Journal of Smart Farming and Food Security
Universiti Malaysia Sabah Press
e-ISSN: 3122-7090
Volume 2, Issue 1, Pages 1–8 (30 June 2026)
DOI: https://doi.org/10.51200/jsffs.v2i1.6603

Research Article
OPEN ACCESS

Short-term amelioration of acidic subsoil using dairy farm effluent compost and humic acid: a laboratory incubation study

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Manuscript received: 7 July 2025 | Accepted: 2 September 2025 | Published online: 2 March 2026

Abstract: Acidic subsoils pose a significant challenge to sustainable agriculture production due to poor nutrient availability and limited productivity, particularly in regions like Malaysia with tropical climates. This incubation study explored the potential of dairy farm effluent compost (DFEC) and humic acid (HA) as organic amendments to ameliorate acidic subsoil, focusing on improving soil chemical properties while reducing fertilizer use. The experiment evaluated five treatments with varying combinations of DFEC, HA, and reduced fertilizer rates (50% and 75%) under controlled laboratory conditions. Soil samples were analysed for pH, organic matter (OM), macronutrient (N, P, K, Ca, Mg) and other selected elements (Al, Fe, Na, Cu, and Zn) concentrations across a 90-day period. The results revealed that while soil pH showed insignificant changes, treatments with DFEC and HA significantly enhanced soil OM and macronutrient levels, particularly N, P, K, and Ca. Treatment 4 (DFEC + HA with 50% fertilizer reduction) was identified as the better combination, demonstrating the best improvements in subsoil nutrient content. Sodium (Na) levels initially increased in DFEC-treated soils but declined over time, possibly driven by decomposition and adsorption processes. Micronutrient dynamics varied, with Al and Fe exhibiting fluctuating trends influenced by soil pH and redox reactions. Trace metals such as Cu and Zn were minimally affected, with Cu concentrations declined possibly due to immobilization processes. In general, study suggest possible long-term benefits of DFEC and HA in promoting nutrient retention, and organic matter enrichment. It provides insights into soil amendment strategies for subsoil rejuvenation, contributing to sustainable agricultural practices in tropical regions. Further research, including pot and field trials, is needed to evaluate the long-term effects and mechanisms in the presence of crops.

Keywords: organic amendments, soil chemical properties, subsoil rejuvenation, sustainable agriculture

1. Introduction

Soil erosion is a pressing global issue that threatens food security, environmental sustainability, and economic stability. Soil erosion depletes fertile topsoil, disrupts nutrient cycles, and causing land degradation, leaving subsoil exposed (Kumar et al., 2024). In regions prevalent in tropical and subtropical climates with acidic subsoil such as Malaysia, the combined effects of erosion and subsoil acidity reduce nutrient availability and disrupt sustainable agricultural practices (Faridah et al., 2022). Acidic subsoils, characterized by low pH and toxic levels of aluminum,

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Citation: Juilih, A. A. Hasbullah, N. A., & Phin, C. K. (2026). Short-term amelioration of acidic subsoil using dairy farm effluent compost and humic acid: a laboratory incubation study. Journal of Smart Farming and Food Security, 2(1), 1–8. https://doi.org/10.51200/jsffs.v2i1.6603

pose challenges for farmers worldwide, especially in regions with high rainfall and intensive farming activities (Zhu & Shen, 2024). Restoring acidic subsoils for growing crops is therefore critical to maximizing land use and ensuring long-term agricultural viability. Addressing this issue requires innovative and sustainable approaches to restore soil health while minimizing environmental impacts.

In Malaysia, acidic subsoils are a common problem due to the country's tropical climate (Faridah et al., 2022), which accelerates soil acidification through leaching and weathering processes. This issue is further worsened by the widespread use of chemical fertilizers, which, while essential for boosting yields, often lead to nutrient imbalances and contribute to environmental pollution (Pandian et al., 2024). The agricultural sector in Malaysia plays a vital role in ensuring food security and supporting livelihoods, making the rehabilitation of degraded soils a critical priority. However, sustainable solutions for soil restoration must balance agricultural productivity with environmentally friendly practices to align with global sustainability goals.

Among various soil amendments, dairy farm effluent compost (DFEC) has emerged as a promising solution. This organic by-product of dairy farming is rich in nutrients, organic matter, and microbial activity, making it suitable for improving soil structure and enhancing soil fertility to support plant growth (Maludin et al., 2019). Dairy farm effluent compost not only reduces soil acidity but also enhances the water-holding capacity and promotes beneficial microbial populations (Smith & Collins, 2007). Its application as an organic amendment aligns with the principles of sustainable agriculture by reducing reliance on chemical fertilizers and recycling agricultural waste.

Another valuable amendment for soil restoration is humic acid (HA), a naturally occurring organic compound derived from the decomposition of plant and animal matter (Gupta et al., 2021). Humic acid possesses unique properties that make it an effective soil conditioner (Bhatt & Singh, 2022), including its ability to chelate harmful metals like aluminum, which are prevalent in acidic subsoils. Additionally, HA improves soil aggregation, enhances nutrient retention, and stimulates root development, thereby creating a more favourable environment for crop growth (Ampong et al., 2022). The synergistic effects of HA with other soil amendments further amplify its restorative potential.

By examining the combined application of DFEC and HA, this laboratory incubation study aimed to determine their effects on selected chemical properties of a weathered acidic subsoil under controlled conditions. By reducing fertiliser application rates to 75% and 50%, the research assessed the potential of these organic amendments to enhance soil nutrients retention and organic matter content, addressing the challenges of subsoil degradation. This investigation provides a foundation for developing sustainable soil management strategies, targeting for improving agricultural productivity, and set pathway for subsequent study to validate these findings in field settings.

2. Materials and Methods

The soil used in this study was Ultisol (Silabukan association), collected from Universiti Malaysia Sabah, Faculty of Sustainable Agriculture in Sandakan, Malaysia (5°55'54.6"N, 118°00'13.9"E). Samples were randomly taken from a depth of 0–15 cm, then air-dried and ground using a rotary trommel. The soil was sieved through a 2 mm mesh for incubation and chemical analysis.

The soil pH was measured using the potentiometric method (Peech, 1965) with a soil-to-solution ratio of 1:2.5 (10 g soil to 25 mL of 0.01 M CaCl2). After shaking the mixture at 180 rpm for 15 minutes using a mechanical shaker, the pH of the suspension was determined using a calibrated digital pH meter (Trans Instruments Professional Benchtop pH meter BP3001, 2019).

DOI: 10.51200/jsffs.v2i1.6603

The Loss-on-ignition method (Piccolo, 1996) was used for quantifying soil OM. A 5 g sample, dried at 60˚C for 24 hours, was weighed and placed in a crucible. It was then incinerated in a muffle furnace at 300˚C for 1 hour and further heated to 550˚C for 8 hours. After cooling in a desiccator, the sample was weighed again. The weight loss represented the OM content, while the remaining weight corresponded to the ash content.

Total nitrogen (N) content was measured using a CHN analyzer (LECO Elemental Analysis by Combustion CHN628, 2012). A 0.2 g sieved soil sample was weighed, wrapped in aluminum foil, and placed into the analyzer. The analyzer provided the percentage of total nitrogen in the sample.

The total element concentrations of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), aluminium (Al), ferum (Fe), sodium (Na), copper (Cu), and zinc (Zn) in the soil were determined using the aqua regia digestion method (Gaudino *et al*., 2007). A 0.5 g soil sample was mixed with 40 mL of aqua regia solution and heated until dry. It was then diluted with 2% HNO3 and filtered through a 0.45 µm hydrophilic filter membrane. The sample was brought to a volume of 100 mL, further diluted by a factor of 30, and analyzed using an ICP-OES analyzer (PerkinElmer Optima 5300DV, 2008). The calculation for nutrient concentration was as follows:

$$\text{ICP value} \times (0.5\ \text{g}\ /100\ \text{mL})\ \text{sample} \times \text{dilution factor}$$

2.1 Incubation study

An incubation study was conducted in the Faculty of Sustainable Agriculture (FSA) laboratory using clear polypropylene containers with modified (perforated) lids to ensure proper aeration while preventing contamination. Each container was filled with 300 g of soil, which was moistened to 60% field capacity overnight. The next day, five treatments (T0 to T4) were surface applied with amendment combinations, as outlined in Table 1, marking the start of the experiment (day zero). For each of the treatments, three sets of experimental units were prepared for destructive sampling at 30, 60, and 90 days of incubation. Each set consisted of treatments in triplicate, arranged randomly using a Completely Randomized Design (CRD).
The recommended fertilizer rates in this study were based on maize plant, consists of 120 kg ha^{-1} of N, 60 kg ha^{-1} of P, and 90 kg ha^{-1} of K, corresponding to 260.87 kg ha^{-1} of urea (46% N), 298.95 kg ha^{-1} of triple superphosphate (TSP, 43.64% P), and 177.73 kg ha^{-1} of muriate of potash (MOP, 83.02% K) as per Muhumed et al. (2014). To prevent excessive nutrient supply from amendments, fertilizer rates were reduced to 75% and 50% for determining optimal effectiveness. Dairy farm effluent compost (DFEC) was applied at a 1:4 ratio, as recommended by Maludin et al. (2019), while humic acid (HA) was applied at 25 kg ha^{-1}, based on Daur & Bakhashwain (2013). Treatment details, including scaled fertilizer rates and amendment applications, are provided in Table 1.

Table 1. Rate of fertilizer and applied amendments.

Tr*	Fertilizer (g plant^{-1})			Fertilizer rate (%)	DFEC:Soil	HA (g plant^{-1})
	Urea	TSP	MOP			
T0	4.89	5.61	3.39	100	-	-
T1	3.67	4.21	2.54	75	-	0.47
T2	3.67	4.21	2.54	75	1:4	0.47
T3	2.45	2.81	1.70	50	-	0.47
T4	2.45	2.81	1.70	50	1:4	0.47

Note: *Tr=Treatment.

At the start of the experiment, the initial weight of each container was recorded. Soil moisture was maintained by checking container weights every 5 days and replenishing water

losses with distilled water. All experimental units were incubated at room temperature (26˚C) and collected on 30, 60, and 90 days. After collected, soil samples were mixed, air-dried, ground, and analysed for soil pH, organic matter (OM), and total element concentrations (N, P, K, Ca, Mg, Al, Fe, Na, Cu, and Zn).

2.2 Statistical analysis

All the data collected was subjected to one-way analysis of variance (ANOVA) to determine any significant differences between treatment means for all parameters measured. Tukey's HSD test was used for post-hoc analysis at a significance level of 0.05 ($p \leq 0.05$). Statistical analysis was performed using IBM SPSS version 26.

3. Results and Discussion

The results in Table 2, Table 3, and Table 4 showed that organic amendments had no significant effect on soil pH throughout the experiment period (90 days). However, all treatments with amendments significantly increased soil OM content throughout the incubation period compared to the control (T0). As shown in Table 2, all treatments (T1-T4) had significantly higher OM levels than the control at Day 30, showing their contribution to improving subsoil OM as found by Rendana et al. (2022). This increase positively impacts soil health and fertility by enhancing water retention and nutrient cycling, aligning with a study by Magdoff & Van Es (2021). The increase on Day 30 resulted from the organic material in the amendments, which provided an easily accessible carbon source for soil microorganisms. However, by Day 90 (Table 4), soil OM levels decreased by 33%, possibly due to decomposition by indigenous microbes. Despite this decline, the subsoil treated with DFEC maintained higher OM levels than the control (T0), highlighting DFEC significant role in maintaining OM in the soil as stated by Maludin et al. (2019).

At Day 30 (Table 2), all treatments significantly increased subsoil macronutrient concentrations compared to control (T0), with DFEC-treated groups (T2 and T4) showing the highest levels of N, P, K, and Ca, followed by HA-treated groups (T1 and T3). This suggests that DFEC and HA improve nutrient availability through enhanced retention and release mechanisms (Hafez et al., 2023; Maludin et al., 2019). Canellas et al. (2015) support that synergistic effect between HA and DFEC was able to chelate released nutrients in the soil. Although macronutrient concentrations decreased over time as seen in Table 2, Table 3, and Table 4, the macronutrient pattern observed across treatments on Day 30, 60, and 90 were consistent, demonstrating the sustained impact of amendments.

The Al and Fe concentrations showed fluctuating patterns during the experiment, increasing significantly between Days 30 and 60 (Table 2 and 3) before decreasing at Day 90 (Table 4). The initial rise in Al concentration may be attributed to desorption triggered by soil pH changes or microbial activity, while its subsequent decline suggests immobilization or precipitation due to microbial processes (Zhang et al., 2023). It also may be linked to interactions with the subsoil matrix, where Al adsorbs onto soil particles and its mobility is influenced by pH (Li et al., 2022). The increase in Fe concentration in T3, T4, and T8 could be linked to redox reactions, as Fe solubility depends on oxidation states, potentially enhanced by amendments or microbial activity (Colombo et al., 2013; O'Loughlin et al., 2021). Fe mobility may also have been influenced by complexation with organic ligands, aided by degradable organic matter in certain treatments.

The addition of DFEC elevated Na levels in T2 and T4 although these gradually declined over time as found by Acharya et al. (2019). The elevated Na concentration in treatments with DFEC was likely due to OM decomposition, which released Na ions into the subsoil. Over time, this concentration decreased as decomposition slowed, with Na being adsorbed onto soil particles or may assimilated by microbes as stated by Li-Xian et al., (2007). Copper initially

found in trace amounts and highest in T0 (control), significantly declined across treatments, nearly reaching zero by Day 90. Regardless of amendments application, Zn which was present in trace amounts showed no significant changes. This was likely due to its low mobility and strong adsorption to soil particles (Kaur et al., 2024). These trends suggest that OM addition and microbial activity influenced the dynamics of these metals (Poveda & Eugui, 2022), with Cu potentially immobilized by microbial processes (Cornu et al., 2017), while Zn behavior remained stable due to its inherent properties.

