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DIETARY PROTEIN REQUIREMENT OF DAPHNIA MAGNA FOR THE IMPROVEMENT POPULATION AND NUTRITIONAL VALUE OF DAPHNIA

Adang Kasmawijaya[1]*, Otie Dylan Soebhakti Hasan[1], Azam Bachur Zaidy[1]

[1]Jakarta Technical University of Indonesia
[*]Email : kasmawijaya.a8k@gmail.com

ABSTRACT

Daphnia magna is one of the live feeds used in aquaculture, but its availability is still limited. The population and nutritional quality of *D. magna* culture depend on the nutritional quality of feed where the feed source is commonly used from agricultural by-products. In contrast, pelleted feed as a source of nutrition in *D. magna* culture is still limited. Evaluation of commercial pelleted feeds with different protein levels and dietary protein requirements of D. magna is the focus of this research. Three treatments with different protein levels of feed with protein levels of 17.83% (P_{20}), 32.35% (P_{30}) and 41.98% (P_{40}) and rice bran as control at a protein level of 8.2% (P_{10}) with five replications for 28 days of rearing with using a completely randomized design. The average weekly population and the highest total biomass of *D. magna* were obtained from *D. magna* fed the P30 (Protein 32.35%) of 7,575 ind. tank^{-1}. week^{-1} and total biomass of 290.26 g.m^{-3} with the feed conversion ratio of 3.85 ± 0.75 and protein efficiency ratio of 0.83 ± 0.14. The highest protein value of *D. magna* was obtained from *D. magna* fed the P_{30} of 3.02% ± 0.20 wet weight. The results showed that feed with a protein content of 32.35% (P_{30}) showed the best results for cultivating *D. magna*. The estimated dietary protein requirement of feed for *D. magna* cultivation using the second polynomial regression analysis of feed protein - population and biomass is 38.8%.

Keywords*: Daphnia magna*, population, biomass, Dietary protein requirement.

INTRODUCTION

Live feed is an important component in fish nutrition, especially for fish in their growth period such as larvae and seeds (Cheban *et al*., 2017; Pangkey, 2009). Some nutrients cannot be produced by the body itself but are needed from feed (exogenous nutrition), especially nutrients from live feed (Cheban *et al*., 2017). Live feed is a source of essential amino acids needed by fish, unsaturated fatty acids, vitamins, minerals, and other components needed for fish growth (Cheban *et al*., 2017). *Daphnia magna* (*D. magna*) is a zooplankton species of the order *Cladocera* (Bogut *et al*., 2010; DeBiase *et al*., 1990; GBIF Secretariat, 2021; Reads, 2020). *D. magna* is a very small crustacean and has a body protected by a kind of transparent shell (transparent carapace) made of a polysaccharide material called chitin and lives evenly distributed in water bodies (El-Feky & Abo-Taleb, 2020). The protein content of *D. magna* reaches 30% - 70% with a calorie level of 333.7 cal (Bogut *et al*., 2010; El-Feky & Abo-Taleb, 2020; Macedo & Pinto-Coelho, 2001).

D. magna is used as a food source in the early stages of rearing fish larvae and several types of ornamental fish (Pangkey, 2009). Several research results using *D. magna* as live food in fish aquaculture include the fry of the carp *Cyprinus carpio* (Bogut *et al*., 2010), larvae of the gourami *Osphronemus goramy* (Fahmi *et al*., 2019), catfish fry *Clarias gariepinus* (Ojutiku, 2008; Prasetya *et al*., 2010), Betta halfmoon *Betta splendens* (Prasetyo *et al*., 2020).

The problem encountered in *D. magna* culture is the low yield of *D. magna* produced from the cultivation carried out (Hasan & Kasmawijaya, 2021). Of course, this affects the need for *D. magna* as live feed along with the increasing number of aquaculture activities where the availability of *D. magna* was fulfilled from nature catches so technological developments in producing *D. magna* are a necessity.

