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Duckweed production on diluted chicken manure

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ABSTRACT. The aim of this study was to test chicken manure as duckweed (*Lemna minor*) fertiliser. Duckweed was grown using three different concentrations (low, medium and high; dilution factors 1:16, 1:12 and 1:8, respectively) of previously solubilised chicken manure. Subsequently, duckweed was evaluated for its fresh and dry biomass production, protein content and protein production capacity. Ammonium-nitrogen (NH_4-N) concentrations increased in all substrates during an experimental week, with the increase being steeper in the treatments with higher chicken manure concentrations. However, duckweed populations were unable to fully utilise all the provided nitrogen. As the concentration of chicken manure increased, growth and protein production decreased. Adding the highest concentration of chicken manure (1:8 dilution) led to nearly complete die-off of the duckweed population. The low concentrated (1:16 dilution) chicken manure fertilisation resulted in acceptable growth (1.85 g dry matter (DM) per m^2 and day) and high crude protein content (42.8% DM). The medium concentration (1:12 dilution) of chicken manure still stimulated growth, although it was significantly lower compared to duckweed grown on the low concentrated poultry manure and declined towards the end of the experiment (0.88 g DM per m^2 and day). The biomass from this treatment also contained slightly lower protein content (40.6% DM). Duckweed cultivated using low and medium chicken manure concentrations produced an average of 0.79 and 0.36 g protein per m^2 and day, respectively. Although solubilised chicken manure can serve as a potential fertiliser for duckweed, balancing the amount of chicken manure necessary to obtain a target NH_4-N concentration when compared to cow or pig slurries is challenging.

Introduction

Nitrogen (N) emissions from agricultural livestock production mostly arise in the form of ammonia (NH_3), nitrogen oxide (NOx) and nitrous oxide (N_2O) (Leip et al., 2015). They are particularly concentrated in areas with high density of agricultural and animal production. Intensive swine or poultry production leads to significant eutrophication of surrounding watersheds due to leaching of nitrogen compounds into groundwater and surface waters (Mallin et al., 2015). Reactive N emissions or losses from agriculture and other anthropogenic sources are associated with high environmental and health-related costs per year, e.g. € 35–230 billion has been reported for Europe (Van Grinsven et al., 2013).

Discussions concerning the increased use of microbial proteins (Leger et al., 2021) to improve N use efficiency in human nutrition are gaining momentum. However, other alternatives for increasing N-recycling, and thus mitigating N emissions have also been studied for many years, and among others, they involve duckweed or water lentil cultures. These small aquatic plants belong to the

family Lemnaceae, comprising 36 species belonging to 5 genera, with a nearly global distribution range, except for the highest latitudes (Bog et al., 2019). Duckweed species have demonstrated high efficiency in nutrient conversion, particularly nitrogen (N), but also phosphorus (P) (Xu and Shen, 2011; Stadtlander et al., 2019). They are among the most rapidly growing vascular plants, making them highly efficient and fast protein producers worldwide, surpassing soybeans several-fold in terms of unit time and area (Xu et al., 2012; Leger et al., 2021; Stadtlander et al., 2022). Duckweeds have been utilised as animal feed for a variety of monogastric animals, including fish (Stadtlander et al., 2023), pigs (Rojas et al., 2014) and poultry (Haustein et al., 1990), and are therefore considered a potential future protein source in animal feed (Sońta et al., 2019).

Globally, poultry, especially broilers and layer hens, are by far the most produced animals in terms of population size and meat production (121 million tons), closely followed by pigs (120 million tons) and cattle (72.4 million tons). Estimating the resulting total amount of globally produced chicken manure is complicated, since manure production depends on several factors, including digestibility, chemical composition of feed and ingredients, as well as production system and moisture content of the manure (Goss et al., 2013; Prado et al., 2022). Feed conversion ratios (FCRs) typically range between 2.00 and 2.45, but also show outliers reaching values as high as 4.35 (Willems et al., 2013). Assuming a global average FCR of 2.40 and excretion of chicken manure relative to feed fed at a ratio of 1:1, it is estimated that approximately 290 million tons of chicken manure are produced annually. Many studies tested sewage sludge (Bonomo et al., 1997; Awuah et al., 2004) or liquid animal manures or slurries, such as cow slurry (Stadtlander et al., 2019; 2022) or pig slurry (Cheng et al., 2002; Xu and Shen, 2011) as a source of nutrients for duckweed production. However, there are only a few scientific reports on the application of chicken manure for fertilising duckweed (Amali et al., 1999; Gena and Sumarsono, 2013).

The objective of this study was to use chicken manure (CM) as a fertiliser for duckweed production, under controlled conditions in a greenhouse, at three different concentrations (low – 1:16 dilution, medium – 1:12 dilution and high – 1:8 dilution), and to evaluate biomass gain, protein content and protein production.

Material and methods

Duckweed

Wild growing duckweed, presumably *Lemna minor* L. (identified under a binocular), was collected live from a spillover pond situated on the premises of the Research Institute of Organic Agriculture (Switzerland). The duckweed was kept in a greenhouse and fertilised twice per week with a commercial liquid NPK-fertiliser (40-20-60 N-P-K, Hauert Zimmerpflanzen, Hauert, Switzerland) to reach ammonium-nitrogen (NH_4-N) concentration of 20 mg/l.