Overall, the prominent effect of DFEC compared to treatments without it could be attributed to differences in decomposition rates, inherent chemical composition, and unique chemical properties. The Al and Fe fluctuations alongside Na, showed the complex interactions between amendments, soil chemistry and perhaps microbial activity. Meanwhile, Cu and Zn concentrations were influenced by microbial immobilization and limited mobility, reflecting a complex metal dynamic in amended soils. Despite declines in nutrient levels over time, the sustained patterns highlight the long-term impact of DFEC and HA in enriching subsoils.

Table 2. Mean values soil pH, soil OM, and total elements content at 30 days of incubation.

Day 30					
Tr*	**T0**	**T1**	**T2**	**T3**	**T4**
pH	5.35±0.12bc	5.23±0.18c	5.56±0.15a	5.32±0.16bc	5.50±0.02ab
OM (%)	1.24±0.14c	1.50±0.08ab	2.16±0.22a	1.56±0.01b	2.23±0.10a
N (%)	0.1204±0.0005d	0.1266±0.0005c	0.1682±0.0023b	0.1262±0.0009c	0.1763±0.0026a
P (ppm)	1.50±0.39d	3.23±0.25b	17.25±0.51a	2.65±0.13c	16.97±0.44a
K (ppm)	24.06±0.47b	23.91±0.83b	36.41±0.66a	23.94±0.29b	35.75±1.14a
Ca (ppm)	37.58±0.60cd	38.61±0.64c	57.39±0.89b	35.78±0.25d	59.09±0.98a
Mg (ppm)	68.21±1.20a	68.11±1.71a	65.34±0.77bc	65.92±0.55b	64.31±1.15c
Al (ppm)	109.11±1.74d	143.37±4.17a	118.44±0.93c	124.68±1.42b	115.11±2.17cd
Fe (ppm)	194.07±3.65e	286.37±5.43a	251.01±2.16b	222.88±2.10d	231.82±2.37c
Na (ppm)	3.42±0.11c	3.17±0.13cd	8.32±0.13a	2.98±0.12d	8.02±0.12b
Cu (ppm)	0.10±0.03a	0.05±0.01b	0.03±0.02c	0.02±0.02d	0.02±0.01cd
Zn (ppm)	0.82±0.04a	0.77±0.01b	0.71±0.03cd	0.66±0.05d	0.73±0.01c

Note: T0=Control (100% FR*); T1=HA (75% FR*); T2=HA + DFEC (75% FR*); T3=HA (50% FR*); T4=HA + DFEC (50% FR*); *Tr=Treatment; FR=Fertilizer rate; Means denoted with different letters indicate significant differences as determined by Tukey's Test at a significance level of $p \leq 0.05$.

Table 3. Mean values soil pH, soil OM, and total elements content at 60 days of incubation.

Day 60					
Tr*	**T0**	**T1**	**T2**	**T3**	**T4**
pH	5.27±0.28ab	5.20±0.22b	5.34±0.21ab	5.02±0.12c	5.48±0.20a
OM (%)	1.03±0.17c	1.42±0.12b	2.11±0.10a	1.36±0.11b	2.13±0.16a
N (%)	0.1202±0.0011d	0.1269±0.0013c	0.1719±0.0023b	0.1246±0.0002cd	0.1753±0.0026a
P (ppm)	2.16±0.33cd	2.02±0.39d	15.93±0.35a	2.71±0.61c	14.62±0.41b
K (ppm)	25.52±0.47cd	23.21±0.35d	38.75±0.93b	26.28±0.69c	41.00±1.21a
Ca (ppm)	37.62±0.04c	38.12±0.18c	61.50±0.91a	41.43±0.42b	61.97±1.19a
Mg (ppm)	66.27±0.79c	66.10±0.34c	66.40±1.05c	71.95±1.33a	69.69±1.49b
Al (ppm)	125.96±1.99ab	114.45±1.12d	118.67±1.81c	127.53±2.94a	128.40±2.80a
Fe (ppm)	225.41±1.46bc	204.64±0.95d	244.61±3.05a	217.52±2.52c	242.33±4.22ab
Na (ppm)	3.44±0.02b	3.17±0.16bc	8.30±0.14a	2.86±0.04c	8.32±0.28a
Cu (ppm)	0.02±0.01a	ND*	ND*	ND*	0.02±0.01a
Zn (ppm)	0.63±0.02b	0.61±0.02bc	0.68±0.01a	0.61±0.01bc	0.67±0.01a

Note: T0=Control (100% FR*); T1=HA (75% FR*); T2=HA + DFEC (75% FR*); T3=HA (50% FR*); T4=HA + DFEC (50% FR*); *Tr=Treatment; FR=Fertilizer rate; ND=Not detected; Means denoted with different letters indicate significant differences as determined by Tukey's Test at a significance level of $p \leq 0.05$.

Table 4. Mean values soil pH, soil OM, and total elements content at 90 days of incubation.

	Day 90				
Tr*	**T0**	**T1**	**T2**	**T3**	**T4**
pH	5.35±0.22ab	5.09±0.16b	5.21±0.16b	5.26±0.20ab	5.51±0.10a
OM (%)	0.89±0.08c	1.08±0.06b	1.82±0.19a	1.16±0.02b	1.69±0.04a
N (%)	0.1191±0.0033b	0.1240±0.0003b	0.1684±0.0005a	0.1233±0.0003b	0.1723±0.0011a
P (ppm)	1.63±0.42c	2.16±0.39c	15.40±0.27b	2.22±0.63c	16.67±0.75a
K (ppm)	29.18±0.78b	25.24±0.67c	39.08±0.73a	25.61±0.56c	38.32±1.00a
Ca (ppm)	39.98±0.65b	37.52±0.45c	60.06±0.64a	36.97±0.33c	62.04±0.99a
Mg (ppm)	74.08±1.90a	67.26±1.38b	65.55±0.90b	66.04±1.22b	66.93±1.16b
Al (ppm)	122.02±3.02bc	128.14±2.92ab	123.47±1.47bc	131.87±2.54a	115.85±2.30c
Fe (ppm)	190.06±3.55c	233.14±3.74b	233.05±1.21b	235.11±2.96ab	236.67±2.66a
Na (ppm)	3.37±0.14b	2.58±0.07c	7.55±0.06a	2.65±0.03c	7.78±0.23a
Cu (ppm)	0.01±0.01a	ND*	ND*	ND*	ND*
Zn (ppm)	0.69±0.02a	0.60±0.01c	0.64±0.01b	0.59±0.03c	0.66±0.03ab

Note: T0=Control (100% FR*); T1=HA (75% FR*); T2=HA + DFEC (75% FR*); T3=HA (50% FR*); T4=HA + DFEC (50% FR*); *Tr=Treatment; FR=Fertilizer rate; ND=Not detected; Means denoted with different letters indicate significant differences as determined by Tukey's Test at a significance level of $p \leq 0.05$.

4. Conclusion

This study highlights the potential of DFEC and HA as effective organic amendments for subsoil rejuvenation in sustainable farming. Treatment 4 (DFEC and HA with a 50% reduction in fertilizer rates) was the best combination, highlighting the role of macronutrient improvement in subsoil restoration. The overall enrichment of subsoil organic matter (OM) and nutrient concentrations shows the benefits of incorporating these amendments, which appear to enhance nutrient retention and availability. As a key factors for supporting sustainable agricultural practices, these findings provide insights into effective strategies for subsoil rehabilitation and sustainable nutrient management, contributing to both local and global efforts to overcome soil degradation. This investigation may contribute to advancing sustainable agricultural practices and developing innovative solutions for soil management challenges. Further research including pot and field tests is necessary to understand these mechanisms fully, particularly in the presence of crops, to establish robust soil amendment strategies for sustainable agriculture.

Acknowledgement

The authors gratefully acknowledge the financial support provided by Universiti Malaysia Sabah (UMS) through Research Grant SDK0079-2019 and SGA0046-2019. The facilities and assistance rendered by the Faculty of Sustainable Agriculture, UMS Sandakan Campus, are also duly appreciated.

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Journal of Smart Farming and Food Security

Research Article

OPEN ACCESS

Universiti Malaysia Sabah Press
e-ISSN: 3122-7090
Volume 2, Issue 1, Pages 9–18 (30 June 2026)
DOI: https://doi.org/10.51200/jsffs.v2i1.6736

Evaluation of antioxidant properties of *Andrographis paniculata* root extracts and their impact on the quality of minced beef

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Manuscript received: 25 August 2025 | Accepted: 1 December 2025 | Published online: 2 March 2026

Abstract: Lipid oxidation remains a major factor affecting the quality and shelf life of meat, leading to undesirable changes in flavour, colour, nutritional value, and consumer acceptance. Synthetic antioxidants such as butylated hydroxytoluene (BHT) are widely used to delay oxidation, their potential health risks have driven interest in natural alternatives. *Andrographis paniculata* (AP), commonly known as “King of Bitters,” is a medicinal herb rich in phenolics, flavonoids, and diterpenoids with strong antioxidant properties. Although AP leaves have been extensively studied, little is known about the bioactivity of its roots and their application in meat preservation. This study aimed to determine the antioxidant potential of AP root extracts and their effects on the quality of minced beef stored at 4°C for seven days. Root extracts were prepared using ultrasound-assisted extraction (UAE) with ethanol and distilled water as solvents. The antioxidant activity of the extracts and marinated beef samples was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, while meat quality was assessed based on pH stability and colour parameters (L*, a*, b*). Results demonstrated that ethanol extracts of AP roots exhibited significantly higher radical scavenging activity (95.38%) compared to distilled water extracts (88.38%). Minced beef marinated with AP extract showed improved antioxidant activity (11.49%) compared with the untreated control (7.74%), though lower than BHT (17.39%). Moreover, AP extract treatments maintained pH values closer to the desirable range, while altering colour characteristics by reducing redness (a*), and yellowness (b*). The findings highlight AP roots as a promising natural antioxidant source that can improve oxidative stability and sensory attributes of meat. Although less potent than BHT, their safety and consumer preference for natural food additives position AP as a valuable candidate for functional meat preservation strategies. Further research should explore optimization of extraction methods, dosage, and synergistic combinations with other plant-based antioxidants.

Keywords: *Andrographis paniculata*, antioxidant activity, DPPH, meat quality, minced beef

1. Introduction

Meat and meat products remain a vital source of high-quality protein, essential amino acids, vitamins, and minerals that are fundamental for human growth and health (Domínguez et al., 2020). With the global population projected to reach 9.7 billion by 2050, meat demand will continue to rise due to urbanisation, rising incomes, and changing dietary habits (OECD/FAO,

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Citation: Jelius, J. M., Rahman, M. J., Kalam, M. A., Bhuiyan, M. S. A., & Aziz, M. F. A. (2026). Evaluation of antioxidant properties of *Andrographis paniculata* root extracts and their impact on the quality of minced beef. Journal of Smart Farming and Food Security, 2(1), 9–18. https://doi.org/10.51200/jsffs.v2i1.6736

2019; USDA, 2024). Poultry, beef, and pork are the most consumed animal proteins worldwide. However, meat is highly perishable and prone to deterioration caused by microbial growth, enzymatic reactions, and oxidative changes that compromise shelf life and consumer acceptance. Among these, lipid oxidation is one of the most damaging processes affecting meat quality. It results in rancid odours, loss of flavour, and depletion of essential fatty acids and vitamins (Falowo et al., 2014). Furthermore, oxidation accelerates colour deterioration by converting myoglobin into metmyoglobin, producing a brownish hue that consumers perceive as spoilage (Bekhit & Faustman, 2005). Minced meat is especially vulnerable because grinding disrupts muscle fibres, exposes tissue to oxygen, and promotes microbial proliferation (Sallam et al., 2004). Thus, controlling oxidation is central to maintaining the safety, quality, and marketability of meat products.

For decades, synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tertiary butylhydroquinone (TBHQ) have been widely applied to slow oxidative processes. Despite their proven effectiveness, increasing evidence links them to potential health risks, including carcinogenicity and mutagenicity (Botterweck et al., 2000). Regulatory scrutiny and growing consumer preference for clean-label products have encouraged research into safer, plant-based alternatives (Aschemann-Witzel et al., 2019). Plants are rich in bioactive compounds such as flavonoids, phenolic acids, alkaloids, and terpenoids that exhibit antioxidant properties through radical scavenging, hydrogen donation, and metal chelation (Tungmunnithum et al., 2018). Numerous plant-derived antioxidants have been tested in meat preservation. Extracts from rosemary, green tea, and grape seed effectively delayed lipid oxidation while maintaining sensory attributes (Ivanov et al., 2025). Similarly, moringa, turmeric, and clove extracts extended shelf life and improved colour stability in meat products (Sultana, 2020; Kulkarni et al., 2021). Nonetheless, many medicinal plants remain underexplored for food applications despite longstanding traditional use.

Andrographis paniculata (AP), commonly known as “King of Bitters” or “Hempedu Bumi,” is a medicinal herb extensively used in Asia for treating fever, infections, and liver disorders (Jarukamjorn & Nemoto, 2008). Its bioactivity is mainly attributed to diterpenoid lactones, particularly andrographolide, as well as phenolic and flavonoid compounds with potent antioxidant and antimicrobial effects (Low et al., 2015; Imran & Shahid, 2020). While most studies have focused on AP leaves and stems, the roots also contain significant levels of antioxidant compounds, including flavonoids and phenolic acids, with potential applications in food preservation (Wu et al., 2008). Evidence of AP’s potential in meat systems is emerging, Fan et al. (2019) demonstrated that AP leaf extracts reduced lipid oxidation in pork during chilled storage, thereby extending freshness. However, little research has evaluated AP root extracts in meat preservation, despite their reported phytochemical richness. Since minced meat is especially prone to oxidation, investigating AP root extracts as natural antioxidants could provide both scientific and practical insights.

