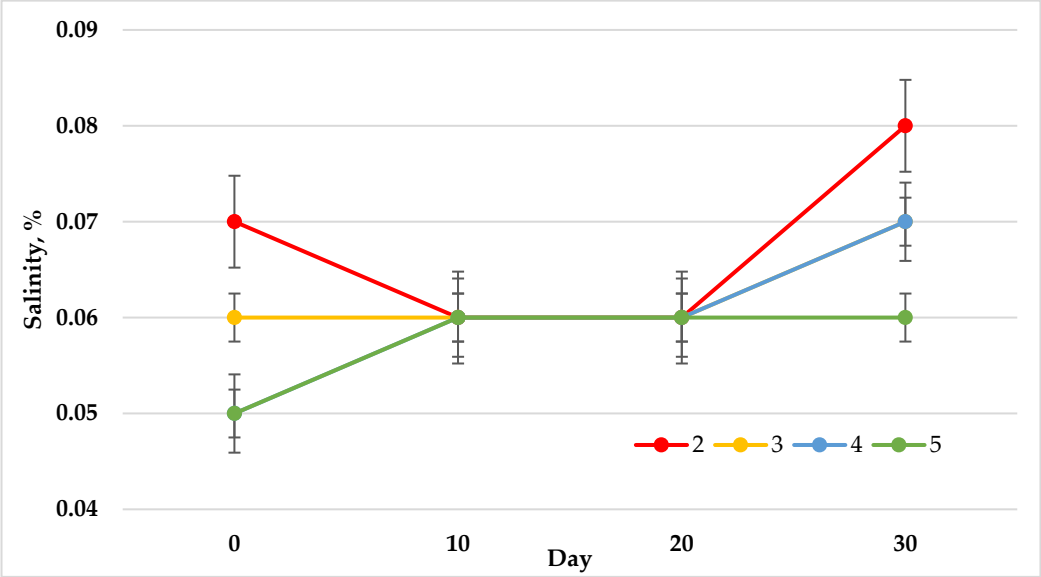

Figure 5. Changes in salt concentration in the growth medium over time (days 0, 10, 20, and 30) in Groups 2–5, %. Legend: slurry concentration in the growth medium: Group 2—1.50%, Group 3—1.00%, Group 4—0.75%, and Group 5—0.50%.

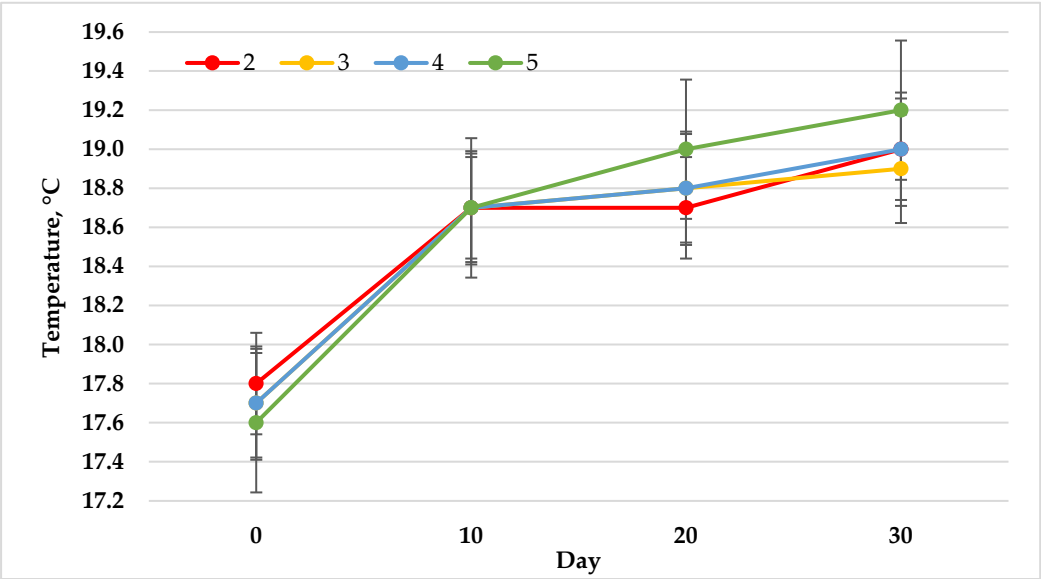

Figure 6. Changes in temperature of the growth medium over time (days 0, 10, 20, and 30) in groups 2–5, °C. Legend: slurry concentration in the growth medium: Group 2—1.50%, Group 3—1.00%, Group 4—0.75%, and Group 5—0.50%.