Experimental setup and chemical analysis

CM was freshly collected once a week from organic layer hens, and 5 kg was mixed on the same day at a ratio of 1:5 (w/v) with water, mechanically stirred for 10–15 min until most of the larger particles disintegrated, and subsequently incubated for 2 h without further stirring. Afterwards, the liquid phase was filtered through a regular 2-mm household sieve, and the filtered solution was diluted at three different concentrations for treatments: 1:16 (low, lCM); 1:12 (medium, mCM) and 1:8 (high, hCM) using tap water. Fifteen boxes, each measuring 36.9 × 26.6 × 14.1 cm (length × width × depth) with a surface area of 981 cm^2 and a maximum volume of 13.8 l, were used for the experiment. Five boxes were assigned to each treatment, and 6 l of the respective CM dilutions were added to each box. Subsequently, all boxes were inoculated with 40 g of fresh matter (FM) of *Lemna minor*, which resulted in approximately 80–85% of surface area coverage and corresponded to 408 g FM m^{-2} of initial density. In parallel, an initial pooled duckweed sample was collected, FM was determined, followed by drying and dry matter (DM) measurements before storage at −20 °C until further analysis. Fresh matter was determined using two different methods, one gentle and one precise. The former was applied at the beginning and during the experiment to minimise stress on the living duckweed fronds by collecting the duckweed and letting it drip on a net until no more water drops were observed (usually 10 min). The precise FM determination method was applied at the final harvest when the entire duckweed was collected from one box and spun twice for 30 revolutions using a household salad spinner before weighing to the nearest 0.1 g. In addition to biomass gain (g), the relative growth rate (RGR) was calculated using the following formula (Ziegler et al., 2014):

$$RGR\ (day^{-1}) = (\ln (W_{end}) - \ln (W_{start})) / d,$$

where: W_{end} – duckweed biomass at the end of the growth period, W_{start} – duckweed biomass at the start of the growth period, and d – elapsed time in days in between both measurements.

The entire experiment was conducted for four weeks (September 24, 2021 till October 21, 2021) in a greenhouse, and boxes were randomly allocated to one of the above-mentioned treatments. Temperature was measured in each box three times a day (9:00, 13:00. and 17:00) and four days a week; pH and total dissolved solids (TDS) were measured once a day (13:00) in three of five boxes per treatment. A Mettler Toledo InLab Expert pH probe (Mettler Toledo, Greifensee, Switzerland) was used to measure pH, and a WTW TetraCon 925 probe was used for TDS determination (Xylem Analytics Germany, Weilheim, Germany). On the first day, oxygen was measured at a depth of approx. 3 cm in three boxes per treatment using a WTW FDO 925 oxygen probe (Xylem Analytics Germany, Weilheim, Germany). However, all substrates in all treatments were found to be anoxic from the beginning of the measurements, and thus oxygen readings were discontinued. At the start and end of each week, substrate depth was measured with a folding rule and evaporation was calculated. The boxes were kept for one week (starting Thursday) before the substrates were replaced. To this end, all duckweed was carefully decanted onto a fine-mesh insect-proof net, allowed to drip for 10 min and weighed. In the meantime, the boxes were cleaned and re-filled with freshly prepared substrates before re-stocking the duckweed populations into their respective boxes. Samples were collected from fresh substrates at the beginning of each week and from the used substrates at the end of each week. At the end of the four-week experiment, all duckweed from all boxes was harvested and centrifuged in a salad-spinner to remove excess water adhering to the fronds for accurate FM determination.

All weekly substrate samples were chemically analysed for total N, NO_3-N,NH_4-N and P_2O_5 according to the methods DIN 38406-5:1993-10 (NH_4-N) and DIN EN ISO 11885; 2009-09 (P_2O_5) (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten, 1976).

Due to the high NH_4-N concentrations and increasing pH over the course of an experimental week, the NH_3 fraction was also calculated based on the following formulas described by Emerson et al. (1975):

$$pK_a = 0.09018 + 2729.92/T,$$

$$f = 1/(10^{pKa - pH} + 1),$$

where: T – temperature in K and f – fraction of NH_4 present as NH_3.

The harvested duckweed was dried at 120 °C for 5 h to determine the DM content by gravimetric difference. For crude protein (CP) determination, dried duckweed was analysed according to the method of Dumas, and total N and CP was calculated using the following conversion factor: N * 6.25 = CP. The total P content in duckweed was also determined according to the method DIN EN ISO 11885:2009-09. Since the available duckweed biomass was limited, the starch content was determined in pooled samples based on the method VDLUFA MB Bd.III 7.2.1:2012 (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten, 1976).

All results, unless otherwise indicated, are presented as mean values per treatment of three (pH and TDS) or five (NH_4-N, P_2O_5, °C, DM, CP) boxes ± standard deviation.