The mechanisms by which AP exerts antioxidant activity are multifaceted. Its bioactive compounds neutralise free radicals, chelate transition metals such as iron and copper, and enhance endogenous antioxidant enzymes, including superoxide dismutase, catalase, and glutathione peroxidase (Low et al., 2015). These mechanisms suggest that AP root extracts could effectively inhibit lipid oxidation, preserve colour stability, and maintain physicochemical quality in meat products. Moreover, AP is culturally familiar and traditionally accepted across many Asian regions, offering advantages for consumer acceptance compared with less familiar botanical sources. Despite this promise, important research gaps persist. Few studies have assessed the antioxidant activity of AP root extracts in food systems, and even fewer have explored their effects on key meat quality parameters such as pH and colour stability. Solvent choice significantly influences the extraction of phenolic compounds, yet limited comparative data exist regarding aqueous versus ethanolic AP root extracts.

DOI: 10.51200/jsffs.v2i1.6736

Additionally, there is a need for direct comparisons between AP extracts and conventional synthetic antioxidants such as BHT to evaluate efficacy in real food matrices.

Therefore, this study aimed to assess the antioxidant potential of *A. paniculata* root extracts and their impact on the physicochemical quality of minced beef during refrigerated storage. Ethanolic and aqueous extracts were evaluated for free radical scavenging activity using the DPPH assay and compared with BHT. Meat quality parameters, including pH and colour (L*, a*, b*), were monitored to determine preservation efficacy. This research seeks to expand knowledge on natural antioxidant applications, supporting industry efforts to replace synthetic additives while meeting consumer demand for safe, functional, and natural meat products.

2. Materials and Methods

2.1 Plant material collection and preparation

Fresh roots of *A. paniculata* were collected from a certified herbal farm in Selangor, Malaysia. The roots were washed thoroughly with running water to remove soil and other debris, then sliced into small pieces for uniform drying. The samples were oven-dried at 50°C for 48 hours until a constant weight was achieved, to reduce moisture content while retaining bioactive compounds. The dried roots were ground into fine powder using a laboratory grinder and stored in airtight amber glass containers at room temperature until extraction.

2.2 Extraction of *Andrographis paniculata* root

Ultrasound-assisted extraction (UAE) was employed for the preparation of root extracts. This technique was chosen due to its efficiency in enhancing the release of bioactive compounds from plant matrices through acoustic cavitation. Approximately 50 g of powdered root sample was mixed with 500 mL of solvent (absolute ethanol or distilled water) at a ratio of 1:10 (w/v). The mixture was subjected to ultrasonic treatment at 20 kHz and 500 W for 30 minutes in an ultrasonic bath. The crude extract was then filtered through Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator at 40°C. The concentrated extracts were reconstituted in a small volume of the respective solvent to a standardized concentration and stored at 4°C until further analysis.

2.3 Preparation of minced beef samples

Fresh minced beef was obtained from a local abattoir and transported to the laboratory under chilled conditions (4°C). All visible fat and connective tissue were trimmed before homogenization to ensure uniformity. Portions of 50 g minced beef were weighed and assigned to three treatment groups: (1) control (distilled water), (2) positive control (butylated hydroxytoluene, BHT), and (3) *A. paniculata* ethanol extract. The extracts or BHT were incorporated at a meat-to-solution ratio of 14:1 (w/v) and mixed thoroughly to ensure even distribution. The samples were packaged in polyethylene bags, sealed to prevent contamination, and stored at 4°C for seven days. Analyses were conducted on days 0, 3, and 7 to assess changes over time.

2.4 Determination of antioxidant activity

The antioxidant activity of both plant extracts and marinated beef samples was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, a widely recognized method for evaluating the free radical scavenging ability of natural antioxidants. For plant extracts,1 mL of extract was mixed with 1 mL of 0.2 mM methanolic DPPH solution. Meanwhile, for minced meat samples, 3 g of meat was homogenized in 80:20 (v/v) methanol:water solution, centrifuged and filtered through Whatman No.1 filter paper. A 200 µL aliquot of the supernatant was diluted with 800 µL of distilled water and then mixed with aforementioned methanolic DPPH solution. The DPPH mixtures (for AP extracts or meat

samples) were vortexed and incubated in the dark at room temperature for 30 minutes, after which absorbance was measured at 517 nm using a UV-vis spectrophotometer. Radical scavenging activity (RSA) was calculated according to the formula:

$$\text{RSA } (\%) = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

where $A_{control}$ represents the absorbance of the DPPH solution without extract, and A_{sample} represents the absorbance of the solution with extract. Higher RSA values indicated stronger antioxidant potential.

2.5 Measurement of pH

The pH of minced beef samples was determined to monitor biochemical stability and microbial activity during storage. Approximately 10 g of each meat sample was homogenized with 50 mL of distilled water using a blender for 1 minute. The pH was measured using a digital pH meter equipped with a penetration electrode, previously calibrated with standard buffers (pH 4.0 and pH 7.0). Measurements were conducted in triplicate for each sample, and mean values were recorded.

2.6 Colour measurement

The colour of the meat samples was evaluated using a Minolta CR-400 chroma meter (Konica Minolta, Japan), which provides values for lightness (L*), redness (a*), and yellowness (b*). Prior to measurement, the instrument was calibrated with a white reference tile. For each sample, three readings were taken from different surface areas to ensure accuracy, and the average value was recorded. Redness (a*) was considered a critical parameter because it reflects myoglobin oxidation, which is closely associated with consumer perception of freshness and acceptability.

2.7 Experimental design and statistical analysis

The study was arranged in a completely randomized design (CRD), with treatments and storage days serving as factors. All analyses were conducted in triplicate, and results were expressed as mean ± standard error of the mean (SEM). Data from antioxidant activity, pH, and colour measurements were subjected to one-way analysis of variance (ANOVA) to determine the effects of treatments. Tukey's honestly significant difference (HSD) test was used for post hoc comparisons, with a significance level set at $p < 0.05$. Statistical analyses were performed using SPSS software (version 25.0; IBM Corp., Armonk, NY, USA).

3. Results and Discussion

3.1 Antioxidant activity of *Andrographis paniculata* root extracts

The antioxidant activity of *Andrographis paniculata* (AP) root extracts was evaluated using the DPPH radical scavenging assay, and the results revealed that the ethanol extract exhibited significantly higher scavenging activity compared to the aqueous extract (Table 1). On day 0, the ethanol extract achieved more than 70% radical inhibition at the tested concentration, which was comparable to the synthetic antioxidant butylated hydroxytoluene (BHT). In contrast, the aqueous extract showed a lower scavenging capacity, with inhibition values below 50%. These results suggest that ethanol was a more effective solvent in extracting phenolic and flavonoid compounds from AP roots, which are responsible for antioxidant properties. This is consistent with previous studies reporting that organic solvents, particularly ethanol and methanol, enhance the solubility of polyphenols and diterpenoids such as andrographolide, thereby improving antioxidant yield (Imran & Shahid, 2020).

The radical scavenging activity of the ethanol extract remained stable throughout the seven-day storage period when incorporated into minced beef, indicating that the active compounds retained their functionality even under chilled conditions. This observation aligns with reports by Fan et al. (2019), who demonstrated that AP extracts reduced lipid oxidation in pork meat during refrigerated storage. Moreover, the stability of AP extract during storage highlights its potential as a natural preservative for meat products, as many plant-based antioxidants are prone to degradation when exposed to environmental conditions such as light, oxygen, and temperature fluctuations (Kulkarni et al., 2021).

The higher efficacy of ethanol extract compared to aqueous extract also confirms earlier reports that non-polar and moderately polar solvents extract higher amounts of bioactive diterpenoids, flavonoids, and phenolic acids from AP roots (Wu et al., 2008). Andrographolide, the major bioactive constituent of AP, has been shown to directly scavenge reactive oxygen species and upregulate endogenous antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) (Low et al., 2015). The strong antioxidant effect observed in this study therefore likely reflects the combined action of these phytochemicals, which contribute to both direct radical scavenging and indirect enhancement of oxidative stability.

Table 1. DPPH free radical scavenging activity (%) of Andrographis paniculata root extracts in ethanol and distilled water.

	AP roots extract solution		
	Ethanol	**Distilled water**	***p*-value**
DPPH (%)	95.38 ± 1.66[a]	88.38 ± 0.63[b]	0.002

DPPH = 2,2-Diphenyl-1-picrylhydrazyl. AP = Andrographis paniculata. The percentage (%) was calculated using the formula equation.

3.2 Antioxidant activity in marinated minced meat

The antioxidant activity of marinated minced beef samples was significantly influenced by the treatments applied shown in Table 2. The control samples, which were treated with distilled water, displayed the lowest radical scavenging activity (RSA), with values decreasing progressively during storage. This reduction reflects the susceptibility of minced meat lipids and pigments to oxidative deterioration when left unprotected, a finding consistent with previous reports highlighting the vulnerability of ground meat to oxidation due to its large surface area and disrupted muscle structure (Sallam et al., 2004).

In contrast, beef samples treated with *Andrographis paniculata* (AP) ethanol extract exhibited significantly higher RSA values compared to the control group ($p < 0.05$). On day 0, the AP-treated meat showed nearly 50% higher antioxidant capacity, and this activity remained relatively stable over the seven-day storage period. Although BHT-treated samples consistently recorded the highest RSA among all treatments, the AP extract demonstrated comparable efficacy, particularly during the early stages of storage. These results suggest that bioactive compounds present in AP roots, particularly phenolics and flavonoids, contributed effectively to suppressing free radical formation in the meat matrix.

Interestingly, the aqueous extract treatment showed moderate RSA activity, higher than the control but lower than ethanol extract, which may be attributed to the lower solubility of certain antioxidant compounds in water. The superior performance of ethanol extract supports previous findings that organic solvents yield higher concentrations of phenolic and diterpenoid compounds responsible for radical scavenging activity (Wu et al., 2008; Imran & Shahid, 2020).

Overall, these findings confirm that AP root extract can enhance oxidative stability in minced beef, thereby reducing lipid peroxidation and extending shelf life. This aligns with earlier studies where natural antioxidants such as rosemary and moringa extracts improved

radical scavenging capacity in meat products (Sultana, 2020; Hernandez-Hernandez et al., 2021).

Table 2. DPPH free radical scavenging activity (%) in minced beef treated with distilled water, BHT, and Andrographis paniculata root extract.

	Treatments			
	T1 (No treatment)	**T2 (BHT)**	**T3 (AP roots)**	***p*-value**
DPPH (%)	7.74 ± 0.85^{a}	17.39 ± 0.35^{b}	11.49 ± 0.02^{c}	0.0441

DPPH = 2,2-Diphenyl-1-picrylhydrazyl. AP = Andrographis paniculata. The percentage (%) was calculated using the formula equation.

3.3 pH stability and changes in marinated minced meat

The pH of minced beef is a critical quality parameter, as it reflects both biochemical stability and microbial activity during storage. In the present study, the initial pH of all minced beef samples across treatments was approximately 5.7, which falls within the normal postmortem range for beef muscle (5.5–5.8). However, distinct differences in pH trends were observed among treatments over the seven-day chilled storage period. Control samples treated with distilled water exhibited a gradual and significant increase in pH, reaching values above 6.0 by day 7. This upward trend is commonly attributed to microbial proliferation and proteolytic activity, which release alkaline compounds such as ammonia and amines into the meat matrix (Sallam et al., 2004). The progressive rise in pH in the control group highlights the vulnerability of untreated minced beef to spoilage under chilled storage conditions.

In contrast, beef samples treated with *Andrographis paniculata* (AP) ethanol extract maintained significantly lower pH values throughout storage ($p < 0.05$), with only slight fluctuations observed. The BHT-treated group showed similar results, reinforcing the role of both natural and synthetic antioxidants in stabilizing meat pH. The aqueous extract of AP roots also slowed pH increases compared to the control, although its effect was less pronounced than that of the ethanol extract. These findings suggest that ethanol extract, with its higher concentration of phenolic and diterpenoid compounds, was more effective in retarding microbial growth and maintaining pH stability.

The observed pH-stabilizing effect of AP extracts may be partly due to their antimicrobial properties, as andrographolide and related phytochemicals are known to inhibit the growth of several Gram-positive and Gram-negative bacteria (Low et al., 2015; Adiguna et al., 2023). By suppressing microbial proliferation, the extracts reduced the accumulation of alkaline by-products, thereby helping to preserve meat quality. Similar outcomes have been reported with other plant-based antioxidants, such as *Moringa oleifera* and turmeric extracts, which delayed pH increases and prolonged the freshness of chilled meat (Sultana, 2020; Hernandez-Hernandez et al., 2021).

Overall, the results confirm that AP root extracts, particularly ethanol extract, effectively stabilized the pH of minced beef during chilled storage. This property, combined with its antioxidant potential, underscores the suitability of AP as a natural preservative for meat products.

3.4 Colour stability and myoglobin oxidation

Colour stability is a critical determinant of consumer acceptance in fresh meat, as it directly reflects the extent of myoglobin oxidation and lipid peroxidation occurring during storage. In this study, significant differences in colour parameters (L*, a*, b*) were observed among treatments ($p < 0.05$), indicating that both synthetic and natural antioxidants influenced the visual quality of minced beef (Table 3).

DOI: 10.51200/jsffs.v2i1.6736

The untreated control group (T1) exhibited the highest lightness (L* = 51.30 ± 0.03) and redness (a* = 11.88 ± 0.13), alongside the highest yellowness (b* = 18.96 ± 0.89). These values suggest that fresh meat initially maintained its expected bright red appearance; however, the elevated L* and b* values also indicate early oxidative changes in surface pigments and lipids. As storage progressed, the control samples showed visible browning, consistent with the accumulation of metmyoglobin, which imparts a brownish discolouration and reduces consumer appeal (Bekhit & Faustman, 2005).

