Manure Based Duckweed Production in Shallow Sink : Effect of Nutrient Loading Frequency on the Production Performance of *Lemna purpusilla*

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ABSTRACT : Effect on the bio-mass and nutrient yield of *Lemna perpusilla* Torry was studied at two nutrient loading frequencies. Six polythene sinks ($6\times2\times0.5$ m^3 area) randomly allocated to two treatments having three sinks in each treatment. Each sink containing $6\times2\times0.35$ m^3 water body, was changed either daily with 12.6 kg anaerobically fermented cattle manure (effluent) or with 76 kg efluent at six-day interval. Media Kjeldhal-N (40 vs. 36 mg/L; $p>0.05$) and NH_3-N (9 vs 7 mg/L; $p<0.05$) concentration were higher in daily charged sinks than those charged at six-day interval. However, temperature (28.48℃ vs. 28.60℃) and pH (6.82 vs. 6.84) were sinilar in both groups of sinks. Biomass (93 vs. 80 $g/m^2/d$) and crude protein (1.24 vs. 1.11 $g/m^2/d$) yield were non-significantly ($p>0.05$) higher in the daily charged than 6-d interval charged ponds. However, dry matter (4.52 vs. 4.15%), organic matter (83 vs. 84%), crude protein (31.4 va. 29.6%) and organic carbon (47 vs. 47%) content were similar in both daily and 6-d interval charged ponds. There were no apparent relationships between the crude protein content of *Lemna* and the media Kjeldhal-N or NH_3-N concentration apparently due to saturated level of nutrient (N in particular). It was concluded that with the level of effluent used for two treatments, nutrient-loading frequency had no significant effect on the biomass and protein yield of *Lemna*. Technique described here can be used for year-round duckweed production from fermented cattle manure for feeding poultry/fish in smallholder production system. *(Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 7 : 1010-1016)*

Key Words : *Lemna*, Effluent, Biomass Yield, Nutritive Value

INTRODUCTION

It is generally observed that duckweed gradually influx into nutrient rich stable water of roadside or homestead ditches and ponds or paddy fields in Bangladesh. These water generally rich in nutrients (1.20-3.42 mg/l of NH_3-N and 20-50 mg/l total N; ADR, 1998) and can support moderate to high level of duckweed production (50-100 g/m^2/day; ADR, 1998). However, from middle of the dry period (January-April) most of these ditches dires off or have too low level of water to support duckweed growth. On the other hand, flooding during the wet period (July-September) inhibit duckweed growth either by flushing away or by diluting the nutrient concentration of duckweed habitat. As a result duckweed is a seasonal crop in this country althought temperature, light intensity and other conditions are favorable for year-round duckweed production. Year-round availability of duckweed is a prerequisite for its use as a source of protein in place of conventional feed stuffs used in poultry feding (Haustein et al., 1989). Despite extensive literature on duckweed habitat and its importance as a source of nutrient for livestock, poultry and fishes, but almost no documented information on year round duckweed production system for feeding livestock and fishes (DRP, 1996; Anh and Preston, 1998). Duckweed production from farm manure in shallow polythene sink in homestead area can ensure year-round duckweed production (ADR, 1998). Biodigester produces N-rich effluent and are a logical source of required nutrient for growing duckweed (Rodriguez et al., 1997). Unpublished work in this laboratory showed that, maximum duckweed (*Spirodel spp.*) biomass production can be achieved at a media N concentration of about 32 mg/l (ADR, 1998) fertilized by anaerobically fermented cow dung. This concentration of N can be achieved by charging the media once with a large does of effluent at the start or by applying the same quantity of effluent at different fractions. In our earlier report (Chowdhury et al., 1999), it was shown that charging media once with anaerobically fermented cattle manure causes gradual reduction in bio-mass yield and nutritive value of duckweed which was attributed to the nutrient depletion of the media. Similar result was also observed with *Lemna* in Vietnam (Rodrigues and Preston, 1996). Hypothesis to be tested here is that nutrient loading frequency has no effect on the biomass and nutrient yield of *Lemna*. It is not sure about the extent of interval of nutrient application that should be used for obtaining right level of nutrient in the media. The present research program has therefore designed with two extreme intervals of nutrient application with the objective of determining the effect of nutrient loading frequency on the bio-mass and nutrient yield of *Lemna*.