Data analysis

All statistical analyses were performed using SPSS 21.0 software (IBM Corporation, Armonk, USA). To analyse the effects of the three treatments on biomass production, a 2-factorial (factor 1 – time, factor 2 – treatment) repeated measures ANOVA was used. The pH and TDS data were analysed using a general linear model using treatments and sampling days as factors, followed by a Bonferroni post-hoc test. To compare the effects of different treatments on P_2O_5 and evaporation, a one-way ANOVA was conducted with treatment as an independent factor. Since Levene's test indicated that the homogeneity of variance was violated for NH_4-N data, a non-parametric Kruskal-Wallis H test was used. The proximate composition of the duckweed was compared between the low and medium CM groups using Student's t-test. A significance level of $P = 0.05$ was applied for all tests.

Results

The measured substrate temperatures ranged from 11.7 °C in the morning to 26 °C in the afternoon (Figure 1). The lowest temperatures were always measured in the morning and the highest in the afternoon. The first week was generally warmer with temperatures up to 26 °C on the first day, while the subsequent three weeks had daily high temperatures ranging between 20.2 and 22.8 °C. The average percentage of evaporated substrate was found to be between 13.9% (mCM) and 15.1% (hCM) over

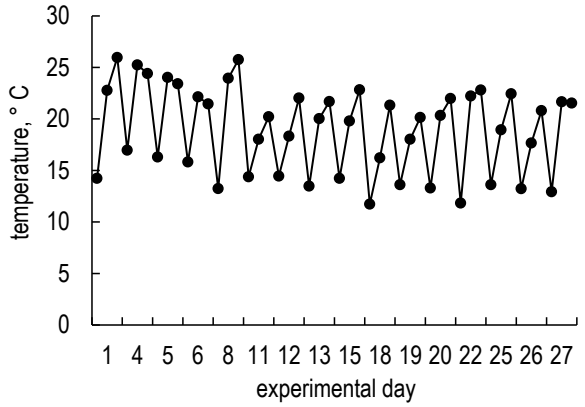

Figure 1. Average substrate temperatures over the four-week period, measured at 9:00, 13:00 and 17:00 every Friday, Monday, Tuesday and Wednesday for 4 weeks

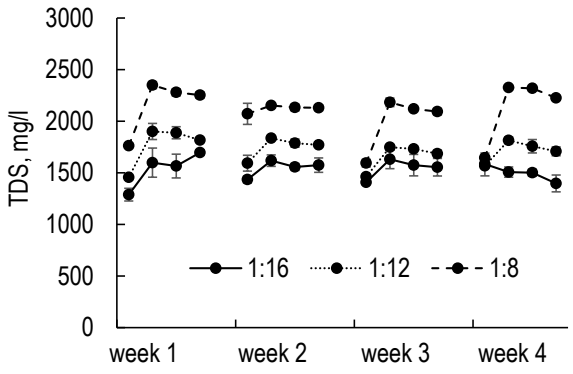

Figure 3. Mean values of total dissolved solid (TDS) in different concentrations of chicken manure. A gap was intentionally left between each experimental week to highlight substrate exchange (n = 3 ± SD)

SD – standard deviation; 1:16 – low concentration of chicken manure at 1:16 dilution, 1:12 – medium concentration of chicken manure at 1:12 dilution, 1:8 – high concentration of chicken manure at 1:8 dilution

the course of four weeks, and did not show any significant differences between the treatments. Only in the last week, a trend towards higher evaporation in the hCM was observed. The average pH values differed between the three treatments, with the low CM concentration tending to show the lowest pH values, and the high CM concentration exhibiting the highest pH values. This pattern was opposite only during the first week of the trial. The pH values varied between 7.23 and 7.50 in the fresh substrates, and from 7.83 to 8.23 in the old substrates at the end of the experimental week, with all treatments showing significant increases (Figure 2, $P < 0.001$).

During the first two weeks of the experiment, no clear pattern was observed between individual treatments, except for a general increase in pH values

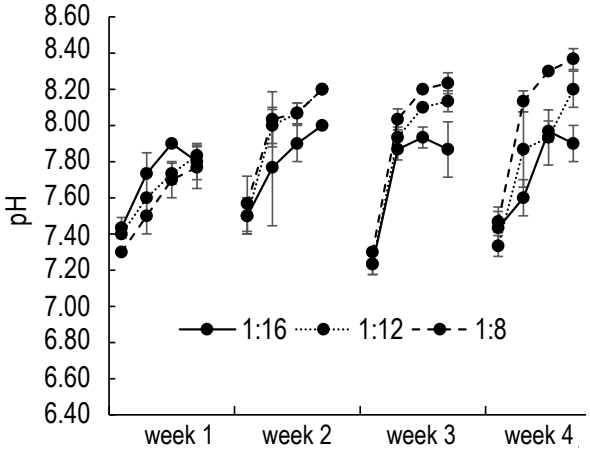

Figure 2. Geometric means for pH values measured in different dilutions of chicken manure. A gap was intentionally left between each experimental week to highlight substrate exchange (n = 3 ± SD)

SD – standard deviation; 1:16 – low concentration of chicken manure at 1:16 dilution, 1:12 – medium concentration of chicken manure at 1:12 dilution, 1:8 – high concentration of chicken manure at 1:8 dilution

over an experimental week. In the last two weeks of the experiments, the lCM treatment exhibited significantly lower pH values compared to the hCM treatment ($P < 0.001$).

