

The Role of Microorganisms in the Application of Various Types of Animal Manure Solutions with Various Concentrations in the Cultivation of Lettuce (*Lactusa sativa* L.)

Djuhari Djuhari¹, Sutoyo², Sugiarto Sugiarto³, Gatra Ervi Jayanti⁴ and Istiyono Kirno Prasetyo⁵

¹Department of Agribusiness, Faculty of Agriculture, Universitas Islam Malang, Indonesia

²Department of Biology, Faculty of Mathematics and Natural Sciences, Jember State University, Indonesia

³Department of Agrotechnology, Faculty of Agriculture, Universitas Islam Malang, Indonesia

⁴Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Islam Malang, Indonesia

⁵Department of Agrotechnology, Faculty of Agriculture, Wisnuwardhana University Malang, Indonesia

Abstract. This study examined the interaction between manure type and concentration on the growth and yield of curly lettuce in a greenhouse, employing a factorial randomized block design. Four manure solutions were tested: goat (M1), cow (M2), chicken (M3), and rabbit (M4), applied at concentrations of 30%, 3%, and 0.3% (v/v). Results showed that microbial content differed by species, with rabbit and cow manures containing higher total fungal and bacterial counts than goat and chicken manures. The application of manure concentrates significantly influenced lettuce performance, improving vegetative growth (leaf number and area) and yield, with an average harvest index of greater than 0.95, which is higher than that of the control. These findings demonstrate that the effectiveness of liquid organic fertilizers depends not only on microbial abundance but also on community composition and proper dosage. Practical implications suggest that goat manure is most effective at a high concentration (30%), cow manure at a medium concentration (\approx 3%), rabbit manure at a low concentration (0.3%), while chicken manure remains relatively stable across concentrations. Selecting the right manure type and dosage enables farmers to harness microbial bioactivity, thereby increasing productivity and reducing their reliance on inorganic fertilizers sustainably. Overall, the study emphasizes the significance of manure type and application level in maximizing curly lettuce yield while promoting sustainable horticultural practices.

¹ Corresponding author: djoe61@unisma.ac.id

Keywords: animal manure, concentration, cultivation, lettuce, microorganism

1 Introduction

Lettuce (*Lactuca sativa* L.) is a horticultural commodity with high economic value and increasing demand, both for household consumption and the modern culinary sector. Lettuce is a leafy vegetable sought after for its nutritional content, particularly vitamin A and vitamin C, dietary fibre, and antioxidants, which are beneficial for health. In urban farming systems, lettuce is often used as a cultivation model due to its relatively short growth cycle and ease of cultivation, both conventionally and hydroponically [1]. However, one of the main obstacles to increasing lettuce productivity is the availability of balanced and environmentally friendly nutrients. The long-term use of inorganic fertilizers is considered to increase production costs, reduce soil fertility, and lead to environmental pollution. Therefore, alternative fertilizers based on organic sources are needed to support plant growth while maintaining the sustainability of agricultural ecosystems [2, 3].

Manure is one of the most commonly used organic material sources in horticultural cultivation. In addition to providing macronutrients such as nitrogen (N), phosphorus (P), and potassium (K), manure also contains micronutrients and various organic compounds that can improve soil structure. Furthermore, manure is rich in microbial communities that play a crucial role in the decomposition of organic matter and the transformation of nutrients. Some commonly found microbial groups include phosphate-solubilising bacteria (*Bacillus* sp. and *Pseudomonas* sp.), nitrogen-fixing bacteria (*Azotobacter* sp. and *Rhizobium* sp.), and decomposing fungi such as *Trichoderma* sp. and *Aspergillus* sp. The presence of these microbes can increase nutrient availability, accelerate mineralization, and stimulate plant growth by producing phytohormones [3].

The type of manure used will affect both the nutritional quality and the diversity of its microorganisms. Chicken manure, for example, is known to have a high nitrogen content, but it also decomposes relatively quickly and has the potential to increase ammonia levels if not properly processed. In contrast, cow manure has a higher fiber and lignin content, resulting in a slower decomposition process; however, it can improve the physical properties of the soil. Goat manure, on the other hand, tends to have a higher potassium and phosphorus content than cow manure, and produces a drier texture, making it easier to process [4]. This difference in composition will affect the growing microbial community and, consequently, the availability of nutrients to plants. Therefore, the choice of manure type needs to be tailored to the specific needs of the plant and the conditions of the cultivated land.