MATERIALS AND METHODS

Sink and nutrient media

Six polythene sinks of (2×6) 12 m^2 surface area

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Table 1. Media characteristics and environmental parameter of sinks at two nutrient loading frequencies

Parameters	Nutrient charging		SED	Significance
	Daily charged	6-d internval		
Temperature (℃)	28.48	28.60	1.99 (Residual *df*=4)	NS
pH	6.82	6.84	0.029 (Residual *df*=4)	NS
Light intensity (Lux)	49000	56500	203.75 (Residual *df*=4)	NS
NH_3-N (mg/L)	9.31	7.16	0.37 (Residual *df*=26)	p<0.05
Total-N (mg/L)	40.36	36.00	4.50 (Residual *df*=26)	NS

NS=Statistically not significant.

and 0.5 m deep water body were used. The bins were placed in an open ground without shed. They were filled with water and effluent to maintain a water depth of 35 cm. The effluent was collected from a fixed dome (Chinese) biodigester fed daily with 100 liters of a mixture of 50 kg fresh cattle manure and 50 kg water. The concentration of N in the effluent varied over the course of experiment with an average of 2.46%.

Design and treatment

There were two treatments replicated three times according to a completely randomized design. The medium for growing the duckwed (*Lemna perpusilla* Torry) was a mixture of effluent and water. *Treatment 1 (Daily charged)*: 3 sinks charged daily each with 12.6-kg effluent to maintain media N concentration of about 32 mg/L. *Treatment 2 (6-d interval charged)*: 3 sinks charged each with 76 kg effluent at six-day interval.

Management of duckweed sink

Initally each sink was filled with tap water up to 35 cm depth and effluent was added at above mentioned rates. *Lemna* (*Lemna perpusilla* Torry) seed was added at the rate of 400 g/m^2. The media was stirred twice daily. Lemna was harvested at 48 h interval and yield was calculated by substracting the inoculum weight from the total biomass. The experiment was conducted during 28th September to 26th October' 1997 having ambient temperature of 27.95-28.5℃ respectively. The trial lasted for 28 days (14 harvests). The pH and temperature of the media were measured by a digital pH meter (Mettler Toledo AG) and light intensity by LUX meter (Lutron LX-101). Root length was measured by extending roots of individual frond and average of five fronds was considered as root length.

Chemical and statistical analysis

The sample of duckweed and media were analyzed for dry matter (DM), organic matter (OM) and nitrogen (N) according to the method of AOAC (1984); The acid detergent fibre (ADF) was determined according to Georing and van Soest (1970). All the data were analyzed by using ANOVA of completely randomized design according to the procedure of Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Media characteristics

Table 1 shows the media characteristics in terms of Kjeldhal-N & NH_3-N concentration, pH and light intensity. Kjeldhal-N & NH_3-N concentration of the media at different harvests are shown figure 1a and 1b. As expected, both Kjeldhal-N (p>0.05) and NH_3-N (p<0.05) concentration were higher in daily charged sinks than those charged at six-day interval. Temperature (28.48℃ vs. 28.60℃) and pH (6.82 vs. 6.84) were similar in both groups of sinks. However, light intensity was slightly higher (p>0.05) in six-day sinks than those charged daily (56500 vs. 49000 Lux). Temperature was within the optimum range (20-30℃) for maximum duckwed growth (Leng et al., 1995). Acidic pH (<7) of the media indicate that $NH_4^+ \leftrightarrow NH_3$ balance shift towards ionized state (NH_4^+) which is the preferred source of N for duckweed (Leng et al., 1992). Light intensity for both groups of ponds was higher in the present trial (49000 to 56500 Lux) than the suggested intensity (12420 Lux) for optimum photosynthesis of *Spirodela polyrrhiza* (Gaponenko and Stazhetshi, 1969). Higher light intensity is associated with high ammonia uptake (Yoshimura, 1952) with consequent increase in rate of multiplication, frond size and frond weight (Ashby and Oxely, 1935).