Figure 4 presents changes in the values of electrical conductance of liquid (EC), i.e., a measure of the total salt concentration in the growth medium. The EC values measured on day "0" and day 30 of the experiment reached 1093 and 1359 µS/cm, respectively, in Group 2 and 1355 and 1591 µS/cm, respectively, in group 5 (difference on day "0": 262 µS/cm, and difference on day 30: 232 µS/cm in favor of Group 5 vs. Group 2). The EC values determined for Groups 2–5 followed a similar tendency of changes over time (Figure 4) and were higher on day 30 than on day "0" (Figure 4).

Figure 5 presents the salinity of the growth media tested. Throughout the experiment, the salt concentration in the growth media used in Groups 2–5 varied insignificantly but remained low and ranged from 0.05 to 0.08%. Though the salinity determined in the growth

media of Groups 2–5 differed in time (Figure 5), it was found to be higher on day 30 than on day "0" in Groups 2 and 5.

Figure 6 presents changes in growth media temperature over the observation period. On day "0", the temperature of the growth media was the lowest (from 17.6 to 17.8 °C), increasing in the subsequent measurements. The greatest ramp increase in media temperature was observed in the first stage of the study, i.e., from the onset of observations until day 10 of experiment (change by 1 °C on average). On day 30, it was higher by 1.3–1.4 °C compared to day "0" and ranged from 18.9 to 19.2 °C in the analyzed groups.

3.3. Chemical Analyses of Duckweed

Tables 2–7 collate the results of chemical analyses made for the proximate composition as well as contents of macroelements, microelements, heavy metals, carotenoids, amino acids, fatty acids, nitrates, and nitrites in duckweed harvested in Groups 2, 3, 4, and 5.

Table 2. Chemical composition of duckweed.

Group	Dry Matter g kg^{-1}	Total Protein	Crude Fat	Crude Ash	Crude Fiber
		g kg DM			
2	53.5 ^{Ab}	417.5 ^{Ab}	32.5 ^{Ab}	232.4 ^{Ab}	82.3 ^{Ab}
3	47.9 ^{b}	360.8 ^{b}	29.9 ^{b}	221.7 ^{b}	87.3 ^{b}
4	42.9 ^{Aa}	348.6 ^{Aa}	26.8 ^{Aa}	215.9 ^{Aa}	90.4 ^{Aa}
5	49.1 ^{a}	373.1 ^{a}	28.2 ^{a}	224.8 ^{a}	86.6 ^{a}
SE	0.80	5.43	0.45	1.14	0.62
p-Value	0.001	0.001	0.001	0.001	0.001

Legend: pig slurry concentration in the growth medium: Group 2—1.50%, Group 3—1.00%, Group 4—0.75%, and Group 5—0.50%. AA—values in the columns with the same letters differ significantly at $p < 0.01$, aa, bb—values in the columns with the same letters differ significantly at $p < 0.05$.

Table 3. Content of macroelements, microelements, and heavy metals in duckweed.

Group	Macroelements				Microelements			Heavy Metals			
	Ca	P	K	Mg	Na	Zn	Cu	Cd	Pb	Al	Cr
	g kg^{-1} DM				mg kg^{-1} DM						
2	3.55 ^{A}	3.95 ^{A}	21.85 ^{A}	4.01 ^{A}	7.54 ^{A}	152.12 ^{A}	3.18 ^{A}	0.05	1.17 ^{A}	151.12 ^{Aa}	0.71 ^{A}
3	4.02	4.12	19.52	3.58	6.24	148.89	4.05	0.05	1.21	142.17	0.75
4	4.41 ^{Aa}	4.68 ^{A}	18.95 ^{A}	3.33 ^{A}	5.98 ^{A}	131.12 ^{A}	4.74 ^{A}	0.04	1.27 ^{A}	134.45 ^{A}	0.79 ^{A}
5	3.89 ^{a}	4.31	19.69	3.52	6.71	138.28	3.41	0.03	1.22	139.12 ^{a}	0.73
SE	0.06	0.05	0.23	0.05	0.12	1.75	0.13	0.01	0.01	1.27	0.01
p-Value	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.060	0.001	0.001	0.001

Legend: slurry concentration in the growth medium: Group 2—1.50%, Group 3—1.00%, Group 4—0.75%, and Group 5—0.50%. AA—values in the columns with the same letters differ significantly at $p < 0.01$, aa—values in the columns with the same letters differ significantly at $p < 0.05$.

