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RESEARCH ARTICLE
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Effects of Manure Type, Fermentation Duration and Bacterial Inoculation on Macronutrient Composition of Fermented Livestock Manure

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ABSTRACT

Livestock manure represents a valuable resource for organic fertilizer production; however, its nutrient availability is often limited without appropriate treatment. This study evaluated the effects of manure type, fermentation duration, and bacterial inoculation on nitrogen (N), phosphorus (P), and potassium (K) contents of fermented manure using a factorial experimental design. Chicken, cattle, and goat manure were fermented for 15 and 30 days with multiple bacterial isolates and an uninoculated control. Nutrient contents were analyzed using three-way analysis of variance to assess main and interaction effects among factors. Bacterial inoculation significantly enhanced N, P, and K contents compared with the control ($P<0.05$), although the magnitude of enhancement varied among bacterial isolates. Manure type strongly influenced fermentation outcomes, with chicken manure exhibiting the highest nutrient enrichment, followed by goat manure, while cattle manure showed comparatively lower responses. Extended fermentation generally increased nutrient availability, particularly in inoculated treatments. Significant interaction effects indicated that bacterial performance was dependent on manure substrate and fermentation duration. Heatmap visualization further highlighted isolate-specific nutrient enhancement patterns across macronutrients. Overall, the results demonstrate that nutrient enrichment during manure fermentation is governed by the combined influence of substrate type, fermentation time, and microbial inoculation. These findings provide practical guidance for optimizing manure fermentation strategies and selecting effective bacterial inoculants to improve the quality and consistency of manure-based organic fertilizers.

Keywords: Manure fermentation, Bacterial inoculation, Nutrient dynamics, Organic fertilizer, Livestock manure.

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INTRODUCTION

The increasing demand for sustainable agricultural practices has intensified interest in organic fertilizers derived from livestock manure. Manure-based fertilizers supply essential macronutrients such as nitrogen (N), phosphorus (P) and potassium (K), while also contributing to soil organic matter and microbial activity (Yan et al., 2023). Despite these benefits, the direct application of raw manure is often constrained by low nutrient availability, nutrient losses, odor emissions, and potential environmental risks, including nutrient leaching and greenhouse gas emissions (Sommer et al., 2021). Fermentation has been widely adopted as an effective approach to improve the agronomic value of manure-based fertilizers. Microbial-driven fermentation processes promote organic matter decomposition and nutrient solubilization, leading to enhanced availability of N, P and K while improving product stability (Prado et al., 2025). Several studies have reported that fermentation duration

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strongly influences nutrient dynamics, with extended fermentation generally associated with greater nutrient release; however, reported outcomes vary considerably across studies (Zhu et al., 2024).

Manure type is a key factor contributing to this variability. Differences in initial chemical composition, organic matter quality, and carbon-to-nitrogen ratios among poultry, cattle, and goat manure result in distinct nutrient transformation patterns during fermentation (Sun et al., 2024). Poultry manure is commonly characterized by relatively high nutrient concentrations, whereas cattle manure often exhibits lower nutrient density and slower mineralization rates. Goat manure typically displays intermediate characteristics, although comparative assessments conducted under identical fermentation conditions remain limited (Muflihayati et al., 2025).

Microbial inoculation has emerged as a promising strategy to further enhance fermentation efficiency and nutrient release. Specific bacterial isolates have been shown to accelerate organic matter degradation and increase nutrient mineralization, thereby improving fertilizer quality (Tian et al., 2023). Nevertheless, many previous studies have evaluated single microbial strains or have examined inoculation effects without accounting for differences in manure substrate or fermentation duration. Consequently, the extent to which bacterial performance is influenced by interactions with substrate type and fermentation time remains insufficiently understood. Although the individual effects of manure type, fermentation duration, and microbial inoculation have been widely investigated, studies integrating these factors within a factorial framework are still scarce (Diepersloot et al., 2021). A comprehensive evaluation of their combined and interactive effects is essential to better understand nutrient dynamics during manure fermentation and to identify bacterial inoculants that perform consistently or exhibit substrate-specific advantages.