Biomass yield

Biomass yield of duckweed at different harvests and their mean is shown in table 2 and figure 2. Biomass yield was higher (p>0.05) in daily charged ponds (93 g/m^2/d) than those charged at six-day interval (80 g/m^2/d). Yield was higher in this trial than our previous trial (63 g/m^2/d) where *Lemna* was grown in small ($0.59 \times 0.54 \times 0.35$ m^3) polythene sinks with fermented cattle manure (Chowdhury et al., 1999). Yield was also higher in this trial than that observed by DRP (1996) in commercial ponds fertilized with human excreta (76 g/m^2/d). In Thailand, yield of *Lemna* in sepatge-loaded earthen ponds found to be

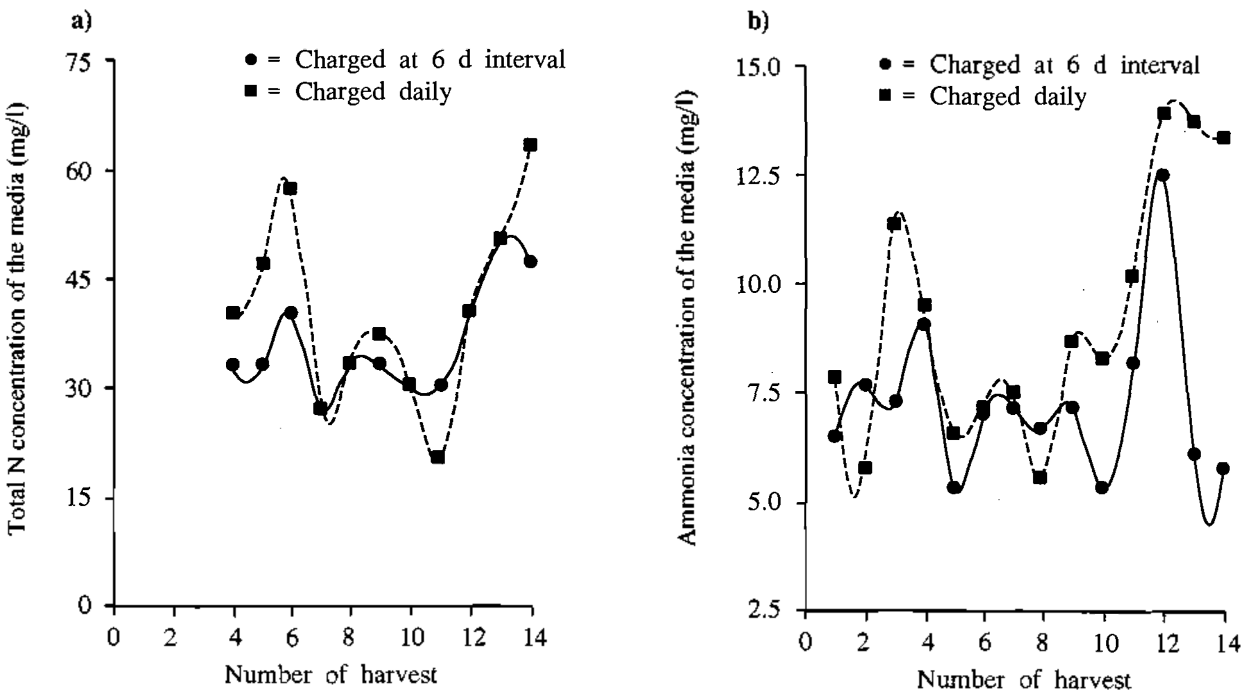


Figure 1. Effect of charging anaerobically fermented cattle manure at two loading frequencies on the media Kjeldhal-N (figure 1a) and NH_3-N (figure 1b) concentration at different harvests. Each point represents the mean of three observations.

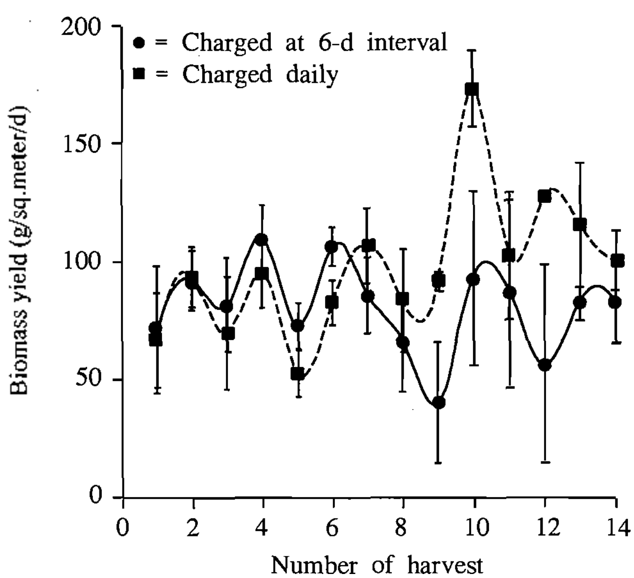


Figure 2. Effect of charging anaerobically fermented cattle manure at two loading frequencies on biomass yield of *Lemna* at different harvests. Each point represents the mean of three observations with vertical bar as standard deviation. Star (*) indicate that biomass yield differe significantly ($p<0.05$) at that particular harvast between the two treatments.

