The Nutrient Content in Liquid Organic Fertilizer (Bio-slurry) and Its Effect on Plankton Abundance and Total Bacteria in Traditional Pond

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ABSTRACT
This research aimed to determine the nutrient content and total bacteria in bio-slurry. In addition, the abundance of plankton, total bacteria and protein content of milkfish cultivated in ponds using bio-slurry were analyzed. The first stage was to make bio-slurry by testing two different main raw materials; cattle manure and chicken manure. They were made in separate containers to compare the nutrients produced. The chopped leaves, rice water stool, probiotics, and molasses were mixed in tank fermentation. After 3 weeks, the bio-slurry was harvested and analyzed for its nitrogen, phosphorus, and potassium (NPK), as well as its bacterial content. Then, the application of a combination of bio-slurry was carried out in traditional ponds. The results showed that the bio-slurry treated with chicken manure contained significantly higher NPK (390±56.57; 2,332.5±1,438.96; 905.5±656.90 pmhos/cm) than the treatment with cattle manure (76.5±4.95; 647.5± 81.32; 264.5±32.53 pmhos/cm). Then, the average data of total bacteria show that bio-slurry from chicken manure had significantly higher numbers (15±4.24 MPN/mL), while for total *Escherichia coli* bacteria was lower ($8.6 \pm 0.57 \times 10^3$ cfu/mL) than those from cattle manure (29.5±6.36 MPN/mL; $2.45 \pm 0.21 \times 10^5$ cfu/mL). After applying to the pond, the abundance and diversity of plankton were monitored. The data showed that organic waste can be presumed to establish a more favorable conditions for the growth of plankton characterized by high nutrient content and less content of pathogenic bacteria. This indicated that animal manure can be utilized in milkfish cultivation because it contains sufficient nutrients and is safe to be used in aquaculture.

Keywords: *Livestock Manure, Liquid Organic Fertilizer, Milkfish, Traditional Pond.*

1. INTRODUCTION

Aquaculture has always faced the challenge of limited availability of lands [1]. With prolonged cultivations in the soil ponds, soil fertility levels decrease with time thus affecting the growth of fish [2, 3]. Pond fertilization proposes a mode to overcome this problem as it can revive and create an ideal environment for fish to live and develop. Fertilization works by stimulating the growth of natural feed in the pond, such as plankton and algae.

The fertilizers used to rejuvenate ponds are of types inorganic or artificial fertilizers, organic fertilizers, or variations of the two. Inorganic or synthetic fertilizers are commonly in the form of urea and TSP, while organic fertilizers are usually made from plant waste or animal waste, i.e., manure or compost. Inorganic fertilizers not rarely create detrimental effects [4], making them less favored. Organic fertilizers provide better outcomes in improving the physical, chemical and biological properties of pond soils.

Animal manures are used as fertilizer because they contain nitrogen (N), phosphorus (P), and potassium (K), abbreviated NPK, which are beneficial for plants and soil fertility. Organic fertilizers are advantageous in that they: 1) improve soil structure, 2) are rich in organic materials that help microbes breed and assist in biological processes, 3) are sustainable and environmentally friendly, 4) can even increase species biodiversity 5) are cheap and easy to make with simple technology [3, 5].

Materials that can constitute organic fertilizers are very diverse, with tailorable physical characteristics and chemical make-up to address the specific needs of the pond to be fertilized. The quality of animal manures varies depending on the quality of the nutrients eaten by the animal. For instance, chickens that are fed with high-quality commercial dietary will produce higher quality manure than animals such as horses and cattle that eat only grass. In addition, the water content in manure also affects its quality. Dry manure will have more concentrated chemical nutrients than the same weight as wet manure. However, within it, the nutrition value may be reduced as they most likely have been exposed to bacteria and other organisms that digest nutritious components and hence degrade their quality.

Organic fertilizer can be made in the form of liquid. Liquid organic fertilizer or bio-slurry has similar advantages to GOF (Granular Organic Fertilizer), namely: ease of use, packaging, and transportation. Another advantage is that the manufacturing process is shorter and easier. In terms of the technique of production and production cost, LOF (Liquid Organic Fertilizer) is very advantageous. The stages involved in producing GOF are very short and simple [6]. Liquid organic fertilizer has organic carbon content: 18.54%, C/N Ratio: 15.32, pH: 8.51 and water content: 15 - 25% all of which, combined, function to replace the role of inorganic fertilizers.