The nutritional requirement for *D. magna* is one of the important components in both biomass production and increasing the nutritional content of *D. magna* produced. The nutritional content of *D. magna* varies depending on the nutritional composition of the feed given (Cheban *et al*., 2017). Feed nutrition for *D. magna* in nature's habitat comes from microalgae such as green algae which has a protein value with a higher level of *D. magna* population development and nutritional content (Alcántara-azuara *et al*., 2015; Cheban *et al*., 2017; Macedo & Pinto-Coelho, 2001). However, there are technical difficulties in providing green algae continuously in *D.magna* mass culture, so an alternative to the use of artificial feed is needed. In some cultivation and research activities that have been carried out, the feed provided is in the form of agricultural and livestock waste such as rice bran, corn flour, soybean flour, (El-Feky & Abo-Taleb, 2020), tofu dregs, and animal manure through fertilizers. fermented bread waste fertilizer (Noviantoro *et al*., 2017), tofu waste (Mujtahidah & Kusuma, 2019), and rice bran with yeast fermentation (Sitohang *et al*., 2012).

The nutritional content of feed from agricultural by-products, one of which is rice bran which is widely used in *D. magna* culture, has a low level of nutrient content when compared to the protein content of green algae where the protein content of rice bran ranges from 9 – 12% (Marbun *et al*., 2018). In aquaculture, the fulfillment of higher protein nutritional needs can use pellet feed with nutrient content ranging from 20 – 50% as an option (Radona *et al*., 2017; Wijaya *et al*., 2015). However, information regarding the effective nutritional content, especially protein in the feed pellets given for *D. magna* culture is still limited. This experiment was to determine and evaluate the protein requirement of feed for *D. magna* which affects the population and nutritional content of *D. magna*.

RESEARCH METHODS

Experimental design

This study relates to the protein requirement of feed for *D. magna*. *D. magna* used as a starter with a size > 1mm came from the hatchery of the Department of Fisheries Extension - Jakarta Technical University of Fisheries, with a stocking density of 6 ind. L^{-1} with a rearing period of 28 days, which every 7 days was partially harvested by 50%. The tank used is an aquarium with the size of 100 x 50 x 35 cm as many as twenty aquariums with a water volume of 150 liters. Each aquarium is given aeration using an aeration pump with a capacity of 140 $l.min^{-1}$ which is divided into twenty aquariums.

The feed used in this experiment used commercial artificial feed for freshwater fish and rice bran as control (P_{10}) containing crude protein levels of 8.2% (P_{10}), 17.83% (P_{20}), 32.35 % (P_{30}), and 41.98% (P_{40}) in five replicates and the nutritional content will be evaluated in the laboratory before use. The nutritional composition of the experimental feed given as a form of treatment to determine the level of feed protein requirements in *D. magna cultivation* can be seen in Table 1.

Feeding for *D. magna* is given by grinding the feed first using a blender until smooth and then filtering it through a ±75 µm sieve which is then dissolved in water to make it easier for *D. magna to* filter the food in its mouth (Burns, 1969). The amount of feed given at the beginning of rearing with the amount of 6 g per day/tank at the *D. magna* population level below 500 ind. $liter^{-1}$ (De Pauw *et al*., 1981), then after the *D. magna* population exceeds 500 ind. $liter^{-1}$, the amount of feed given using the formula calculations (De Pauw *et al*., 1981).

Table 1. Nutritional composition of experimental feed

Nutrients composition (%, dry weight)	Experimental feed			
	P_{10}	P_{20}	P_{30}	P_{40}
Crude protein	8.2	17.83	32.35	41.98
Crude lipid	5.9	3.9	5.64	5.07
Ash	42.18	13.28	10.12	9.89
Carbohydrate[1)]	43.72	64.99	51.89	43.06

[1)] Carbohydrate (%) = 100 – (protein + lipid + ash)%

Population and Biomass of *D. Magna*

In calculating the population, it is done by taking samples from the rearing tank using the volumetric method. Population measurements were conducted every 7 days with the number of samples being taken 3 (three) times for each sampling. The sampling begins with stirring the rearing tank first evenly and then using a 1000 mL measuring tank. The total population of *Daphnia sp* is calculated using the formula (Mujtahidah & Kusuma, 2019; Rahayu & Piranti, 2009) as follows:

$$a = b\ x \left(\frac{p}{q}\right) \quad (1)$$

Where a = Number of individual *Daphnia sp*. on a culture medium (ind. L^{-1})
b = Average number of Daphnia from samples
p = volume of culture medium (L)
q = volume of sample bottle (L)

The calculation of biomass is conducted every 7 days by harvesting in each tank then 50% is stored and the other half is returned to the tank. *D. magna* is harvested using a sieve and the water content is reduced by attaching tissue to the bottom of the filter until the water can be absorbed then the wet weight of *D. magna is measured* using a digital scale with an accuracy of 0.01g.

