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GROWTH AND PRODUCTION OF LESSER DUCKWEED (*Lemna minor*) IN DIFFERENT MANURE SOLUTIONS AND CONCENTRATIONS

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ABSTRACT

This study was done at the glass house of University of Diponegoro, Semarang. The research was about investigating the performance of *Lemna minor* from Lemna spp. of Lemnaceae family when grown for 14 days under 3 different manure types and concentration levels. The beef cattle manure, chicken manure and dairy cattle manure were mixed with 30 liters tap water in a triplicate media concentration of 0g/l, 5g/l and 10 g/l. In addition to the manures 1 kg top soil was added in all experimental units and 20 g of Lemna minor was planted as the initial plant weight. The manure type and manure concentration level were arranged in a 3x3 factorial completely randomized design arrangement and mean comparisons was done with Duncan Multiple Range Test. The results in ANOVA showed that there was significant interaction (F=6.31, p=.0023) as well as significant main effects (F=21.51, p=.0001; F=52.78, p=.001) for FY. For the GR the manure type x concentration level interaction was significant (F=6.30, p=.0024) and type of manure and level of concentration main effects were also significant (F=21.45, p=.0001; F=52.63, p=.0001). Significant interaction was also noticed for CPC (F=28, p=.0001) and significant main effects of manure and concentration (F=72.64, p=.0001; F=29.12, p=.0001). The average pH of the manure solutions before planting and after harvesting was 6.07 and 6.48 respectively. Prior to preparing the mediums duplicate samples of various manure as well as top soil were tested for Kjeldahl Nitrogen on dry matter basis.

Key words: Lemnaceae, Lemna minor, Lemna spp., manure

INTRODUCTION

The traditional methods of raising farm animals have been replaced by modern intensive farming mechanisms which involve large animal farms and confined feeding systems. This transformation has made animal industries to produce meat, milk and egg at large scale in relatively shorter periods with minimum operational cost hence the industries are destined to be successful. However, the major draw-back to these concentrated facilities is the production of large volumes of manure that must be disposed into the environment without compromising environmental damage and human health.

Most of the large commercial poultry and livestock industries do not have enough farm land to spread the staggering amount of manure generated and store liquid manure in artificial lagoons and use conventional methods to purify the wastewater. Manure provides an excellent source of organic fertilizer due to the presence of macro and micro nutrients that can help enhance plant growth and production. Furthermore, the organic matter residual can improve soil physical and chemical properties, however, over application of manure will result in nutrients accumulating in the soil surface and during the rainy seasons it is inevitable that the nutrients will end up in the aqua systems through run-off and leaching. Furthermore, when the manure storage facilities are not maintained the liquid manure will seep out of the tanks and into the environment (Barker and Walls, 2002). It is inevitable that when manure reach the waterbodies the accumulation of nutrients will cause cultural eutrophication in surface water and contaminate portable underground drinking water and cause health problems like blue-baby syndrome. Therefore, in order to prevent negative effects of livestock waste it is proper to utilize the organic nutrients contained in the wastewater thus making the pollutants become assets instead of becoming costly liabilities.

One simple method of utilizing the contaminants in the wastewater is by employing aquatic plants. Among the few aquatic macrophytes used Lemna minor from duckweed group of plants is the best alternative due to its special adaptive characteristics. Duckweed from the *Lemna spp.* of the Lemnaceae Family is a free-floating fresh water microscopic plant. Lemna minor does not have true leaves or stems however, as a single body a leaf like structure which performs both the function of the leaves and stems (Cheng and Stomp, 2009). Though duckweeds are flowering plants however, the form of reproduction is exclusively through budding (asexual), daughter fronds form from parent fronds and then split and form new colonies (Goppy, 2003).

Duckweeds thrive in quiescent and waters high in nutrients especially nitrogen and phosphor compounds and can be found in lakes, ponds, ditches and brackish estuaries. Duckweed can. These aquatic plants can adapt to a wide range of temperatures ranging from 6-33 ^{o}C (Reid JR. 2004) and able to tolerate extreme ph of 3.5-10 (Glavas, 2010), however, the ideal pH range for optimum growth for duckweed is 6.5 to 7.5 range (Leng *et al*., 1995).

The by-product biomass of duckweed is regarded as an excellent feed or feed component due to the high nutritional composition and low fiber content. Though duckweed protein content depends on the aqua ecosystem the plant is growing however, when grown in high nutrient conditions the crude protein content will be between 35 to 43% range (Cheng and Stomp, 2009). This phenomenal protein content of duckweed is compared against alfalfa meal (20%) and soybean (41.7%) (Hillman, 1978, in the citation of Cheng and Stomp, 2009).