The BHT-treated group (T2) showed slightly lower L* (49.80 ± 0.95) and a* (9.26 ± 0.09) values compared to the control, suggesting that the synthetic antioxidant slowed myoglobin oxidation but did not fully prevent it. The reduction in redness values confirms that oxymyoglobin was gradually oxidised to metmyoglobin during storage, although at a slower rate than in untreated samples. These findings are consistent with previous reports that BHT effectively delays but cannot completely suppress pigment degradation under refrigerated conditions (Falowo et al., 2014).

Interestingly, the *Andrographis paniculata* root extract treatment (T3) displayed the lowest L* (39.41 ± 0.86) and a* (7.71 ± 0.55) values, alongside reduced b* (16.02 ± 0.65) compared to both BHT and control groups. The lower L* values indicate a darker surface appearance, possibly due to the presence of plant-derived pigments and polyphenolic compounds that interacted with meat proteins. The reduced redness values (a*) suggest partial oxidation of oxymyoglobin; however, the overall lower b* values imply reduced lipid oxidation and secondary yellow-brown pigment formation. This pattern indicates that AP extract slowed the browning process while maintaining a darker but more stable colour profile compared to untreated samples.

The ability of AP root extract to stabilise colour is attributed to its high content of phenolics and diterpenoid lactones, such as andrographolide, which are known to scavenge reactive oxygen species and inhibit lipid peroxidation (Imran & Shahid, 2020). By limiting the generation of lipid peroxides, the extract reduced oxidative stress on myoglobin, thereby slowing the transition from oxymyoglobin to metmyoglobin. Similar findings have been reported with rosemary, moringa, and clove extracts, which improved redness retention and suppressed browning in meat systems (Sultana, 2020; Kulkarni et al., 2021). The preservation of colour in meat treated with AP extract agrees with previous findings where natural antioxidants delayed pigment oxidation. Kulkarni et al. (2021) reported that clove extract-maintained redness and delayed browning in buffalo meat patties, while Kandeepan et al. (2020) demonstrated similar effects with grape seed extract in goat meat. The comparable efficacy of AP ethanol extract to BHT observed in this study provides strong evidence for its potential as a natural alternative to synthetic antioxidants in meat preservation.

Table 3. Physicochemical properties of minced meat. Values are mean ± SD, and means with various superscript letters differ substantially.

	Meat quality parameters			
Treatment	**pH**	**L***	**a***	**b***
T1 (no treatment)	6.22 ± 0.04^{a}	51.30 ± 0.03^{a}	11.88 ± 0.13^{a}	18.96 ± 0.89^{a}
T2 (BHT)	6.45 ± 0.08^{c}	49.80 ± 0.95^{b}	9.26 ± 0.09^{b}	17.00 ± 0.09^{b}
T3 (AP roots)	6.29 ± 0.05^{b}	39.41 ± 0.86^{c}	7.71 ± 0.55^{b}	16.02 ± 0.65^{c}
p-value	0.00576	0.0182	0.0002	0.00357

T1: No treatment, T2: BHT (butylated hydroxytoluene), T3: Roots extraction. The percentage (%) was calculated using the formula equation.

Overall, the results demonstrate that AP root extract exerted a measurable effect on colour stability, comparable to that of BHT, though with distinct differences in L*, a*, and b* values.

While redness values were slightly lower in AP-treated samples, the darker and less yellow appearance may still be favourable, especially in processed meat products where consumer expectations for bright red colour are less stringent. Importantly, the ability of AP extract to inhibit both lipid oxidation and pigment degradation supports its potential as a multifunctional natural preservative in meat systems.

The results of this study collectively indicate that AP root ethanol extract is effective in enhancing the oxidative stability, pH stability, and colour retention of minced beef during chilled storage. These benefits are comparable to those achieved with the synthetic antioxidant BHT, highlighting AP as a viable natural alternative for the meat industry. The study also provides evidence that ethanol extraction is superior to aqueous extraction in recovering bioactive compounds with antioxidant activity. The findings contribute to the broader body of literature on natural antioxidants for meat preservation. Over the past decade, researchers have increasingly emphasized the importance of replacing synthetic additives with plant-based alternatives to address consumer demand for clean-label products (Aschemann-Witzel et al., 2019). Recent work has confirmed the potential of various plant extracts, including rosemary, green tea, turmeric, clove, and moringa, to improve oxidative stability in meat (Hernandez-Hernandez et al., 2021; Sultana, 2020; Kulkarni et al., 2021). The present study extends this knowledge base by introducing *Andrographis paniculata* roots as a novel source of antioxidants with proven efficacy in meat systems.

Importantly, the multifunctional properties of AP combining antioxidant and antimicrobial activities provide additional advantages for food preservation. Unlike some natural extracts that primarily target lipid oxidation, AP extract also contributes to microbial inhibition, which is critical for extending shelf life and ensuring safety. This dual functionality has been highlighted in other medicinal plants used in food preservation, but few studies have systematically evaluated AP in this context. However, several limitations must be acknowledged. The study was conducted under controlled laboratory conditions, and the applicability of AP root extracts in commercial meat processing and packaging systems remains to be tested. The sensory characteristics of AP-treated meat, including flavour and aroma, were not evaluated in this work and warrant further investigation, as consumer acceptance ultimately determines market viability. In addition, variability in phytochemical composition due to plant origin, environmental conditions, and extraction methods must be addressed through standardized protocols to ensure consistent quality and efficacy of AP extracts for industrial applications.

4. Conclusion

This study evaluated the antioxidant potential of Andrographis paniculata (AP) root extracts and their effects on the quality of marinated minced beef during chilled storage. The results demonstrated that ethanol extracts of AP roots exhibited strong radical scavenging activity, significantly higher than aqueous extracts, and comparable to the synthetic antioxidant butylated hydroxytoluene (BHT). When incorporated into minced beef, the ethanol extracts effectively suppressed lipid oxidation, stabilized pH, and maintained colour quality over seven days of storage. The pH stability observed in AP-treated samples suggests that the extract not only acted as an antioxidant but also exerted antimicrobial effects, limiting microbial growth and the accumulation of alkaline spoilage compounds. In terms of colour, AP extract slowed the oxidation of myoglobin and reduced the formation of yellow-brown pigments, although treated meat appeared darker compared to controls. While this may slightly affect consumer perception of fresh meat, the stable colour profile remains advantageous in processed meat products.

Overall, the findings highlight the multifunctional preservative role of AP root extracts, which combine antioxidant and antimicrobial properties to enhance meat quality and shelf life. As consumer demand for clean-label and natural food additives continues to grow, AP roots

represent a promising alternative to synthetic antioxidants. Nonetheless, further studies are needed to standardize extraction methods, assess sensory attributes, and evaluate performance in commercial processing environments. In conclusion, AP root extract shows considerable potential as a natural preservative for meat systems, offering a safer and sustainable option for improving oxidative stability and extending the shelf life of minced beef.

Acknowledgement

The authors wish to express their sincere appreciation to Dr. Ismail Fitry Bin Mohammad Rashedi, Miss Faridah Mohd Razali, Mrs. Suraya Binti Saad, and Dr. Muhammad Nizam Bin Hayat for their invaluable technical assistance and dedicated support throughout the course of this research project.

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Journal of Smart Farming and Food Security
Universiti Malaysia Sabah Press
e-ISSN: 3122-7090
Volume 2, Issue 1, Pages 19–27 (30 June 2026)
DOI: https://doi.org/10.51200/jsffs.v2i1.7453

Research Article
OPEN ACCESS

Phenology of flower and fruit formation progression in *Musa acuminata* cv. Cavendish

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Manuscript received: 11 January 2026 | Accepted: 31 March 2026 | Published online: 1 April 2026

Abstract: Phenology is the study of plant life cycle stages and seasonal activities observed throughout the year. *Musa acuminata* cv. Cavendish bananas are valued for their resistance to pests and diseases, as well as their health benefits. This study was conducted at the Faculty of Sustainable Agriculture to determine the stages of flower and fruit development in Cavendish bananas, aiming to enhance yield through improved agricultural practices. Five main parameters were measured, including daily observations of each phase of banana fruit development. The number of days each bract takes to open and naturally fall off was recorded, starting from when each bract fully opens until it drops. The time required for each phase to form was recorded, the number of hands (banana clusters) was counted for each bunch, and finally, the harvested bananas were weighed to determine the average weight of each finger. The results indicate that Cavendish bananas grown in Sabah take approximately 89 days to complete the flower and fruit development cycle, producing 103–105 bracts and passing through 12 distinct growth stages. This development period aligns with findings from South Africa, where Cavendish bananas mature in 85–100 days after flowering. Variation in banana fruit maturity is influenced by environmental factors, such as climate, which affect the growth cycle.

Keywords: bracts, Cavendish banana, fruit development, phases, phenology

1. Introduction

Banana (*Musa* spp.) belongs to the Musaceae family. Musa acuminata is widely distributed, and Malaysia is considered the main origin of *Musa acuminata* (Mathew & Nagi, 2017). Bananas are tropical plants originating from Southeast Asia and play an important role in the global agricultural sector. This plant thrives in tropical areas, making it easy to cultivate in various locations with suitable climates. Today, bananas are extensively grown worldwide, especially in tropical regions, with major producers and exporters including countries in Latin America, the Caribbean Islands, and Asia. In Asia, major banana-producing countries include the Philippines, India, Thailand, Indonesia, and Malaysia. In Malaysia, banana cultivation is widespread due to its adaptability to various soil types and tropical climates.

Bananas are available year-round because this crop does not depend on a specific season. This makes it a continuous and easily accessible food source. Bananas are also a very versatile fruit. They can be eaten fresh as table fruit, cooked in various dishes, or processed to produce products such as banana chips, smoked bananas, banana flour, and more. However, their uses

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Citation: Suriyah, A. F. M., & Mijin, S. (2026). Phenology of flower and fruit formation progression in *Musa acuminata* cv. Cavendish. Journal of Smart Farming and Food Security, 2(1), 19–27. https://doi.org/10.51200/jsffs.v2i1.7453

depend on the cultivar grown, as different banana varieties have distinct textures, flavours, and sweetness levels that are suitable for different purposes.

Popular cultivars in Malaysia include Cavendish, Pisang Emas, Pisang Raja, and Pisang Rastali (Mohamad & Aman, 2009). Globally, bananas are typically triploid (containing three sets of haploid chromosomes and usually sterile), resulting from the crossing of two types of bananas, *Musa acuminata* (genome AA) and *Musa balbisiana* (genome BB), producing various genome variations such as AAA (Cavendish banana), AAB (Pisang Raja), and ABB (Pisang Awak). Traditionally, Musaceae species have been classified based on combinations of morphological, phenological, and floral characteristics (Wikantika et al., 2021). In 2017, banana cultivation in Malaysia covered 34,894.06 hectares, yielding 350,492.59 metric tonnes. For the export market, the total export value of bananas in 2016 was RM34 million, with a production yield of 25,101.46 metric tonnes. This amount increased to RM40 million in 2017, with 27,450 metric tonnes exported (Mulia, 2019).

In Malaysia, bananas such as Cavendish, Mas, and Rastali can be eaten directly. Bananas such as Raja, Tanduk, Nipah, and Pisang Awak are often made into banana chips or fried bananas. Cavendish, Mas, and Berangan bananas are usually exported and commercialized overseas. Bananas are categorized as climacteric fruits, meaning they ripen or mature after being harvested, or continue the ripening process even after being picked (Febri, 2023). In Malaysia, most farmers prefer banana cultivation due to high market demand, and the more stable prices it offers compared to other crops. However, many farmers consider banana cultivation high-risk, as bananas are said to require a long period, about 10 months, to reach harvest. This directly leads to various issues, such as disease outbreaks and animal disturbances, which can affect crop yield (Mahmud, 2022).

Banana phenology is the study of changes and developmental phases from the early stages to maturity (Weinert & Simpson, 2016). It involves monitoring key growth stages, including bud emergence, leaf formation, flowering, fruit development, and fruit ripening. Understanding the banana phenology cycle enables producers to optimize management and plan planting and harvesting dates within the studied period. Moreover, phenology is important for understanding how environmental factors such as temperature, sunlight, and humidity affect the banana life cycle. By knowing the correct timing for each growth phase, farmers can plan agronomic activities such as pruning, fertilizing, and pest control more effectively. This can increase both the yield and quality of bananas. Banana phenology also helps determine the optimal harvest time. Harvesting at the right time ensures bananas reach the desired maturity level. For example, bananas harvested too early may lack the desired sweetness and texture, while those harvested too late may be easily damaged and have a shorter shelf life. Leaf emergence rates are important in subtropical areas as they inform growers when management practices such as fertilisation and leaf removal should be carried out (Weinert & Simpson, 2016). However, detailed phenological information on banana cv. Cavendish flower and fruit development under Sabah climatic conditions remains limited. Therefore, this study was conducted to determine the developmental and maturity stages of Cavendish banana flowers and fruits.

2. Materials and Methods

2.1 Location and duration of the study

The study was conducted at the Banana Plot at Faculty of Sustainable Agriculture, Universiti Malaysia Sabah, Sandakan, Sabah located at latitude 5°55'50.3"N and longitude 118°00'31.1"E. The region experiences an average temperature range of 24°C to 35°C. The study began in June and continues until November 2024, lasting six months. Observations were conducted on twelve different banana plants.