With the advancement of agricultural technology, manure is not only applied in solid form but is also processed into liquid fertilizer. Liquid manure has advantages because roots more easily absorb its nutrients, can be applied through foliar sprays, and has a faster effect on plant growth [5]. However, the effectiveness of liquid fertilizers is highly dependent on the concentration applied. A concentration that is too low can reduce the effectiveness of the fertiliser, while a concentration that is too high can potentially cause toxicity or inhibit plant growth. Therefore, determining the optimal concentration of various types of liquid manure is key to achieving maximum results.

Various previous studies have reported the benefits of using liquid manure on horticultural plant growth. For example, the application of liquid chicken manure at a concentration of 20–40% can increase plant height, leaf number, and fresh weight of water spinach [6]. Meanwhile, liquid cow manure has been reported to enhance the vegetative growth of chilli peppers and increase leaf chlorophyll content [3]. However, a knowledge gap remains regarding the role of microorganisms in various types of manure solutions at

different concentrations, particularly in lettuce cultivation. However, lettuce is a leafy crop that is highly responsive to nutrient availability, making research on this commodity crucial for providing a more comprehensive understanding.

Furthermore, the integration of liquid manure with the natural microorganisms it contains aligns with the principles of sustainable agriculture and supports national policies aimed at developing local organic fertilizers. This research is also relevant to the direction of modern agricultural development, which prioritizes input efficiency, food safety, and environmental friendliness. In an academic context, this research is expected to contribute to the body of knowledge on the relationship between soil microbiology, liquid organic fertiliser technology, and horticultural plant physiology.

This study aims to examine the role of microorganisms in various types of manure solutions (chicken, cow, goat, and rabbit) at different concentrations on the growth and yield of lettuce (*Lactuca sativa* L.). The results are expected to provide scientific recommendations on the most effective types and concentrations of liquid manure, as well as serve as a basis for developing microorganism-based liquid organic fertilisers to support sustainable horticultural cultivation.

2. Materials and Methods

2.1 Research Location and Time

The research was conducted in the greenhouse of the Faculty of Agriculture at the Universitas Islam Malang, East Java, Indonesia. Research activities were conducted from early March to late May 2025, encompassing the preparation of the animal manure solution, the preparation of the growing medium, the application of treatments, maintenance, and harvesting of the lettuce.

2.2 Materials and Equipment

The primary materials used in this study were animal manure (from goat, cow, chicken, and rabbit), molasses, and water. These three ingredients were combined into a solution with a composition of 300 g of animal manure, 500 cc of molasses, and 500 cc of water. The solution was fermented for 21 days. The test plants consisted of lettuce (*Lactuca sativa* L.) seeds of the curly green variety, along with a solution of manure from four livestock species: goats (M1), cows (M2), chickens (M3), and rabbits (M4). Liquid manure was obtained by fermenting the animal manure with the addition of water (a 1:1 w/v ratio). The solution concentrations used were 30% (K1), 3% (K2), and 0.3% (K3) (v/v). The growing medium used was a mixture of soil and compost in a 2:1 ratio, which was then sterilized by drying for 3 days to reduce pathogen contamination. Other additional materials included clean water for irrigation, 30 x 30 cm polybags, and a botanical insecticide (neem leaf extract) to prevent pests. In addition, the equipment used included plastic buckets for fermenting the liquid fertilizer, measuring cups, digital scales, a sprayer, a tape measure, a ruler, a digital camera for documentation, and stationery for data recording.

2.3 Experimental Design

This study employed a factorial Randomised Block Design (RBD) with two treatment factors: four types of manure solutions (M): M1 (cow manure), M2 (goat manure), M3

(chicken manure), and M4 (rabbit manure). Factor 2: Manure solution concentration (K): K1: 30% (v/v), K2: 3% (v/v), and K3: 0.3% (v/v). The combination of these two factors resulted in 12 treatment combinations. Each treatment was replicated three times, resulting in 36 experimental units. Each experimental unit consisted of five plants, resulting in a total of 180 plants observed.

