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Effect of Poultry Excreta on Water Quality and Daphnia *Magna* Production in Chlorella Powder Medium

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Abstract: *Daphnia magna* Straus, 1820, plays a significant role in food supply for fish farming; thus, it is important to formulate a medium that can increase the *D. magna* population. This can be achieved by supplying a direct nutrient for *D. magna* while supporting the growth of the phytoplankton and zooplankton population. However, at certain levels, the nutrient source addition adversely affects the culture medium. This study aims to investigate the effects of different concentrations of poultry excreta included in a culture medium enriched with chlorella powder on the biomass production of *D. magna*. An experiment was conducted by adding poultry excreta at four concentrations (0, 2, 4, and 6 $g \cdot L^{-1}$) to the chlorella powder culture medium. The culture was maintained for 15 days, and samples were collected on days 0, 5, 10, and 15 to analyze the *D. magna* population, water pH, and concentrations of ammonia, nitrate, and dissolved oxygen (DO). Furthermore, a statistical evaluation was conducted using one-way analysis of variance in a completely randomized research design. The results showed that an increase in the poultry excreta concentration reduced the water quality ($P < 0.01$), as indicated by the water pH and ammonia, nitrate, and DO concentrations. In addition, a longer duration of the experiment substantially improved the qualitative parameters evaluated. Similarly, the population of *D. magna* was significantly affected ($P < 0.01$) by both factors. In conclusion, the addition of poultry excreta decreased the water quality of the chlorella powder medium. The water quality improved on prolonged days; thus, the highest population of *D. magna* was achieved on day 15 using 2 $g \cdot L^{-1}$ of poultry excreta.

Keywords: *Daphnia Magna*, Daphnia production, Chlorella powder medium, poultry excreta, water quality.

家禽排泄物对小球藻粉培养基水质和大溞产量的影响

摘要：大型水蚤，1820年，在鱼类养殖的食物供应中发挥着重要作用；因此，重要的是配制可以增加大麦格纳菌种群的培养基。这可以通过为D.麦格纳提供直接养分同时支持浮游植物和浮游动物种群的生长来实现。然而，在某些水平上，添加营养源会对培养基产生不利影响。本研究旨在研究富含小球藻粉的培养基中不同浓度的家禽排泄物对大麦格纳生物量产生的影响。通过向小球藻粉末培养基中添加四种浓度（0、2、4和6g•升-1）的家禽排泄物进行了一项实验。培养物维持15天，并在第0、5、10和15天收集样品以分析D.麦格纳种群、水的酸碱度值以及氨、硝酸盐和溶解氧(做)的浓度。此外，在完全随机的研究设计中使用方差的单向分析进行了统计评估。结果表明，家禽排泄物浓度的增加降低了水质（磷<0.01），如水的酸碱度值和氨、硝酸盐和溶解氧浓度所示。此外，更长的实验持续时间大大改善了评估的定性参数。同样，这两个因素都显着影响了D.麦格纳的种群（磷<0.01）。总之，家禽排泄物的添加降低了小球藻粉末培养基的水质。水质在延长的日子里有所改善；因此，使用 2 g•升-1 的家禽排泄物在第 15 天实现了最大数量的D.麦格纳。

关键词：大型水蚤，水蚤产量，小球藻粉培养基，家禽排泄物，水质。

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1. Introduction

Fish farming requires specific culture techniques as well as live food organisms to serve as feed, including zooplankton, which is naturally used in aquaculture. *Daphnia magna* Straus is considered an important zooplankton species in freshwater ecosystems, owing to its ability to control microbial populations and serve as a food supply for fish [1]. These cladocerans exhibit superior nutritional and physical properties in terms of particle size, digestibility, and essential nutrient content, which meet the requirements for the growth and reproduction of larvae and fish [2]. In addition, *Daphnia sp*. contains several enzymes, such as proteinases, peptidases, amylases, lipases, and cellulase, which can serve as digestive enzymes for fish larvae [3]. Therefore, the production of *Daphnia sp*. biomass has attracted considerable research interest over the decades, particularly in terms of their feeding techniques and nutrient requirements.

