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Effects of Chicken Manure Dose and Application Timing on the Productivity and Microbiological Safety of Lettuce (*Lactuca sativa* L.)

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Abstract

Organic lettuce is widely regarded as a healthier alternative because it is free of chemical contaminants. Chicken manure is commonly used as an organic fertilizer because of its high nutrient content, which is readily available to plants. However, applying manure poses a risk of pathogenic bacterial contamination. This study aimed to evaluate the effects of different chicken manure doses and application times, as well as their interactions, on lettuce (*Lactuca sativa* L.) productivity and microbiological safety. The experiment was conducted from March to May 2023 in Cianjur, West Java, using a factorial randomized block design (RBD) with three replications. Treatments included a control and two factors: (1) manure dose (10, 20, and 30 t/ha), and (2) application times (1, 2, and 3 weeks before transplanting). Microbiological analysis of harvested lettuce leaves was performed in the laboratory to assess contamination levels of *Escherichia coli*, *Salmonella* spp., and *Shigella* spp. Manure doses significantly influenced all growth and yield parameters but had no significant effect on quality variables, including flavonoid, nitrate, and total chlorophyll content. Application times showed no significant effect on any measured parameter. The interaction between dose and time was also not statistically significant for most variables. The highest productivity and marketable yield were observed at the 30 t/ha dose. *Salmonella* spp. was not detected in any samples; however, *E. coli* and *Shigella* spp. were present in all samples at levels exceeding the Indonesian National Standard (SNI) for food safety. In addition to the required dose of chicken manure, to minimize microbial contamination, fully decomposed manure is recommended for application, as it can be applied to land shortly before planting. The use of microbial-free water for washing vegetables is recommended, including in further research.

Keywords: food safety, microbial hazard, organic farming

Introduction

Lettuce is commonly consumed fresh (raw) and is rich in nutrients such as calcium, phosphorus, iron, and vitamins A, B, and C (Shi et al., 2022). Fresh vegetables typically contain higher nutrient levels, as cooking and heating processes can lead to nutrient loss (Aisyah et al., 2015). However, consuming raw vegetables also poses a risk of contamination that can negatively impact human health. For instance, pesticide residues may accumulate in the body and potentially cause degenerative diseases such as cancer, leukemia, and asthma (Kim et al., 2017).

Organic farming presents an alternative approach to producing pesticide-free vegetables. Nonetheless, fresh organic produce may be at higher risk of contamination by pathogenic bacteria, which can endanger consumer health (Jensen et al., 2013). Lettuce, in particular, is highly susceptible to contamination due to its broad, uneven leaf surfaces, which facilitate the attachment of parasites, and its short stems, which increase contact with soil and irrigation

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water (Al Nahhas & Aboualchamat, 2020). Contamination sources include organic fertilizers that may harbor pathogenic bacteria such as *Escherichia coli, Salmonella* spp., and *Shigella* spp. (Johannessen et al., 2005). These bacteria can cause foodborne illnesses (infectious or toxic diseases transmitted through food consumption) such as colitis, diarrhea, typhoid fever, and dysentery (Shehu et al., 2014). Even though these bacteria can be harmful to humans, they have many strains, and not all are harmful.

Good Agricultural Practice (GAP) implementation in organic lettuce cultivation is essential to ensure product safety. Chicken manure is widely used as an organic fertilizer due to its higher content of macro- and micronutrients compared to other types of animal manure (Drozdz et al., 2020). However, it must undergo appropriate treatments, such as anaerobic decomposition, composting, or incineration, before application (Ishimori et al., 2017). Untreated chicken manure may contain various pathogenic microorganisms that pose serious health risks to consumers. Several studies have reported the risk of pathogenic bacterial contamination in vegetables fertilized with chicken manure (Yang et al., 2016). Although *E. coli, Salmonella* spp., and *Shigella* spp. are considered human pathogens, they are not pathogenic to plants. To minimize this risk, manure application must follow five key principles: the right type, the correct dose, the right time, proper placement, and the correct method (Dewi et al., 2022). Among these, the appropriate combination of dose and application time not only supports optimal plant growth and yield but also plays a crucial role in reducing the presence of harmful pathogens. Timely application promotes composting, which can deactivate pathogens while enriching the soil with organic matter (Chen & Jiang, 2014).