2.4 Research Implementation

Descriptive Research

The method used was Viable Plate Count. The basic principle is that one living microbial cell will grow and form a colony on a suitable solid medium. Therefore, the number of colonies grown on a plate is considered representative of the number of viable microbes in the initial sample, and the results are expressed as Colony Forming Units (CFU) per mL or per gram.

Experimental Research

- a. Seed and Planting Media Preparation:
Lettuce seeds were sown in seedling trays containing a soil: compost (2:1) medium until 14 days after sowing (DAS). Healthy seedlings with uniform growth were transferred to polybags containing the main growing medium.
- b. Application of Manure Solution
The manure solution was fermented for 14 days and then diluted according to the treatment concentrations (30%, 3%, and 0.3% v/v). The application was carried out by watering the growing medium at a dose of 100 ml per plant every 7 days, starting 7 days after planting (DAS) and continuing until harvest.
- c. Plant Maintenance
Maintenance included watering with clean water as needed, manual weeding, and pest control using botanical insecticides when necessary.
- d. Observation Variables
Observations were made on the following lettuce plant growth and yield parameters: Vegetative growth: plant height (cm), number of leaves (leaflets), leaf area (cm²), leaf color (green score using a SPAD meter). Yield variables: total fresh weight per plant (g), fresh economic weight (g), dry economic weight (g), and root length (cm). Growth observations were conducted every 7 days from 7 Days After Transplanting (DAP) until harvest, while yield variables were observed at harvest.

2.5 Data Analysis

The observation data were analyzed using analysis of variance (ANOVA) in accordance with the factorial RAK experimental design at a 95% confidence level. If there was a significant interaction between treatments, an Honestly Significant Difference (HSD) test was conducted at the 5% level.

3. Results and Discussion

3.1 Characteristics of Animal Manure Solution

The results of laboratory analysis using the Viable Plate Count method for the total number of fungi and bacteria are presented in Table 1.

Table 1. Average total fungal and bacterial counts in animal manure concentrate fermented for more than 21 days.

No.	Manure Concentrate	Fungus Count (CFU/ml)	Bacteria Count (CFU/ml)
1.	M1 = Goat	31.33×10^2	39.33×10^7
2.	M2 = Cow	35.67×10^2	42.33×10^7
3.	M3 = Chicken	25.00×10^2	26.67×10^7
4.	M4 = Rabbit	35.00×10^2	44.33×10^7

Differences in the total number of fungi and bacteria in animal manure solutions are generally influenced by the nutrient composition, fibre content, moisture content, and the physiological characteristics of the animal's digestive tract. Cow and rabbit manure are known to have high crude fiber content because both animals are herbivores with fermentative digestive systems. In cows, the fermentation process occurs in the rumen, whereas in rabbits, it takes place through hindgut fermentation in the cecum. Both systems produce manure residue rich in undigested fiber, lignocellulose, and other organic compounds that serve as ideal substrates for microorganism growth [7]. Furthermore, cow and rabbit manure tend to have higher water content than goat and chicken manure. These humid conditions strongly support the colonization and proliferation of bacteria and saprophytic fungi. Fungi, such as *Aspergillus*, *Penicillium*, and *Mucor*, are often found to be more dominant in manure with high cellulose content, while cellulolytic bacteria, including *Bacillus* and *Cellulomonas*, also thrive on fibre-rich substrates [8, 9]. Therefore, cow and rabbit manure solutions offer a more potent medium for providing a higher total microbial population than other types.