Table 4. Content of α-tocopherol and carotenoids in duckweed.

Group	α-Tocopherol	β-Carotene	α-Carotene	Violaxanthin	Zeaxanthin	Lutein
	mg kg^{-1} DM					
2	67.2 ^{A}	327.2 ^{Aa}	21.1 ^{Aa}	249.8 ^{Aa}	30.7 ^{B}	579.6 ^{Ab}
3	66.4	324.8	20.4 ^{a}	241.2 ^{a}	31.9	574.2 ^{b}
4	64.9 ^{A}	312.5 ^{A}	19.9 ^{A}	231.8 ^{Ab}	32.8 ^{AB}	568.8 ^{Aa}
5	66.5	319.5 ^{a}	20.7	244.7 ^{b}	30.7 ^{A}	576.5 ^{a}
SE	0.19	1.18	0.10	1.37	0.19	0.83
p-Value	0.001	0.001	0.001	0.001	0.001	0.001

Legend: slurry concentration in the growth medium: Group 2—1.50%, Group 3—1.00%, Group 4—0.75%, and Group 5—0.50%. AA, BB—values in the columns with the same letters differ significantly at $p < 0.01$, aa, bb—values in the columns with the same letters differ significantly at $p < 0.05$.

Table 5. Amino acid composition of duckweed protein (g/100 g protein).

Amino Acid	Group				SE	*p*-Value
	2	3	4	5		
Alanine	2.77	2.70	2.69	2.75	0.01	0.057
Arginine	3.03	3.01	3.01	3.02	0.01	0.844
Aspartic acid	3.61	3.56	3.55	3.58	0.01	0.184
Glutamine acid	6.31	6.29	6.27	6.30	0.01	0.678
Glycine	2.73	2.72	2.71	2.72	0.01	0.739
Histadine	0.82	0.81	0.79	0.81	0.01	0.629
Isoleucine	2.02	1.99	1.99	2.01	0.01	0.355
Leucine	4.09 ^{A}	4.02	4.00 ^{A}	4.05	0.01	0.009
Lysine	2.58 ^{Ab}	2.33 ^{b}	2.21 ^{Aa}	2.44 ^{a}	0.03	0.001
Methionine	0.75	0.73	0.71	0.74	0.01	0.306
Phenylalanine	2.22 ^{Ab}	2.09 ^{b}	2.01 ^{Aa}	2.19 ^{a}	0.02	0.001
Proline	1.16	1.13	1.12	1.15	0.01	0.276
Serine	2.26	2.22	2.20	2.24	0.01	0.073
Theronine	1.85 ^{a}	1.78	1.75 ^{a}	1.80	0.10	0.016
Tryptophan	0.31	0.28	0.27	0.30	0.01	0.384
Tyrosine	1.81	1.77	1.75	1.78	0.01	0.091
Cysteine	0.37	0.35	0.34	0.36	0.01	0.602
Valine	2.55 ^{Aa}	2.42 ^{a}	2.40 ^{A}	2.48	0.01	0.002

Legend: slurry concentration in the growth medium: Group 2—1.50%, Group 3—1.00%, Group 4—0.75%, and Group 5—0.50%. AA—values in the rows with the same letters differ significantly at $p < 0.01$, aa, bb—values in the rows with the same letters differ significantly at $p < 0.05$.

Table 6. Fatty acid composition of duckweed as a percentage of total fatty acids (%).