Some previous studies have indicated the effect of adding organic substrates, such as wheat bran, soybean powder [4], tofu waste [5], and animal wastes [6], to the culture medium. Animal wastes can supply nutrients for the proliferation of phytoplankton and bacteria, which serve as feed for *Daphnia sp*. However, certain levels of animal wastes in the culture medium tend to produce toxic effects on the reproduction of *Daphnia magna* Straus, 1820 [6].

This toxicity can be avoided by developing numerous culture media (e.g., chlorella powder) with the aim of directly supplying the relevant nutrients [4] [7], [8], especially essential amino acids and unsaturated fatty acids [9]. However, it is necessary to develop a medium that can adequately support the growth of both phytoplankton and zooplankton [10] [11]. Therefore, this study aims to determine the optimum concentration of poultry excreta that needs to be added to chlorella powder-enriched cultures to support the production of *D. magna*.

2. Materials and Methods

2.1. Materials

Daphnia magna Straus, 1820, was originally isolated from the river area of South Kalimantan, followed by its reservation and maintenance in the laboratory. Conversely, the poultry excreta were obtained from a local broiler farm, and the *Chlorella Sp*. powder was purchased from Ugoplanktonshop (Central Java, Indonesia).

2.2. Procedure for *D. Magna* Culture

An experiment was conducted by adding four concentrations (0, 2, 4, and 6 $g \cdot L^{-1}$) of poultry excreta, which was dried and ground through a 1-mm mill size. These samples were individually incorporated into a 60 × 40 × 30 cm^3 chamber containing 20 L of tap water, with each level replicated three times. The experimental medium was aerated for eight days, followed by inoculation with 30 $heads \cdot L^{-1}$ of *D. magna* or 600 heads per chamber; then, the chlorella powder was added at 1000 mg per day. Next, 500 mL of the water sample was collected on days 0, 5, 10, and 15 for determining the microorganism population and water quality, including pH, as well as the concentrations of ammonium, nitrate, and dissolved oxygen (DO).

2.3. Data Analysis

The data were statistically evaluated using the analysis of variance (one-way ANOVA) with a completely randomized research design. All statistical analyses were conducted using SPSS 22.0 software (IBM, USA).

3. Results

3.1. Water Quality

The concentration of poultry excreta added to the chlorella powder culture medium significantly ($P < 0.01$) affected the medium pH according to the *D. magna* culture period (Table 1). On day 0, the poultry excreta levels resulted in a relatively similar pH of culture media, ranging within a neutral pH (6.95–7.32). Then, higher amounts of poultry excreta increased the water pH water on day 5. However, on days 10 and 15, the medium without poultry excreta resulted in the highest pH value (8.39). Meanwhile, the media with poultry excreta addition had slightly neutral pH values, except for the poultry excreta at 6 $g \cdot L^{-1}$ (pH 8.01).

Table 1 Effects of poultry excreta on the pH of chlorella powder culture medium

	Excreta levels ($g.L^{-1}$)			
Days	**0**	**2**	**4**	**6**
0	7.32±0.61	7.25±0.01	7.13±0.04	6.95±0.35
5	6.98±0.01^{c}	6.08±0.01^{a}	6.95±0.02^{b}	7.21±0.01^{d}
10	8.39±0.01^{d}	7.20±0.01^{a}	7.23±0.01^{b}	8.01±0.01^{c}
15	8.39±0.01^{d}	7.20±0.01^{a}	7.23±0.01^{b}	8.01±0.01^{c}

$^{a, b, c, d}$ Means with different superscripts in the same row are significantly different ($P < 0.01$)

The amount of poultry excreta had a significant ($P < 0.01$) positive effect on ammonium concentration in the experimental medium, as shown in Table 2. Subsequently, a decline in the ammonia concentration was observed when extending the culture period.