Therefore, this study investigated the combined effects of manure type, fermentation duration, and bacterial inoculation on the macronutrient composition of fermented manure using a factorial experimental design. By examining nutrient responses across different livestock manures and fermentation periods and by evaluating the performance of multiple bacterial isolates relative to an uninoculated control, this work provides insight into substrate-dependent and time-dependent nutrient enhancement patterns. The results are expected to support the optimization of manure fermentation strategies and contribute to the development of more efficient and sustainable organic fertilizers.

MATERIALS & METHODS

Experimental Design and Treatments

The experiment was conducted at the Laboratory of Feed and Waste Valorization, Faculty of Animal Science, Universitas Hasanuddin, located in Makassar, South Sulawesi Province, Indonesia. The laboratory is geographically situated at approximately 5°08′52″ S latitude and 119°25′58″ E longitude. This study investigated the influence of microbial inoculation on nutrient dynamics during manure fermentation using a factorial arrangement with three sources of variation. The substrates represented three livestock manure types (chicken, cattle, and goat manure), which were fermented for two incubation periods (15 and 30 days) and subjected to fourteen microbial treatments consisting of an uninoculated control and thirteen rumen-derived bacterial isolates coded A–M. The experimental units were arranged in a completely randomized manner, and each treatment combination was conducted three independent experimental replications, with each replicate prepared and fermented separately to ensure experimental independence. The primary response variables were macronutrient contents, namely nitrogen (N), phosphorus (P), and potassium (K), determined from the fermented products at the end of each incubation period.

Preparation and Standardization of Bacterial Inoculum

Bacterial isolates (A–M) originating from rumen fluid were maintained on general-purpose growth medium and revived prior to fermentation trials. The thirteen isolates were selected from a larger collection of rumen-derived bacteria based on their distinct colony morphology and growth performance during preliminary screening, in order to represent diverse microbial functional potentials involved in organic matter degradation. For inoculum preparation, each isolate was cultured separately in liquid nutrient medium under aerobic conditions and incubated at 30 ± 2°C for 24h to obtain actively growing cells. Culture density was standardized to an approximate cell concentration equivalent to 10^8 CFU mL^{-1} based on turbidity adjustment (optical density approach) to minimize variation in inoculum load among treatments. Inocula were prepared fresh and used immediately to ensure high viability and consistent metabolic activity at the start of fermentation (Subramaniam et al., 2018). The isolates used in this study had not been molecularly identified but were phenotypically characterized based on growth behavior under laboratory conditions.

Fermentation Procedure and Process Control

Fermentation was conducted under aerobic conditions in laboratory-scale containers designed to permit gas exchange. Prior to inoculation, each manure substrate was homogenized to reduce within-sample heterogeneity and adjusted to a target moisture content of approximately 60%, which is commonly considered suitable for sustaining microbial activity during aerobic fermentation of organic residues. For inoculated treatments, standardized bacterial cultures were applied at a fixed rate (0.3% v/w relative to substrate mass) and mixed thoroughly to achieve uniform distribution throughout the substrate matrix; the control treatment underwent the same handling and mixing steps without addition of bacterial inoculum (Lee et al., 2020).

All experimental units were incubated at ambient laboratory temperature (approximately 28–32 °C). To maintain aerobic conditions and promote consistent decomposition, the substrates were manually turned at regular intervals (every five days). Throughout fermentation, qualitative process indicators (odor, color, and texture) were monitored to track progression and detect potential process deviations such as anaerobic zones or abnormal microbial growth. Fermentation was terminated at 15 days and 30 days according to the assigned treatment duration, representing an early and a more advanced stage of aerobic manure fermentation, respectively, as commonly applied in laboratory-scale studies to capture short-term and extended microbial effects on nutrient dynamics (Du et al., 2023).

Sample Preparation

At the end of each fermentation period, fermented material from each replicate container was sampled using a representative approach by collecting subsamples from multiple points within the container (e.g., upper, middle, and lower layers) and combining them into a composite sample for that replicate. Composite samples were homogenized to ensure representativeness and then air-dried to constant weight to minimize moisture-related analytical variation. Dried samples were ground using a laboratory grinder and passed through a 2-mm sieve to obtain uniform particle size, thereby improving digestion efficiency and analytical precision. Prepared samples were stored in clean, sealed containers at room temperature and analyzed within an appropriate timeframe to avoid changes due to post-processing moisture uptake (Lyu et al., 2022).