2.2 Banana fruit development phase

The development of the banana fruit follows a series of stages, starting from flower formation to maturity. Banana cv. Cavemdish trees were marked, and each tree's flower was tagged and observed for changes until the fruit was matured. After fruit set, observations were carried out every day. During these observations, visual changes in morphology and colour were recorded. Digital images of the transitional phase were captured with a smartphone. These stages include flower formation, pollination, fruit enlargement, and fruit ripening, which eventually lead to harvest readiness. Each of these phases is crucial in determining the yield and quality of the banana fruit (Fabro-Realin et al., 2022).

2.3 Observation of banana flower bract opening and shedding

Observation began with the emergence of the banana flower from the centre of the banana stem, designated as day one, and continued until the banana heart shed its final bract. Each bract was recorded at different stages of development. To ensure accuracy, smartphone images were used to track and document the bract-opening process (Waniale et al., 2021).

2.4 Duration of each growth phase

The duration of each phase was observed from flower formation as a day one until the fruit matured. Observations were carried out every day. The transitional phase was counted manually (Fabro-Realin et al., 2022). Four-year-old trees were marked, and each tree's inflorescence was randomly tagged and observed for changes until the fruit was set and matured. After fruit set, observations were carried out every seven days. During these observations, visual changes in morphology and colour were recorded. Digital images of the transitional phase were captured with a camera (DSLR-A200, Sony, Japan).

2.5 Calculation of the total number of banana hands

The total number of banana hands was calculated after a bunch of bananas was harvested. The bunch was cut, and each hand was counted and labelled. Each hand was recorded through photos taken with a smartphone. The total number of hands for each bunch was then manually counted and recorded as data (Pinang, 2020).

2.6 Calculation of the weight of cavendish bananas

The ripe bananas were weighed using a scale. The weight of the bananas was recorded, and this data was used for comparison with the weight from other plants (Suwito & Daud, 2024). Each fruit weight was recorded, and the average weight was calculated using a formula:

$$\text{Average weight} = \frac{\text{Total weight of the bananas}}{\text{Total number of hands obtained per bunch}}$$

3. Results and Discussion

3.1 Banana fruit development phase

Throughout the study, the development of the Cavendish banana flower and fruit showed distinct changes across 12 phases, from phase 1 to phase 12. The process began with the emergence of the flower at the centre of the pseudostem, which then developed into a bunch of fruit. Figure 1 illustrated the progression in the development of the Cavendish banana flower and fruit throughout the study.

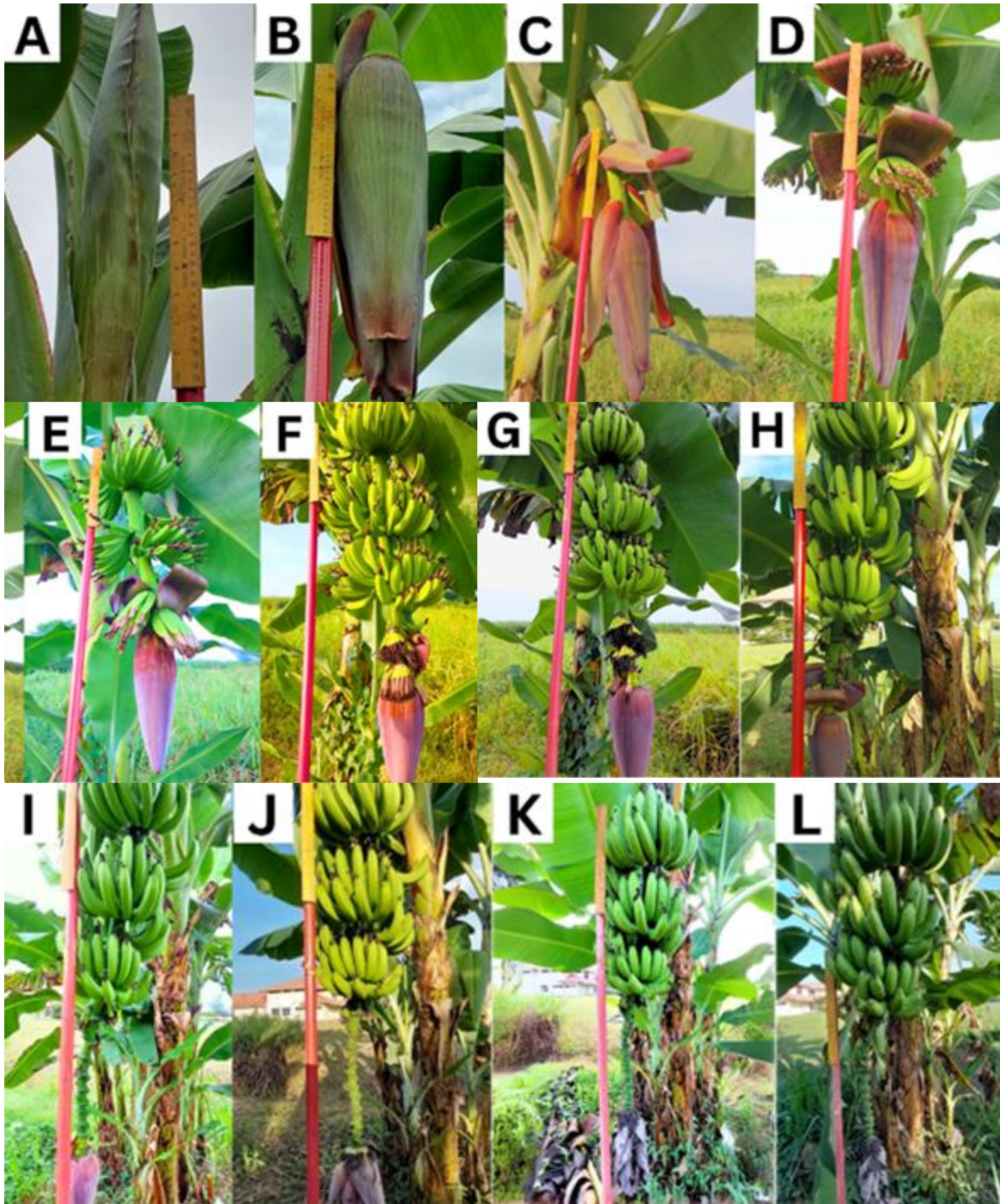

Figure 1. Banana cv. Cavendish fruit development phases from phase 1 to phase 12.

Figure 1(A) in phase 1 showed the beginning of stem formation, which served as the main support structure for the banana plant. This stem provided stability to the plant, allowing it to support the weight of the leaves, flowers, and fruits that would develop in the following phases. The pseudostem is the part of the banana plant that appears like a stem, consisting of a soft central core or false stem wrapped with leaf sheaths. These leaf sheaths detach from the stem and become banana leaves when mature (Paull & Duarte, 2025). This phase is the optimal time to apply fertiliser to the banana plant. At this early stage, the banana fruit was undergoing active growth and required adequate nutrient support, such as potassium fertiliser, to ensure optimal development and produce high-quality fruit (Robinson & Saúco, 2010). The use of appropriate fertiliser at this stage increased fruit size, bunch strength, and overall crop quality.

DOI: 10.51200/jsffs.v2i1.7453

In Phase 2, Figure 1(B), the flower or inflorescence showed changes distinct from Phase 1. The immature inflorescence was protected by a purple bract, giving it the appearance of a large bud, and was called the 'bell' (Christelová et al., 2016). The inflorescence was initially upright but soon drooped and pointed downward. This occurred due to the size and weight of the flower, the growth of the peduncle (flower stalk), and the geotropic effect. The geotropic effect played a role, as the plant tended to grow towards gravity, causing the inflorescence to point downward as the flower stalk developed (Doreen, 2024).

Each flower in the inflorescence was protected by purple bracts, which served as shields for the developing flowers in Figure 1(C) (Phase 3). The bracts opened sequentially, with the bracts at the base of the inflorescence opening first and progressing towards the tip. These bracts curved backward (reflexed) before eventually falling off completely (Christelová et al., 2016). The female flowers were clearly visible, appearing green in colour. At the same time, the peduncle began to elongate, with the stalk emerging from the pseudostem to support the developing inflorescence (Robinson & Saúco, 2010). This process exposed the female flowers or other structures within the bracts, creating space for further development, such as fruit formation.

In phase 4, Figure 1(D) the partially rolled bracts, which served as a protective covering, began to reveal the female banana flowers, closely arranged in two layers. This indicated that the banana flowers were developing and starting to create spacing between each other. Additionally, the banana flowers hanging at the end of the cluster showed that their flower stalks had elongated and become heavier due to the formation and development of the flowers and fruits (Robinson & Saúco, 2010).

In Figure 1(E) (Phase 5), many physical changes occurred in the bunch and hands of the banana. The main flower stalk continued to elongate, and some of the female flower bracts began to fall off naturally (Doreen, D.A, 2024). The banana hands expanded, allowing space for fruit formation, while the flower tips darkened. Additionally, the bracts that had opened continued to produce new flowers, which eventually developed into fruit. This process demonstrated how the female flowers systematically developed into bananas through growth and complex structural changes.

In phase 6, Figure 1(F), the female flowers began to curve slightly upwards, and flowers appeared between the nodes of the female and male flowers, containing intermediate structures. These flowers were hermaphroditic, with short ovaries that did not develop into edible fruit. Typically, the appearance of hermaphroditic flowers signalled that the bract would no longer produce female flowers (no further fruit would be produced), and male flowers would dominate the next stage of development. The male flowers remained within the bell-shaped structure (flower bud structure) with their bracts throughout the life of the inflorescence. However, in commercial practice, the flower buds were usually removed to prevent continuous meristem growth and elongation of the peduncle axis (Summerville, 2024).

Moreover, in phase 7, Figure 1(G), the bracts and flowers opened and expanded sequentially, starting from the part of the bracts and flowers closest to the stem (proximal) and moving towards the more distant part (distal). This process caused the peduncle, the flower stalk, to continue elongating. This ensured that the inflorescence developed in an orderly manner, with bracts and flowers opening one by one, while the elongating peduncle supported the upward movement of the inflorescence. Over several weeks, the banana fruit gradually curved upwards due to negative geotropism, a response to gravity regulated by auxin hormones (Summerville, 2024).

In Phase 8, Figure 1(H), the banana fingers clearly displayed their upward growth direction (a manifestation of negative geotropism) (Fabro-Realin et al., 2022), and the pulp filling became more pronounced. This process demonstrated the physiological development of the banana fruit due to the imperfect ovary of the flower (parthenocarpic flower, which produces fruit without

fertilisation). The rachis (flower stalk) supporting the banana bunch underwent significant elongation, resulting in a clearer separation between the male and female flowers. This elongation of the rachis increased the distance between each flower, creating a more organised arrangement and facilitating the pollination process. The male flowers, located at the top of the bunch, became further separated from the female flowers, which were located at the bottom of the bunch (Doreen, 2024).

Furthermore, in Phase 9, in Figure 1(I), the banana fruit became more curved compared to the previous phase. In this phase, the structure of the banana fruit cluster or bunch was explained more clearly. First, the stem containing two rows of female flowers was known as the 'hand', with each 'hand' containing a group of 'fingers'. These fingers were individual bananas that developed from the female flowers and, when combined, formed the entire 'bunch' of bananas. The edible banana bunch consisted of several hands arranged spirally on the thick banana stem (peduncle). The number of hands in each bunch and the number of fingers on each hand were determined early in flower formation, depending on the number of female flowers formed on the banana stem. This process is influenced by several factors, including the banana genome group, the crop cycle and growth period, environmental temperature affecting flower formation, plant health, and the management of banana cultivation and care (Robinson & Saúco, 2010).

From phase 10 in Figure 1(J), the bracts on the banana no longer fall off naturally. Instead, they remain attached to the flower stalk until they eventually decay or dry out. This occurs because the bracts no longer play a role in fruit development. However, bracts that do not fall off can interfere with the maturation process by causing excessive moisture or providing a site for the growth of fungi and bacteria.

In Figure 1(K) for phase 11, the banana bunch approaches maturity, and this phase is called the "Green Life," referring to the period after the banana harvest when the fruit remains green and has not yet fully ripened. At this stage, the bananas show no signs of maturity, and the pulp remains firm and green. This continues until the fruit enters the climacteric phase, marked by a change in fruit colour to yellow and the softening of the pulp.

Finally, for Figure 1(L), phase 12 was the maturity phase, during which the banana fruit reached full maturity, with the skin turning yellow and the pulp becoming softer. The banana peel had turned yellow, which was an early sign that the banana had reached maturity and was ready for harvesting. Additionally, the maturity of the fruit could be determined using the banana maturity index, according to the Department of Agriculture, which was at index 3. This index was used to determine the stage of maturity of the banana before it was harvested or marketed.

3.2 The number of days for the banana flower bract to open and fall

Table 1 shows the development and emergence of the banana inflorescence (flower). Each bract (a leaf-like structure surrounding the flower) opened sequentially, with each bract opening approximately one day apart. This resulted in a continuous or gradual effect on the flowering and fruiting process of the banana plant (Robinson & Saúco, 2010).

Banana bract opening began in phase 2 and continued until phase 12, while in phase 1, the bracts remained closed within the pseudostem. Starting from phase 2, the bracts began to open five days after the appearance of the banana flowers, but during this phase, no bracts naturally fell off. The number of days required between all the phases varied. This can be observed in phases 3 and 4, where the bracts opened within a short period of 9 to 11 days, with the bracts beginning to fall off between days 11 and 12. The duration for the opening and shedding of the bracts gradually increased during the middle phases (phases 5 to 7), taking 13 to 21 days for the bracts to open and 13 to 22 days for them to fall off, starting from the day the first flower appeared. In the following phases (phases 8 and 9), the opening period increased to 34 to 50 days, with shedding occurring between 36 and 46 days. In phases 10 to 12, the duration of bract

DOI: 10.51200/jsffs.v2i1.7453

opening continued to increase from 67 to 86 days, but during this phase, no bracts fell off naturally. Only 48 bracts fell off naturally, while the remaining bracts remained attached to the rachis until the banana flower rotted or dried. Day 86 marked the last day of natural bract opening, and the total number of bracts ranged from 100 to 110 for Cavendish bananas. From day 87 to 89, no new bracts opened. Overall, the table showed that bananas required more time to mature as they approached full maturity.