Table 2 Effects of poultry excreta on ammonium concentration ($mg.L^{-1}$) in chlorella powder culture medium

	Excreta levels ($g.L^{-1}$)			
Days	**0**	**2**	**4**	**6**
0	0.23±0.19	1.14±0.01	1.68±0.01	2.64±0.02
5	0.28±0.01^{a}	0.64±0.10^{b}	2.24±0.10^{c}	0.52±0.01^{b}
10	0.26±0.01^{b}	0.19±0.01^{a}	0.32±0.01^{c}	0.37±0.01^{d}
15	0.26±0.01^{b}	0.19±0.01^{a}	0.32±0.01^{c}	0.37±0.01^{d}

a, b, c, d Means with different superscripts in the same row are significantly different ($P < 0.01$)

The concentration of poultry excreta conferred a significant effect on the concentration of nitrate present in the experimental medium ($P < 0.01$), as shown in Table 3. However, a decline in the concentration was observed during a prolonged period of exposure.

Table 3 Effects of poultry excreta on the nitrate concentration ($mg.L^{-1}$) of chlorella powder culture medium

	Excreta levels ($g.L^{-1}$)			
Days	**0**	**2**	**4**	**6**
0	3.00±0.00	39.60±0.00	54.60±1.00	30.40±0.20
5	0.28±0.01^{a}	0.64±0.10^{b}	2.24±0.10^{c}	0.52±0.10^{b}
10	3.35±0.28^{b}	4.80±0.10^{c}	4.80±0.10^{c}	0.10±0.00^{a}
15	3.50±0.10^{b}	4.80±0.10^{c}	4.80±0.10^{c}	0.10±0.00^{a}

a, b, c Means with different superscripts in the same row are significantly different ($P < 0.01$)

The DO content on day 5 was significantly affected by the concentration of poultry excreta ($P < 0.01$), although a reduction in the concentration was subsequently observed on days 10 and 15 ($P > 0.05$). This study showed increased DO concentrations after prolonged days of culture which were not significantly affected by the concentration of poultry excreta on day 10 and 15 (Table 4).

Table 4 Effects of poultry excreta on DO ($mg.L^{-1}$) of chlorella powder culture medium

	Excreta levels ($g.L^{-1}$)			
Days	**0**	**2**	**4**	**6**
0	7.80±0.10	7.20±0.10	7.30±0.10	7.20±0.10
5	5.50±0.10^{b}	7.70±0.10^{a}	7.60±0.10^{a}	4.20±0.10^{c}
10	7.03±0.87	7.03±0.97	7.83±0.38	7.03±0.87
15	7.03±0.87	7.03±0.97	7.83±0.38	7.03±0.87

a, b, c Means with different superscripts in the same row are significantly different ($P < 0.01$)

3.2. *D. Magna* Population

Table 5 shows the significant effect of poultry excreta concentration on the *D. magna* population on day five ($P < 0.05$). This was very significant at day 10 ($P < 0.01$), and it was the reverse on day 15 ($P > 0.05$). Despite the relatively higher population in medium, without the treatment, a subsequent decline was observed after prolonged period of exposure. However, the presence of poultry excreta increased the *D. manga* population in the following days, with the highest obtained on day 15, using a concentration of 2 $g.L^{-1}$.

Table 5 Effects of poultry excreta on *D. Magna* population ($head.L^{-1}$)

	Excreta levels ($g.L^{-1}$)			
Days	**0**	**2**	**4**	**6**
0	300±0	300±0	300±0	300±0
5	1,044±315^{b}	255±93^{a}	99±61^{a}	865±245^{b}
10	888±305^{l}	611±224^{kl}	362±30^{k}	835±159^{l}
15	876±208	1,488±917	836±378	782±34

a, b Means with different superscripts in the same row are significantly different ($P < 0.01$)
k, l Means with different superscripts in the same row are significantly different ($P < 0.05$)

4. Discussion

Daphnia is capable of tolerating a wide range of pH (6.5–9.5), although 7.2–8.5 was identified as the optimum value for the population growth of most species [1]. A study by [12] demonstrated 7.9–8.3 as an optimum pH range for the growth and reproduction of *D. magna*, as values lesser than 4.55 and more than 10.13 were assumed to decrease survival and reproduction.