The TDS patterns followed the expected clear dependency on the concentration of chicken manure. The treatment with lCM concentration (1:16) showed the lowest TDS values throughout the experiment, while the treatment with hCM concentration (1:8) had the highest TDS values (Figure 3, $P < 0.001$). Similar to the pH, the TDS values decreased for all treatments with each substrate exchange, but rapidly increased again to relatively stable values. This increase was more pronounced with increasing concentrations of chicken manure. NH_4-N concentrations increased several-fold in individual treatments during each experimental week. In fresh substrates, they ranged from 11.2 mg/l in the lCM treatment to 42.5 mg/l in the hCM treatment. At the end of each experimental week, NH_4-N levels increased to a maximum of 41.9 mg/l in the lCM and 122.9 mg/l in the hCM treatment (Figure 4, $P < 0.001$).

During the first week, the estimated NH_3 concentrations reached a maximum of 1.90 mg/l in the lCM treatment, and 2.91 mg/l in the hCM NH_3 treatment. During the four experimental weeks, NH_3 in the lCM treatment did not increase significantly above 2 mg/l, while in weeks 3 and 4, NH_3 concentrations in the mCM and the hCM treatments reached values of 10 mg/l and above, with hCM peaking at 12.89 mg/l NH_3 in week 3. It was clearly visible that NH_3 levels increased dramatically during an experimental week for the two more concentrated chicken manure fertilisers (Figure 5).

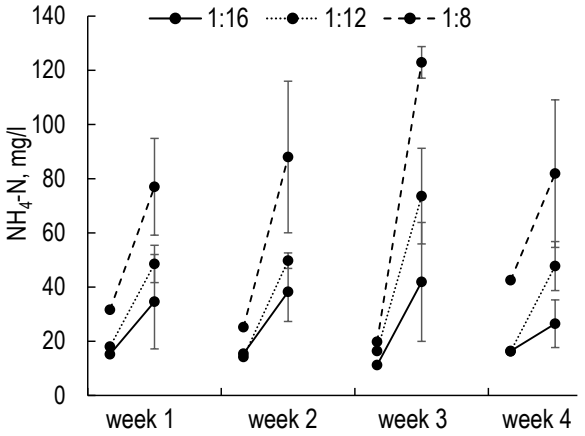

Figure 4. Mean ammonium-nitrogen (NH_4-N) concentrations in different dilutions of chicken manure at the start and end of each experimental week. A gap was intentionally left between each experimental week to highlight substrate exchange (n = 5 ± SD).

SD – standard deviation, 1:16 – low concentration of chicken manure at 1:16 dilution, 1:12 – medium concentration of chicken manure at 1:12 dilution, 1:8 – high concentration of chicken manure at 1:8 dilution

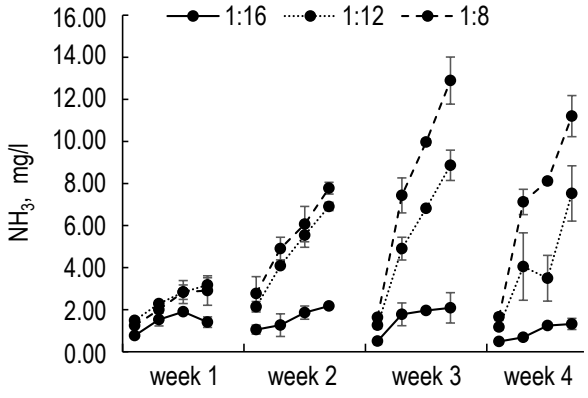

Figure 5. Mean ammonia (NH_3) concentrations in different dilutions of chicken manure. A gap was intentionally left between each experimental week to highlight substrate exchange (n = 3 ± SD)

SD – standard deviation, 1:16 – low concentration of chicken manure at 1:16 dilution, 1:12 – medium concentration of chicken manure at 1:12 dilution, 1:8 – high concentration of chicken manure at 1:8 dilution

Phosphorous (P_2O_5) showed relatively high variability between the treatments, but generally a clear pattern with the lowest concentrations in the lCM treatment and highest concentrations in the hCM treatment was observed (Figure 6). However, concentrations from the beginning to the end of the experimental week differed between individual weeks. In the second week, almost no change in P_2O_5 levels were recorded; in the third week, P_2O_5 concentrations increased in the mCM and hCM treatments, but slightly decreased in the lCM treatment. Ultimately, during the last week, phosphorus levels strongly declined in all treatments.