Calculation of biomass production using the following formula (De Pauw *et al*., 1981):

$$W = \frac{(Wt\text{-}Wo)}{L} \times 1000 \quad (2)$$

Where W = Total weight gain ($g.m^{-3}$)
W_0 = initial weight (g)
W_t = Final weight (g)
L = volume of water (L)

Calculation of *Feed Conversion Ratio* (FCR) using the following formula (Tanjung *et al*., 2020):

$$FCR = \frac{Total\ Feed\ (g)}{\text{Total biomass (g)}} \quad (3)$$

Calculation of *Protein Efficiency Ratio* (PER) using the following formula:

$$PER = \frac{\text{Weight gained } (g)}{\text{Total protein in feed (g)}} \quad (4)$$

Proximate analysis of experimental feed and *D. magna*

The feed used was evaluated for its nutritional composition in the laboratory before being used. At the end of the rearing, the experimental results of *D. magna* were taken as much as 15 g (wet weight) and frozen for evaluating its nutritional content. The test is carried out based on the Indonesian National Standard (SNI) by following the *method of Official Methods Chapter 4* (AOAC, 2005). Protein content was evaluated using the *Kjeldahl method*, ash content using an ashing furnace at 600 ^{o}C for 4 hours, lipid content using *Soxhlet fat extraction*, fiber content using an electric furnace at 600 ^{o}C for 1 hour, and moisture content evaluated using an oven at 65 ^{o}C for 24 hours.

Water quality

During the rearing period, the water quality in the rearing tank was measured periodically. Water changes are conducted every 2 days with an amount of 25% of the total volume. Parameters measured included temperature and oxygen using an *oxygen meter* (Lutron DO-5510), pH was measured using a pH meter (Pen type PH-009 (1) A) while ammonia, nitrite, and nitrate were evaluated in the laboratory using the *spectrophotometric method* at the end of the experiment.

Statistic analysis

The data was evaluated using *One-way ANOVA* and *Least Significance Difference* (LSD) to determine the level of differences between experimental feed treatments with a probability of 5% ($p < 0.05$) using a linear equation model (Steel & Torrie, 1980). Regression analysis was used to evaluate the dietary protein requirement of feed on the population, biomass, and nutritional value of *D. magna*.

RESULTS

The condition of water quality in tanks for 28 days of rearing presented in Table 2. The water quality parameters in the experimental tanks during the rearing of *D. magna* were in optimal conditions for all treatments. Based on several previous studies (Ebert, 2005; El-Feky & Abo-Taleb, 2020; Herawati *et al*., 2018; Tanjung *et al*., 2020), the optimal condition of water quality parameters for *D. magna* cultivation temperature of 23 ^{o}C to 28 ^{o}C, pH of 6.5 to 8.5, dissolved oxygen is above 3.0 mg.L^{-1}. Meanwhile, the total ammonia level in the P_{10} treatment was outside the optimal range, that is > 0.2 mg.L^{-1} (Delbare & Dhert, 1996; Herliwati *et al*., 2021), for nitrite and nitrate are in the optimal range.