Conversely, goat and chicken manure have characteristics that are less conducive to microbial growth. Goat manure tends to be drier and denser, with a fiber content that is less degradable, resulting in lower surface contact with the environment and lower relative humidity. These conditions limit microbial colonisation, particularly that of fungi, which require a moist substrate for mycelial growth. Chicken manure also differs in its nutrient composition, as chickens are omnivores with a non-fermentative digestive tract. Chicken manure typically contains high levels of nitrogen and ammonia, which contribute to a more alkaline pH and natural antimicrobial properties. High levels of ammonia have been shown to suppress the growth of certain fungi and inhibit the survival of sensitive bacteria [10]. Furthermore, chicken manure tends to mix with urine (dropping), which accelerates decomposition but also increases the levels of compounds toxic to microbes [11]. This condition results in a relatively lower total number of fungi and bacteria growing in chicken manure compared to rabbit and cow manure. Therefore, differences in animal digestive systems, nutrient levels, moisture content, and faecal chemistry significantly influence the total number of microbes that thrive in each animal's manure.

3.2 Effect of Animal Manure Concentrate on Plants

Experimental results on curly lettuce plants indicate an interaction between the type of animal manure concentrate and its application concentration on plant growth and yield, as shown in Table 2. The differences in curly lettuce's response to the type and concentration of animal

manure solution are closely related to the chemical composition, nutrient content, and availability of functional microbes in each concentrate. Rabbit manure at a concentration of 30% showed the best results in terms of leaf number and leaf area because goat manure has a relatively low nitrogen and phosphorus content and a dry, hard physical structure. This causes nutrient release to be slow, so high concentrations are required to achieve optimal nutrient availability for lettuce vegetative growth [12]. Therefore, low concentrations are unable to provide sufficient nutrients for leaf formation, while high doses (30%) support optimal growth.

Like goat manure, cow manure is most effective at a 30% concentration because it is rich in cellulolytic and lignocellulolytic microorganisms, which play a role in the decomposition of organic matter. Cow manure has a relatively moderate macronutrient content and a high water content, so higher concentrations of cow manure can provide sufficient nitrogen, phosphorus, and potassium for plants [3]. As for chicken manure concentrate, the insignificant results at various concentrations can be explained by its high inorganic nitrogen and ammonia content. Therefore, despite varying doses, lettuce showed no significant differences due to physiological tolerance thresholds. Similar findings have been reported, indicating that chicken manure has the potential to increase growth; however, excessive doses can easily lead to toxicity and nutrient saturation [13].

Table 2. The average number of leaves and leaf area due to the effect of giving the type of animal dung solution and application concentration at 4 weeks after planting (WAP)

Treatment	the number of leaves (strands)	leaf area (cm ²)
M1K0	6.11 ab	87,08 abc
M1K1	8.33 c	122,68 c
M1K2	7.78 c	105.09 abc
M1K3	7.33 abc	120.50 abc
M2K0	7.33 abc	89.90 abc
M2K1	8.00 c	110.20 abc
M2K2	8.22 c	126.13 c
M2K3	7.44 bc	121.27 bc
M3K0	6.22 ab	73.18 ab
M3K1	7.78 c	105.64 abc
M3K2	7.78 c	115.12 abc
M3K3	7.89 c	116.63 abc
M4K0	5.89 a	71.84 a
M4K1	7.33 abc	106.78 abc
M4K2	8.33 c	105.39 abc
M4K3	7.33 abc	177.55 d
BNJ 5%	1.48	49.27

In line with its effect on plant growth, the tested treatments also showed a similar effect on yield. The combination of concentrate type and application concentration can increase the yield of curly lettuce. As shown in Table 3, the effects of these treatments are consistent with those in Table 2. The results show that the combination of concentrate type and application concentration not only affects vegetative growth but also significantly impacts curly lettuce yield. An average harvest index of more than 0.95 indicates high biomass allocation efficiency from vegetative to economically viable parts (leaves). This is relevant to the findings reported that the use of liquid organic fertilizer based on animal manure can increase photosynthesis efficiency and assimilate translocation, thereby increasing the proportion of

marketable crops [14] This increase in productivity is influenced by the macronutrient content (N, P, K) and micronutrients (Ca, Mg, Zn) in the animal manure concentrate, as well as the role of microbes that accelerate mineralization and nutrient availability. Therefore, the appropriate application of animal manure solution can increase the harvest index while maintaining yield quality.