This study aims to evaluate the effects of varying chicken manure doses and application times, as well as their interaction, on lettuce growth, productivity, and pathogenic bacterial contamination. The findings are expected to identify the optimal dose and application time that support lettuce growth and high productivity while ensuring the produce remains free from pathogenic contamination, thus safe for consumption.

Material and Methods

This research was conducted from March to May 2023 in Cianjur, West Java, Indonesia (coordinates: -6.763, 107.063). The experimental site is situated at an altitude of 1,110 meters above sea level and characterized by latosol soil. The region receives an average annual rainfall of 1,000–1,500 mm. Soil and chicken manure analyses were carried out at the Laboratory of the Department of Agronomy and Horticulture, IPB University. The experiment used a factorial randomized complete block design with two treatment factors. The first factor was the chicken manure dose, consisting of three levels; D1: 10 t/ha, D2: 20 t/ha, D3: 30 t/ha, and control (without manure) The second factor was the application time of the chicken manure; A1: 1 week before transplanting, A2: 2 weeks before transplanting, and A3: 3 weeks before transplanting. Each treatment combination was replicated three times, resulting in a total of 30 experimental plots. Each plot measured 1 m × 10 m, with a height of 30 cm, and a 30 cm distance between plots.

Chicken manure was applied to each plot according to the assigned doses and application times. Lettuce seeds were germinated in trays containing a medium of cocopeat and burnt rice husks, and seedlings were raised in a simple bamboo-and-plastic nursery for 3-4 weeks before transplanting at a spacing of 20 cm × 20 cm. Given the rainy season and moderate-to-high rainfall intensity during the experiment, supplemental irrigation was provided as needed. No chemical agents were used for weeding or pest control throughout the study.

Observations were conducted during both the pre-harvest and post-harvest phases. Pre-harvest observations were carried out at 10, 20, and 30 days after transplanting (DAT). The observed variables included: growth parameters (plant height, number of leaves, leaf area, relative growth rate, net assimilation rate, fresh weight

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per plant, and dry weight per plant), production parameters (total fresh and dry weight per plant, fresh and dry weight of roots and leaves per plant, percentage of leaf weight to total plant weight, productivity per plot, and marketable yield), and quality parameters (flavonoid content, nitrate content, total chlorophyll content, leaf greenness level, and level of bacterial contamination).

Lettuce harvesting was conducted 30-35 DAT by carefully uprooting the entire plant, including roots, while wearing gloves to maintain hygiene. Samples designated for laboratory analysis were packed in food-grade plastic bags and stored in a cooling box during transportation.

The leaf nitrate content and greenness were measured immediately after harvest in the field using the LAQUAtwin Horiba Nitrate Meter and SPAD-502 Chlorophyll Meter. Flavonoid and total chlorophyll analyses were conducted at the Postharvest Laboratory, Faculty of Agriculture, IPB University, while bacterial contamination analysis was performed at the IPB Culture Collection Laboratory. Pathogenic bacteria were isolated from harvested lettuce leaves and cultured on selective media, namely Eosin Methylene Blue Agar (EMBA) for *Escherichia coli*, *Salmonella-Shigella* Agar (SSA) for *Salmonella* spp., and *Shigella* spp., using the streak plate method. Bacterial identification was based on colony morphology characteristics on selective media, where *Escherichia coli* showed a metallic green sheen on EMBA, while *Salmonella* spp. produced colorless colonies with black centers and *Shigella* spp. formed colorless colonies on *Salmonella–Shigella* Agar (Sivalingam et al., 2024).

Data Analysis

Data were analyzed using the *F* test, *t* test, and LSD test at a 5% significance level with the Statistical Tool for Agricultural Research (STAR) software.

Results and Discussion

Varying doses of chicken manure significantly enhanced growth and production but did not alter quality variables. Furthermore, application timing and its interaction with dose remained non-significant for all parameters, except for leaf count at 20 DAT.

Plant Growth

Application of 30 t/ha chicken manure significantly increased plant height by 10.26% to 52.48% compared to the control and lower doses at 30 DAT, while control plants (0 t/ha) consistently showed the lowest heights (Table 1). This result is in line with Cera (2022), who reported a significant effect of manure doses on lettuce height starting at 10 DAT. The increased plant height is likely due to improved macro- and micronutrient availability, which promotes vegetative growth.