Table 2 Water quality parameters of D. magna culture

Water quality parameters	Experimental Feed[1]			
	P_{10}	P_{20}	P_{30}	P_{40}
Temperature (^{o}C)	23.3 - 24.8	23.3 - 24.8	23.3 - 24.5	23.3 - 24.5
pH	8.18 - 8.40	8.25 - 8.38	7.90 - 8.35	7.80 - 8.28
Dissolved oxygen/DO (mg. L^{-1})	4.20 - 6.70	4.40 - 6.70	4.20 - 6.60	3.90 - 7.00
Total Ammonia / TAN (mg. L^{-1})	0.295 - 0.355	0.162 - 0.165	0.138 - 0.26	0.097 - 0.165
Nitrite / NO_2 (mg.L^{-1})	0.015 - 0.055	0.135 - 0.525	0.154 - 0.925	0.044 - 0.221
Nitrate / NO_3 (mg.L^{-1})	6.767 - 6,847	6,647 - 6,854	6.851 - 7.153	6,244 - 8,689

[1] Experimental feed protein 8.2% (P_{10}), feed protein 17.83% (P_{20}), feed protein 32.35% (P_{30}), feed protein 41.98% (P_{40})

During 28 days of rearing *D. magna* fed with different protein feeds (P_{10}, P_{20}, P_{30}, and P_{40}) showed significant results both in terms of population and biomass as shown in Table 3. Cultivation of *D. magna* with a high protein feed resulted in a higher population and biomass every week. Significantly ($p < 0.05$) the highest number of populations resulted from feeding with the protein content of P_{30} and P_{40} compared to the control. Every week, the number of populations at P_{30} and P_{40} produced reached 4.8 times more than P_{10} in week 1 and 1.6 times more in week four. Based on the *LSD test*, the experimental feed with a protein content of 30% (P_{30}) and a protein content of 40% (P_{40}) was not significantly different from the population of *D. magna* (Table 3).

The 2nd Polynomial regression analysis showed a strong correlation between feed protein content and *D. magna* biomass, R^2 was 0.9859 (Figure 1). Based on the research data, by looking at the following equation: $Y = -4.7944x^2 + 371.88x + 282.94$ that the dietary protein requirement of feed needed for *D. Magna* cultivation is 38.8% and can produce an average population of 7.494 ind/tank.

Table 3. Population and total biomass of *D. magna* (wet weight) with experimental fed every week.

Experimental fed[1]	Population ($ind.L^{-1}$)				Total Biomass[2] ($g.m^{-3}. week^{-1}$)			
	week 1	week 2	week 3	week 4	week 1	week 2	week 3	week 4
P_{10}	1.640 ± 3.5 ^{a}	1,430 ± 1.8 ^{a}	4,490 ± 4.1 ^{a}	4,490 ± 4.1 ^{a}	12.8 ± 3.7 ^{a}	11.16±1.84 ^{a}	35.05 ± 4.34 ^{a}	38.72±4.71 ^{a}
P_{20}	5.270 ± 10.8 ^{b}	3.920 ± 4.6 ^{b}	5,150 ± 8.1 ^{ab}	5,150 ± 8.1 ^{ab}	41.14 ± 13.33 ^{b}	30.6±4.86 ^{b}	40.2 ± 8.46 ^{ab}	47.62 ± 7.69 ^{ab}
P_{30}	7,800 ± 17.6 ^{bc}	5,760 ± 7.2 ^{cd}	8.150 ± 16.1 ^{c}	8.150 ± 16.1 ^{c}	60.89 ± 18.47 ^{c}	44.96±7.58 ^{cd}	63.62 ± 16.88 ^{c}	67.06 ± 13.88 ^{c}
P_{40}	7,800 ± 11.7 ^{bcd}	5,000 ± 17.9 ^{bc}	8,600 ± 20.7 ^{cd}	8,600 ± 20.7 ^{cd}	60.90 ± 12.22 ^{cd}	39.03 ± 18.75 ^{bc}	67.14 ± 21.63 ^{cd}	61.67 ± 5.5 ^{cd}

The average value in the same column that has the same letter notation is not statistically significant ($p > 0.05$).
[1] Experimental feed protein 8.2% (P_{10}), feed protein 17.83% (P_{20}), feed protein 32.35% (P_{30}), feed protein 41.98% (P_{40})
[2] Biomass harvested is 50% of the total wet weight of *D. magna*.