Table 2. Biomass yield (g/m²/d) of *Lemna* in different harvests at two nutrient loading frequencies

Harvests	Nutrient charging		SED	Significance
	Daily charged	6-d interval	(Residual *df*=26)	
1st	66	71	15.8	NS
2nd	93	91	15.6	NS
3rd	69	81	18.0	NS
4th	94	109	11.7	NS
5th	52	72	8.0	NS
6th	82	106	7.15	NS
7th	106	85	12.9	NS
8th	83	65	30.2	NS
9th	91	40	27.4	NS
10th	117	92	23.1	NS
11th	102	86	27.6	NS
12th	127	56	24.8	NS
13th	115	82	2.5	NS
14th	100	82	12.4	NS
Average	93	80	29.3	NS

NS=Statistically not significant.

varying from 39 to 63 g/m²/d (Edwards et al., 1992). However, in Vietnam (Anh and Preston, 1998; Anh et al., 1997; Rodriguez et al., 1996) biomass yield of *Lemna* grown with fermented pig manure recorded to be higher biomass yield (90-120 g/m²/d) than the present trial with fermented cattle manure. No apparent relationship was observed between the biomass yield and Kjeldhal N (figure 3) or ammonia N (figure

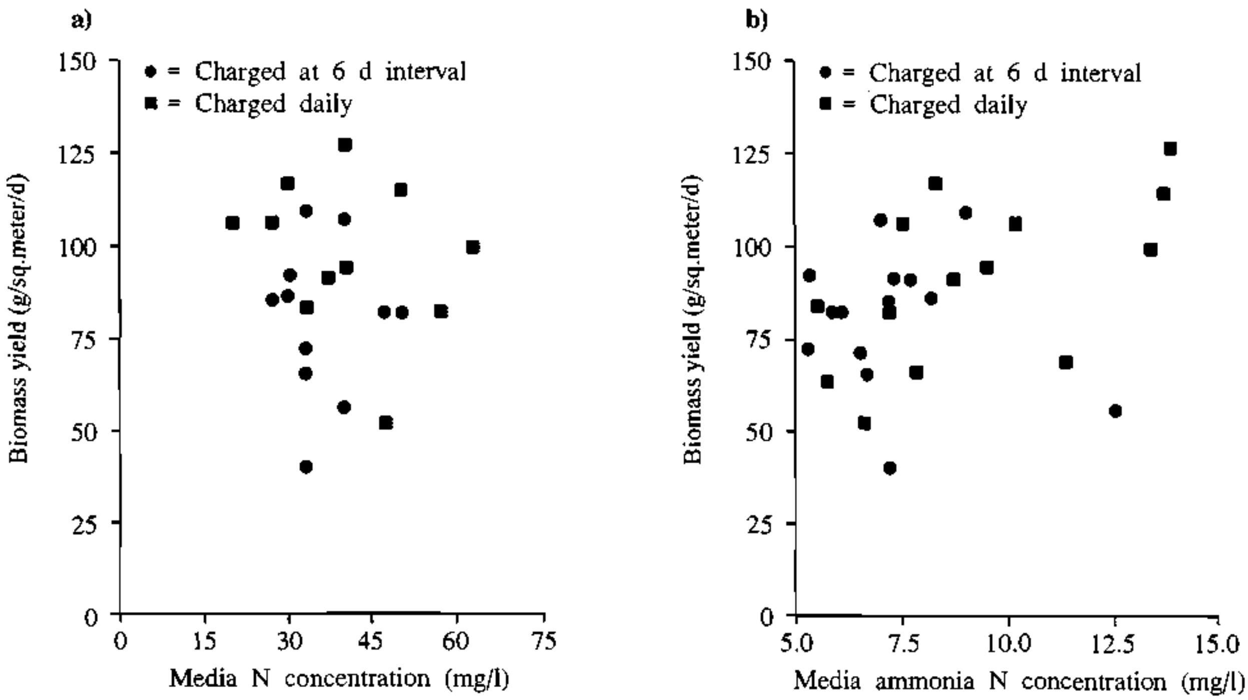


Figure 3. Relationships between the biomass yield of *Lemna* and media Kjeldhal N (figure 3a) or NH_3-N (figure 3b) concentration. Each point represents the mean of three observations.