Items	Group				SE	*p*-Value
	2	3	4	5		
10:0	0.8	0.6	0.4	0.5	0.06	0.055
14:0	2.4	2.3	1.9	2.1	0.07	0.079
16:0	31.1 ^{a}	30.7	29.4 ^{a}	30.1	0.15	0.036
17:0	15.4 ^{Ab}	14.8 ^{a}	13.2 ^{Aa}	14.1 ^{b}	0.19	0.001
18:0	5.3 ^{Aa}	4.8	4.0 ^{A}	4.3 ^{a}	0.13	0.002
20:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
22:0	1.1	1.0	0.6	0.8	0.07	0.087
18:1-c9	4.3 ^{a}	4.0	3.4 ^{a}	3.7	0.11	0.015
18:1-c11	1.8 ^{A}	1.4	0.9 ^{A}	1.1	0.10	0.008
18:2-c9	17.7 ^{Aa}	16.8 ^{b}	15.4 ^{Ab}	16.1 ^{a}	0.20	0.001
18:3	35.2 ^{Aa}	34.1	33.0 ^{A}	33.6 ^{a}	0.19	0.001
20:1	1.1	0.9	0.6	0.8	0.06	0.118
20:5	0.7	0.6	0.3	0.4	0.05	0.071
Σ SFA	56.1 ^{Aa}	54.3 ^{b}	49.5 ^{Ab}	51.9 ^{a}	0.54	0.001
Σ MUFA	7.2 ^{A}	6.3 ^{a}	4.9 ^{Aa}	5.6	0.21	0.001
Σ PUFA	53.6 ^{Aa}	51.5 ^{b}	48.7 ^{Ab}	50.1 ^{a}	0.41	0.001

n.d.—not detected. Legend: slurry concentration in the growth medium: Group 2—1.50%, Group 3—1.00%, Group 4—0.75%, and Group 5—0.50%. AA—values in the rows with the same letters differ significantly at $p < 0.01$, aa, bb—values in the rows with the same letters differ significantly at $p < 0.05$.

The proximate chemical composition of duckweed from Groups 2–5 was similar and comparable (Table 2). The highest contents of protein, lipids, and ash were determined in duckweed from Group 2, and the lowest were found in that from Group 4 ($p < 0.001$). The difference between the highest (Group 2) and the lowest (Group 4) protein content was 68.9 g/kg DM ($p < 0.001$). Opposite observations were made for crude fiber. Its highest content was found in duckweed from Group 4, and the lowest content was found in that from Group 2, with the difference amounting to 8.1 g/kg DM ($p < 0.001$).

Table 7. Contents of various nitrogen species in duckweed.

Group	$N\text{-}NO_3$	$N\text{-}NH_4$
	mg N/g DM	
2	0.099 ^{Aa}	0.141
3	0.056 ^{Ab}	0.074 ^{A}
4	0.086 ^{b}	0.141
5	0.069 ^{a}	0.167 ^{A}
SE	0.01	0.01
p-Value	0.001	0.001

Legend: slurry concentration in the growth medium: group 2—1.50%, group 3—1.00%, group 4—0.75%, and group 5—0.50%. AA—values in the columns with the same letters differ significantly at $p < 0.01$, aa, bb—values in the columns with the same letters differ significantly at $p < 0.05$.

The highest K content was determined in duckweed from Group 2 ($p < 0.001$), whereas contents of the other assayed macroelements (Ca, P, and Mg) were similar among the groups, with the lowest Ca ($p < 0.001$) content noted in Group 2 and the lowest Mg ($p < 0.001$) content noted in Group 4 (Table 3). Duckweed was rich in Zn, the highest content of which was determined in plants grown in Group 2 ($p < 0.001$). Contents of heavy metals in duckweed from Groups 2–5 were similar, whereas significant differences were noted between Groups 2 and 4 in terms of Pb, Al, and Cr contents ($p < 0.01$).

Table 4 collates the contents of α-tocopherol and carotenoids in duckweed. Differences in their contents noted between the groups were negligible. High contents per mass unit were determined for lutein and β-carotene. Statistically significant differences in the content of α-tocopherol and carotenoids were found between Groups 2 and 4 ($p < 0.001$).

Table 5 presents the fatty acid profile of duckweed grown on the media with pig slurry addition (Groups 2–5). The test material was found to contain both exogenous amino acids (lysine, methionine, threonine, leucine, isoleucine, valine, tryptophan, and phenylalanine) and endogenous amino acids (glycine, alanine, arginine, aspartic acid, glutamic acid, serine). Higher contents of amino acids were determined in duckweed from Group 2 compared to Groups 3–5, and lower contents were found in Group 4 compared to Groups 2–3 and 5. Statistically significant differences ($p < 0.01$) in the content of amino acids were found between Groups 2 and 4 for leucine, lysine, phenylalanine, and valine.