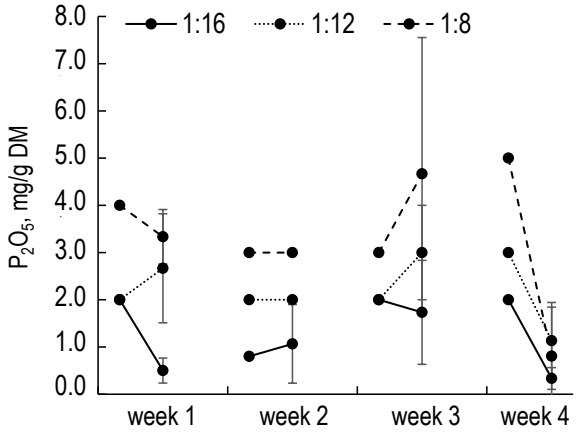

Figure 6. Mean phosphorous (phosphorous pentoxide, P_2O_5) concentrations in different dilutions of chicken manure at the start and end of each experimental week. A gap was intentionally left between each experimental week to highlight substrate exchange (n = 5 ± SD)

SD – standard deviation, DM – dry matter, 1:16 – low concentration of chicken manure at 1:16 dilution, 1:12 – medium concentration of chicken manure at 1:12 dilution, 1:8 – high concentration of chicken manure at 1:8 dilution

After the second week, the hCM treatment exerted a severe negative effect on duckweed populations and instead of growing, duckweed biomass started to decline. In contrast, duckweed populations on mCM and lCM concentrations showed an increase in biomass. By the end of the four-week experiment, almost the entire duckweed population in the hCM treatment perished, and the harvested biomass was too small in all replicates to be further analysed, even when pooled. For duckweed cultured on mCM concentration, an initial faster growth was observed, which also started to decline in the last week before harvest. Only in the lCM treatment, a continuous biomass growth was observed, although during the last week, a slower biomass gain was recorded (Figure 7). The calculated RGR showed a clear decline over the four-week period after the initial peak at week 1. The biomass reduction was more pronounced in the mCM and especially hCM treatments, with RGR in the hCM treatment becoming negative as early as week 2. The RGR for the mCM treatment, became slightly negative only in week 4. Duckweed in the lCM treatment showed a slightly positive RGR in week 4, although growth slowed significantly. The highest RGR was recorded in the lCM treatment in week 2 (RGR = 0.07), the lowest in the hCM treatment in week 4 (RGR = −0.23).

In the entire four-week trial, duckweed biomass in the lCM treatment gained 173% in fresh weight, duckweed in the mCM treatment gained 81% in fresh weight, while duckweed in the hCM treatment

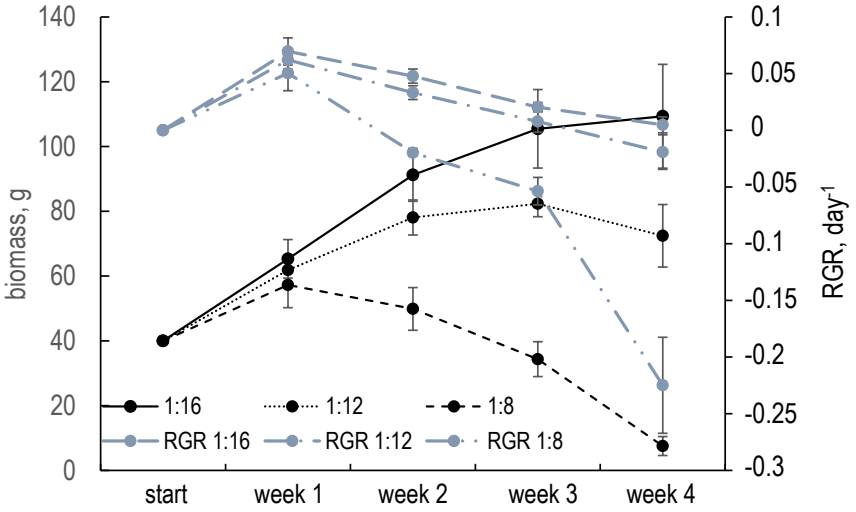

Figure 7. Growth and relative growth rate (RGR) curves for *Lemna minor* growing with diluted chicken manure for 4 weeks (n = 5 ± SD)
SD – standard deviation, 1:16 – low concentration of chicken manure at 1:16 dilution, 1:12 – medium concentration of chicken manure at 1:12 dilution, 1:8 – high concentration of chicken manure at 1:8 dilution

lost 81% of its fresh biomass (Figure 8). The estimated duckweed coverage in the hCM treatment was reduced to approximately 15%, coverage in the mCM treatment was 90–95%, and for the lCM treatment, a coverage of 95–100% was estimated. A repeated measures ANOVA revealed no differences between duckweed growth in the lCM and mCM treatments (P = 0.136), but it was significantly different compared to the hCM treatment ($P < 0.001$ for lCM and $P = 0.002$ for mCM, respectively).

The DM content of the initially pooled duckweed sample was estimated at 6.3%, CP content at 30.1% DM, and P concentration at 1.54% DM. Starch was not analysed in the initial sample. For both the lCM and mCM treatments, concentrations of all measured chemical parameters increased during the four weeks of the trial.

The dry matter content increased to 7.32 ± 0.16% and 7.42 ± 0.11 % for the lCM and mCM treatments, respectively, and did not differ between the two groups. Crude protein increased to 42.8 ± 0.73% DM in the lCM group, and to 40.6 ± 0.63% DM in the mCM group, and differed statistically between both treatments. The P content increased to 2.59 ± 0.34% DM (lCM) and 3.18% ± 0.13% DM (mCM) and was also statistically different between the treatments. The starch content in the pooled samples from the lCM treatment constituted 1.37% DM and 1.50% DM in the mCM treatment. Proximate chemical data are shown in Table 1.