Table 3. Nutrient content of *Lemna* at two nutrient loading frequencies

Nutrient content (%)	Nutrient charging		SED (Residual *df*=26)	Significance
	Daily	6-d interval		
Dry matter	4.52	4.15	0.2449	NS
Organic matter	83.41	84.51	1.638	NS
Crude protein	31.39	29.62	2.02	NS
Ash	15.17	15.48	0.4509	NS
Organic carbon*	47.12	46.96	0.305	NS

* Organic carbon per cent estimated as (100-% Ash)/1.8 (Edwards et al., 1992).
NS=Statistically not significant.

3b) concentration of the media. This is similar to our previous observation (Chowdhury et al., 1999) but not in accordance with the observation of Rodriguez and Preston (1996). They showed that biomass yield was linearly related with the media N concentration. Over the normal range (15-150 mg N/L), media N concentration is positively correlated with the biomass yield (BLRI, 1997; Rodriguez and Preston, 1996). In this trial, media Kjeldhal N concentration ranges between 27-47 (mean 38) mg/L for ponds charged at 6 d interval and 20-63 (mean 40) mg/L with ponds charged daily, which are with in the optimum range. This may indicates that other macro-or micro-nutrients rather than N, is probably limiting in this system of cattle manure based duck- weed production.

Nutrient content

Table 3 shows the nutrient content of duckweed. Average dry matter, organic matter and ash content were similar in either systems of nutrient loading. Crude protein content at different harvests shows marked variation within the treatment over the experimental period (figure 4) but average crude protein content was nearly similar ($p>0.05$) in daily charged ponds (31.39%) and those charged in 6 d interval (29.62%). Further, there was no significant relationship between the crude content of *Lemna* and media Kjeldhal N (figure 5a) or NH_3-N (figure 5b) concentration. This is quite different from the observation that crude protein content of *Lemna* is linearly related to the N concentration of the media (Rodriguez and Preston, 1996). Leng et al. (1994) showed that crude protein content of duckweed increases linearly from traces concentration of ammonia and reaches asymptotic value at media Kjeldhal N concentration of about 15 mg/L Leng et al. (1994). In the persent trial media NH_3-N or Kjeldhal N concentration in either frequencies of nutrient loading were in the range of 5.3-12.5 mg/L and 20-65 mg/L respectively, which are almost at the saturation levels. Thus absence of relationship between crude protein content of Lemna and media Kjeldhal N or NH_3-N concentration could be expected.

Crude protein content of duckweed was positively

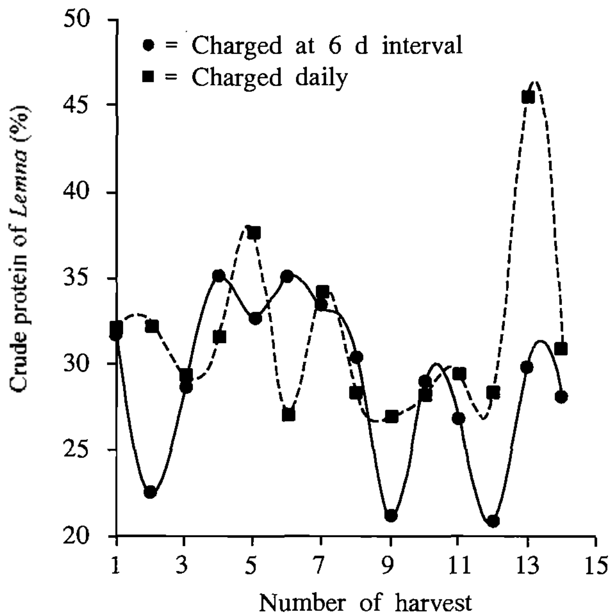


Figure 4. Crude protein content of *Lemna* at differnt harvests, cultivated at two nutrient loading frequencies. Each point represents the mean of three observations.

observation of Rodriguez and Preston (1996), who showed that crude protein content of duckweed is negatively correlated with its root length. This is probably because root length of duckweed may not necessarily be related to the media N concentration alone particularly when media N level is about 30 mg/L which is well above the saturation level of 15 mg/L (Leng et al., 1994).

