Providing feed from a mixed of chicken broth and chicken manure with different dosages on nutritional content *Daphnia magna*

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**Abstract.** Live feed is one of the most important factors of the success of fish farming, especially in fish hatchery activities. Live feed that has the potential to support cultivation activities is *Daphnia magna*. One of the problems on *Daphnia magna* culture is feed availability that can meet the nutrients in *Daphnia magna*. The aim of this research was to study the effect of providing feed from combination of chicken broth and chicken manure on the nutritional value of *Daphnia magna*. This study was experimental using the RAL method consisting of 4 treatments with 5 replications, namely control treatment (P0) 7.5 mL/L dose of feed, (P1) 2.5 mL/L dose of fermented feed, (P2) 5 mL/L dose of fermented feed and (P3) 7.5 mL/L dose of fermented feed. Data analysis was processed using ANOVA and Duncan. The result of this study has a significant effect (P<0.05) on nutritional content of *Daphnia magna* with the best dose in P3 which can affect the highest protein (51.98%) and highest fat (12.17%) of *Daphnia magna*. The highest fiber (13.32%) of *Daphnia magna* was found in the P1 treatment and the highest carbohydrates (37.51%) of *Daphnia magna* was found in the P0 treatment.

1. **Introduction**

Zooplankton are the first level consumers. The existence of zooplankton in the waters is very important because of its role as a liaison between primary producers and other biota at a higher trophic level [1]. Zooplankton also has a role as live feed [2]. The existence of feed is needed in aquaculture and fish hatchery activities because of the nutrients contained therein [3].

Live feed is one of the important factors for the success of fish farming, especially in fish hatchery activities [4]. The availability of live feed must be adequate in terms of quantity, quality and sustainability in order to fulfill cultivation activities. Live feed that has the potential to support cultivation activities is *Daphnia magna*. *Daphnia magna* is a zooplankton that is non-selective filter feeder, including crustaceans with relatively small body sizes ranging from 0.3 - 1 mm [5].
These organisms are generally used as live feed for freshwater fish larvae. The advantage of *Daphnia magna* that has a size that fits the mouth of the fish larvae [6]. Another advantage is that it is easy to cultivate and has a high nutritional content [7]. The problems of *Daphnia magna* culture is the lack of feed sources that can meet the needs of *Daphnia magna*. The nutrients contained in *Daphnia magna* are highly dependent on the food contained in the culture medium.

In general, *Daphnia magna* culture only uses chicken manure as feed for *Daphnia magna*. Cultivating *Daphnia magna* with chicken manure will result in a high content of ammonia in the rearing media because chicken manure contains high enough ammonia so that it can interfere with *Daphnia magna* in the metabolic process and absorption of nutrients [8].

The organic material that can be used as a source of feed for *Daphnia magna* culture in this study is chicken broth. Chicken broth is a processed product that is often found in the community. Chicken broth is obtained from the process of boiling chicken which is liquid and yellowish in color [9]. As a by-product, this chicken broth is rarely consumed and utilized, so its presence is considered as waste around industrial sites. Chicken broth is obtained from the process of boiling chicken which is liquid and yellowish in color [9]. The high organic matter content in chicken broth has the potential to be used as feed and a source of nutrition for *Daphnia magna*. According to [10], *Daphnia magna* can be cultured on a suspension of abundant organic particles and bacteria. *Daphnia magna* is a non-selective filter feeder where it can be fed a variety of organic materials that can increase the nutritional value in *Daphnia magna* [11].

2. Materials and methods
This research was conducted for 5 months from March 01 to July 30, 2021. The culture of *Daphnia magna* was carried out at the Laboratory of Anatomy and Aquaculture, Faculty of Fisheries and Marine Affairs, Universitas Airlangga. Proximate analysis was carried out at the Nutrition Laboratory, Faculty of Public Health, Universitas Airlangga.

This study uses several materials for proximate analysis. The culture of *Daphnia magna* uses an aquarium with a size of 40x30x30 cm³ as many as 20, 5 L jars to make feed, petri dishes, basins (plastic containers), spoons, thermometers, measuring cups, pH meters, DO meters, ammonia test kits, cameras, stationery, microscopes, aerators, sample bottles, dropper pipettes, sample plastics and filters.

The experiment used 4 treatments with 5 replications. The design used was a Completely Randomized Design (CRD), with feeding from a mixture of chicken broth and chicken manure on *Daphnia magna* with different concentrations.