There was no significant interaction between dose and application time on leaf number at 10 and 30 DAT (Table 2), but a significant interaction was found at 20 DAT (Table 3). The increase in leaf number is a crucial indicator of the effectiveness of chicken manure, as leaves play a central role in chlorophyll production and are essential for photosynthesis and the formation of photosynthates that drive plant growth and development (Jeje et al., 2023).

The chicken manure dose had a significant effect on leaf area at 10 and 20 DAT but showed no significant difference at 30 DAT (Table 4), likely because leaf area had reached its maximum at that stage of growth. The application of 30 t/ha of manure increased leaf area by 99.65% to 151.70% compared to the control. This enhancement is attributed to the presence of nitrogen (N) and phosphorus (P) in chicken manure, which are essential nutrients for vegetative growth, particularly in promoting leaf expansion (Islam et al., 2020).

Treatment with chicken manure at 30 t/ha produced the highest fresh weight per plant across all observation periods (Table 5), consistent with Bhatta (2022), who reported that chicken manure significantly increased lettuce fresh and dry weights compared with other chemical and organic fertilizers. This increase is likely due to increased nutrient availability,

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which promotes vegetative growth. Doses significantly affected dry weight per plant at 20 and 30 DAT (Table 5), with 30 t/ha increasing dry weight by 495.8%. Faster decomposition of chicken manure increased nutrient availability, supporting photosynthesis and carbohydrate accumulation rates, and increasing plant fresh and dry weights (Buhaerah et al., 2017).

Table 1

Effect of Chicken Manure Dose and Application Timing on Lettuce Plant Height

Treatments	Plant height (cm)		
	10 DAT	20 DAT	30 DAT
Control	5.83 ^{x}	6.87 ^{x}	8.07 ^{x}
Chicken manure dose (t/ha)			
10	7.64$^{b\,y}$	8.64$^{b\,y}$	9.90$^{b\,x}$
20	7.87$^{b\,y}$	9.16$^{b\,y}$	11.16$^{ab\,x}$
30	9.13$^{a\,y}$	10.52$^{a\,y}$	12.30$^{a\,y}$
F test	*	*	*
Application time (weeks before transplanting)			
1	9.46	11.20	11.20
2	7.73	9.12	11.03
3	8.40	9.74	11.12
F test	ns	ns	ns

Notes. DAT: days after transplanting.
*: significant ($p < 0.05$); ns: not significant ($p > 0.05$). Values with the same letter in the same column do not differ significantly ($p < 0.05$) based on the LSD test (a, b) and *t* test (x, y).

Table 2

Effect of Chicken Manure Dose and Application Timing on Lettuce Leaf Number

Treatments	Plant height (cm)		
	10 DAT	20 DAT	30 DAT
Control	5.83 ^{x}	6.87 ^{x}	8.07 ^{x}
Chicken manure dose (t/ha)			
10	7.64$^{b\,y}$	8.64$^{b\,y}$	9.90$^{b\,x}$
20	7.87$^{b\,y}$	9.16$^{b\,y}$	11.16$^{ab\,x}$
30	9.13$^{a\,y}$	10.52$^{a\,y}$	12.30$^{a\,y}$
F test	*	*	*
Application time (weeks before transplanting)			
1	9.46	11.20	11.20
2	7.73	9.12	11.03
3	8.40	9.74	11.12
F test	ns	ns	ns

Notes. *: significant ($p < 0.05$); **: very significant ($p < 0.01$); ns: not significant ($p > 0.05$). Values with the same letter within the same column do not differ significantly ($p < 0.05$) based on the LSD test (a, b) and *t* test (x, y).

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Table 3

Interaction Effect of Chicken Manure Dose and Application Timing on the Number of Lettuce Leaves at 20 DAT

Treatments	Dose of chicken manure		
	10 t/ha	20 t/ha	30 t/ha
	Number of leaves		
1 week before transplanting	3.2^{b}	3.4^{b}	4.6^{a}
2 weeks before transplanting	3.6^{a}	3.8^{a}	3.9^{a}
3 weeks before transplanting	3.2^{b}	4.1^{a}	4.0^{a}
CV (%)	10.5		

Note. Values with the same letter in the same column do not differ significantly ($p < 0.05$) based on the LSD test..