Determination of Nitrogen, Phosphorus and Potassium

Macronutrient contents were quantified using standardized wet-chemistry procedures widely applied in compost and organic fertilizer characterization. Total nitrogen (N) was determined using the Kjeldahl method, which includes digestion of organic nitrogen to ammonium followed by distillation and titration/ quantification. Total phosphorus (P) was measured after wet digestion and quantified using a molybdenum blue spectrophotometric method, where absorbance was read at the appropriate wavelength and calculated against calibration standards. Potassium (K) was determined following acid digestion and quantified using a flame photometer. For quality assurance, reagent blanks and calibration standards were included in each analytical batch, and measurements were performed consistently across all samples. Results were expressed on a dry-weight basis in mg kg^{-1} (Garcia et al., 2018; Wang et al., 2020).

Statistical Analysis

Each replicate container was treated as an independent experimental unit in all statistical analyses. Prior to statistical analysis, the dataset was screened for completeness, transcription errors, and consistency of numeric formats, including standardization of decimal separators where applicable. For each response variable (N, P, and K), effects of manure type, fermentation duration, microbial treatment, and their interactions were evaluated using a three-way analysis of variance (ANOVA). Model assumptions were assessed using residual diagnostics for normality and homogeneity of variance; where needed, appropriate data transformations were applied to meet ANOVA assumptions. When significant effects were detected ($P<0.05$), pairwise comparisons among levels were performed using Tukey's Honestly Significant Difference (HSD) test. Given the non-normal distribution and high variability observed in the pooled dataset, descriptive summaries were reported as median (interquartile range, IQR) for overview tables, while treatment-level comparisons were presented as means with appropriate measures of variability in tables and Fig.s used for inferential comparisons.

Ethical Approval

The experimental protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Andalas University (Approval Number: 0376/UN.16.2/KEP-FK/2025) and was conducted in accordance with national guidelines for the care and use of experimental animals. Efforts were made to minimize animal discomfort and to ensure adherence to ethical standards throughout the study.

RESULTS

Overview of Fermentation Effects on Nutrient Composition

The overall distribution of nitrogen (N), phosphorus (P) and potassium (K) contents across major treatment groupings is summarized in Table 1. Due to high variability and non-normal data distribution, results are presented as median and interquartile range (IQR). Overall, the uninoculated control exhibited the lowest nutrient levels across all macronutrients, whereas pooled inoculated treatments (A–M) showed higher median values of N, P, and K, indicating a general enhancement of nutrient content following bacterial inoculation. Fermentation duration and manure type further influenced nutrient distributions. Samples fermented for 30 days generally exhibited higher median nutrient contents than those fermented for 15 days, although overlapping IQRs suggested treatment-dependent responses. Among manure types, chicken manure consistently showed the

Table 1: Overall summary of nitrogen (N), phosphorus (P), and potassium (K) contents (mg kg^{-1} dry weight) across treatment groupings. Values are presented as median and interquartile range (IQR)

Grouping	n	Nitrogen (median (IQR))	Phosphorus (median (IQR))	Potassium (median (IQR))
Control (no inoculum)	18	2.0 (1.0–5.0)	2.0 (1.0–7.0)	4.0 (3.0–20.0)
Inoculated (A–M, pooled)	234	35.0 (18.0–61.0)	49.0 (26.0–86.0)	118.0 (60.0–190.0)
Fermentation 15 days	126	29.0 (13.0–50.0)	41.0 (19.0–70.0)	98.0 (44.0–160.0)
Fermentation 30 days	126	38.0 (17.0–60.0)	52.0 (22.0–90.0)	125.0 (58.0–193.0)
Chicken manure	84	45.0 (30.0–68.0)	60.0 (42.0–98.0)	138.0 (108.0–220.0)
Cattle manure	84	6.0 (3.0–9.0)	8.0 (5.0–12.0)	20.0 (14.0–29.0)
Goat manure	84	23.0 (11.0–38.0)	32.0 (16.0–57.0)	76.0 (37.0–129.0)

highest concentrations of N, P, and K, followed by goat manure, while cattle manure exhibited the lowest values. These overall patterns provide a contextual framework for the factor-specific analyses presented in the subsequent sections.