Table 3. Average total fresh weight of plants, economic fresh weight, and harvest index due to the effect of application of animal manure solution type and application concentration at harvest time

Treatment	Total Fresh Weight of Plants (g)	Economic Fresh Weight (g)	Harvest index
M1K0	85.40 ab	81.07 ab	0.95 ab
M1K1	253.73 b	245.17 b	0.97 b
M1K2	135.93 ab	129.90 ab	0.5 ab
M1K3	15283 ab	146.20 ab	0.96 ab
M2K0	105.03 ab	99.47 ab	0.95 ab
M2K1	169.83 ab	163.67 ab	0.96 ab
M2K2	201.40 ab	195.27 ab	0.97 b
M2K3	204.70 ab	196.07 ab	0.96 ab
M3K0	67.43 a	64.73 a	0.96 ab
M3K1	165.30 ab	157.70 ab	0.95 ab
M3K2	176.30 ab	164.30 ab	0.93 a
M3K3	255.90 b	245.97 b	0.96 ab
M4K0	87.43 ab	82.33 ab	0.93 ab
M4K1	176.23 ab	168.17 ab	0.96 ab
M4K2	228.03 b	219.63 b	0.96 ab
M4K3	216.07 b	207.37 b	0.96 ab
BNJ 5%	144.06	139.16	0.035

The relevance of these results is also supported by recent studies that emphasize the role of organic fertilizer in enhancing yield efficiency compared to unfertilized controls. Research by [14] demonstrated that the application of liquid organic fertilizer increased the harvest index of leafy vegetables by 10–15% compared to unfertilized treatments, primarily due to the combination of nutrients and microbial activity that enhanced nutrient availability. Therefore, the relevance of this research emphasizes that the use of animal manure solutions serves not only as a nutrient source but also as a natural bioactivator that supports growth, increases yield, and ensures the sustainability of the curly lettuce production system.

4. Conclusion

Based on the research results, it can be concluded that the characteristics of microorganisms in animal manure concentrate differ according to species, with rabbit and cow manure having higher total bacterial and fungal counts than goat and chicken manure. The effect of the type of manure concentrate and its application concentration is reflected in curly lettuce cultivation, where the application of animal manure concentrate increased vegetative growth (in terms of number and leaf area) and crop yields, with an average harvest index of >0.95, which is higher than that of the control. These findings emphasise that the effectiveness of liquid organic fertiliser is determined not only by the abundance of microbes but also by the quality of the microbial community and the optimal application concentration.

Practical implications: For curly lettuce cultivation, goat manure is effective at high concentrations (30%), cow manure at medium concentrations ($\pm 3\%$), rabbit manure at low concentrations (0.3%), while chicken manure is relatively stable at various concentrations. By selecting the right concentrate and dosage, farmers can harness the bioactivity of natural microbes to increase productivity while reducing their dependence on inorganic fertilisers in a sustainable manner.

This research was funded by the Universitas Islam Malang (UNISMA), through the UNISMA Institutional Grant for the 2025 fiscal year. We would like to express our gratitude to the Rector and Head of the UNISMA Research and Community Service Institute.