Table 4

Effect of Chicken Manure Dose and Application Timing on Lettuce Leaf Area

Treatments	Plant height (cm)		
	10 DAT	20 DAT	30 DAT
Control	6.74^{x}	10.41^{x}	16.99^{x}
Chicken manure dose (t/ha)			
10	$10.41^{b\,x}$	$13.01^{b\,x}$	30.22^{x}
20	$9.10^{b\,x}$	$16.39^{ab\,x}$	31.30^{x}
30	$15.87^{a\,x}$	$20.78^{a\,y}$	42.75^{y}
F test	*	*	ns
Application time (weeks before transplanting)			
1	12.37	18.36	33.89
2	10.86	15.78	28.55
3	12.14	16.03	41.83
F test	ns	ns	ns

Notes. *: significant ($p < 0.05$); ns: not significant ($p > 0.05$). Values with the same letter within the same column do not differ significantly ($p < 0.05$) based on the LSD test (a, b) and *t* test (x, y).

The application of chicken manure at 30 t/ha produced the highest RGR at 10-20 DAT but the lowest at 20-30 DAT (Table 6), which was associated with increased shading, higher respiration, and leaf senescence (Zuffo et al., 2019). During 10–20 DAT, this treatment resulted in significantly higher NAR (99.76%–324.46%) than the control and other doses, whereas at 20–30 DAT, NAR did not differ significantly among doses. NAR is influenced by leaf area, arrangement, and angle, and assimilate partitioning (Schneider et al., 2018).

Productivity

Application of chicken manure at 30 t/ha dose produced the highest fresh and dry weight per harvested plant, which were not significantly different from those produced by 20 t/ha treatment (Table 7). At this rate, chicken manure can supply approximately 789 kg N, 1,347 kg P_2O_5, and 981 kg K_2O/ha, sufficient to meet lettuce nutrient requirements for optimal growth and productivity.

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Table 5

Fresh and Dry Weight per Plant by Various Doses and Application Times of Chicken Manure

Treatments	Fresh weight per plant (g)			Dry weight per plant (g)		
	10 DAT	20 DAT	30 DAT	10 DAT	20 DAT	30 DAT
Control	3.36^{x}	5.00^{x}	21.58^{x}	0.24^{x}	0.37^{x}	1.20^{x}
Chicken manure dose (t/ha)						
10	10.86$^{b\ x}$	14.67$^{b\ x}$	51.86$^{b\ x}$	0.61^{x}	1.00$^{b\ x}$	2.63$^{b\ x}$
20	14.87$^{b\ y}$	18.43$^{b\ y}$	80.38$^{ab\ x}$	0.87^{y}	1.25$^{b\ y}$	3.81$^{a\ y}$
30	28.34$^{a\ y}$	31.94$^{a\ y}$	122.61$^{a\ y}$	1.16^{y}	2.22$^{a\ y}$	5.59$^{a\ y}$
F test	**	**	*	tn	**	*
Application time (weeks before transplanting)						
1	18.42	21.40	84.63	1.02	1.57	4.18
2	18.10	21.85	85.40	0.79	1.48	3.72
3	17.54	21.79	84.81	0.82	1.42	4.14
F test	ns	ns	ns	ns	ns	ns

Notes: *: significant ($p < 0.05$); **: highly significant ($p < 0.01$); ns: not significant ($p > 0.05$). Values with the same letter in the same column do not differ significantly ($p < 0.05$) based on the LSD test (a, b) and *t* test (x, y).

Table 6

Effect of Chicken Manure Dose and Application Timing on Lettuce Relative Growth Rate and Net Assimilation Rate

Treatments	Relative growth rate (g/g per day)		Net assimilation rate (g/cm^2 per day)	
	10-20 DAT	20-30 DAT	10-20 DAT	20-30 DAT
Control	0.040	0.116	0.001^{x}	0.007^{x}
Chicken manure dose (t/ha)				
10	0.041^{b}	0.111	0.003$^{b\ y}$	0.009^{x}
20	0.039^{b}	0.100	0.003$^{b\ y}$	0.010^{x}
30	0.080^{a}	0.088	0.006$^{a\ y}$	0.011^{y}
F test	*	ns	**	ns
Application times (weeks before transplanting)				
1	0.043	0.094	0.003	0.010
2	0.062	0.099	0.005	0.010
3	0.055	0.105	0.004	0.011
F test	ns	ns	ns	ns

Notes. *: significant ($p < 0.05$); **: highly significant ($p < 0.01$); ns: not significant ($p > 0.05$). Values with the same letter in the same column do not differ significantly ($p < 0.05$) based on the LSD test (a, b) and *t* test (x, y).