Nutrient yield

Although dry matter (4.3 vs. 3.90 $g/m^2/d$) and organic matter (3.75 vs. 3.21 $g/m^2/d$) yield were significantly ($p<0.05$) higher but the crude protein yield (1.238 vs. 1.223 $g/m^2/d$) was only marginally higher ($p>0.05$) in the daily charged ponds than 6-d interval charged ponds (table 4). Compared to that of the six days charged ponds crude protein yield was lower ($p>0.05$) during the initial 7 harvests in daily charged pods but was higher ($p>0.05$) in the last 7 harvests (figure 7). This is probably because nutrient (N in particular) concentration was initially lower in the daily charged ponds (see figure 1a). Dry matter

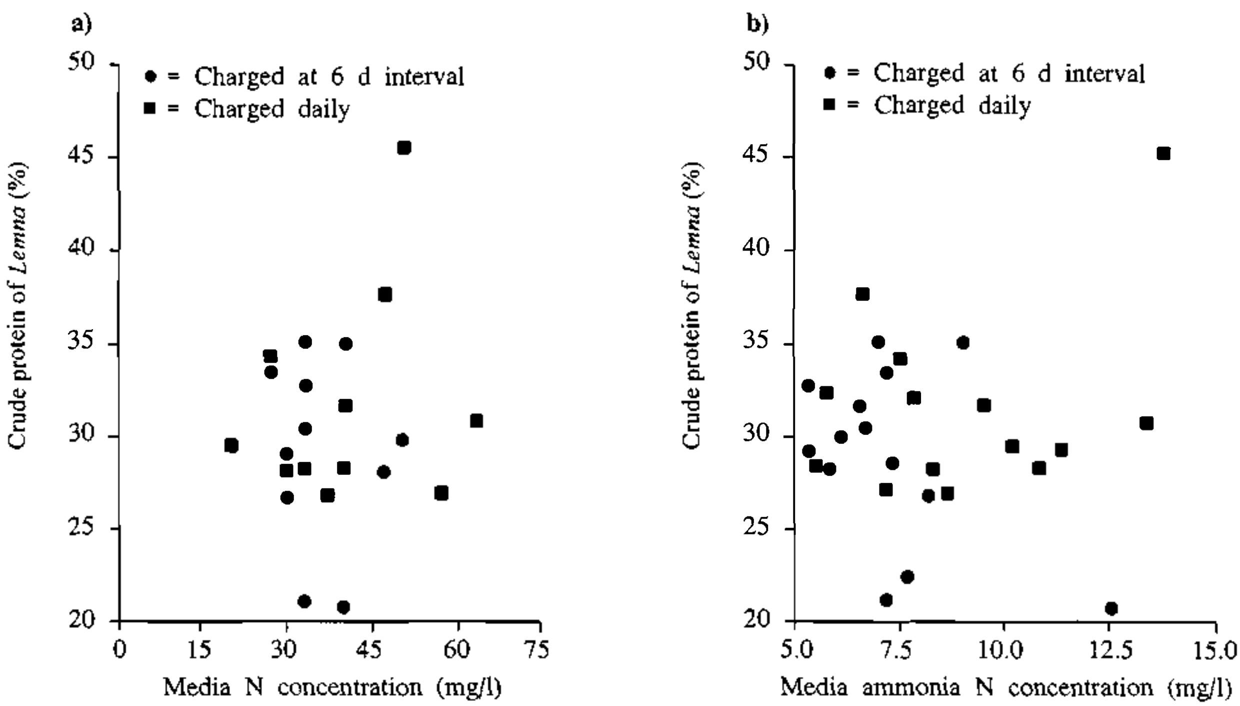


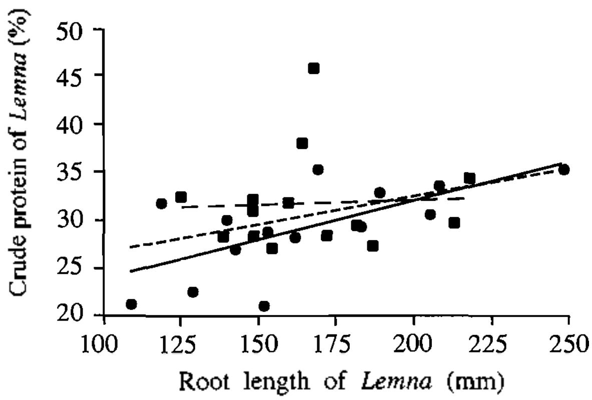
Figure 5. Relationships between the crude protein content of *Lemna* and media Kjeldhal N (figure 5a) or NH_3-N (figure 5b) concentration. Each point represents the mean of three observations.