Effect of Manure Type on N, P and K Contents

The effect of manure type on nitrogen (N), phosphorus (P), and potassium (K) contents of fermented manure is presented in Fig. 1A–C. Analysis of variance showed that manure type had a significant effect on the concentrations of all three macronutrients ($P<0.05$). Across treatments, fermented chicken manure consistently exhibited the highest nutrient contents, whereas cattle manure showed the lowest values, with goat manure displaying intermediate levels.

Nitrogen content differed significantly among manure types ($P<0.05$; Fig. 1A). Chicken manure produced significantly higher N concentrations than both goat and cattle manure. Goat manure also differed significantly from cattle manure, which consistently exhibited the lowest nitrogen content. Phosphorus content was also significantly affected by manure type ($P<0.05$; Fig. 1B). Fermented chicken manure resulted in significantly higher P concentrations compared with goat and cattle manure. Goat manure showed intermediate phosphorus levels and differed significantly from cattle manure. Similarly, potassium content varied significantly among manure types ($P<0.05$; Fig. 1C). Chicken manure showed the highest K concentrations following fermentation, which were significantly greater than those observed in goat and cattle manure. Goat manure also exhibited significantly higher K content than cattle manure.

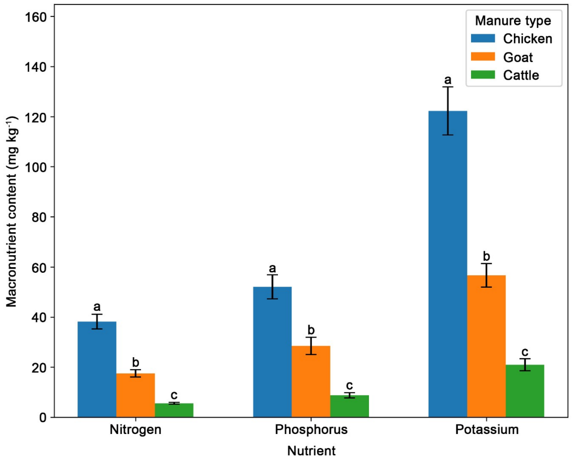


Fig. 1: Effect of manure type on macronutrient contents of fermented manure (mg kg^{-1} dry weight). Bars represent mean values pooled across fermentation durations and microbial treatments. Different superscript letters indicate significant differences among manure types based on Tukey's HSD test following three-way ANOVA ($P<0.05$).

Effect of Fermentation Duration on Nutrient Dynamics

The effect of fermentation duration on nitrogen (N), phosphorus (P), and potassium (K) contents of fermented manure is presented in Fig. 2A–C. Statistical analysis showed that fermentation duration significantly affected all three macronutrients ($P<0.05$). Overall, extending the fermentation period from 15 to 30 days resulted in higher nutrient contents across treatments. Manure fermented for 30 days consistently exhibited higher concentrations of N, P, and K than manure fermented for 15 days. This pattern was observed across manure types and microbial treatments, although the magnitude of increase varied among treatments, indicating that prolonged fermentation generally enhanced macronutrient availability.

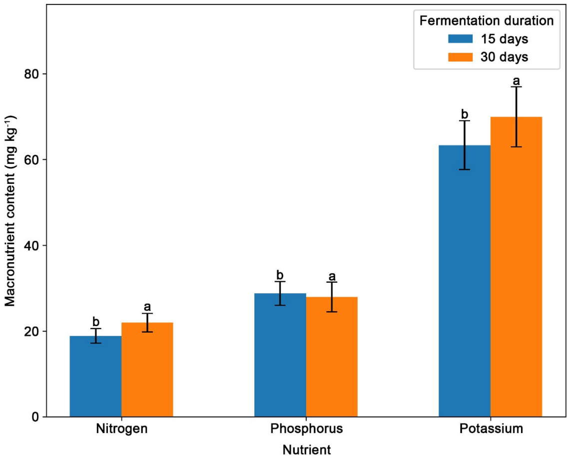


Fig. 2: Effect of fermentation duration on macronutrient contents of fermented manure (mg kg^{-1} dry weight). Bars represent mean values pooled across manure types and microbial treatments. Different superscript letters indicate significant differences between fermentation durations based on Tukey's HSD test following three-way ANOVA ($P<0.05$).