Table 1. The number of days required for the banana bract to open and fall off after the banana flower appeared.

Phase	*Day of bract opening	**Day of bract fall
1	0	0
2	5	0
3	9	11
4	11	12
5	13	13
6	15	16
7	21	22
8	34	36
9	50	46
10	67	-
11	83	-
12	86	-

Note: *The bract stopped opening from day 87 to 89; **On day 48, only 48 bracets fell off naturally. The remaining bracts stayed attached to the rachis until the flower buds rotted or dried out.

3.3 Duration required for each phase to form

Table 2 shows the duration (number of days) required for the banana fruit to reach maturity in each phase, with 12 phases in total, each having a different duration before reaching maturity. In phase 1, the process took 5 days, starting from the first day the banana flower emerged in the middle of the pseudostem until it transitioned to phase 2. Between phases 2 and 6, each phase required only about 2 days to form, making this the shortest period in the entire maturation process. In this study, the appearance of hermaphroditic flowers (flowers that do not produce fruit) in phase 6 marked the appropriate time to cut the banana flower (male bud). Cutting the banana flower focused the plant's energy and nutrients on the growth and development of the banana fruit, ensuring higher-quality yields. Phase 7 then took around 6 days to complete before transitioning to the next phase. The number of days required for the maturation process began to increase from phase 8 to phase 11.

Phase 8 took 13 days to complete, while phases 9 to 11 took longer than the other phases, between 16 and 17 days. In the final phase, Phase 12, the banana ripening process took 6 days, during which the bananas began to show colour changes from green to yellowish green, indicating that the fruit was nearly fully ripe. This entire process showed the development of the fruit from the early phases to the final stage, providing important information to understand the duration and characteristics of each maturation phase. This information was useful for crop management and more accurate harvest timing predictions (Mathew & Nagi, 2017). In conclusion, the time require for a bunch of Cavendish bananas to reach maturity in Sabah was around 85 to 89 days (2 to 3 months). This period was like South Africa, where banana maturity was reached within 85 to 110 days (2 to 3 months) after the emergence of the inflorescence (Robinson & Saúco, 2010).

Table 2. The number of days required for each phase to form.

Phase	Duration of each phase to form
1	5
2	2
3	2
4	2
5	2
6	2
7	6
8	13
9	16
10	17
11	16
12	6
Total number of days	89

3.4 Calculation of the total number of banana hands

The study found that each Cavendish banana produced an average of 7 to 8 hands per bunch, with about 12 to 14 fingers in each hand. However, this number shows a difference compared to the data released by Bojonegoro University in Indonesia, which recorded 8 to 13 hands per bunch, with each hand consisting of 12 to 22 fingers (Suwito & Daud, 2024).

3.5 Calculation of the weight of cavendish bananas

Table 3 shows the average weight per finger for 3 banana plants. The harvested and ripened banana bunches were using a scale to determine the average weight of the bananas. Based on Table 3, Plant 1 recorded an average hand weight of 0.079 kg, Plant 2 recorded 0.101 kg, and Plant 3 recorded 0.135 kg. Overall, the average hand weight of Cavendish bananas in Sabah was around 0.1 kg. This difference was not very significant compared to the average hand weight in neighbouring Indonesia, which recorded around 0.15 kg per hand (Suwito & Daud, 2024). In conclusion, this occurred because the tropical climate in Sabah and Indonesia is similar. Although there is a slight difference in the hand weight between the two locations, the Cavendish banana yield in Sabah is still within a similar range as in neighboring Indonesia and remains at a satisfactory level.

Table 3. Average weight of a Cavendish banana.

Banana plant	Total weight per bunch (kg)	Total number of fingers (per bunch)	Average weight of a finger (kg)
Plant 1	6.30	80	0.079
Plant 2	8.79	87	0.101
Plant 3	14.85	110	0.135

4. Conclusion

In conclusion, the banana cv Cavendish fruit exhibits 12 growth phase before the fruit matured or reaches physiological maturity. The process begins with the bract opening, followed by the development of the flowers and fruit, and concludes with the maturation. Banana cv. Cavendish takes approximately 89 days from fruit set until maturation. Identifying the growth phase and changes during flowering and fruiting provides growers with invaluable information to optimize production planning. Furthermore, this study provided farmers with useful data to determine the optimal time to cut the banana flower, ensuring that fruit growth and development were not disrupted. Understanding the timing and progression of these critical stages throughout

the banana life cycle is essential for maximizing yield and ensuring efficient harvest cycles. These findings are a valuable resource for the banana industry, supporting sustainable farming practices and promoting the production of high-quality banana cv. Cavendish in Sabah and beyond.

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Journal of Smart Farming and Food Security
Research Article
Universiti Malaysia Sabah Press
OPEN ACCESS
e-ISSN: 3122-7090
Volume 2, Issue 1, Pages 28–37 (30 June 2026)
DOI: https://doi.org/10.51200/jsffs.v2i1.7329

Enhancing Japanese quail growth performance and egg quality through effective microorganism water supplementation in diet

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Manuscript received: 29 January 2026 | Accepted: 10 April 2026 | Published online: 17 April 2026

Abstract: Effective Microorganisms (EM) are probiotic mixtures of beneficial bacteria that improve gut health by reducing harmful pathogens to improve growth performance. EM support overall health and egg production in avians by balancing intestinal microflora. This study investigated the impact of EM supplementation via drinking solution on the growth performance and egg quality of quails. A total of 64 quails were divided into four treatment groups, with each group receiving a different concentration of EM in their drinking water. Growth performance, such as feed intake, body weight gain, and feed conversion ratio (FCR), were monitored weekly for seven weeks. Egg quality parameters, including egg yolk color, shell thickness, albumen height, and Haugh unit, were evaluated during the final week of the study. The results demonstrated that quails supplemented with EM exhibited improved growth performance; with a significant reduction ($p < 0.05$) in FCR and enhanced weight gain compared to the control group. In terms of egg quality, EM supplementation led to improve the yolk pigmentation and albumen height, resulting in higher Haugh unit scores. Shell thickness was also positively influenced ($p < 0.05$) by the EM concentrations. The findings indicate that EM supplementation in quail diets can enhance both growth performance and egg quality, making it a profitable strategy for sustainable poultry farming. Future studies could explore the long-term effects of EM and its influence on other physiological and reproductive traits.

Keywords: egg quality, effective microorganism, feed conversion ratio, growth performance, quail feed supplementation

1. Introduction

The demand for poultry products has been rising due to population growth and changing dietary habits. While chickens dominate the poultry industry, quails (*Coturnix coturnix japonica*) are gaining popularity for their rapid growth, early maturity, high egg production, and resilience to diseases (Cheong et al., 2016). Quails require less space, consume less feed, and produce nutrient-rich meat and eggs, making them an attractive option for both small-scale and commercial farmers (Jumadin et al., 2022). However, quail farming remains underutilized due to limited research and farmer awareness (Wong et al., 2025). Expanding research on quail farming and nutritional strategies is essential to optimize production efficiency and enhance food security and productivity (Juan, 2026).

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Citation: Maitel V. H., Mohd Yaakub, N., Huda, N., Bhuiyan, M. S. A., & Rasid, R. A. (2026). Enhancing Japanese quail growth performance and egg quality through effective microorganism water supplementation in diet. Journal of Smart Farming and Food Security, 2(1), 28–37. https://doi.org/10.51200/jsffs.v2i1.7329

DOI: 10.51200/jsffs.v2i1.7329

One promising approach to improving feed efficiency, growth performance, and egg quality is the supplementation of Effective Microorganisms (EM) in poultry diets. EM consists of beneficial microorganisms such as lactic acid bacteria, yeast, and photosynthetic bacteria that enhance digestion, improve nutrient absorption, and support immune function (Gnanadesigan et al., 2014). These microorganisms help maintain a balanced gut microbiota, suppress harmful bacteria, and promote overall gut health, which is crucial for efficient feed utilization and improved productivity (Gesek et al., 2018; Xiang et al., 2019)

Studies have demonstrated that EM supplementation positively impacts poultry performance, including weight gain, feed conversion ratio (FCR), and egg production (Biswas et al., 2015). Utami & Akbar (2025) has utilised EM in their *Leucaena leucocephala* leaf meal for laying quail. The findings found that EM assisted the leaf fermentation process and improved egg productivity with significantly better egg yolk color.

Probiotics in poultry diets enhance intestinal morphology, increasing villus height and crypt depth, which leads to improved nutrient absorption (Gesek et al., 2018; Nur Azri et al., 2018; Ahmad et al., 2022). Additionally, probiotics have been reported to improve egg quality parameters such as yolk pigmentation, albumen height, shell thickness, and Haugh unit scores. These preferences are both increases market demand as well as consumer preference (Neijat et al., 2020; Atsbeha & Hailu, 2021).

Quail eggs are valued for their rich nutritional profile, containing high levels of proteins, essential amino acids, vitamins, and minerals (Shalome et al., 2021). They are associated with various health benefits, including improved immune function and reduced risk of chronic diseases due to their antioxidant properties. The internal and external qualities of poultry eggs, such as yolk color, shell thickness, albumen height, and Haugh unit, are key determinants of egg quality, and these parameters can be influenced by dietary modifications, including probiotics like EM (Atsbeha & Hailu, 2021; Yitbarek, 2023). Both quantity and quality of quail eggs relied on gut health and nutrient utilization ability through EM supplementation.

However, more studies are required to examine the effects of EM supplementation on quails in order to establish the effective EM concentration towards growth performance and egg quality. This study aims to investigate the effects of EM supplementation on the growth performance and egg quality of quails. Specifically, it would evaluate the impact of different EM concentrations in drinking water on key performance indicators; including feed intake, body weight gain (BWG), and FCR. Additionally, egg quality parameters such as yolk pigmentation, shell thickness, albumen height, and Haugh unit was assessed.

The findings would contribute to sustainable poultry farming practices and provide recommendations for optimizing quail diets. The results of this study could benefit quail farmers and poultry nutritionists seeking to enhance production efficiency and product quality. If proven effective, EM supplementation could serve as a natural and cost-effective alternative to conventional growth promoters, reducing reliance on antibiotics and mitigating antibiotic resistance in poultry farming. Improved growth performance and egg quality in quails could also enhance the commercial viability of quail farming, encouraging wider adoption and diversification in the poultry industry. While probiotics have been extensively studied in poultry, research on quails remains explorable. This study seeks to bridge this knowledge gap by evaluating the effects of EM on quail production, contributing valuable insights for improving quail farming practices and promoting sustainable poultry production.

2. Materials and Methods

2.1 Study area

This research was conducted in the Poultry Research Facility at the Faculty of Sustainable Agriculture (FSA), Universiti Malaysia Sabah, Sandakan, Sabah. The quails were kept in a controlled setting in separate cages for the duration of the study. Each cage had sufficient space,

feeders, and nipple drinkers to ensure easy access to food and water. All cages and equipment were thoroughly cleaned and disinfected before the quails were introduced. The environment was maintained through regular waste removal and routine upkeep to ensure optimal conditions throughout the study.

2.2 Experimental animal and dietary treatment

A total of 64-day old quail chicks were bought from Department of Veterinary. The experimental groups were including one control group and three treatment groups to study growth performance and egg quality using commercial EM supplementation (EM4, BH Farm) in quail diets. The treatment groups received the baseline diet with 0.5%, 1.0%, and 1.5% of EM solution supplementation via filtered water, whereas the control group would receive the basal diet without any EM supplementation as in Table 1. The quails were rando
y allocated to these groups to reduce bias, and each group was closely observed to ensure similar conditions throughout all groups.

Table 1. Experimental cages and animal managements.

Treatment	EM supplementation level (%)	Number of quails	Duration (weeks)
Control (T1)	0	16	8
Treatment 1 (T2)	0.5	16	8
Treatment 2 (T3)	1.0	16	8
Treatment 3 (T4)	1.5	16	8

2.3 Experimental design

This study used a Completely Randomized Design (CRD), this study aims to evaluate the impact of varying levels of EM supplementation on both the quality of quail eggs and their growth performance. Basal diet was prepared and fed to quails for a total of eight weeks, with the diet adjusted based on the age of the birds as recommended by National Research Council (1994). The diet was divided into two phases; a starter diet for the first five weeks, followed by a grower diet for the remaining three weeks. Each treatment consisted of four replicates, with each cage received 150 g of feed. The unsexed quails were monitored regularly to ensure uniform feed intake and growth. The starter diet was formulated to meet the nutritional requirements of young quails, while the grower diet was designed to support the birds' growth during the later stages of development. The dietary formulations for both phases were based on established recommendations for quail nutrition. The EM supplementation was prepared and incorporated into the quails' drinking water according to the experimental design (Utami & Akbar, 2025). A commercial EM product was diluted with water to achieve the required concentrations of 0%, 0.5%, 1.0%, and 1.5%. For each treatment group, 600 mL of water was provided in week 2, 1200 mL from week 3 to 5, and 2400 mL from week 6 to 8. The respective volume of EM product was added using a syringe to each water volume, ensuring the correct concentration for each treatment group. The EM solutions were offered *ad libitum*, and their concentrations were routinely monitored (morning and afternoon) daily and adjusted as necessary to maintain consistency throughout the study, accounting for any changes in ambient conditions or water consumption rates (Rahman et al., 2019).