*Daphnia magna* feed is made from chicken manure obtained from chicken farmers in the Surabaya area and weighed as much as 60 grams, chicken broth comes from chicken claw factory waste in the Pemon area, Surabaya and is used as much as 225 mL. The two ingredients are mixed in a jar and stirred. Add 1 L of water and stir gently. After thoroughly mixed, the jars containing a mixture of chicken manure and chicken broth were fermented using 5 mL of EM4 bacteria and kept closed for 14 days. It aims to decompose organic elements and grow several microorganisms such as bacteria and phytoplankton in the feed mixture.

Determination of the dose of feed for *Daphnia magna* in this study was modified from [6] with a feed dose of: (P1) 2.5 mL/L from a mixture of 50 mL chicken broth and 15 grams of chicken manure; (P2) 5 mL/L of a mixture of 75 mL of chicken broth and 15 grams of chicken manure; (P3) 7.5 mL/L of a mixture of 100 mL of chicken broth and 15 grams of chicken manure into a jar that had previously been fermented with probiotic bacteria EM4 in 1 liter of water and (P0) 7.5 mL/L of feed from 15 grams of chicken manure as much as 15 grams put into a jar as much as 1 L, as a control.

The *Daphnia magna* used was purchased directly from Daphnia magna cultivators located in the Sukodono area, Sidoarjo Regency, then acclimatized and cultured in an aquarium measuring 50x40x30
cm³ for 3 days so that the size is uniform. As a test, 20 glass aquariums measuring 40x30x30 cm³ were used, filled with 20 liters of water and given 1 point of aeration in each aquarium. Each aquarium was labeled P0, P1, P2, P3 and repeats 1, 2, 3, 4 and 5. *Daphnia magna* was stocked in 20 treatment aquariums with an initial density of 100 ind/L for each.

The feed derived from the solution of chicken manure and chicken broth was filtered before being fed into the test aquarium. The solution of chicken manure and chicken broth that has been fermented is given according to Label P1: given fermented feed from chicken broth and chicken manure as much as 2.5 mL/L; Label P2 is given fermented feed from chicken broth and chicken manure as much as 5 mL/L; label P3 given fermented feed from chicken broth and chicken manure as much as 7.5 mL/L. As for the aquarium labeled P0 is a control containing 7.5 mL of chicken manure. Feed is given once every three days in the morning and afternoon at 07.00 and 15.00 WIB.

Water quality measurements include temperature, DO, pH and ammonia. Measurements were made every day at 07.00 WIB. Temperature measurement is done with a thermometer, DO measurement is done with a DO meter and pH measurement is done with a pH tester. and measurement of ammonia with NH3/NH4 Test kit.

*Daphnia magna* that has been cultured for 14 days is harvested and then tested with the proximate analysis method which includes protein, fat, fiber, ash and carbohydrates levels [12]. To be able to compare the nutritional content of *Daphnia magna*, a test of the nutritional content of the feed was also carried out to determine the role of feed on the nutritional content of *Daphnia magna*. The high and low nutrient content in *Daphnia magna* is influenced by the feed in which there are nutrients dissolved in the water.

Data from the proximate analysis of *Daphnia magna* consisting of protein, fat, fiber, ash and carbohydrates were presented as mean ± standard deviation. Then, these values were compared using independent t-test analysis, at p<0.05 using SPSS version 22 software.

**3. Result and discussion**

The feed ingredients in the form of a mixture of chicken broth and chicken manure contain nutrients including protein, fat, fiber and carbohydrates that can be utilized by *Daphnia magna* as a source. According to [6], the nutrients needed by *Daphnia magna* can come from various sources, namely from suspended organic matter and bacteria derived from feed given to culture media. Based on the proximate analysis of feed (Table 1), obtained from a mixture of chicken broth and chicken manure has nutrients including protein, fat, fiber, ash and carbohydrates. The difference in the nutritional content of feed is very dependent on the composition of the feed itself [13].
Table 1. Feed Nutrient Content Based on Dry Ingredients

| Component (%) | Feed Ingredients |
|---------------|------------------|
| P0            | P1               | P2       | P3       |
| Protein       | 43.40            | 52.66    | 54.10    | 60.95    |
| Fat           | 7.46             | 21.50    | 21.66    | 20.36    |
| Fiber         | 6.02             | 8.50     | 7.74     | 6.80     |
| Ash           | 11.28            | 7.38     | 7.58     | 6.54     |
| BETN          | 31.84            | 9.96     | 8.92     | 5.35     |

The protein content in P0 feed converted into dry matter was 43.40% and fat content was 7.46%, protein content in P1 feed was 52.66% and fat was 21.50%, protein content in P2 feed was 54.10% and fat was 21.66% and protein content at P3 60.95% and fat 20.36%. The nutritional content of P3 based on protein and fat makes P3 is feed with the highest nutrient content than P0, P1 and P2, because at P3 the concentration of chicken broth added was increasing. P0 feed has the lowest nutrient content. This is because the feed at P0 is only made from chicken manure. The addition of chicken broth in this feed mixture will increase the nutritional value of the feed.