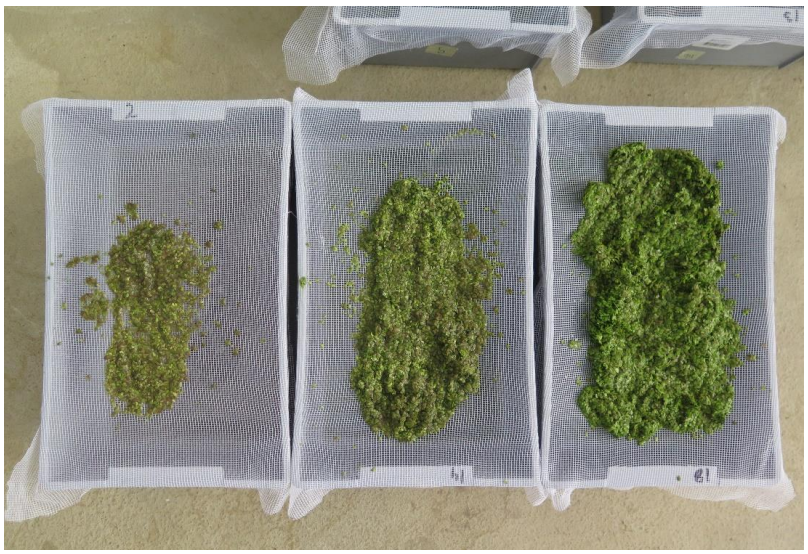

Figure 8. Final harvested fresh *Lemma minor* biomass from one replicate each for high (1:8, left), medium (1:12, middle) and low (1:16, right) chicken manure concentrations

Table 1. Dry matter, protein, phosphorous and starch content of *Lemma minor* grown for four weeks on different dilutions of chicken manure (n = 5 ± SD)

Item	Initial duckweed⁺	lCM (1:16)	mCM (1:12)	hCM (1:8)	*P*-value
Evaporation, %	-	14.3 ± 2.19	13.9 ± 1.60	15.1 ± 1.01	0.669
DM, %	6.30	7.32 ± 0.16	7.42 ± 0.11	n.d.	0.290
CP, % DM	30.1	42.8 ± 0.73*	40.6 ± 0.63	n.d.	0.001
Phosphorous, % DM	1.54	2.59 ± 0.34*	3.18 ± 0.13	n.d.	0.015
Starch⁺, % DM	n.d.	1.37	1.50	n.d.	
g DM/m²/day	-	1.85 ± 0.43*	0.88 ± 0.26	n.d.	0.003
g CP/m²/day	-	0.79 ± 0.18*	0.36 ± 0.11	n.d.	0.004

SD – standard deviation, DM – dry matter, CP – crude protein, lCM (1:16) – low concentration of chicken manure at 1:16 dilution, mCM (1:12) – medium concentration of chicken manure at 1:12 dilution, hCM (1:8) – high concentration of chicken manure at 1:8 dilution, ⁺ - pooled samples, n.d. – not determined, * indicates significant difference between lCM and mCM (t-test, $P < 0.05$)

Fresh and dry biomass production was significantly higher (fresh: 25.2 ± 5.83 g/m²/day; dry: 1.85 ± 0.43 g/m²/day) in the lCM group compared to the mCM group (fresh: 11.8 ± 3.52 g/m²/day; dry: 0.88 ± 0.26 g/m²/day). Similarly, CP production per m² and day was significantly higher in the lCM group (0.79 ± 0.18 g/m²/day) compared to the mCM group (0.36 ± 0.11 g/m²/day) (Table 1).

Discussion

Chicken manure is often used as an agricultural or aquaculture fertiliser, especially in tropical and subtropical countries (Ghosh and Chattopadhyay, 2005; Green, 2022). However, in EU and other European regions with intensive animal production, excessive amounts of animal manures and slurries can cause problems due to strict regulations regarding permitted N loads in agricultural areas (Loyon, 2017). To mitigate N emissions, farmers must implement various manure and slurry treatment methods, such as aerobic treatment, anaerobic digestion, composting, or the addition of biochar during composting. These approaches aim to reduce N emissions, both through volatilisation and leaching into groundwater, and adhere to the maximum allowable N application levels for cultivated fields (Janczak et al., 2017; Loyon, 2017). Direct application of large amounts of chicken or other bird manures to fields is associated with a risk of soil salinisation, even in areas with sufficient rainfall (Li-Xian et al., 2007).

The use of duckweed to directly recover and transform residual N from chicken manure into plant proteins has not been reported as frequently as the application of cow or pig slurries. Thus far, to the authors' knowledge, only two studies have been published (Amali et al., 1999; Gena and Sumarsono, 2013) using chicken manure as a fertiliser for duckweed cultivation. Amali et al. (1999) used 0.5, 1.0 and 2.0 g/l of chicken manure to grow *L. minor*. These authors reported that all chicken manure treatments yielded more duckweed biomass compared to the tap water control, but the differences between chicken manure treatments were not statistically significant. Biomass gain or RGR have not been reported. On the other hand, Gena and Sumarsono (2013) explored the use of fresh beef cattle, pig and chicken manures in *L. minor* cultivation at concentrations of 0, 5 and 10 g/l. The latter treatment yielded the highest biomass, growth rates and crude protein content (29.2% DM).