Effect of Bacterial Inoculation on Nutrient Enhancement

The effect of bacterial inoculation on nitrogen (N), phosphorus (P), and potassium (K) contents of fermented manure is presented in Fig. 3A–C. Analysis of variance indicated that bacterial treatment had a significant effect on all measured macronutrients ($P<0.05$). The uninoculated control consistently exhibited the lowest nutrient contents, whereas all inoculated treatments resulted in higher N, P, and K concentrations to varying degrees. Nitrogen content differed significantly among treatments ($P<0.05$; Fig. 3A). Several bacterial isolates produced substantially higher N concentrations than the control, indicating effective enhancement of nitrogen availability during fermentation. Differences among isolates were evident, with some isolates forming a high-performing group, while others showed moderate increases relative to the control. Phosphorus content was also significantly influenced by bacterial inoculation ($P<0.05$; Fig. 3B). Inoculated treatments generally exhibited significantly higher P concentrations than the control. However, the magnitude of phosphorus enhancement varied among isolates, suggesting isolate-specific differences in phosphorus mobilization during the fermentation process. Similarly, potassium content varied significantly among bacterial treatments ($P<0.05$; Fig. 3C). Several isolates resulted in pronounced increases in K concentration compared with the uninoculated control, while other isolates produced intermediate responses. The consistently lower K content observed in the control highlights the contribution of bacterial inoculation to potassium availability. A heatmap summarizing the relative performance of bacterial treatments, including the uninoculated control and isolates A–M, is shown in Fig. 4. The heatmap provides a comparative visualization of N, P, and K contents pooled across manure types and fermentation durations. The uninoculated control formed a distinct low-intensity cluster across all three nutrients, reflecting its consistently low nutrient values. In contrast, several bacterial isolates exhibited high color intensity across multiple nutrients, indicating superior overall performance. Other isolates showed moderate intensity patterns, suggesting selective enhancement of one or two macronutrients.

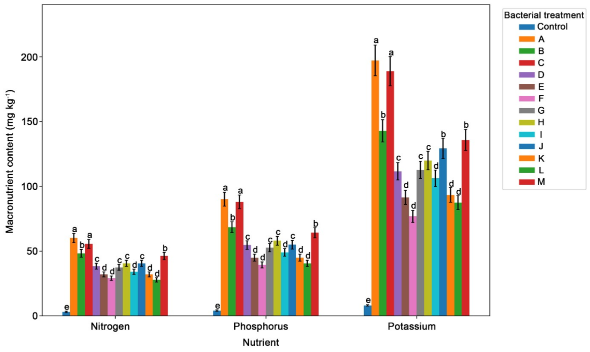


Fig. 3: Effect of bacterial inoculation on macronutrient contents of fermented manure (mg kg^{-1} dry weight). Bars represent mean values pooled across manure types and fermentation durations. Different superscript letters indicate significant differences among bacterial treatments based on Tukey's HSD test following three-way ANOVA ($P<0.05$).

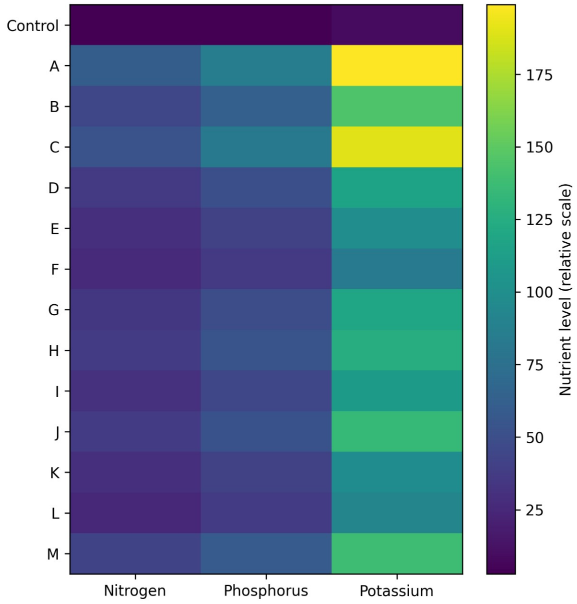


Fig. 4: Heatmap visualization of nitrogen (N), phosphorus (P), and potassium (K) contents (mg kg^{-1} dry weight) of fermented manure as affected by bacterial treatments, including the uninoculated control and bacterial isolates (A–M). Color intensity represents relative nutrient levels pooled across manure types and fermentation durations.