Table 2. Nutrient Content of Daphnia magna Converted from Dry Ingredients

| Component (%) | Daphnia magna |
|---------------|---------------|
| P0            | P1            | P2       | P3       |
| Protein       | 35.08 ± 0.56a | 46.99 ± 0.46c | 45.00 ± 0.57b | 51.98 ± 0.35d |
| Fat           | 6.16 ± 0.27a  | 8.06 ± 0.38b | 11.47 ± 0.42c | 12.17 ± 0.57d |
| Fiber         | 13.32 ± 0.59c | 8.72 ± 0.43b | 5.14 ± 0.24a  | 4.75 ± 0.27a  |
| Ash           | 21.24 ± 0.33d | 12.34 ± 0.22a | 18.79 ± 0.16b | 19.94 ± 0.69c |
| Carbohydrate  | 37.51 ± 0.46d | 32.60 ± 0.59c | 24.72 ± 0.38b | 15.90 ± 0.66d |

Note: values are average with standard deviation of five-unit replicates. Different superscripts within a row indicate significantly different at p<0.05.

The nutritional content of Daphnia magna was examined by proximate analysis. From the results of the average proximate analysis of protein (Table 2), it is known that feeding with different doses of a mixture of chicken broth and chicken manure affects the protein content of Daphnia magna. The nutritional content of Daphnia magna in P1 treatment was greater than P2. This is because the absorption of different nutrients due to water quality in P1 is more optimal than water quality in P2. According to the theory, the amount of nutrient content in the feed will affect the nutritional content of Daphnia magna. These results indicate that the amount of nutrient content in feed that comes from different doses affects the nutritional content of Daphnia magna [14]. The fat content of Daphnia magna in each treatment showed differences. According to [12], protein content is related to fat content, the less fat content, the more protein content. The high and low fat content in Daphnia magna in this study was influenced by the nutrients present in the feed given. The higher the dose of the feed mixture of chicken broth and chicken manure, the higher the fat content in Daphnia magna. The higher the protein content and the lower the lipid content [15].

Protein is the main source of energy which is composed of C, H, O, and N atoms while carbohydrates and fats do not contain N atoms (nitrogen) in which there are amino acids that are needed by fish [16]. Amino acids are simple organic materials which are nutrients needed by Daphnia magna in the early
stages of life. Essential amino acids are needed by *Daphnia magna* for processes in the body as transmission of genetic information for growth, maintaining cells, energy sources, forming new tissues, and as forming enzymes and hormones for metabolic processes in Daphnia's body. Proteins and fats will be digested, absorbed and metabolized after which they are converted into useful energy [17]. Fat is a source of high energy, apart from being a source of energy, it is also a source of essential fatty acids, among others, to maintain the integrity of cell membranes, as a precursor to prostaglandins, prostacyclin, thromboxane and leukotrienes [18].

In addition to protein and fat, carbohydrates are also an important component. The carbohydrate value in *Daphnia magna* depends on the feed. The higher the other nutritional components, the lower the carbohydrate content and vice versa [19]. [20] explained that carbohydrates are a source of energy for growth and metabolism. Carbohydrates are absorbed in the intestinal wall in the form of monosaccharides, namely glucose, fructose and galactose. Glucose is the end result of the digestion of high levels of carbohydrates, a form of food that circulates in the blood and can produce energy for growth. According to the theory, the more suspended organic matter content, the lower the carbohydrate content in *Daphnia magna*. Carbohydrate content is also influenced by the presence of bacteria that live in the feed [20].

The highest fiber content in *Daphnia magna* was found in P1 with an average value of 13.32% and the lowest in P3 with an average value of 4.75%. The fiber content in P2 and P3 was not significantly different but the fiber content in P2 and P3 was significantly different at P1. According to the theory, the lower the crude fiber value, the higher the carbohydrate value [21]. Crude fiber is an organic material, part of carbohydrate nutrients that are not easily soluble in water [22]. Crude fiber consists of cellulose, hemicellulose and lignin, most of which cannot be digested and are blockers [23]. The absorption of crude fiber in *Daphnia magna* is influenced by several factors, including fiber content in feed, crude fiber composition and activity of microorganisms [24].