The three different treatments applied in this study led to highly diverse responses in *L. minor* populations. The lCM treatment appeared to provide acceptable growth and biomass gain compared to the mCM and hCM treatments (Figure 8). However, when comparing the growth rate and biomass production of *L. minor* in the lCM treatment with other reported experiments aimed at recovering N from animal manures, it becomes evident that biomass production in the current study was lower than reported elsewhere. Maximum DM production of 5.7 g/m²/day was reported for *Spirodela polyrhiza* and 5.8 g/m²/day for *Landoltia punctata* grown on diluted organic cow slurry (Stadtlander et al., 2022). Devlamynck et al. (2021) reported a similar maximum DM production of 6.1 g/m²/day for *L. minor* produced with the addition of pig slurry. A higher production rate was reported for *S. polyrhiza* grown on diluted swine slurry, reaching values of 10.1 g/m²/day (Xu et al., 2012). Earlier studies from the US and Israel recorded even higher values – 12.7 g/m²/d for *Spirodela punctata* grown in the US on cattle manure dilutions (Mestayer et al., 1984), and 14.8 g/m²/day for *L. gibba* in Israel cultivated with municipal wastewater (Oron et al., 1987). These studies demonstrated significantly higher

DM production rates compared to the amount of DM obtained in this study for lCM and mCM (1.85 and 0.88 g/m^2/day, respectively). The CP content of duckweed from both surviving chicken manure treatments were at the higher end of the reported spectrum, exceeding 40% DM. Most studies reported CP contents of duckweed between 20% and 40% (Leng et al., 1995; Xu et al., 2012; Stadtlander et al., 2022), with only one study reporting a high CP content of 45.5% (Mbagwu and Adeniji, 1988). All treatments in the present study showed similar RGR after one week of culture, with values ranging from 0.05 to 0.07. However, RGRs quickly decreased with increasing CM concentrations, the decline being slower in the lCM treatment compared to the mCM and hCM treatments. Nevertheless, all observed RGRs were significantly below the potential maximum RGR of 0.42 reported for *L. minor* (Ziegler et al., 2014).

A TAN (NH_4-N + NH_3-N) concentration of 19 mg/l was indicated as optimal for overall CP production utilising *S. polyrhiza* and *L. punctata* (Stadtlander et al., 2022). The initial NH_4-N concentration in the lCM treatment was expected to have a similar value, while treatments with higher concentrations should contain higher NH_4-N levels. However, during successive experimental weeks, NH_4-N concentrations increased rapidly to values ranging from 40 mg/l in the lCM treatment to even more than 120 mg/l in the hCM treatment. These high NH_4-N values, along with increasing pH values, as well as correspondingly high NH_3 levels in the hCM treatment, could be considered the main reason for the collapse of the respective *L. minor* population and the decreasing RGR in the lCM and especially mCM treatments. Körner et al. (2001) reported a decrease in duckweed growth as a result of increasing NH_3 concentrations, as well as toxicity effects starting from 1 mg/l up to a maximum tolerance level of 8 mg/l. In the current study, only the lCM treatment showed stable NH_3 levels ranging from 0.5 to 2.1 mg/l, while in the second week, both the mCM and hCM treatments reached values between 6 and 7 mg. In the third week, both higher concentrated treatments exceeded 8 mg/l with the hCM treatment even reaching nearly 13 mg/l. One possible solution to prevent such soaring NH_3 concentrations could be acidifying the substrates to keep pH at a maximum value of 7.5 or lower, thereby reducing the risk of high NH_3 accumulation. This strategy has also been proposed and applied for conventional animal slurries to prevent NH_3 volatilisation (Kupper et al., 2020; Prado et al., 2022). Evaporation most likely contributes to the increase in NH_3 and NH_4-N concentrations, although its magnitude cannot be estimated without further analysis of uric acid and NH_3 levels and NH_3 volatilisation. Although no statistical differences in average evaporation over the entire four-week period were found (Table 1), there was a trend towards higher evaporation rates in the hCM treatment in the last week, which likely exacerbated the already high NH_3 concentrations in this treatment.

The sharp increase in NH_4-N levels in all treatments was most likely caused by high concentrations of uric acid, which is the largest N fraction in chicken manure, accounting for about 40–70% of total N (Murakami et al., 2011). Uric acid and urea can be converted to NH_4 by various microbes equipped with ureases (Lori et al., 2018). This strong microbial activity has not been anticipated in our study and reveals an important challenge when using chicken manure as fertilizer in duckweed systems. Unlike other slurries, the adjustment and maintenance of an initial NH_4-N concentration close to the targeted level becomes more complex, as the rate of transformation from uric acid to NH_4 is not known in advance. In contrast, cow or pig slurries, which primarily contain NH_4 as their principal nitrogen fraction, can be more easily adjusted to obtain a desired NH_4-N concentration (Ndegwa et al., 2008; Prado et al., 2022). The overall patterns of changes in NH_4-N levels in this study were therefore completely different compared to the typical NH_4-N concentration development reported in studies utilising pig or cow slurries, where NH_4-N levels steadily declined over time (Cheng et al., 2002; Xu and Shen, 2011; Stadtlander et al., 2019) instead of increasing rapidly.