Duckweed was also analyzed for fatty acid composition (Table 6). The contents of SFAs, MUFAs, and PUFAs were comparable (similar) in duckweed from all analyzed groups (Groups 2–5). Different highly statistically significant differences were found between Groups 2 and 4 for the discussed groups of fatty acids ($p < 0.001$).

Table 7 presents the contents of various nitrogen species in duckweed and indicates that the content of ammonia nitrogen ($N\text{-}NH_4$) was higher than that of nitrate nitrogen ($N\text{-}NO_3$). The highest ammonia nitrogen content was determined in duckweed from Group 5, and the lowest was found in that from Group 3 ($p < 0.01$). In the case of nitrate nitrogen, its highest content was noted in duckweed from Group 2 and the lowest was found in that from group 3 ($p < 0.001$).

4. Discussion

Duckweed, the growth rate of its green mass, and its ability to absorb and accumulate nutrients from various growth media, have been discussed in the literature before [14,34–38]. Duckweed was grown on culture media prepared under laboratory conditions [14,35], wastewater [34,37], and animal excreta [36,38], like the present study.

In the present study, the course of duckweed growth observed in Groups 2–5 was deemed appropriate. Our previous study [11] with effluent from a biorefinery demonstrated that its small addition (0.39%, 0.60%, and 0.78% concentration in the growth media) had a positive effect on the growth of duckweed (*Lemna minuta*). Stadtlander et al. [39] drew a similar conclusion from their study with bovine slurry, wherein a decreasing concentration of the natural fertilizer promoted higher yields of duckweed (*Spirodela polyrhiza*

and *Lemna punctata*) fresh mass. As noticed by the aforementioned authors, duckweed growth decline could have been mainly due to the use of high slurry concentrations. The slurry has a high concentration of NH_3, exerting a toxic effect on live organisms, duckweed included. In addition, high slurry concentration in the growth media may contribute to the unfavorable increase in their pH—towards alkaline values—and these high pH values also inhibit duckweed growth. In the present study, between days 20 and 30 of observations, a moderate pH increase was noted in Group 5, which ultimately led to duckweed yield decrease compared to groups 2–4, which is consistent with the findings reported by other authors [39–41].

The appropriate growth of duckweed and its capability to accumulate nutrients are affected by multiple factors. One of the key factors is the already mentioned pH. As demonstrated by Ullah et al. [40], the pH optimal for growth and maximal green mass yield of duckweed (*Lemna minor*) is 7 ± 1, whereas pH values exceeding 8 and lower than 4 were observed to inhibit duckweed growth. In the present study, the pH values either fell within the recommended range or were exceeded periodically (in Group 5). Similar conclusions regarding acidification of growth and culture media used to produce duckweed (*L. minor*) were reported by Jones et al. [41]. They demonstrated that pH > 8.2 inhibited its growth and that better yields could be achieved at pH < 8.

The total content of compounds dissolved in water (TDS) and the electrical conductance of liquid (EC) should be considered together. The TDS index is based on the electrical conductance measurement; therefore, when EC values decrease/increase, the TDS values respectively do the opposite, i.e., increase/decrease. The EC values are additionally affected by temperature. Conductance can only be recorded when inorganic metal ions, such as N, P, K, Ca, and Mg, are present in the solution. In a study by Wendeou et al. [42], the best growth of duckweed (*S. polyrhiza*) was observed at EC values of 800, 1200, and 1400 µS/cm. In the present research, the values of the EC index were similar, indicating good conditions for duckweed growth, which indeed grew well as seen by its new, large, green leaves. Similar observations related to the content of soluble compounds and the electrical conductance of the medium were made by Iqbal et al. [43]. They recorded the best growth of duckweed (*L. minor*) and its nutrient accumulation capability at an EC value of the growth medium approximating 1000 µS/cm.

Salinity, which is a measure of the salt concentration in a solution, provides information about the mass of all dissolved substances, excluding gases, colloids, suspended solids, and organic matter. The results of investigations conducted by Tkalec et al. [44], Wendeou et al. [42], and Ullah et al. [40] indicate that excessively high salinity of the culture/growth medium negatively affects the growth and proliferation of duckweed green mass. In the present research, the level of media salinity was low; no problems were observed in the subsequent stages of the experiment (days 10, 20, 30)—namely, no inhibition of duckweed growth. The worse duckweed growth results recorded in Group 5 could be due to the aforementioned increased pH and a too low nutrient concentration (concentration 0.50%) in the growth medium used.