2.4 Variables and Sampling

Quail's body weight gain was calculated as the mean weight recorded during treatment period for growth performance. The amount of feed given to each quail group was measured regularly, ensuring reliable tracking of consumption. Any leftover feed was gathered and weighed daily. The daily feed intake for each quail group was calculated by deducting the weight of the leftover

DOI: 10.51200/jsffs.v2i1.7329

feed from the original amount given throughout the experimental period. The formulation to determine Feed Conversion Ratio (FCR) as shown in Equation 1 (Varkoohi et al., 2010) is the proportion of feed consumed over a specified period relative to the weight of eggs produced during that period:

$$FCR = \frac{\text{Total feed consumed}}{\text{Total weight gain}} \qquad \text{Equation 1}$$

Parameters such as egg weight were analysed and measured analytical scale with 0.01g deviation. Measurements were conducted in a controlled environment to minimize external factors affecting accuracy. The eggshell thickness was measured using an eggshell thickness gauge (Models 3001, DIG) as done by Zhu et al. (2022). The thickness was recorded in micrometres, and the average of the three measurements was calculated to represent the overall eggshell thickness for each egg. The tester probe (ORKA Digital Haugh Tester) was placed at the thickest part of the albumen to measure the albumen height, and at the thickest part of the yolk to measure the yolk thickness. (Renukadevi et al., 2018). The Haugh unit (HU) was calculated using the Equation 2 introduced by Raymond Haugh in (Haugh, 1937).

$$HU = 100 \log (H + 7.57 - 1.7W^{0.37}) \qquad \text{Equation 2}$$

Egg yolk color was measured manually using the Roche Yolk Colour Fan (RYCF). The color of the yolk was visually matched to the closest shade on the RYCF, and the corresponding number was recorded as the yolk color score. (Sünder et al., 2022). For pH measurement, the yolk and white (albumen) of the egg were prepared by separating egg yolk and the white before placed into Falcon tube. A pH meter (Eutech Instruments, pH 2700, USA) was used to determine the mean pH of the egg white and yolk. (Aygün & Olgun, 2019).

2.5 Statistical analysis

The data were analysed using one-way analysis of variance (ANOVA) with Minitab 16.2.3 (2010). Post-hoc comparisons were performed using Tukey's test at a 95% confidence level ($p < 0.05$) to determine significant differences between means.

3. Results and Discussion

The purpose of this study was to evaluate the addition Effective Microorganisms (EM) to quail diets at different concentrations (0%, 0.5%, 1.0%, and 1.5%) affected egg quality, feed efficiency, and growth performance. The findings are provided in terms of their effects on important parameters such as growth performance, feed conversion ratio (FCR), feed and water intake, and egg internal and external quality.

3.1 Growth performance

Growth performance measures the total efficacy of dietary treatments in promoting body weight gain and health in quails. The mean body weight increased steadily across all treatments (T1, T2, T3, and T4) from week two to week eight. At week eight, T4 (1.5% EM) had the highest mean body weight (248.44 g), followed by T3 (240.81 g), T2 (239.94 g), and T1 (219.88 g). However, statistical analysis showed no significant differences in mean body weight between the treatments for weeks two to seven ($p > 0.05$).

At week eight, T4 was significantly different from T1 ($p < 0.05$), while T3 and T2 were statistically comparable to both T4 and T1. This indicates that EM supplementation did not significantly affect body weight in the early weeks but showed a trend for higher body weight at higher EM levels (1.5%) by the end of the study as shown in Figure 1.

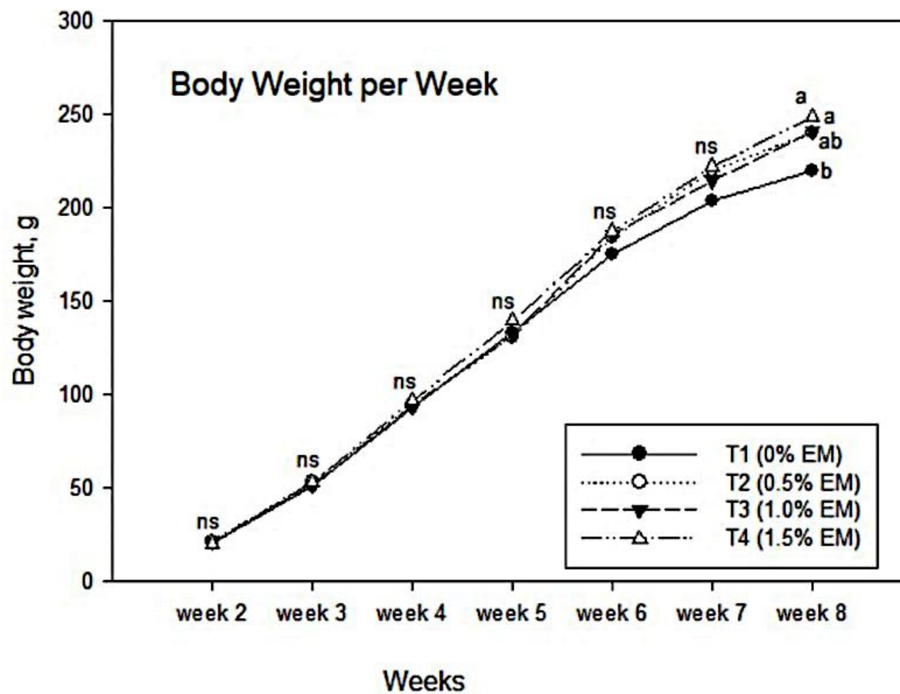

Figure 1. Quail's weekly body weight gain (BWG).

EM supplementation considerably improved quail growth performance, with T4 (1.5% EM) resulting in the highest body weight gain by week 8. This is consistent with the findings of Ahmad et al. (2022), who showed that probiotics improve gut health and nutrient absorption, especially under stressful environmental conditions, by increasing villus height and crypt depth. These morphological enhancements are directly proportional to the increased surface area available for nutrient absorption, resulting in optimal growth rates. Furthermore, probiotics such as *Bacillus subtilis* and *Lactobacillus spp*. have been proven to alter the gut microbiota, increasing the number of beneficial bacteria while reducing pathogenic germs (Aziz Mousavi et al., 2018; Deng et al., 2020). This microbial balance enhances the intestinal environment, enabling more efficient digestion and nutritional utilisation. Probiotics' production of short-chain fatty acids (SCFAs) helps to increased energy metabolism and feed efficiency, resulting in higher growth rates.

Additionally, while T4 showed the greatest growth, T2 (0.5% EM) had the best feed conversion ratio (FCR), implying that mild EM supplementation will not bring extra benefits, thus may be more cost-effective for farmers looking to balance growth and efficiency. Aziz Mousavi et al. (2018) reported similar findings, emphasising the economic benefits of adjusting probiotic dosage to meet specific production goals. These findings highlight the potential of EM as an antibiotic alternative, hence promoting sustainable poultry farming practices.

3.2 Feed conversion ratio

Table 2 showed the effects of EM supplementation on feed and water intake, as well as FCR was demonstrated on how even low concentrations can improve resource utilisation while preserving productivity. Feed intake observed to be consistent across treatments, showing no significant differences ($p > 0.05$). Water intake tended to decrease slightly with higher EM concentrations, which might indicate improved nutrient utilization. The FCR pattern was similar to Utami & Akbar (2025) revealed no significant differences across the experimental groups. This is likely because of the basal diet met the standard nutritional requirements for

laying quail. Abou-Kassem et al. (2020) reported no significant difference (p>0.05) observed in quails fed with *Bacillus toyonensis* and *Bifidobacterium bifidum*. However, FCR was recorded differently based on experimental period. In this study, FCR was lowest in T2 (0.5% EM) at 3.36 ± 0.87, suggesting that this treatment was the most potential in converting feed into body mass or egg production and cost-effective supplementation level.

Table 2. Feed and water intake with feed conversion ratio (FCR) by different treatments.

Parameters	**T1 (0% EM)**	**T2 (0.5% EM)**	**T3 (1.0% EM)**	**T4 (1.5% EM)**
Feed intake (g)	122.8a ± 8.78	107.9 a ± 6.60	119.3 a ± 6.41	122.8 a ± 7.91
Water intake (mL/bird)	435.9 a ± 79.2	414.1 a ± 64.4	376.5 a ± 57.9	399.4 a ± 71.3
FCR	3.80 a ± 1.13	3.36 a ± 0.87	3.58 a ± 0.75	3.53 a ± 0.62

Note: Values are presented as mean ± standard deviation. Same letter denotes mean values are not significantly different $p > 0.05$ at the same row.

Supplementing EM considerably increased the feed conversion ratio (FCR) in quails, with T2 (0.5% EM) having the lowest FCR (3.36 ± 0.87). This suggests that even small amounts of EM can improve feed efficiency by optimising feed conversion into body mass or egg production. Consistent with these findings, Aziz Mousavi et al. (2018) found that probiotics such Bacillus subtilis increase FCR by altering gut microbiota, lowering pathogenic bacteria, and promoting beneficial microbe development. These modifications lead to enhanced nutrition uptake and utilisation. The observed FCR benefits can be related to probiotics' ability to increase the synthesis of digestive enzymes such amylase and lipase, which aid in the breakdown of feed into absorbable nutrients (Deng et al., 2020). Furthermore, probiotics generate short-chain fatty acids (SCFAs), which improve energy metabolism and nutrition absorption (Aziz Mousavi et al., 2018). The small decrease in water intake seen at higher EM concentrations could possibly reflect greater feed digestibility and less need for additional water to process nutrients.

The decrease in feed intake observed in Treatment 2 (T2), which was positioned closer to the light source, can be attributed to temperature stress. Extreme temperatures are known to negatively impact feed consumption, as highlighted by Nawab et al. (2018). Despite this reduction in feed intake, the growth trend from Treatment 1 (T1) to Treatment 4 (T4) still showed an increase. This suggests that the beneficial effects of EM supplementation on nutrient utilization were able to offset the negative impact of the reduced feed intake in T2.

Interestingly, although T2 exhibited the best feed conversion ratio (FCR), this can be explained by the fact that EM supplementation may enhance the efficiency of feed usage, leading to better growth even with less feed consumed. This observation aligns with the findings of Jha et al., (2020), which emphasize the positive effects of using probiotics, such as EM, at moderate doses to improve growth performance and feed utilization in poultry farming. Therefore, despite the temperature-related decrease in feed intake, the superior FCR in T2 demonstrates the significant role of EM supplementation in enhancing the efficiency of nutrient absorption and utilization.

3.3 Egg internal and external quality

The addition of Effective Microorganisms (EM) at various concentrations resulted in considerable improvements in key egg quality indices, including yolk colour, albumen weight, and Haugh unit as shown in Table 3. The data show that EM supplementation had no significant effect (p>0.05) on the pH of either yolk or albumen, as all treatments (T1-T4) had similar values. Egg weight increased with higher EM concentrations, with T4 (1.5% EM) producing the heaviest eggs (10.05 ± 0.28). Higher EM concentrations led to a small increase in yolk

colour intensity, with T4 (1.5% EM) producing the brightest colour (11.67 ± 0.18). Higher concentrations, specifically T4 (1.5% EM), resulted in a considerable increase in yolk colour intensity (11.67 ± 0.18). These findings are consistent with research demonstrating probiotics' potential to increase carotenoid metabolism and bioavailability, resulting in more excellent yolk pigmentation (Mazanko et al., 2018; Abd El-Hack et al., 2020). Vibrant yolk colour is highly desired in the market because it increases consumer attractiveness and perception of egg quality. This indicates that EM may have a good influence on yolk colour, potentially improving its physical and nutritional value.

Table 3. Effects of different EM percentage supplementation on quail internal and external egg quality.

Parameters	T1 (0% EM)	T2 (0.5% EM)	T3 (1.0% EM)	T4 (1.5% EM)
pH (Yolk)	6.32a ± 0.04	6.36a ± 0.03	5.76a ± 0.008	6.28a ± 0.039
pH (Albumin)	9.57a ± 0.03	9.58a ± 0.03	9.61a ± 0.008	9.62a ± 0.014
Yolk Colour	10.83a ± 0.29	11.17a ±0.38	11.33a ± 0.37	11.67a ± 0.18
Egg weight (g)	9.02a ± 0.33	9.46a ± 0.29	9.52a ± 0.18	10.05a ± 0.28
Albumen height (mm)	4.65b ± 0.27	4.66b ± 0.22	4.99b ± 0.25	6.06a ± 0.20
Haugh unit	92.10b ± 1.50	91.92b± 1.09	93.52b ± 1.31	98.32a ± 0.88
Yolk height (mm)	8.97b ± 0.42	9.32b ± 0.23	9.71ab ± 0.39	10.90a ± 0.29
Egg shell thickness (mm)	0.17b ± 0.01	0.21ab ± 0.01	0.22a ± 0.01	0.21ab ± 0.01

Note: Values are presented as mean ± standard deviation. Same letter denotes mean values are not significantly different $p > 0.05$ at the same row.

Higher albumen quality (98.32 ± 0.88) in T4 suggests that EM supplementation improves egg freshness and structural integrity. Similar findings were reported by Neijat et al., (2020) and Utami & Akbar (2025), probiotics specifically *Bacillus subtilis* and *Lactobacillus spp*., improve nutrient absorption, resulting in higher albumen height and quality. Albumen weight and Haugh unit also improved significantly (p<0.05), indicating better egg freshness and albumen quality, with T4 again outperforming other treatments. Yolk height showed a similar trend, with T4 having the highest value (10.90 ± 0.29), suggesting enhanced yolk structure.

The processes underlying this improvement are most likely related to probiotics' ability to promote nitrogen absorption and protein synthesis, both of which are required for albumen formation (Applegate et al., 2010). These findings underline the importance of EM supplementation in enhancing several aspects of egg quality, making it a valuable tool for improving egg production efficiency and satisfying market needs. Enhanced yolk color, albumen height, and shell strength contribute to higher-quality eggs, which are preferred by customers and function better in transport and storage.