Due the duckweeds doubling time of 16 hours to 2 days (Leng *et al*., 1995) and ease of harvesting the biomass pollutants can be removed regularly and

permanently. This growth rate is faster than any other higher plants, and more loosely resembles the exponential growth of unicellular algae than that of any higher plants (Willett, 2005). Duckweed used for municipal wastewater became effective within 7-10 days and produced a minimal biomass of 36.6 tons dry weight/ha/year (Zhao *et al*. 2012). Duckweed can be employed as a post-treatment mechanism before the effluent can be discharged into the environment or used to irrigate crop farming (Bejarano, 2005).

Objectives and Benefits of The Research

The prime objective of this research is to observe in which manure medium and concentration level will suit Lemna minor growth and production. To utilize the nutrients contained in animal manure by employing duckweed in which the organic nutrients especially the nitrogen compounds are captured by Lemna minor and turn the once thought of as pollutants into a nutritious protein source for birds and animals. The benefits of the research were to establish the knowledge and understanding that Lemna minor crude protein as well as adaptation depends on the environment conditions the plants are growing. Moreover, nutrients contained in animal manure can be recycled through Lemna minor growth hence, preventing the nutrients from damaging the environment as well as human health and welfare.

Aims of The Research

The prime aim of this trial is to investigate the performance of Lemna minor when grown in different animal manure concentrations and solutions. The performance of Lemna minor is tried for its crude protein content, growth rate and fresh biomass yield.

MATERIALS AND METHODS

Location

This research was conducted at the glass house of University of Diponegoro, Semarang, from June to August 2012. The time period of Lemna minor growth was 14 days at which the plant biomass was harvested and the parameters determined.

Research Materials

Fresh beef cattle, chicken and dairy cattle manure and were collected at various farms at University of Diponegoro while top soil was gathered at campus vicinity and sun dried at the university glass house for 3 days. Fresh lesser duckweed plant was collected at the nearby lake and separately grown to increase plant

population up to 540 g in order to cater the 27 containers. The circular containers used for growing Lemna minor had a surface area of 0.12 m^2 with a volume of 30 liters.

Research Procedure

The various livestock manure (beef cattle, chicken and dairy cattle) were sun dried for 3 days at the university glass house. The samples were dried again for constant weight in the oven at 105 ^oC for 3-4 hours and tested for Kjeldahl Nitrogen. The manures were mixed with tap water for the triplicate media concentration of 0 g/l, 5 g/l and 10 g/l. Top soil of 1 kg was added in all experimental units and the initial plant population was 20 grams in all units, while evaporated water was replaced with equal amount of tap water. Media pH was taken before planting and after harvesting with a pH paper.

Research Design and Statistical Hypothesis

This is a 3 x 3 factorial (α: beef cattle; chicken; dairy cattle and β: 0 g/l; 5 g/l; 10 g/l) completely randomize design with 3 replicates.

Criteria for testing hypothesis

The hypothesis was tested with analysis of variance (ANOVA), while mean comparison after significant treatment noticed in the ANOVA was done using Duncan Multiple Range Test (DMRT).

Analytical Procedures and Measurement Methods

Fresh Yield and Growth Rate

At the end of the growing period of 2 weeks the plant biomass was harvested and plant fresh yield (weight), growth rate of the colonies biomass were determined. After harvesting the Lemna minor biomass was blotted on a blank A4 paper before weighed to determine the fresh yield. The fresh yield was then used to calculate the crop growth rate (CGR) of the plant population in each experimental unit. The CGR was calculated using the formula as stated by Hunt (2003); [(W2 – W1) / (T2 – T2)], where CGR is the crop growth rate; W1 and W2 are the initial and fresh weight of Lemna minor plants, while T1 and T12 are the initial and final time (period the plants were allowed to grow) in days.

Kjeldahl Nitrogen

The nitrogen content of Lemna minor was determined after drying at 60 ^{o}C by the ISO method of Kjeldahl nitrogen. Crude protein content of Lemna minor was then estimated by multiplying the KN by 6.25. The Formula adopted as follows;

$$CPC = \frac{(A - B) \times C \times D \times E}{F} \times 100\%$$

Where;

A = Titration volume of sample
B = Titration volume of blank
C = Nitrogen Hydrochloric acid
D = Molecular weight of nitrogen (0.014)
E = Estimated protein content of plants (6.25)
F = Weight of Sample (dry basis in grams)

RESULTS AND DISCUSSION

Fresh Yield (FY) and Growth Rate (GR)