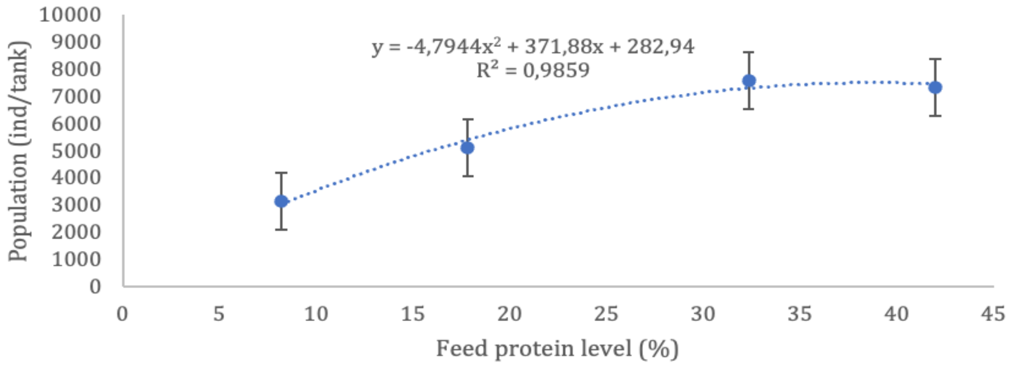

Figure 1. The 2nd polynomial regression analysis between dietary protein level (%) and population of *D. magna* (Y_{max} value, where X value = 38.8%).

Feeding with protein 32.35% (P_{30}) and protein 41.98% (P_{40}) significantly different ($p < 0.05$) showed a higher amount of biomass of *D. magna* every week when compared to the control. Table 3 shows that feed proteins P_{30} and P_{40} produced higher biomass of *D. magna* and lower feed conversion ratio compared to control with feed protein 8.2% (P_{10}). Based on the *LSD test* conducted, the experimental feed with a protein content of 32.35% (P_{30}) and a protein content of 41.98% (P_{40}) was not significantly different from the biomass production of *D. magna* (Table 4).

Table 4. Total Biomass (wet weight), total feed and feed conversion in *D. magna* culture.

	Experimental Feed[1]			
Parameter	P_{10}	P_{20}	P_{30}	P_{40}
Total Biomass[2] ($g.m^{-3}$)	123.13 ± 15.21	193.86 ± 24.74	290.26 ± 48.69	277.11 ± 37.51
Feed ($g.l^{-1}.day^{-1}$)	0.04	0.04	0.04	0.04
Total feed ($g.m^{-3}$)	1080	1080	1080	1080
FCR	9.03 ± 1.18	5.71 ± 0.85	3.85 ± 0.75	3.99 ± 0.59
PER	1.37 ± 0.17	1.00 ± 0.13	0.83 ± 0.14	0.62 ± 0.08

[1] Experimental feed protein 8.2% (P_{10}), feed protein 17.83% (P_{20}), feed protein 32.35% (P_{30}), feed protein 41.98% (P_{40}).
[2] Total biomass 4 weeks of harvested *D. magna biomass.*

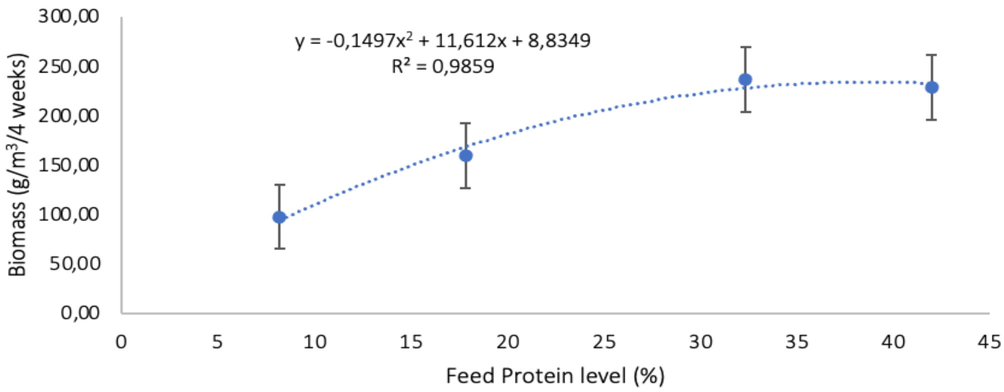

Figure 2. The 2nd polynomial regression analysis between dietary protein level (%) and biomass of *D. magna* (Y_{max} value, where X value = 38. 8%).