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Table 7

Fresh and Dry Weight of Lettuce Plant at Harvest (35 DAT) with Various Doses and Application Times of Chicken Manure

Treatments	Fresh weight per plant (g)	Dry weight per plant (g)
Control	17.03 x	3.02 x
Chicken manure dose (t/ha)		
10	66.83b y	8.87b y
20	186.78a y	23.81a y
30	224.58a y	28.23a y
F test	**	**
Application times (weeks before transplanting)		
1	131.47	17.00
2	144.84	17.24
3	201.88	26.67
F test	ns	ns

Notes: **: very significant ($p < 0.01$); ns: not significant ($p > 0.05$). Values with the same letter in the same column do not differ significantly ($p < 0.05$) based on the LSD test (a, b) and *t* test (x, y).

Fresh and dry root weights at harvest were higher at 20 t/ha than 10 t/ha and comparable to 30 t/ha, which produced the highest root biomass (Table 8). This response is associated with P content in chicken manure, which supports carbohydrate formation and translocation, as well as in root nodulation and development (Islam et al., 2020). The 30 t/ha dose also resulted in the highest leaf fresh and dry weights, similar to 20 t/ha (Table 8), in line with the favorable response of leafy vegetables to organic manure, increasing yields and nutritional quality (Hossain & Ryu, 2017).

The 20 t/ha dose produced the highest proportion of fresh and dry leaf weight relative to total plant weight, comparable to 30 t/ha (Table 9). As lettuce is harvested for its leaves, sufficient N availability is key to maximizing fresh and dry leaf weights (Wardany & Anjarwati, 2020).

Manures at 30 t/ha produced the highest productivity per ha and marketable yield (Table 10), with productivity increasing by 36.34% to 71.17% and marketable yield increasing by 47.13% to 78.16%. Increasing the organic fertilizer dose significantly enhanced lettuce yield, as lettuce responds well to organic fertilization (Altuntaş et al., 2022). However, application times had no significant effect on either productivity or marketable yield. There was no significant interaction between dose and time in affecting lettuce productivity or marketable yield.

Quality

Flavonoid and nitrate contents were lower in control plants than in chicken manure-treated plants (Table 11), but were not significantly affected by manure dose, application timing, or their interaction. This may be related to rainy-season conditions with low light intensity, which can limit flavonoid accumulation (Cho et al., 2023). In addition, increased soil pH following chicken manure application may reduce flavonoid concentrations (Alomari et al., 2024; Huang et al., 2019).

Chicken manure doses did not affect chlorophyll content or leaf greenness (Table 11), likely because additional N beyond optimal levels does not further increase chlorophyll (Shi et al., 2024). Moreover, higher temperatures at harvest and postharvest may have enhanced respiration and chlorophyll degradation, reducing photoassimilate accumulation (Silva et al., 2017).

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Table 8

Fresh and Dry Weight of Lettuce Roots and Leaves with Various Doses and Application Times of Chicken Manure

Treatments	Root weight at harvest (g)		Leaf weight at harvest (g)	
	Fresh weight	Dry weight	Fresh weight	Dry weight
Control	2.64 x	0.91 x	14.39 x	2.11 x
Chicken manure dose (t/ha)				
10	6.93 b y	1.81 b y	59.90 b y	7.06 b y
20	13.70 a y	3.17 a y	173.12 a y	20.64 a y
30	17.04 a y	3.62 a y	207.54 a y	24.61 a y
F test	**	*	**	**
Application times (weeks before transplanting)				
1	10.94	2.63	120.53	14.37
2	10.11	2.15	134.73	15.09
3	16.63	3.83	185.25	22.84
F test	ns	ns	ns	ns

Notes: *: significant ($p < 0.05$); **: highly significant ($p < 0.01$); ns: not significant ($p > 0.05$). Values with the same letter in the same column do not differ significantly ($p < 0.05$) based on the LSD test (a, b) and *t* test (x, y).