The analysis of variance (Table 1) with $\alpha = 5\%$ level significance shows that the two IVs have significant interactions (F=6.31, p=.0023) as well as significant main effects (F=21.51, p=.0001; F=52.78, p=.001) for FY hence, the quantity of Lemna minor biomass as FY after day 14 varied in different concentrations and manure types. The GR at $\alpha = 5\%$ level significance (Table 3) the manure type x concentration level treatment effects had significant interactions (F=6.30, p=.0024) and type of manure and level of concentration main effects were also significant (F=21.45, p=.0001; F=52.63, p=.0001). The mean comparison with DMRT for FY (Table 2) and GR (Table 4) indicates that means with same letters represent no significant effects while different letters shows significant treatments effects. The graphs in Fig 1. and Fig. 2 for FY and GR respectively, shows that growth rate is proportional to biomass yield. The close relationship between GR and FY is influenced by the nutrient condition of the aqua ecosystem the plants are growing. As pointed out by Leng (1999), in nutrient-rich conditions the colonies of duckweed will undergo rapid growth rate and this is the stage where plant population also increases to its highest. According to the graphs the growth rate was phenomenal in the 5 g/l chicken manure media culture and this is the period where highest plant yield was recorded (See Table 2). When the growth rate declined in the 10 g/l concentration the biomass yield also decreased.

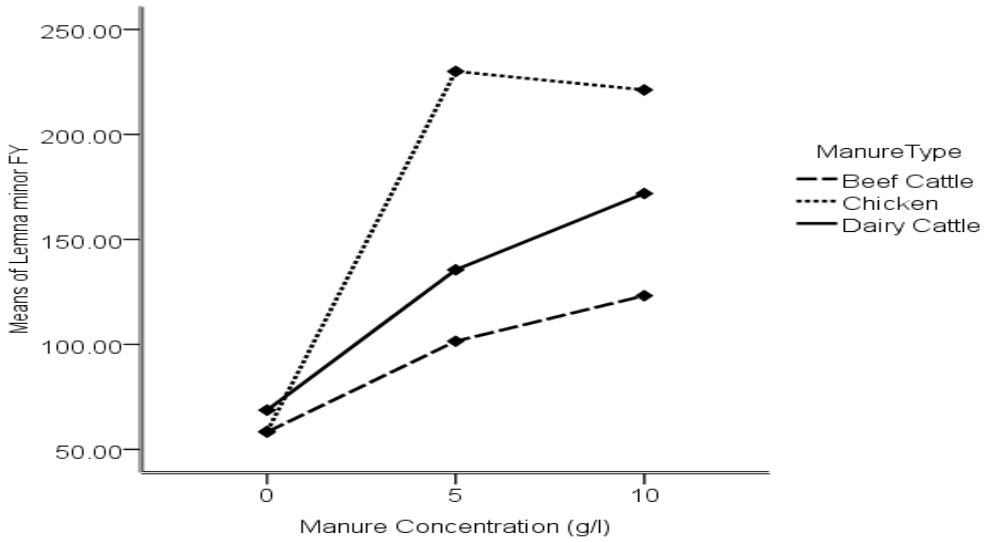

Fig. 1 Influence of Manure Type and Manure Concentration Level on Fresh Yield of Lemna mina minor

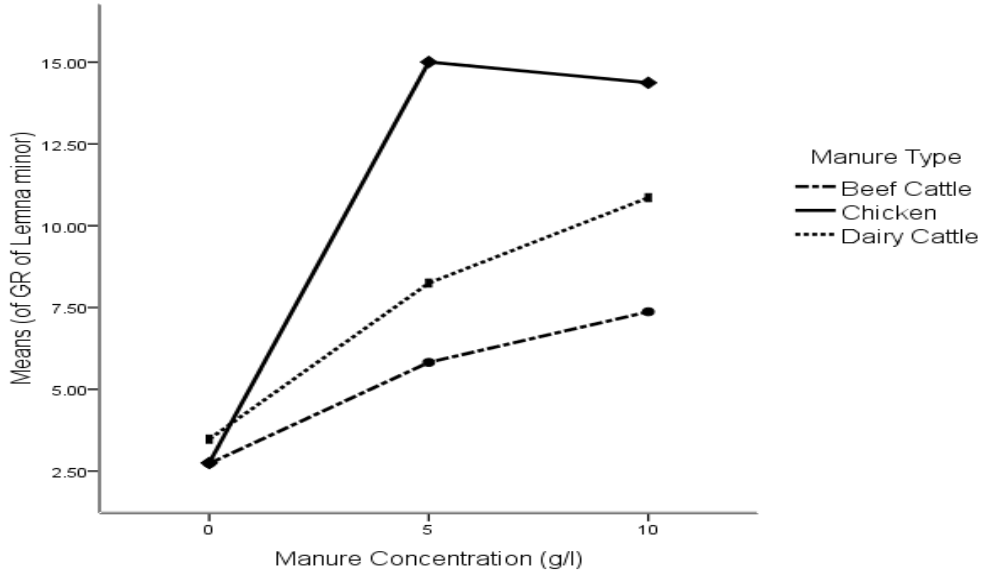

Fig 2. Relationship between the Manure Type and Concentration Level on Growth Rate of Lemna minor