Based on the above, research was carried out to compare the nutrition composition of bio-slurry with the main components being chicken manure this research is considered important to be carried out to compare the nutrient composition of bio-slurry with a main composition comprising of chicken and cattle manures to increase cultivation pond fertility as indicated by a presence of phytoplankton and bacteria.

2. MATERIALS AND METHODS

This research entails details and stages of the experiment as follows:

2.1. Time and Research Location

This research was conducted from April to September 2021, which consisted of two main stages of activities, namely field and laboratory activities. Field activities involved the making of liquid organic fertilizers, fish rearing and water sampling in the earth ponds of the farmer community in Mondoe Village, South Pallangga District, South Konawe Regency, SE-Sulawesi, Indonesia. Laboratory activities were conducted at 1) the Environmental Laboratory of the Environmental Agency of SE-Sulawesi Province for NPK analysis; and 2) Laboratory of Fisheries and Marine Sciences, Halu Oleo University, Kendari, Indonesia for plankton observations and protein analysis.

2.2. Tank Fermentation Design

The equipment prepared to produce the liquid organic fertilizer was a clean plastic tank or barrel with a volume of 200 L equipped with a tight cover, 1 m of transparent aerator hose (approximately 0.5 cm in diameter), and a 1 L plastic bottle. A hole the size of the aerator hose was made on the tank cover. At the bottom of the tank (approximately 25 cm from the bottom) a sieve was installed to retrieve pure manure. In addition, the tank was also equipped with a tap for the convenient harvest of the fertilizer (Figure 1).

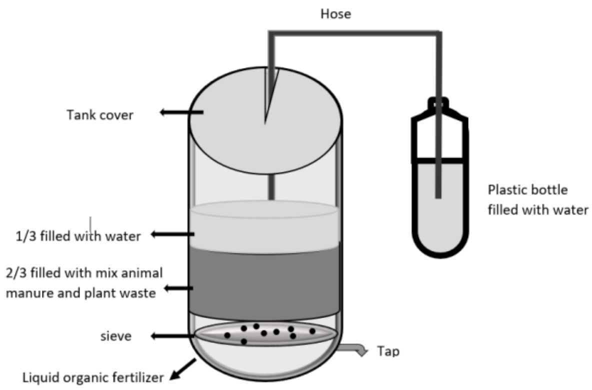

Figure 1. Fermentation tank.

2.3. Liquid Organic Fertilizers (LOF) Production

The first step in making organic fertilizer is the production of compost. Compost was made using the decomposing solution EM4. The microbes contained in EM4 are used to accelerate the fermentation and decomposition of the manure into compost. The following ingredients were used: 2 sacks of chicken manure or cattle manure, 1 sack of bran, 30 kg of forage (straw, banana stem, legume leaves), 2 L of molasses, 200 mL of bio-activator (EM4), 25 L volume of rice water and clean water as much as 1/3 of the volume of the tank.

In this study, two types of organic fertilizer were made; one fertilized was made using chicken manure and the other using cattle manure. Chicken manure and cattle manure were dried and then comminute to minimize or eliminate lumps. Other organic ingredients such as leaves, corn cobs and banana stalks were cut or chopped into small pieces. All ingredients were mixed and then put into a barrel and added with water. The composition of the ingredients involved 2 parts organic matter and 1 part water. The bio-activator, EM4 and 5 L of molasse were combined and mixed until evenly distributed. This bio-activator solution was then added into the barrel containing the raw material for the fertilizer and all were mixed until evenly distributed.

The barrel was tightly closed and a hose was inserted through the hole made on the barrel cover. The inlet where the hose entered the tank was glued together so that there were no air gaps. The other end of the hose went into the bottle that had been filled with water. The reaction would take place anaerobically. The function of the hose was to stabilize the temperature of the mixture by removing the gas produced without having to let air from outside enter the barrel.

After 21 days the maturity level of the fertilizer was checked by opening the lid of the tank and smelling the mixture. If odour is similar to that of beer, the mixture is ready.