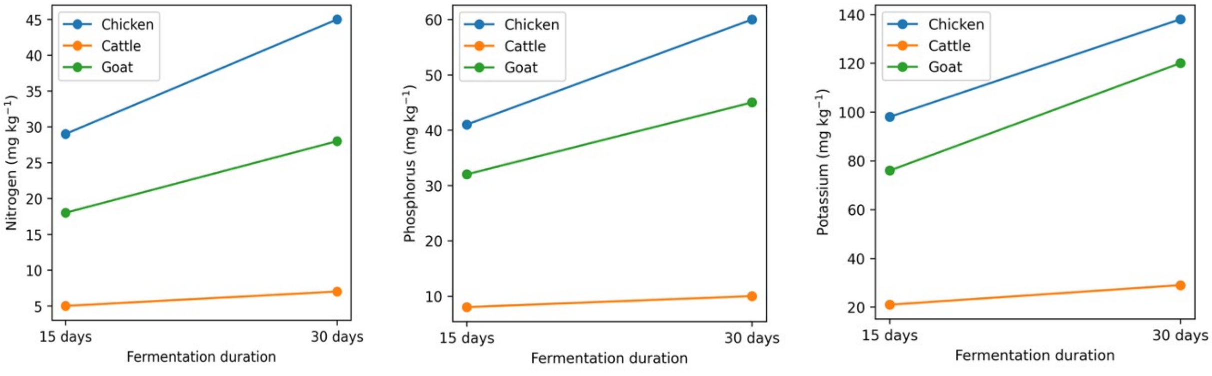
Interaction Effects among Manure Type, Fermentation Duration and Bacterial Treatment

The interaction effects between manure type and fermentation duration on nitrogen (N), phosphorus (P), and potassium (K) contents are shown in Fig. 5A–C. Three-way analysis of variance indicated a significant interaction between manure type and fermentation duration for all macronutrients ($P<0.05$), demonstrating that nutrient responses to extended fermentation differed among manure substrates. Across all nutrients, increasing fermentation duration from 15 to 30 days resulted in higher N, P, and K contents in chicken, goat, and cattle manure; however, the magnitude of increase varied by manure type. Chicken manure consistently showed the strongest positive response to prolonged fermentation, goat manure exhibited an intermediate response, and cattle manure displayed the smallest increase. These patterns indicate that although extended fermentation generally enhanced macronutrient availability, the extent of enhancement was strongly substrate-dependent.

DISCUSSION

The results of this study demonstrate that manure fermentation outcomes are governed by the combined effects of manure type, fermentation duration, and bacterial inoculation rather than by any single factor alone. Across all analyses, bacterial inoculation consistently enhanced nitrogen (N), phosphorus (P), and potassium (K) contents compared with the uninoculated control, confirming the central role of microbial activity in nutrient transformation during manure fermentation. These findings align with previous studies reporting improved nutrient availability following microbial-assisted fermentation of organic waste materials (Lei et al., 2019; Zhao et al., 2024). Similar enhancements in macronutrient availability following microbial-assisted manure fermentation have been reported in poultry, cattle, and mixed livestock wastes under both aerobic and semi-aerobic systems (Sowmeya & Sathiavelu, 2024; Ilyina et al., 2022; Dey et al., 2025). Recent studies further emphasize that microbial inoculation not only accelerates organic matter decomposition but also promotes nutrient solubilization through enzymatic mineralization pathways (Li et al., 2021; Mustafa et al., 2025).