Physicochemical factors affect the growth and development of organisms, while rapid and strong changes in abiotic factors can inhibit these processes. One of the important factors is temperature, which has a significant impact on the growth, development and metabolism of organisms, as it determines the rate and amount of absorbed and accumulated nutritionally important nutrients. Different duckweed species have adapted to a broad range of ambient temperatures from 5 to 35 °C [45]. According to Vymazal [46], the optimum temperature of the growth medium for duckweed production should be between 20 and 30 °C. Air temperature, which was stable in the present study, and—more importantly—water temperature or culture medium/growth medium temperature under experimental conditions are important for the organisms living in water and partly on its surface. The study conducted by Chakrabarti et al. [20] demonstrated that the temperature of the growth medium enriched with manure or chemical fertilizers was lower than 18.5 °C, which inhibited duckweed (*L. minor*) growth. Intensive growth of duckweed was observed

again when it increased to 19.4 °C. In the present study, the temperature of the growth media at the beginning of observations was relatively low (17.6–17.8 °C) for the growth needs of duckweed; hence, the increase in its green mass in the first stage of the experiment was slow. On day 10 of observations, the growth media temperature was 18.7 °C and in the final phase, i.e., on day 30, it was higher by 0.2–0.5 °C and amounted to 18.9–19.2 °C. This ensured a good growth of green mass, without compromising the values of the remaining important parameters tested, i.e., media pH, TDS, EC, and salinity.

Quantitative and qualitative parameters were assessed in duckweed from the *Lemnaceae* family, which is used as human food [32] as well as feedstuffs for animals [19,20]. The chemical composition of duckweed depends on many factors, including the type of growth medium, species of duckweed, place of cultivation, availability of nutrients, and environmental conditions [47]. These factors allow modifying duckweed composition through the use of various types, concentrations, and solution forms of the growth media. In a study by Devlamynck et al. [48] with pig slurry used as a growth medium, the protein content of duckweed (*L. minor*) was approximately 35% that of dry matter. In another study by Mohedano et al. [49] investigating duckweed (*Lemna punctata*) growth media with animal excreta, the manure was first subjected to biofermentation and then, the leachate was discharged to the retention tank, from where it was pumped to the ponds where duckweed was grown. The media used were fed with 1 m^3/day of leachate, and the crude protein content in the produced duckweed ranged from 28 to 35%. In the present study, the protein content of the duckweed produced was higher or comparable to the results reported by the aforementioned authors [49]. Our previous study [11] showed that biogas plant effluent could also serve as a good medium for the growth of duckweed (*L. minuta*) and allowed us to conclude that the installation used would enable the recovery of valuable fertilizing materials (struvite and ammonia) and the production of high-quality animal feed on the leachate. Compared to our previous study [11], the present research results indicate a comparable or higher content of protein, fat, fiber, and ash in the produced plant material—duckweed. The study conducted by Stadltander et al. [39] with duckweed (*Spirodela polyrhiza* and *Lemna punctata*) produced using bovine slurry also confirmed a high content of crude protein per mass unit, i.e., from 30 to 38 g 100 g^{-1} DM. Duckweed produced in the present growth study had a high total protein content, which indicates its potential suitability for commercial production and use for feedstuff-production purposes; however, the variability of results reported in the available literature [50,51] makes this area ripe for further research.