correlated (r^2=0.42) with the root length of duckweed in 6 days charged ponds but no such relationship was observed for daily charged ponds (figure 6). Positive or absence of relationship was also observed in previous trial in the laboratory (Chowdhury et al., 1999). However, this is completely different from the and crude protein yield in this trial is quite comparable to our earlier observations (Chowdhury et al., 1999). Protein yield of DW is much higher than the conventional livestock fodder, e.g. maize, nepier grass. For example, annual CP yield of maize (fodder) under Bangladesh conditions found to be 0.81 Ton/Hac

Table 4. Average dry matter, organic matter and crude protein yield (g/m^2/d) of *Lemna* at two nutrient loading frequencies

Parameters	Nutrient charging		SED (Residual *df*=26)	Significance
	Daily	6-d interval		
Dry matter yield	4.30	3.90	0.0712	$p<0.05$
Crude protein yield	1.238	1.112	0.1038	NS
Organic matter	3.75	3.21	0.0852	$p<0.05$

NS=Statistically not significant.



• = 6 days {Y=15.61+0.081(X); (r=0.65; n=14; t=3.02; p<0.05)}
▪ = Daily {Y=30.34+0.008(X); (r=0.04; n=14; t=0.146, p=0.88
Combined: Y=20.66+0.058(X); (r=0.38; n=28; t=2.10; p<0.05

Figure 6. Relationships between the root length and crude protein content of *Lemna* cultivated in polythene sinks, nourished by anaerobically fermented cattle manure, charged at two loading frequencies. Solid, dashed and doted line indicate linear regression between the root length and crude protein content of 6-d charged and combined (6-d & daily charged) respectively.

(Huque et al., 1995) while corresponding values for the Lemna in the present trial were approximately 4.5 Ton/Hac respectively. Another advantage of duckweed is that it can be harvested throughout the year in 24 to 48 hour interval which is not possible for traditional fodder. Thus recycling anaerobically fermented farm manure through DW production can ensure year round protein rich forage supply for livestock.

Implication

Provided other physiological conditions are conductive, productivity of duckweed depends on

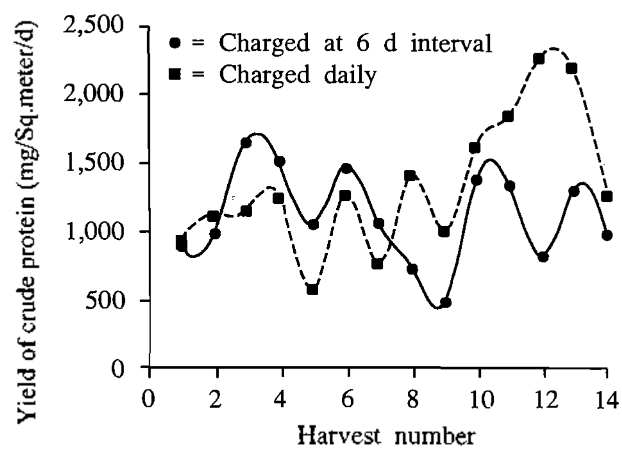


Figure 7. Crude protein yield of *Lemna* at different harvests, cultivated at two nutrient loading frequencies. Each point represents the mean of three observations.

media nutrient concentration. Under the present experiment conditions, nutrient-loading frequency had no significant effect on the biomass and protein yield of *Lemna*. *Lemna* produced from fermented cattle manure contain 30% curde protein; has a better array of essential amino acids, than most vegetable protein and more closely resembles animal protein than other vegetable proteins. Thus it can be used as protein supplement in chicken, duck and fish diet. Despite huge potentiality of its use as protein supplement, especially for small-holder poultry/fish production, its utilization is still limited partly due to its fluctuating avalilability. Method described here can be used for year-round duck weed production from fermented cattle manure. For year-round production of duckweed in small polythene sink following management practice can be suggested :

1) A $6\times2\times0.5$ m^3 polythene sink is filled with water up to 35 cm.
2) An initial charge of 76 kg of effluent.
3) Charge either daily with 12.5 kg or with 75 kg in 6 day interval.
4) Harvest *Lemna* at 24-48 hour interval.

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