Differences among manure types were pronounced and consistent across nutrient parameters. Chicken manure exhibited the highest nutrient contents, followed by goat manure, while cattle manure generally showed the lowest values. This pattern reflects inherent differences in initial substrate composition, including organic matter quality, nutrient density, and carbon-to-nitrogen ratios (Muflihayati et al., 2025). Poultry manure is known to contain higher readily mineralizable nitrogen and phosphorus fractions, which can be rapidly transformed during fermentation, whereas cattle manure often contains more recalcitrant organic compounds that slow nutrient release (Kacprzak et al., 2023). Goat manure displayed intermediate behavior, suggesting a balance between nutrient availability and organic matter stability.



(A) (B) (C)

Fig. 5: Interaction effects of manure type and fermentation duration on macronutrient contents of fermented manure: (A) nitrogen, (B) phosphorus, and (C) potassium (mg kg^{-1} dry weight). Lines represent mean values pooled across microbial treatments and are intended to illustrate interaction trends. Significant interaction effects were identified using three-way ANOVA ($P<0.05$).

The superior performance of chicken manure observed in this study can be attributed to several biochemical and structural characteristics. Poultry manure typically contains higher proportions of uric acid, readily hydrolysable proteins, and inorganic phosphorus fractions, which are more rapidly mineralized by microbial enzymes during fermentation (Chang et al., 2022; He et al., 2024). In contrast to ruminant manure, poultry manure lacks extensive lignocellulosic bedding material, resulting in lower structural complexity and greater accessibility of nutrients to microbial communities. Additionally, the relatively low carbon-to-nitrogen ratio of chicken manure creates favorable conditions for microbial growth and enzymatic activity, thereby accelerating nutrient transformation processes during fermentation (Cai et al., 2024). Fermentation duration also played a significant role in shaping nutrient dynamics. Extended fermentation generally resulted in higher nutrient contents, indicating progressive mineralization and solubilization of organic-bound nutrients over time. This trend is consistent with earlier reports showing that longer fermentation periods allow microbial communities to more fully decompose complex organic substrates, thereby increasing nutrient availability (Salangsang et al., 2022). This pattern is consistent with findings from recent aerobic and semi-aerobic manure fermentation studies, which reported increased nitrogen and phosphorus availability with longer incubation periods due to sustained microbial degradation of complex organic substrates (Xiao et al., 2022; Ma et al., 2025). Similar trends were also observed in poultry manure fermentation systems, where extended processing time enhanced enzymatic activity and nutrient solubilization efficiency (Mohamed et al., 2022; Pagliarini et al., 2024).

However, the magnitude of nutrient enhancement observed in the present study varied among manure types and was notably greater for chicken manure than for cattle manure. This contrasts with reports from anaerobic cattle manure fermentation systems, where prolonged fermentation did not consistently increase phosphorus or potassium availability, likely due to substrate recalcitrance and slower organic matter turnover (Baba et al., 2022; Mindari et al., 2024). These differences suggest that fermentation outcomes are strongly influenced by both substrate composition and process configuration, particularly oxygen availability and microbial community structure.

The pronounced effects of bacterial inoculation observed in this study underscore the importance of microbial selection in optimizing fermentation performance. Although all inoculated treatments outperformed the control, clear differences were evident among bacterial isolates, as illustrated by both statistical comparisons and heatmap visualization. These isolate-specific responses suggest functional diversity among the bacterial treatments, potentially related to differences in enzymatic capabilities, nutrient solubilization pathways, or adaptability to specific manure substrates (Hatamzadeh et al., 2025). At the biological level, such isolate-specific variability is likely driven by differences in metabolic pathways and extracellular enzyme production among rumen-derived bacteria. Individual isolates may vary in their ability to produce key enzymes such as proteases, phosphatases, and carbohydrate-degrading enzymes, which directly regulate nitrogen mineralization, phosphorus solubilization, and potassium release during organic matter decomposition (Butkovich et al., 2025; Eom et al., 2025). In addition, differences in growth kinetics, substrate affinity, and tolerance to fluctuating oxygen availability and moisture conditions can influence colonization efficiency and persistence within the manure matrix. Isolates capable of rapid establishment and sustained metabolic activity are therefore more likely to exert pronounced effects on nutrient transformation, whereas less adaptable strains may show limited or inconsistent performance depending on substrate characteristics and fermentation duration. Similar isolate-dependent responses have been reported in previous studies evaluating microbial consortia and single-strain inoculants in organic waste fermentation systems (Dahdah et al., 2022).