Table 1. Analysis of variance for FY

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Manure Type	2	25938.70296	12969.35148	21.51	< 0.0001
Concentration Level	2	63651.70296	31825.85148	52.78	< 0.0001
Manure*Concentration	4	15228.53926	3807.13481	6.31	< 0.0023

Testing Interaction

Table 2. Duncan Multiple Range Test for FY

Duncan Grouping	Mean	N	MC
A	230.00	3	M2C2
A	221.00	3	M2C3
B	171.83	3	M3C3
BC	135.50	3	M3C2
C	123.17	3	M1C3
CD	101.50	3	M1C2
D	68.67	3	M3C1
D	58.50	3	M2C1
D	58.27	3	M1C1

Means with the same letter are not significantly different

Table 3. Analysis of variance for GR

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Manure	2	132.5580222	66.2790111	21.45	< 0.0001
Concentration	2	325.2173556	162.6086778	52.63	< 0.0001
Manure*Concentration	4	77.8206889	19.4551722	6.30	< 0.0024

Table 4. Duncan Multiple Range Test on GR

Duncan Grouping	Mean	N	MC
A	15.003	3	M2C2
A	14.370	3	M2C3
B	10.857	3	M3C3
BC	8.247	3	M3C2
C	7.370	3	M1C3
CD	5.823	3	M1C2
D	3.477	3	M3C1
D	2.750	3	M2C1
D	2.733	3	M1C1

Means with the same letter are not significantly different.

Crude Protein Content

The crude protein content of Lemna minor is dependent on the nutrient availability of the aqua system the plants are growing. As stated by the Leng *et al*. (1995), the protein content of duckweeds grown in a nutrient scarce condition will be at 15 to 25% range while in a rich nutrient ecosystem the protein will be between 35 to 43%. Cheng and Stomp, (1999) also stated duckweeds having protein content range of 15 to 45%. In this trial the highest protein content on average was 29. 19 in 5 g/l chicken manure solutions. Therefore, chicken manure has more available nitrogen which can support duckweed growth and production and enable the plants to synthesize ideal protein content in its biomass. The analysis of variance (Table 5) with α = 5% level significance indicates that the manure x concentration has significant interaction (F=28, p=.0001) and significant main effects of manure and concentration (F=72.64, p=.0001; F=29.12, p=.0001).

Table 5. Analysis of variance for CPC

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Manure	2	686.1071372	343.0535686	72.64	< 0.0001
Concentration	2	274.9891801	137.4945900	29.12	< 0.0001
Manure*Concentration	4	528.8635244	132.2158811	28.00	< 0.0001

The mean comparison (Table 6) result indicate that the treatment difference between 10 g/l and 5 g/l were significant as well as the rest of the concentrations. The means with same letters shows that the mean differences are not statistically significant therefore, for CPC the only two chicken manure solutions that showed significant effect are 5g/l and 10 g/l.

Table 6. Duncan Multiple Range Test for CPC

Duncan Grouping	Mean	N	MC
A	29.181	3	M2C3
B	21.731	3	M2C2
C	10.754	3	M3C2
C	9.013	3	M1C2
C	8.854	3	M3C1
C	8.502	3	M3C3
C	7.427	3	M1C1
C	6.990	3	M1C3
C	6.681	3	M2C1

Means with the same letter are not significantly different.

pH of manure mediums

The pH of the various manure concentrations were taken twice before planting Lemna minor and after harvesting of the biomass at day 14. The mean pH values before and after was 6.07 and 6.44 respectively. The increase in the pH of the cultured media indicates that duckweed can raise the pH an acidic wastewater to a near neutral pH.

CONCLUSION

The following conclusions were drawn from this experiment : Chicken manure has higher protein availability to support duckweed growth and production. This is evident in the high Lemna minor crude protein content as well as growth rate and biomass yield. The manure concentration of 5 g/l is the appropriate dose for the GR and plant population in chicken manure solutions. While the 10 g/l concentration suited CPC of Lemna minor. Duckweed adaptability depends on the nutrient density of the aqua environment as well as factors such as space availability and overcrowding of colonies.

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