The 2nd Polynomial regression analysis showed a strong correlation between feed protein content and *D. magna* biomass, the correlation coefficient R^2 was 0.9859 (Figure 2). Looking at the following equation: $-0.1497x^2 + 11.612x + 8.8349$, that the dietary protein requirement of feed is 38.8% and can produce an average amount of biomass of 234.02 g/m^3.

The highest protein content of *D. magna protein* (dry weight) based on the proximate test conducted was 49.73% or 3.3% (wet weight) and 9.89% lipid or 0.66% (wet weight) at treatment feed protein 32.35 % (P_{30}). Meanwhile, the lowest protein content of *D. magna* was 40.38% or 2.48% (wet weight) and 4.93% lipid or 0.29% (wet weight) in treatment P_{10}. The composition of the nutritional content of *D. magna* is presented in Table 5.

The protein level of feeds had a significantly different (*p < 0.05*) on the protein content of *D. magna.* Table 3 shows the differences in the protein content of *D. magna* in the fed of protein feed 8.2% (P_{10}) with the fed of protein feed 17.83% (P_{20}), 32.35% (P_{30}) and 41.98% (P_{40}). The Protein content of D. magna fed P_{20} is 12%, P_{30} is 18% and P_{40} is 17% higher when compared to the protein content of D. magna fed P_{10}. Based on the *LSD test* conducted, treatment P_{30} (protein feed 32.35%) was significantly different from treatment P_{20} (protein feed 17.83%) but not significantly different from treatment P_{40} (protein feed 41.98%) while P_{20} and P_{40} were not significantly different (Table 3).

Polynomial regression analysis showed a strong correlation between the protein content of the feed and the protein content of *D. magna* where the correlation coefficient R^2 was 0.9952 (Figure 3).

By looking at the equation where: $y = -0.0007x^2 + 0.0484x + 2.2287$, it can be predicted that the feed protein requirement (Y_{max}) of 34.6% can produce an average protein content of 3.07%.

Table 5. Nutritional composition (%, wet weight) D. magna

Nutritional composition (%)	Nutritional composition of *D. magna* fed experimental feed[1]			
	P_{10}	P_{20}	P_{30}	P_{40}
Moisture	93.76 ± 0.24	93.79 ± 0.36	93.73 ± 0.34	93.99 ± 0.28
Crude protein	2.57 ± 0.08 [a]	2.88 ± 0.14 [b]	3.02± 0.20 [cd]	2.99 ± 0.17 [bc]
Crude lipid	0.29	0.5	0.66	0.42
Ash	1.16	0.91	0.76	0.79
Carbohydrate [2)]	2.22	1.92	1.83	1.81

The values in the rows that have the same letter notation are not statistically significant (*P>0.05*).
[1)] Experimental feed protein 8.2% (P_{10}), feed protein 17.83% (P_{20}), feed protein 32.35% (P_{30}), feed protein 41.98% (P_{40}).
[2)] carbohydrate (%) = 100 – (protein + lipid + ash)