P_2O_5 concentrations also differed significantly from what would normally be expected from duckweed-slurry systems. Typically, P levels decrease over time, as do NH_4-N concentrations (Cheng et al., 2002; Xu and Shen, 2011; Stadtlander et al., 2019). Contrary to NH_4-N concentrations observed in this study, no clear patterns were discernible for P levels, thus neither a general increase over the experimental week, nor a clear decrease across the three treatments was observed. Instead, ambiguous changes in P_2O_5 concentrations were recorded for individual treatments. P_2O_5 concentrations in individual treatments displayed varying patterns during the experimental weeks. In the most concentrated CM treatment (hCM), P_2O_5 levels mostly declined during an experimental week. Conversely, in the medium and low CM treatments, there were occasional increases, particularly in weeks 1–3.

Although we do not have data showing weakly phosphorus uptake by duckweed, sedimentation is likely playing a significant role, along with microbial activity or other mechanisms that may influence P_2O_5 concentrations. During the last experimental week, all treatments showed a strong decline in P_2O_5 concentrations, while the *L. minor* population in the treatment with the highest CM concentration was already severely depleted. It is therefore safe to rule out significant P uptake by duckweed as the sole cause of the fluctuations. However, the medium CM treatment resulted in a significantly higher P content in the biomass compared to the low CM treatment, despite producing less fresh and dry matter overall. This indicates a higher P-uptake by duckweed in the medium CM treatment. It is worth noting that in a study with sterilised medium resembling pig slurry, treatments without duckweed did not show significant changes in P concentrations over time (Cheng et al., 2002). This observation suggests that microbial activity may be a key driver for the fluctuations in P_2O_5 concentrations rather than sedimentation. However, in a non-sterile medium with a high content of organic material, sedimentation likely contributes to reduced P concentrations, in addition to uptake by duckweed or microbial activity. Another challenge associated with using chicken manure as a fertiliser for duckweed is its high salt content. Total dissolved solids (TDS), measured as mg/l in our experiment, were numerically equal to electrical conductivity (EC) expressed as µs/cm, both of which serve as indicators of salt content in aquatic solutions (Walton, 1989). Therefore, we decided to measure and report only TDS values. At the start of the experiment, TDS values ranged between 1298 mg/l in the lCM treatment and 1595 mg/l in the hCM treatment, and at the end of an experimental week, they varied between 1697 mg/l in the lCM treatment and 2227 mg/l in the hCM treatment. Chicken manure (49 g/kg on average) contained more than double the amount of soluble salts compared to pig manure (20.6 g/kg) and consisted mainly of potassium and sodium sulphates and chlorides (Li-Xian et al., 2007). Duckweed species are generally known to be sensitive to high salt concentrations, although there are some more tolerant clones (Sree et al., 2015, de Morais et al., 2019).

For *L. minor*, stress-induced growth inhibition depends not only on the total salt concentration (in terms of EC or TDS), but also on the type of salt, e.g. NaCl alone or in combination with Na_2SO_4 and K_2SO_4 (Lambert et al., 2021). Studies have shown that *L. minor* exhibits signs of reduced growth at electrical conductivities (EC) of around 3500 µS/cm, corresponding to a TDS of approx. 3500 mg/l (Lambert et al., 2021). The TDS values observed in the hCM in the current study (up to 2350 mg/l) were lower than this threshold, suggesting that factors other than high salinity were contributing to the decline of the duckweed population in this group. Under salinity stress, duckweed species have been observed to develop higher starch concentrations, making them attractive for potential use in bio-energy and bio-fuel production (Sree et al., 2015, de Morais et al., 2019). The high TDS concentrations in our study might have contributed to the low growth rates in the lCM and mCM treatments, although only low starch concentrations up to 1.50% DM were measured for both treatments (Table 1). It is important to note that different clones of the same duckweed species may exhibit variable responses to salinity stress, including differences in starch accumulation (Sree et al., 2015). Therefore, relying solely on starch concentration as an indicator of salinity stress might not be universally applicable.

Conclusions

While chicken manure (CM) can generally be considered a viable fertiliser for duckweed, the results of our study reveal challenges associated with applying CM for this purpose. Nutrient dynamics in CM appears more complicated compared to cow or pig slurries, particularly with respect to NH_4-N concentrations. Since NH_4 is the preferred N source for duckweed, gradual and controlled transformation of CM urea or uric acid to NH_4 would be beneficial, providing a longer-term nitrogen supply for the plants. However, the rate of this conversion needs to be controllable to keep NH_4 levels within an optimum range. The lowest CM concentration (1:16 dilution) used in this study resulted in lower but generally comparable growth and CP production compared to studies using cow or pig manure. In contrast, medium (1:12) and highest (1:8) concentrations resulted in insufficient growth or even the die-off of the duckweed population. Therefore, future studies should focus on lower CM concentrations and a thorough analysis of the transformation dynamics of uric acid to NH_4. Furthermore, a prolonged experimental period would be beneficial in order to elucidate potential long-term effects exerted by relatively high CM salt concentrations on the production and proximate composition of duckweed species, including starch content.

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Conflicts of interest

The Authors declare that there is no conflict of interest.

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