The liquid and the dregs would be separated because the barrel was equipped with a filter. The dregs could later be used as solid organic fertilizer. The liquid that had passed the filter was put in a plastic jerry can and then tightly closed. The liquid organic fertilizer was produced, ready to use, and can be used for up to 6 months.

2.4. Pond Preparation and Maintenance

The preparation of the earth entails the improvement or refurbishing of pond components, namely embankments, sluice gates, caren and channels, as well as management of pond subgrade. Subgrade management was intended so that natural food (valves) can thrive. After plowing, drying, and liming (with a dose of CaO 600 ppm per ha), the next stage was then fertilizing the subgrade.

Initial fertilization was carried out with inorganic fertilizer SP-36 at a dose of 300 kg/ha over the entire surface of the pond. Next, water was put into the pond until a depth of about 5 – 10 cm and the water was let to evaporate. Subsequently, fertilization with LOF of 12 l/ha was done over the entire pond evenly. Lastly, the pond was irrigated in stages, up to a depth of 10 cm, then let stand and growth of valves were observed. Repeated fertilization was done after a month of maintenance on water media.

2.4. Observed Parameters

2.4.1 NPK content in Cattle and Chicken Manure

Measurement of NPK parameters was carried out in the cattle and chicken manure after fermentation for 21 days. NPK contents in the sample were determined by measuring concentration using a flame photometer (SNI 2803:2010).

2.4.2 Enumeration and Identification of Plankton

Analysis of plankton was done through a sampling of 50 L of water that was filtered and extracted to obtain 100 mL plankton sample using a net. As much as 2-4 drops of Lugol solution were used to preserve the sample. The concentrated plankton sample that had been preserved was examined using a Sedgewick-Rafter counting cell (SR-cell) under a compound binocular microscope (Olympus) at the laboratory of the Faculty of Fisheries and Marine Sciences at Halu Oleo University. The quantitative value of phytoplankton present in the sample was determined using the formula proposed by APHA (1989), whereby the Diversity index (H') and (E) were calculated using the Shannon-Wiener index [7].

2.4.3 Bacterial Count in Liquid Organic Fertilizers

Total Bacteria Count was determined using the Standard Plate Count (SPC) method, which is a method of estimating the total number of bacteria contained within a sample growing under the aerobic conditions on standard methods agar.

Samples of LOF of cattle and chicken manures were serially diluted. As much as 0.1 mL of the samples were taken and plated onto TSA medium in a petri dish, then incubated for 48 hours at 35°C. Total bacteria was calculated by multiplying the number of bacteria by the diluent factor and expressed in units of CFU ml^{-1}. While, the number of *Escherichia coli* bacteria was calculated using the method of Most Probable Number (MPN-Durhan) then further test on *E. coli* media for confirmation.

2.4.4 Protein Content Analysis

After a maintenance period of 3 months, samples of milkfish reared in the earth ponds were taken. Muscles on the dorsal part of the fish were collected for further analysis using the Kjeldhal method (AOAC, 1970)

2.5 Data Analysis

All data obtained were analyzed descriptively.

3. RESULTS AND DISCUSSION

From the tests carried out on samples of cattle and chicken manure, data on nutrients and bacteria content were obtained as follows.