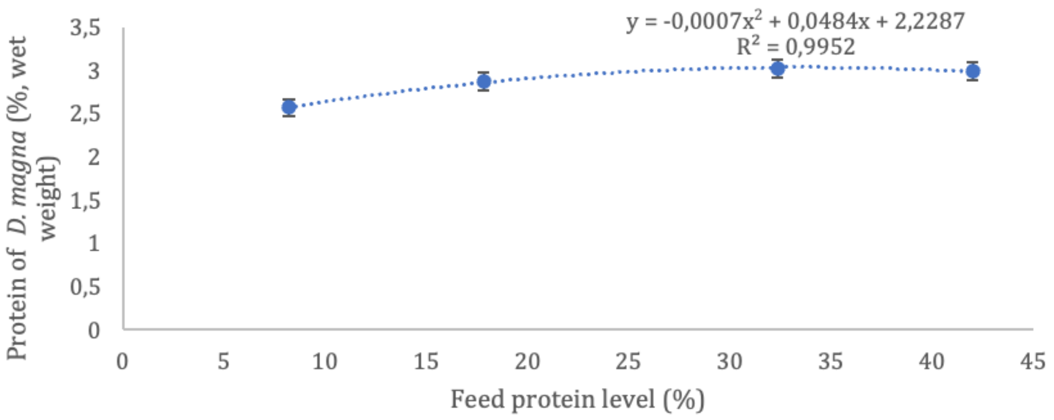

Figure 3. The 2nd polynomial regression analysis between dietary protein level (%) and protein content of D. magna (wet weight) (Y_{max} value, where the value of X = 34.6%).

DISCUSSION

The increase in ammonia levels resulted from the decomposition of nitrogen compounds (Herliwati *et al*., 2021). According to Yang *et al*. (2012) and Serra *et al*. (2019), ammonia has a bad impact on *D. magna* and is even deadly at certain levels. The research of Herliwati *et al*. (2021) showed an increase in ammonia concentration led to a decrease in the growth and reproduction rate of *D magna*. The higher the ammonia value, the higher the mortality rate will be (Serra *et al*., 2019).

This study is a form of evaluation of the application of commercial pellets as an alternative feed source with better nutritional levels. Research on pelleted feed used as a source of nutrition for *D. magna* culture is still extremely limited. Several studies on Daphnia related to the nutritional sources used came from natural feed such as microalgae (Cheban *et al*., 2017) as well as agricultural by-products such as rice bran, corn flour, soybean flour (El-Feky & Abo-

Taleb, 2020) and tofu dregs (El-Feky & Abo-Taleb, 2020; Mujtahidah & Kusuma, 2019) to determine the best source of nutrients to produce the best population and biomass of *D. magna*.

D. magna has the characteristics of a high reproduction rate, fast growth and suitable for cultivation. The proper amount of protein nutrition will determine the effectiveness of the biomass produced (Cheban *et al*., 2017). Natural food for *D. magna* is plankton and algae with a protein content of 30% – 50% (Cheban *et al*., 2017). Based on this study dietary protein requirement for *D. magna* is 38.8%.

Based on research on nutrient enrichment through rice bran fermentation for *D. magna* conducted by Herawati *et al*., (2017, 2018) and Sitohang *et al*. (2012) showed that one of the factors that affected the population, biomass and nutrient content of *D. magna* production is the nutrients in the feed given. This is in line with research that has been conducted where higher protein content in feed has a significant effect on population and biomass.

Monitoring and evaluation related to total protein and lipid in types of feed that will be given to *D. magna* needs to be done. This is because the chemical nutrient content contained in *D. magna* is influenced by the quality of the feed source provided (Cheban *et al*., 2017). Based on this study the highest protein and lipid content of *D. magna* was obtained from the experimental fed of protein feed 32.35% (P_{30}) and protein feed 41.98% (P_{40}). These results are in line with several studies that have focused on improving the nutritional quality of *D. magna* produced using the fermentation method where increasing the nutritional quality of the fermented rice bran affects the nutritional quality of *D. magna* (Damle & Chari, 2011; Herawati *et al*., 2017).

The nutritional quality of feed for *D. magna* cultivation is not only limited to protein and lipid as macronutrients, but micronutrient considerations such as essential amino acids have an influence where the provision of essential amino acids can increase production, reproduction and hatching rate (Fink *et al*., 2011). In their habitat, *D. magna* gets a source of nutrition from microalgae, bacteria and other plankton which have micronutrients such as amino acids (Fink *et al*., 2011; Lari *et al*., 2018). This study has not explored the data up to micronutrients so further investigation is needed to determine the level of amino acids contained in pellet feed and evaluate the effect of adding amino acids, especially essential amino acids in *D. magna cultivation*.

CONCLUSION

D. magna produced from experiments using pelleted feed with different protein content showed a significant increase in the population and biomass produced and had better nutritional content. Pellet feed can be used as an alternative source of nutrition for *D. magna* culture. The estimated protein level required for *D. magna* culture for population and biomass in this study was 38.8%.

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