This study showed a decline in the medium pH at the initiation of experiment, which was simultaneous with the increase of concentration of poultry excreta and an extended period of exposure. The responses were possibly linear or quadratic. However, the water pH was within a range that was suitable for *D. magna*, which is optimal for the commencement of growth and reproduction in experimental medium using poultry excreta concentration of 2 $g.L^{-1}$.

The ammonium content in water was produced from the decomposition of nitrogen compounds. Hence a higher supply results in the generation of more ammonium. This is known to confer detrimental and lethal effects on *Daphnia* at high amounts, according to [13], in a study that showed a decline in growth and reproduction after increasing the concentration from 0.366 to 0.581 $mg.L^{-1}$. Furthermore, the organic nitrogen compound used in this study was supplied by the chlorella powder and poultry excreta.

This study showed an increase in ammonium content of the experimental medium, which occurred as a response to the elevated poultry excreta concentration. This value decreased after a prolonged day of culture, reaching 0.37 $mg.L^{-1}$ on day 15, with 6 $g.L^{-1}$ of poultry excreta. Furthermore, the maximum concentration required for *D. magna* to grow and reproduce was surpassed, based on the limit of <0.2 mg.L^{-1} [14]. Therefore, the addition at 2 and 4 g.L^{-1} treatment is expected to fulfil the nitrogen requirement for the growth and reproduction of *D. magna*.

Table 3 shows the presence of high nitrate content in the medium at the initiation of the experiment using poultry excreta, which subsequently decreased over a prolonged exposure period. One experiment reported 462 mg.L^{-1} as the lethal concentration for *D. magna* production; hence the values recorded in this study were below the toxic level [15]. However, the lowest quantity was identified in treatments with a 6 g.L^{-1} concentration.

The addition of poultry excreta was perceived to cause a decline in the DO content of the experimental medium. Low oxygen concentration (below 3.5 mg.L^{-1}) reduced growth rate, body size, and egg size of *Daphnia sp*. [16]. Smaller adult body sizes in hypoxia could be an adaptive response of *Daphnia sp*. to improve hypoxia tolerance.

The results showed higher concentrations after prolonged exposure, which were not significantly affected by poultry excreta on days 10 and 15 (Table 4). The decrease in DO on day five is possibly affiliated with the high microbial activity observed during the degradation of organic matter. As shown by [17], the decomposition of organic materials mediated by bacteria results in high respiration in the water. However, the DO content in this study was still in the optimum range required for the growth of *D. magna*.

Based on the initial water quality parameters, the experimental mediums with poultry excreta demonstrated worse outcomes than the control, especially with the ammonium (Table 2), nitrate (Table 3), and DO concentration (Table 4). These tend to decrease in content after five days, except for the DO, which increased. Low quality of food and environmental conditions contributed to reduced lifespan and fertility of *D. magna* [18]. *Daphnia sp*. is a sensitive and responsive species to water quality and environmental health [1]. Therefore, sources and levels of food inclusion in culture mediums should create a better environment for the growth and reproduction of *D. magna*.

5. Conclusion

Nutrient enrichment could change the water environment. This study indicated poultry excreta levels had a significant impact on pH, oxygen conditions, and other water quality parameters that determine *D. magna* growth and reproduction. Higher levels of poultry excreta caused a decline in the water quality of mediums enriched with chlorella powder. This was, however, in the tolerable range for the growth and reproduction of *D. magna*. In addition, the quality parameters were observed to have improved after prolonged days of culture, which was followed by an increasing *D. magna* population, with the highest amount reported on day 15, using a poultry excreta concentration of 2 g.L^{-1}.

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