In the present study, the contents of macroelements, microelements, and heavy metals determined in duckweed produced on growth media with different concentrations of pig slurry turned out to be lower than those in the research by Devlamynck et al. [48]. The regulation of the European Commission [52] specifies the maximum levels of certain contaminants, including heavy metals, in various foodstuffs. In the group of food products including leafy vegetables and seaweed, the highest permissible levels are 0.10 for lead and 0.20 mg/kg fresh weight for cadmium. Like in the present study, Devlamynck et al. [48] also performed their experiment with pig slurry, but it had been first subjected to centrifugation and bio-treatment. In our previous experiment [11], the contents of mineral elements and heavy metals in duckweed (*L. minuta*) were similar to those reported in the present study. In another experiment carried out by Appenroth et al. [32], wherein duckweed (genus *Wolffia*) was grown on a medium prepared under laboratory conditions (KNO_3, KH_2PO_4, K_2HPO_4, $MgSO_4$, $Ca(NO_3)_2$, H_3BO_3, $ZnSO_4$, Na_2MoO_4, $MnCl_2$, Fe(III)NaEDTA, EDTA-Na_2), the contents of Ca, P, K, Cd, and Pb in the plant material were higher than in the present study. It should be noted, however, that different *Wolffia* species used for the study had various contents of minerals even though they were grown under the same conditions. This finding allows us to conclude that there is a need and even a necessity to control the heavy metal content of commercially produced duckweed for feed or nutritional purposes considering the variety of duckweed species and different types and concentrations of growth media used to this end.

Carotenoids are an important group of compounds responsible for the pigmentation of plants and animal products (egg yolk, broiler carcass) that also exhibit antioxidant properties. The analyzed duckweed was found to contain six representatives of this group of compounds, i.e., α-tocopherol, β-carotene, α-carotene, violaxanthin, zeaxanthin, and lutein. These carotenoids occur naturally in feedstuffs for animals. For instance, carrot contains β-carotene and α-carotene, alfalfa contains lutein and zeaxanthin, whereas marigold flower and maize both contain lutein and zeaxanthin [53]. These compounds are nutritionally important for livestock; hence, their presence in duckweed produced in the present research additionally confirms its usefulness for animal feeding purposes. Investigations conducted by Appenroth et al. [32,54] and our previous study [11] demonstrated similar levels of the analyzed carotenoids. According to Polutchko et al. [55] and Stewart et al. [56], duckweed production can provide a significant amount of green mass rich in nutrients, containing an attractive mixture of carotenoids and polyphenols, which supports its viability as a feed supplement for animals.

Duckweed is also a source of amino acids. Their average and diversified contents were determined in the duckweed samples analyzed in the present study. In turn, Stadtlander et al. [39] showed higher contents in duckweed (*L. punctata* and *S. polyrhiza*) produced on media with a cattle slurry addition compared to our own research. The cited authors showed no tendencies of changes in the contents of amino acids in the plant material samples. No similar relationships were found in the present study either, as the contents of amino acids were similar in all analyzed groups. Alike results were reported by Chakrabarti et al. [20]. The present study results confirm the value of duckweed as a source of valuable amino acids.

The fatty acid profile of the analyzed duckweed samples seems interesting because the contents of SFAs and PUFAs turned out to be comparable. According to previous studies, the content of SFAs was usually lower than that of PUFAs [20,32]. The results of a study conducted by Appenroth et al. [32] show the following percentages of individual groups of fatty acids in the total fatty acid profile: SFA—33.9, MUFA—3.5, and PUFA—62.6 (% fatty acid methyl esters). Chakrabarti et al. [20] also determined a lower content of SFAs (22.72% of total fatty acids) and a higher content of PUFAs (63.38% of total fatty acids).

High levels of nitrates in plants can indirectly lead to increased intake of nitrites and N-nitroso compounds, increasing the risk of development of human and animal diseases [57]. According to standards in force, the permissible content of nitrates in feed materials ingested with via feed by ruminants is 9.3 g NO_3 kg^{-1} DW, whereas in for human diets, it is 46 g NO_3 kg^{-1} DW [52,58]. In a study by Devlamynck et al. [57], the nitrate content of duckweed was higher than in the present study. These authors [57] demonstrated that the level of nitrates increased with the increasing content of macronutrients in the growth medium. Similar correlations were noted in our own research, which indicates the need to precisely control the quality of the media used for duckweed production.

5. Conclusions

The study results demonstrated that duckweed can grow well on pig slurry as a growth medium. Its highest yields were noted at pig slurry concentrations of 1.50%, 1.00%, and 0.75%. Appropriate environmental conditions for duckweed growth (pig slurry concentrations, pH, TDS, EC, salinity, temperature) allowed the production of plant material featuring acceptable composition and optimal contents of nutritionally important components. It may be concluded that while maintaining a closed circuit of duckweed production and use in the agricultural environment by harnessing animal excreta (pig slurry) for its production, and by ensuring specified (optimal) conditions for its growth, it is feasible to obtain feed material meeting basic quality standards.

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