Table 9

Percentage of Leaf Weight at Harvest Relative to Total Harvest Weight of Lettuce with Various Doses and Application Times of Chicken Manure

Treatments	Percentage of leaf fresh weight (%)	Percentage of leaf dry weight (%)
Control	84.24 x	69.94 x
Chicken manure dose (t/ha)		
10	89.64 b y	79.85 b y
20	92.31 a y	86.22 a y
30	92.55 a y	87.10 a y
F test	**	**
Application times (weeks before transplanting)		
1	90.80	82.81
2	92.53	86.70
3	91.17	83.65
F test	ns	ns

Notes. **: very significant ($p < 0.01$); ns: not significant ($p > 0.05$). Values with the same letter in the same column do not differ significantly ($p < 0.05$) based on the LSD test (a, b) and *t* test (x, y).

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Table 10

Lettuce Productivity and Marketable Yield with Different Chicken Manure Doses and Application Times

Treatments	Productivity (t/ha)	Marketable yield (t/ha)
Control	1.55 ^{x}	0.90 ^{x}
Chicken manure dose (t/ha)		
10	2.44$^{c\ x}$	1.69$^{c\ x}$
20	3.78$^{b\ y}$	2.74$^{b\ y}$
30	5.39$^{a\ y}$	4.10$^{a\ y}$
F test	**	**
Application times (weeks before transplanting)		
1	4.15	3.04
2	3.61	2.68
3	3.86	2.59
F test	ns	ns

Notes. *: significant ($p < 0.05$); **: highly significant ($p < 0.01$); ns: not significant ($p > 0.05$). Values with the same letter in the same column do not differ significantly ($p < 0.05$) based on the LSD test (a, b, c) and *t* test (x, y).

Table 11

Flavonoid, Nitrate, and Chlorophyll Content, and Leaf Greenness of Lettuce with Various Doses and Application Times of Chicken Manure

Treatments	Flavonoid content (mg QE/g)	Nitrate content (ppm)	Chlorophyll content (mg/g)	Leaf greenness
Control	16.66	1,667	5.59	11.93
Chicken manure dose (t/ha)				
10	18.84	1,922	6.58	14.33
20	19.02	1,744	6.96	13.75
30	19.34	2,356	6.84	14.87
F test	ns	ns	ns	ns
Application times (weeks before transplanting)				
1	18.19	2,122.22	6.55	14.18
2	19.26	1,577.78	6.81	13.91
3	19.75	2,322.22	7.03	14.86
F test	ns	ns	ns	ns

Note. *: ns: not significant ($p > 0.05$) at 5% LSD test.

Soil and chicken manure samples were analyzed in advance to characterize their microbiological profiles. The analysis results indicated that the *E. coli* bacteria count in both the soil and chicken manure samples was relatively low. However, several *Salmonella* spp. and *Shigella* spp. were detected in both samples (Table 12).

E. coli and *Shigella* spp. were detected in lettuce samples, whereas *Salmonella* spp. was not detected. Although soil and manure initially showed low *E. coli* counts, contamination

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occurred in nearly all lettuce samples, except at 30 t/ha applied 2 WBT (Table 13). The lowest *E. coli* count was observed at 20 t/ha applied 3 WBT (5.0 × 10^1 CFU/g), while the highest occurred at 10 t/ha applied 1 WBT (8.5 × 10^4 CFU/g). The persistence of *E. coli* on lettuce despite low soil and manure levels is consistent with surface contamination reported by Rodrigues et al. (2014). Environmental conditions such as high rainfall and humidity, low light intensity, and reduced temperatures likely favored *E. coli* survival and spread via surface runoff and water percolation into deeper soil layers and groundwater (Honorine et al., 2013; Sharma et al., 2019).

Salmonella spp. was detected in soil but not in lettuce leaves (Table 13), likely due to bacterial inactivation under rainy-season conditions. High moisture levels can reduce *Salmonella* survival compared with dry conditions (Sharma et al., 2019). In addition, the use of composted chicken manure may have further minimized contamination risk, as composting produces organic amendments free of *Salmonella* (Soobhany, 2018).

Shigella spp. counts were higher in chicken manure than in soil samples (Table 12) and were detected at even higher levels in all lettuce leaves (Table 13). The highest leaf contamination occurred at 20 t/ha applied 2 WBT (6.0 × 10^6 CFU/g), while the lowest was observed at 30 t/ha applied 3 WBT (1.0 × 10^6 CFU/g). Larger, denser leaves produced at higher manure doses may limit bacterial penetration into inner leaf tissues, thereby indirectly reducing contamination risk.