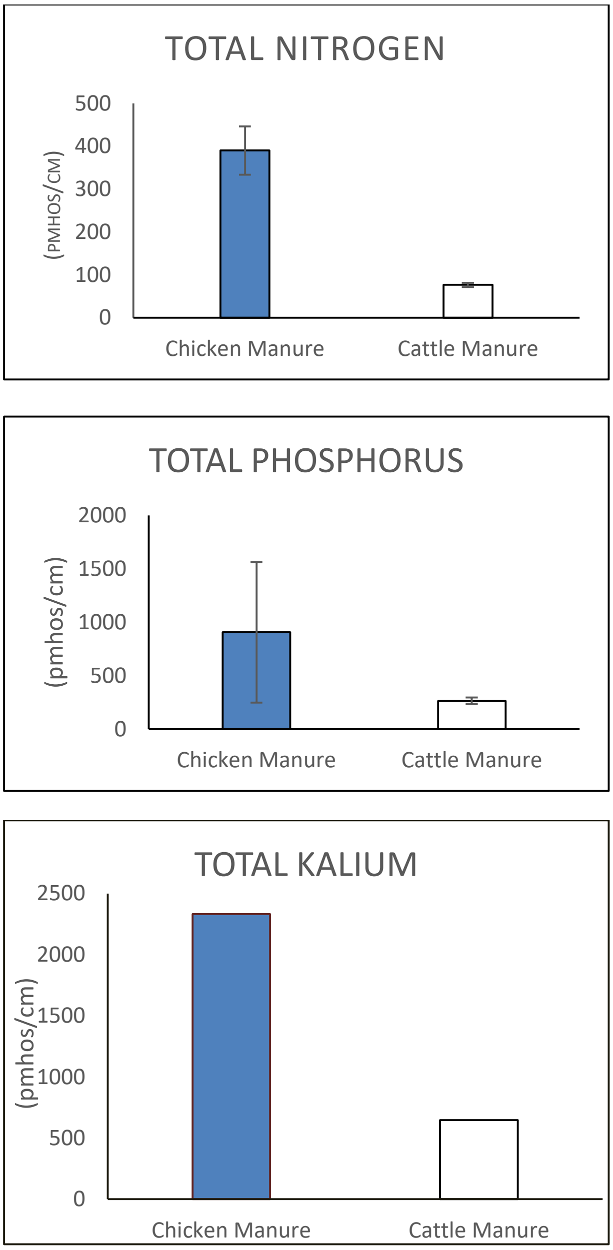

Figure 2. Comparison of total NPK of cattle and chicken manure of liquid fertilizer.

Figure 2 shows that the nutrient content, especially NPK, was significantly different between the two types of animal manure. The NPK nutrient content in chicken manure was 4-5 times higher than that of cattle manure. Bacterial population data also showed higher in chicken manure than cattle manure (Figure 3). In contrast, the pathogenic bacteria, *E. coli*, was lower in chicken manure (Figure 4).

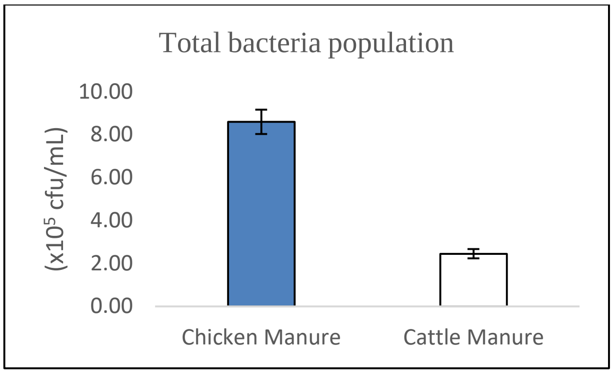

Figure 3. Comparison of total bacteria of cattle and chicken manure in liquid fertilizer.

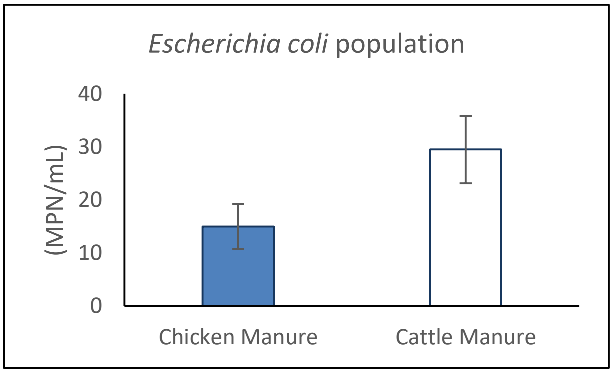

Figure 4. Comparison of *E. coli* bacteria of cattle and chicken manure in liquid fertilizer.

Nutrient content and friendly bacteria are important points in analysis of the resulting LOF. Considering not only for fish growth, but also for environmental health and safety for consumers.