The interaction analyses further revealed that bacterial performance was not uniform across manure types or fermentation durations. Certain isolates exhibited strong nutrient enhancement in chicken and goat manure but showed limited effects in cattle manure, particularly at shorter fermentation durations. These findings indicate that microbial effectiveness is context-dependent and influenced by substrate characteristics and temporal dynamics. Similar interaction effects have been reported in composting and anaerobic fermentation studies, where microbial activity was modulated by substrate composition and process duration (Fisgativa et al., 2018). This highlights the importance of matching bacterial inoculants to specific manure types and fermentation conditions rather than assuming universal effectiveness. The heatmap analysis provided an integrative overview of isolate performance across multiple nutrients, complementing the quantitative results obtained from ANOVA and pairwise comparisons. By simultaneously visualizing N, P, and K responses, the heatmap revealed distinct performance clusters among bacterial treatments and clearly differentiated inoculated treatments from the uninoculated control. Such multivariate visualization approaches have been increasingly recommended for complex biological datasets to facilitate interpretation of treatment patterns and functional diversity (Wu et al., 2018).

Despite the strengths of this study, several limitations should be acknowledged. The fermentation experiments were conducted under controlled laboratory conditions, which may not fully capture environmental variability encountered under field-scale or on-farm applications. In addition, nutrient assessments were limited to macronutrients (N, P, and K), and did not include micronutrients, organic carbon fractions, or microbial community dynamics, which could provide deeper insight into fermentation mechanisms. Furthermore, although multiple bacterial isolates were evaluated, the functional traits and taxonomic identities of these isolates were not explored in detail. Future studies integrating molecular characterization of microbial communities and field validation trials would strengthen the applicability of the findings. Nevertheless, the results of this study have important practical implications. The clear differentiation among manure types, fermentation durations, and bacterial treatments provides a basis for tailoring fermentation strategies to specific substrates. The identification of high-performing bacterial isolates and the demonstration of substrate-dependent responses offer valuable guidance for the development of more efficient, targeted, and sustainable manure-based organic fertilizers. By optimizing microbial inoculation and fermentation conditions, this approach has the potential to enhance nutrient recovery from livestock waste and contribute to environmentally sound nutrient management practices in agriculture.

Conclusion

This study demonstrates that the macronutrient composition of fermented manure is strongly influenced by the combined effects of manure type, fermentation duration, and bacterial inoculation. Overall, microbial inoculation substantially enhanced nitrogen, phosphorus, and potassium contents compared with the uninoculated control, with inoculated treatments exhibiting markedly higher nutrient levels, while extending fermentation from 15 to 30 days consistently increased nutrient availability. Chicken manure showed the greatest nutrient enrichment, followed by goat manure, whereas cattle manure exhibited more limited responses. Clear differences among bacterial isolates and significant interaction effects indicate that microbial performance is context-dependent and influenced by both substrate characteristics and fermentation time. Collectively, these findings highlight the importance of selecting appropriate bacterial inoculants and optimizing fermentation duration, and suggest that this approach can be practically applied in farm-scale manure management systems to improve nutrient recovery and support more sustainable agricultural nutrient management.

DECLARATIONS

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Conflict of Interest: The authors declare no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript

Data Availability: Data presented in this study will be available on a fair request to the corresponding author

Ethics Statement: The experimental protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Andalas, Indonesia (Approval No. 376/UN.16.2/KEP-FK/2025).

Author's Contribution: JM contributed to the conceptualization of the study, the implementation of the experiments, the data collection, the analysis of the research results, and the writing of the initial draft of the manuscript. II contributed to the design of the research methodology, supervision of the research activities, and the review and refinement of the manuscript. SS contributed to the laboratory analysis of macronutrient composition, data validation, and interpretation of the research results. AA contributed to the processing and statistical analysis of the data, the presentation of the results, and the editing of the manuscript. II contributed to the discussion of the research results, the development of the theoretical framework, and the critical review of the manuscript. LORM contributed to the general conceptualization of the study, oversight of the overall research process, and the final approval of the manuscript for publication.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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