Ash is an organic substance left over from the combustion of an organic material. Ash content is a mixture of inorganic or mineral components contained in a material [25]. Feeding of a mixture of chicken broth and chicken manure with different doses showed a difference in the ash content of *Daphnia magna*. Feed with high ash content will affect the ash content of *Daphnia magna*. Based on table 2, treatment P0 (control) had feed ash content of 11.18% which in turn affected the ash content of *Daphnia magna* with an average value of 21.24%. In treatment P2 had the highest feed ash content of 7.58%. However, in *Daphnia magna*, it is known that the highest ash content is in P3 with an average value of 19.94%. This is because the higher the dose of feed, the inorganic components will increase which is then represented by the ash content in the body of *Daphnia magna*.

[26] stated that *Daphnia magna* reared in water containing suspended organic matter and minerals selected absorption and ingestion of food particles. Foods found in the environment can support the rapid development of *Daphnia magna* if the food is sufficient. The higher the digestibility level of daphnia, the higher the nutritional content because properly digested feed will affect the quality of the nutrients in the body [27]. In addition, habitat also affects differences in the nutritional quality of *Daphnia magna*. Because *Daphnia magna* is a non-selective filter feeder, its nutritional value can vary.
Table 3. Water Quality

| Water Quality Parameters | P0                | P1                | P2                | P3                |
|-------------------------|-------------------|-------------------|-------------------|-------------------|
| Temperature (°C)         | 23.1 – 29.8       | 23.1 – 29.7       | 23 – 29.7         | 23 – 29.7         |
| Dissolved Oxygen (DO)   | 4.07 – 4.28       | 4.03 – 4.29       | 4.0 – 4.27        | 4.13 – 4.29       |
| pH                      | 7.2 – 8.1         | 7.2 – 8.1         | 7.2 – 8.2         | 7.2 – 8.2         |
| Ammonia                 | 0.05 - 1          | 0.03 – 0.3        | 0.03 – 0.5        | 0.03 – 0.5        |

Another thing that affects the nutritional content of Daphnia magna is the water quality of the rearing culture media [28]. Water quality plays an important role in the process of nutrient absorption as well as the growth and reproduction of Daphnia magna. If the water quality of the maintenance media is appropriate, it will support the cultivation of Daphnia magna. [29] states that cultivated organisms will eat as much feed as they get and will grow rapidly if the water quality is good and suitable for life, on the contrary if the water quality is poor, it will cause the cultured organisms to be stressed and disturbed so that their nutrient absorption will be reduced [30].

Feeding of a mixture of chicken broth and chicken manure with different doses in each treatment affected the quality of the Daphnia magna rearing media. The results of water quality measurements at P0 showed that the results were not optimal on the Daphnia magna rearing media. This is because in the P0 maintenance media the feed given comes from chicken manure only. Chicken manure contains high enough ammonia so that it affects the water quality of the Daphnia magna rearing media [31]. The results of water quality measurements were in the optimal range on the rearing media fed P1 and P3 feeds. In P2, the water quality of the maintenance media is less than optimal. Therefore, the absorption of protein by Daphnia magna at P2 was less than optimal so that the protein content was lower than that of P1. Water quality parameters measured during the study were temperature, pH, dissolved oxygen (DO) and ammonia. The results of water quality measurements during maintenance if adjusted to optimal conditions are classified as optimal. According to [32], Daphnia magna can survive at temperatures of 25-30°C. The optimum dissolved oxygen concentration for Daphnia magna culture is above >3.0 mg/l. Daphnia magna requires an alkaline pH between 7.0 to 8.6 [7]. According to [33] the ammonia content that can be tolerated by Daphnia magna ranges from 0.35 - 0.61 ppm.

4. Conclusion

The use of feed derived from a mixture of chicken broth and chicken manure for Daphnia magna culture showed a difference. Utilization of this feed mixture will result in better nutritional value of Daphnia magna. The more doses of the feed mixture given, can produce the higher nutritional value of Daphnia magna. In addition, feed from a mixture of chicken broth and chicken manure can reduce environmental pollution caused by waste so that it can be used and reduce the value of feed production in Daphnia magna culture.

5. References

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6. Acknowledgment
It is with grateful gratitude that we acknowledge the publication support provided by the Research and Knowledge Acquisition Plan (RKAT) of the Faculty of Fisheries and Marine and the funds provided for the instrument laboratory.