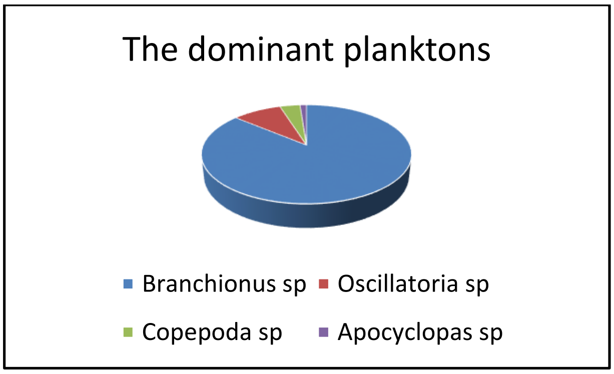

Figure 5. The dominant plankton species found in water pond fertilized by bio-slurry.

Table 1. The abundance and diversity of plankton in ponds in the third month after dispersal of bio-slurry compared to ponds that used solid organic fertilizer.

Parameters (cell.L-1)	Test Results	References (Yusnaini & Nur [11])
Plankton abundance	3,276	1,008
Plankton Diversity Index	0.6571	0.7184
Plankton Evenness Index	0.2991	0.3269

Based on the analysis of the data obtained from both the chicken manure and cattle manure fertilizers for nutrients content, the bio-slurry made from chicken manure contained significantly higher NPK than the one made from cattle manure. While, bio-slurry made from cattle manure contained higher *E. coli* but lower bacteria content compared to the one made from chicken manure. However, results showed bacteria content in the fertilizer had no significant effect on the quality of the fish produced.

Organic fertilizers are very beneficial for increasing agricultural production both in quality and quantity, reducing environmental pollution, and sustainably improving land quality. The use of organic fertilizers in the long term can increase land productivity and prevent land degradation.

Several studies have described how fertilizers work, in which they involve; 1) a process of decomposition and release of nitrogen, phosphorous and potassium elements from the organic fertilizers to the surround providing nutrients to phytoplankton consumed by the growing fish; 2) release of other elements within the organic fertilizer, in this case animal manure, that provides more source of nutrition as well as a surface for microbes to attach. These organisms would provide food for fish, though not all that is produced by said organisms may be consumed by the fish; and 3) production of "green manure" in the form of non-digested food contained within the manure itself that can be consumed by the fish, thereby providing nutrition and increase fish production [8, 9].

The results of phytoplankton abundance analysis were 3,276 (cell.L-1) (Table 1). This value was higher than water in ponds that only used solid organic fertilizers [11]. The abundance of plankton could change everytime because they were consumed by fish and depended on the growth of plankton and the environment. This is consistent with [12], who claimed that the phytoplankton composition and distribution would change at multiple levels in response to changes in environmental conditions; physically, chemically, and biologically. One of the environmental factors which influenced phytoplankton abundance was nutrients, with nitrate serving as a limiting factor.

After LOF was spread in ponds, there was three highest density of plankton which accounted by Branchionus sp, followed by *Oscillatoria* sp and *Copepoda* sp, while the smallest number was zooplankton *Apocyclopas* sp (Figure 5). Milkfish is an omnivorous species. However, they tend to be herbivorous. From the digestion observation in milkfish, it was known that the type of plankton preferred was *Pleurosigma* sp. and *Oscillatoria* sp [13]. While, for the larval stage, the species of *Nannochloropsis* sp. and zooplankton rotifer (*Brachionus* sp.) were two plankton species which were chosen as the primary food source of milkfish [14]. From the description above, it can be concluded that LOF was made from animal manure as its main ingredient would make grow the preferred plankton species and provided a source of nutrients consumed by fish.

The protein content of milkfish from ponds in this study was quite rich in protein (51.14%). Compared with nutrient composition analysis of milkfish from ponds in Pangkep Regency, South Sulawesi, it only contained 24.18% protein [15]. The nutritional content of fish depends on internal and external factors.

4. CONCLUSION

Ponds fertilizing with organic fertilizer made from fermented chicken manure can be presumed to establish more favorable conditions for the growth of plankton characterized by high nutrient content and less content of pathogenic bacteria. Plankton would provide a source of nutrients consumed by fish so that it is rich in protein.

AUTHORS' CONTRIBUTIONS

All authors contributed in giving ideas, conducting research, data analysis, and writing article.

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