Table 12

Soil and Chicken Manure Microbiological Analysis

Samples analyzed	Parameter (CFU/g)		
	Escherichia coli	*Salmonella* spp.	*Shigella* spp.
Soil	<10	5.7 x 10^3	3.4 x 10^4
Chicken manure	<10	5.7 x 10^3	8.1 x 10^4

Note. Laboratory test results from IPB Culture Collection.

Table 13

Lettuce Microbiological Analysis

Treatments	Parameter (CFU/g)		
	Escherichia coli	*Salmonella* spp.	*Shigella* spp.
Control (0 t/ha)	1.0 x10^4	<10*	3.3 x10^6
Chicken manure (t/ha)			
10 t/ha, 1 WBT	8.5 x1^{0}4	<10*	3.0 x10^6
10 t/ha, 2 WBT	1.4 x10^3	<10*	3.2 x10^6
10 t/ha, 3 WBT	7.3 x10^4	<10*	3.5 x10^6
20 t/ha, 1 WBT	2.0 x10^2	<10*	3.4 x10^6
20 t/ha, 2 WBT	9.0 x 10^2	<10*	6.0 x10^6
20 t/ha, 3 WBT	5.0 x 10^1	<10*	3.5 x10^6
30 t/ha, 1 WBT	3.3 x10^3	<10*	1.4 x10^6
30 t/ha, 2 WBT	<10*	<10*	1.6 x10^6
30 t/ha, 3 WBT	1.5 x10^3	<10*	1.0 x1^{0}6

Notes. Data were not statistically analyzed. WBT: weeks before transplanting.
*: no bacterial growth detected.

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Bacterial transmission occurs mainly through surface attachment and root uptake (Burris et al., 2020), and lettuce with loose, folded leaves is particularly susceptible to contamination via splashed soil particles (Amedor et al., 2015; Llera et al., 2022). The persistence of *Shigella* spp. is further supported by its high environmental adaptability (Henriquez et al., 2024).

Generally, *E. coli, Salmonella* spp., and *Shigella* spp. are primarily found on plant surfaces, such as leaves and fruits, and are not pathogenic to plants. Some strains of *E. coli* and *Salmonella* can occasionally internalize into plant tissues through stomata, wounds, or roots, although such internalization is rare, transient, and does not cause systemic infection (Jacob et al., 2024; Mina & Deering, 2025). *Shigella* spp. could also be found in leaf and damaged cells in both leaves and roots (Jo et al., 2019).

According to SNI 7388:2009, which sets the Maximum Limits for Microbial Contamination in Food, the acceptable limit for *Salmonella* spp. in fresh vegetables is negative per 25 g. Since *Salmonella* spp. was not found in any of the lettuce leaf samples, it can be concluded that the tested lettuce complies with the SNI standard for *Salmonella* contamination. However, *E. coli* and *Shigella* spp. contamination were present in almost all lettuce samples. The acceptable *E. coli* contamination limit for fresh vegetables is <3 MPN/g (equivalent to 6.3 CFU/g), meaning that only the sample treated with 30 t/ha of chicken manure and applied 2 WBT met the standard. However, this sample cannot be considered pathogen-free as it was still contaminated with *Shigella* spp., while the standard for *Shigella* spp. contamination in fresh vegetables is absent (negative), as it's a zero-tolerance pathogen due to its low infectious dose.

Conclusions

The results of this study demonstrate that the dose of chicken manure significantly affected all growth and production variables but had no significant effect on the quality variables. The application time of chicken manure did not influence any of the observed parameters. The interaction between dose and application time did not affect any variables, except the number of leaves at 20 days after transplanting (DAT). No *Salmonella* spp. colonies were found in any of the lettuce samples, but *E. coli* and *Shigella* spp. colonies were present in amounts exceeding the standards set by SNI. It is recommended that treated water be used for irrigation to reduce the risk of waterborne contamination, thereby providing a more accurate assessment of contamination risks from chicken manure. Future research should consider conducting studies in both the rainy and dry seasons to examine potential differences in the species and numbers of pathogenic bacteria in organic lettuce fields. A two-week interval between chicken manure application and transplanting is recommended to minimize the risk of *E. coli* contamination while maintaining soil fertility.

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