Reference

1. N. Nurhidayati, M. Machfudz, A. Basit, Yield and nutritional quality of green leafy lettuce (*Lactuca sativa* L.) under soilless culture system using various composition of growing media and vermicompost rates. *Caraka Tani: Journal of Sustainable Agriculture*, **36**, 2 (2021). <https://doi.org/10.20961/carakatani.v36i2.46131>
2. T. Junaidi, SY. Tyasmoro, Effect of Planting Media and Dose of Goat Manure on the Growth and Yield of Lettuce in the Urban Farming System. *Plantropica: Journal of Agricultural Science*. **7**, 2 (2022). DOI:[10.21776/ub.jpt.2022.007.2.4](https://doi.org/10.21776/ub.jpt.2022.007.2.4)
3. C. Suriani, SFA. Limbong, FA. Nasution, The Influence of Liquid Organic Fertilizer from Cow Manure on the Growth and Production of Lettuce (*Lactuca sativa* L.) Var Kriebo. *BIOLINK (Jurnal Biologi Lingkungan Industri Kesehatan)*. **10**, 1 (2023).. DOI: [10.31289/biolink.v10i1.9647](https://doi.org/10.31289/biolink.v10i1.9647)
4. H. Bamdad, S. Papari, G. Lazarovits, F. Berruti, F. Soil amendments for sustainable agriculture: Microbial organic fertilizers. *Soil Use and Management* **38**, 1 (2022). <https://doi.org/10.1111/sum.12762>
5. T. Phibunwatthanawong, N. Riddech, Liquid organic fertilizer production for growing vegetables under hydroponic condition. *International Journal of Recycling of Organic Waste in Agriculture*, **8**, 369-380 (2019). <https://doi.org/10.1007/s40093-019-0257-7>
6. AF. Aziez, DS. Utami, CF. Putra, Liquid Organic Fertilizer Alcohol Waste Enriched Manure Effect on Growth and Yield of Chili (*Capsicum Annum* L.). *Agricultural Science*. **5**, 1 (2021). <https://doi.org/10.55173/agriscience.v5i1.63>
7. V. Fasake, K. Dashora, A sustainable potential source of ruminant animal waste material (dung fiber) for various industrial applications: A review. *Bioresource Technology Reports*. **15**, 100693 (2021). DOI:[10.1016/j.biteb.2021.100693](https://doi.org/10.1016/j.biteb.2021.100693)
9. H. Duan, M. Ji, A. Chen, B. Zhang, J. Shi, L. Liu, J. Sun, Evaluating the impact of rice husk on successions of bacterial and fungal communities during cow manure composting. *Environmental Technology & Innovation*. **24**, 102084 (2021). <https://doi.org/10.1016/j.eti.2021.102084>
10. O. Zapata Martínez, A. Serrano, MM. Aguilera Flores, DK. Villa-Gómez, Rabbit manure as a potential inoculum for anaerobic digestion. *Journal of Environmental Science and Health, Part A*. **54**, 9 (2019). <https://doi.org/10.1080/10934529.2019.1610293>
11. RÖ. Sürmeli, A. Bayrakdar, BA. Calli, Removal and recovery of ammonia from chicken manure. *Water science and technology*. **75**, 12 (2017). DOI:[10.2166/wst.2017.116](https://doi.org/10.2166/wst.2017.116)

12. M. Kacprzak, K. Malińska, A. Grosser, J. Sobik-Szołtysek, K. Wystalska, D. Drózdź, E. Meers, Cycles of carbon, nitrogen, and phosphorus in poultry manure management technologies—environmental aspects. *Critical Reviews in Environmental Science and Technology*. **53**, 8 (2023). <https://doi.org/10.1080/10643389.2022.2096983>
13. IM. Mesa, YP. Situmeang, AANM. Wirajaya, IGB. Udayana, MS. Yuliartini, Utilization of rabbit manure and biochar chicken manure and its effect on the growth and yield of pakchoy plants. In Proceeding of the 2nd Annual Conference of Science and Technology (ANCOSSET 2020), 28 November 2020, Malang, Indonesia. *Journal of Physics: Conference Series*. **1869**, 1 (2021). DOI [10.1088/1742-6596/1869/1/012045](https://doi.org/10.1088/1742-6596/1869/1/012045)
14. E. Kantikowati, Y. Yusdian, C. Suryani, Chicken manure and biofertilizer for increasing growth and yield of potato (*Solanum tuberosum* L.) of Granola varieties. In Proceeding of the International Seminar and Congress of Indonesian Soil Science Society 2019 5–7 August 2019, Bandung, West Java, Indonesia. *IOP Conference Series: Earth and Environmental Science*. **393**, 1 (2019). DOI [10.1088/1755-1315/393/1/012017](https://doi.org/10.1088/1755-1315/393/1/012017)
15. Peiris, P. U. S., & Weerakkody, W. A. P. (2015, April). Effect of organic based liquid fertilizers on growth performance of leaf lettuce (*Lactuca Sativa* L.). In the paper of the International conference on agricultural, ecological and medical sciences (AEMS-2015) April 7-8, 2015, Phuket, Thailand. Retrieved from <https://iicbe.org/upload/9745C0415010.pdf>