The quality features of eggs, such as egg weight, yolk height, and shell thickness, were positively affected by EM supplementation. T4 (1.5% EM) produced the largest eggs (10.05 ± 0.28 g) and thickest yolks (10.90 ± 0.29 mm). These data imply that EM improves protein and nutrition deposition in eggs, resulting in larger and more structurally stable eggs. Probiotics have been proven in studies to improve protein synthesis and nutrition retention, resulting in higher egg weight and yolk height (Siadati et al., 2018; Neijat et al., 2020).

The improvement in shell thickness at T3 (1.0% EM) highlights the role of probiotics in optimizing calcium metabolism for eggshell development. Probiotics like *Lactobacillus spp.* and *Bacillus subtilis* enhance calcium retention, strengthening the shell and reducing breakage rates, which is beneficial for transportation and storage (Duskaev et al., 2020). However, the modest decrease in shell thickness at T4 (1.5% EM) suggests that excessively high EM

concentrations may not be as effective for shell strength. Shell thickness was highest in T3 (1.0% EM), indicating this concentration might optimize shell strength. However, the decrease in shell thickness at T4 (1.5% EM) indicates that too high EM concentrations may affect calcium metabolism.

This result is consistent with previous research demonstrating that excessive probiotic administration might sometimes result in reduced returns in specific metrics (Xiang et al., 2019). The beneficial impacts on egg weight, yolk height, and shell strength shown in this study show that EM could improve both the interior and exterior properties of eggs. These enhancements lead to increased market value and consumer pleasure, making EM a promising addition for poultry husbandry.

4. Conclusion

Based on the findings of this study, Effective Microorganism (EM) supplementation does not improve FCR but significantly improved growth performance at the end of 8th week compared to control group. We could observe EM supplementation positively influenced egg quality by enhancing yolk pigmentation, albumen height, and Haugh unit scores, as well as shell thickness. Future studies should need separating sex among treatments, increase the number of animals and replications. Exploring the application of EM in other poultry species, such as ducks and chickens, could further validate its effectiveness across different farming systems.

Acknowledgement

We were grateful to our colleagues and collaborators for their insightful discussions and feedback that enriched the quality of this work.

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Journal of Smart Farming and Food Security
Universiti Malaysia Sabah Press
e-ISSN: 3122-7090
Volume 2, Issue 1, Pages 38–48 (30 June 2026)
DOI: https://doi.org/10.51200/jsffs.v2i1.7393

Research Article
OPEN ACCESS

Effects of chicken manure dose and Eco Farming spray intensity on growth and ear traits of baby corn (*Zea mays* L.)

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Manuscript received: 21 February 2026 | Accepted: 13 April 2026 | Published online: 24 April 2026

Abstract: Organic nutrient management is important in baby corn because this crop has a short production cycle and requires rapid early growth and timely reproductive development. However, information on the combined use of chicken manure and repeated Eco Farming liquid organic fertilizer spraying under field conditions remains limited. This study evaluated the effects of chicken manure dose, Eco Farming spray intensity, and their interaction on the growth and ear traits of baby corn using a 3 × 3 factorial randomized block design with three replications. Chicken manure was applied at 0, 4.5, and 5.5 kg bed^{-1}, whereas Eco Farming was applied as 0, six, or seven foliar sprays. Plant height and leaf number were recorded at 15, 25, and 35 days after planting (DAP), whereas days to female flowering, husk weight, cob weight, and cob length were measured at harvest. Chicken manure significantly increased plant height at 25 and 35 DAP, whereas Eco Farming had no significant main effect on plant height. Significant interaction effects were detected for plant height at 25 DAP and for leaf number at 15 and 25 DAP, indicating that early vegetative responses depended on the combination of both inputs. Eco Farming significantly accelerated female flowering and increased husk weight and cob length, with seven sprays producing the best response. Cob weight was not significantly affected by either factor or their interaction. Overall, chicken manure more consistently supported vegetative growth, whereas intensified Eco Farming spraying improved flowering earliness and market-relevant ear traits in baby corn.

Keywords: foliar nutrient application, integrated nutrient management, silk emergence, sustainable agriculture

1. Introduction

Maize (*Zea mays* L.) is one of the most widely cultivated cereal crops and remains a strategic commodity for food, feed, and agro-industry in many regions (Erenstein et al., 2022). In addition to grain production, maize also has horticultural value when harvested at an immature stage prior to pollination and kernel filling. At this stage, the developing ear is marketed as baby corn, typically harvested shortly after silk emergence and consumed as a whole tender cob (Yeasmin et al., 2024). Baby corn production is attractive because it offers a short production cycle, diversified market channels, and potential income opportunities for farmers, particularly where intensive or peri-urban cultivation systems are developing.

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Citation: Zahra, F. A., Syamsia, S., Halim, I., Mado, I., & Rosanna, R. (2026). Effects of chicken manure dose and Eco Farming spray intensity on growth and ear traits of baby corn (*Zea mays* L.). Journal of Smart Farming and Food Security, 2(1), 38–48. https://doi.org/10.51200/jsffs.v2i1.7393

DOI: 10.51200/jsffs.v2i1.7393

A distinctive feature of baby corn is its narrow harvest window and reliance on rapid early growth to support ear initiation and elongation within a limited time. Consequently, crop performance is highly sensitive to early vegetative vigor and to nutrient availability during the transition from vegetative growth to reproductive development. Plant height development and leaf formation are important indicators of canopy establishment and photosynthetic capacity that can affect assimilate supply to developing ears. In parallel, quality-related traits such as cob length and husk characteristics influence market acceptance and economic value. Recent evidence suggests that agronomic management can shape not only yield components but also nutritional-quality indicators of baby corn, indicating that production strategies should consider both productivity and market-relevant quality (Haque et al., 2024).

Nutrient management is therefore a key leverage point for improving baby corn performance. While mineral fertilizers can increase productivity, there is growing interest in integrating organic inputs that may support crop nutrition while improving soil quality and long-term fertility. Organic amendments can contribute organic matter and stimulate soil biological activity, potentially enhancing nutrient cycling and availability across the season (Anshori et al., 2022). Among commonly available organic resources, chicken manure is frequently highlighted as a nutrient-rich amendment that supplies essential macronutrients and can promote vegetative growth indicators such as plant height and leaf number in maize systems (Rasool et al., 2023; Essilfie et al., 2024). Improvements in soil physical structure and nutrient retention associated with organic amendments may be particularly relevant for short-duration crops like baby corn, where timely nutrient supply is critical for rapid canopy development.

In addition to soil-applied amendments, liquid organic fertilizer (LOF) and foliar-based nutrient strategies have been explored to address short-term nutrient limitations during periods of high demand. Reported responses in maize depend on fertilizer formulation, dose, timing, and application frequency (Zaki & Ahmed, 2023; Ssemugenze et al., 2025). However, the effectiveness of foliar-applied nutrients depends on their absorption through the leaf surface and subsequent translocation within the plant, processes that remain complex and not fully predictable (Fernández & Brown, 2013). Eco Farming is a liquid organic fertilizer typically applied via repeated spraying, and such practices may influence both vegetative processes and phenological progression. For baby corn, management interventions that support early vigor and synchronize flowering and ear development may translate into improved cob attributes and more consistent harvest scheduling (Yeasmin et al., 2024).

Despite the reported benefits of chicken manure and foliar or liquid fertilizer inputs (Zaki & Ahmed, 2023; Essilfie et al., 2024; Yeasmin et al., 2024), evidence remains limited regarding their combined use under comparable management conditions, particularly in terms of whether they produce additive benefits or interaction effects. This gap is important because soil-applied amendments and repeated foliar applications may act through different pathways: manure can strengthen baseline nutrient supply and soil condition, whereas LOF sprays may provide timely supplementation at critical growth stages. Studies in maize indicate that integrating soil fertilisation with foliar liquid fertilizer can improve nutrient uptake and stabilize yield responses compared with single-input approaches, supporting the plausibility of complementarity or interaction between the two strategies (Adeniyan et al., 2016; Onte et al. 2025). Clarifying these effects is necessary to develop practical and efficient organic nutrient-management options for baby corn production.

Accordingly, this study aimed to evaluate (i) the effect of chicken manure dose, (ii) the effect of Eco Farming spraying intensity, and (iii) their interaction on baby corn growth and ear traits. We hypothesised that responses would vary across manure doses and spraying intensities, and that combined application could generate interaction effects on key vegetative and market-relevant indicators.

2. Materials and Methods

2.1 Study site and experimental period

The field experiment was conducted from January to March 2024 in Dusun Borong Bulo, Bontoala Village, Pallangga District, Gowa Regency, South Sulawesi, Indonesia. The experimental site was located at 5°12'58.15"S, 119°25'41.78"E. Field activities included land preparation, treatment application, planting, crop maintenance, growth observations, and harvest at the baby corn stage.

2.2 Plant materials and inputs

Baby corn seeds of the Exotic Pertiwi F1 variety were used in this study. This variety was selected because Exotic Pertiwi F1 is a superior hybrid sweet corn cultivar that is resistant to rust, leaf blight, and downy mildew, and is well adapted to lowland conditions. The organic inputs tested were chicken manure and Eco Farming liquid organic fertilizer (LOF). The Eco Farming product used was Eco Farming Premium, produced by PT Bandung Eco Sinergi Teknologi (PT BEST), and was applied using a 2-L manual pressure hand sprayer (Torab brand). Chicken manure was applied as dry manure mixed with rice husk used as poultry litter in broiler chicken houses.

2.3 Land preparation and crop establishment

The field was first cleared manually of weeds and crop residues. The soil was then loosened using hand tools, and planting beds were formed. Each bed measured 300 cm × 30 cm (0.90 m^2). Beds were spaced 75 cm apart, while the distance between blocks was 100 cm. Planting holes were prepared manually on each bed according to the designated spacing. Two seeds were dibbled into each planting hole, and seedlings were thinned to one plant per hole at 7 days after planting (DAP). Replanting was carried out when necessary, up to 14 DAP to maintain a uniform plant stand.

2.4 Treatments

The experiment consisted of two factors arranged in a 3 × 3 factorial pattern. Factor A was chicken manure dose: A0 = 0 kg bed^{-1}, A1 = 4.5 kg bed^{-1}, and A2 = 5.5 kg bed^{-1}. Because each bed had an area of 0.90 m^2, these rates are equivalent to approximately 0, 50.0, and 61.1 t ha^{-1}, respectively. The hectare conversion was calculated from the plot area and is numerically correct. These rates represent relatively high-input organic treatments intended to test crop response under intensive organic nutrient supply conditions. Factor E was Eco Farming spray intensity: E0 = no spraying, E1 = six sprays at 5, 10, 15, 20, 25, and 30 DAP, and E2 = seven sprays at 5, 10, 15, 20, 25, 30, and 35 DAP.

2.5 Eco Farming preparation and application

Eco Farming was prepared by dissolving one tube (30 g) of the product in 1 L of water, preferably coconut water or sugarcane water, and allowing the solution to stand for at least 15 min before use as a starter solution. For field spraying, 25–50 mL of the starter solution was mixed with 15–20 L of water. In each application, Eco Farming solution was sprayed at 30 mL $plant^{-1}$ onto the leaves and stems. Based on this volume, the cumulative spray volume was 180 mL $plant^{-1}$ for E1 and 210 mL $plant^{-1}$ for E2 over the full treatment period. Spraying was conducted uniformly on the leaf and stem surfaces until the assigned volume was completely applied.

2.6 Experimental design and plot layout

The study used a 3 × 3 factorial randomized block design with three replications (blocks) to account for field variability (e.g., soil heterogeneity and micro-topography), generating nine

DOI: 10.51200/jsffs.v2i1.7393

treatment combinations per block (27 plots in total). Each plot consisted of one bed measuring 300 cm × 30 cm (bed area = 0.90 m^2). Beds were spaced 75 cm apart, and the distance between blocks was 100 cm. Plants were spaced 30 cm within each bed (in-row) and 75 cm between beds (inter-row), resulting in nine plants per bed and a total population of 243 plants across the experiment. Treatment combinations were randomly assigned to beds within each block. Plant spacing was set to reflect standard baby corn management practices (Saptorini & Sutiknjo, 2021; Jena et al., 2025).

2.7 Crop management

Chicken manure was applied according to treatment during plot preparation and placed in the planting holes before sowing. Eco Farming was sprayed according to the assigned treatment schedule. Routine crop management included manual weeding and irrigation according to field conditions. No pesticide was applied during the experiment.

2.8 Observations and measurements

Plant height and leaf number were recorded at 15, 25, and 35 DAP. Plant height was measured from the soil surface to the tip of the highest leaf, while leaf number was counted as the number of fully expanded leaves per plant. Days to female flowering were recorded as the number of days from planting to first silk emergence. Husk weight, cob weight, and cob length were measured at harvest. Harvesting was carried out at the baby corn stage, namely 1–3 days after silk emergence, when the ears were still tender and kernels had not yet developed. Measurements were taken from five centrally located plants per plot to minimize border effects. The sample plants were labeled at the beginning of the observation period.

2.9 Statistical analysis

All variables were analyzed using two-way analysis of variance (ANOVA) under a randomized block design, with chicken manure dose and Eco Farming spray intensity as fixed factors and block as a random factor. When significant main effects or interactions were detected, treatment means were compared using Duncan's Multiple Range Test (DMRT) at the 5% significance level.

3. Results and Discussion

3.1 Plant height

Plant height increased progressively from 15 to 35 DAP, indicating continued vegetative development across treatment combinations. At 15 DAP, no significant effects were detected for chicken manure dose, Eco Farming spray intensity, or their interaction. The overall model was not significant ($F = 1.535$; $p = 0.214$), and the main effects of chicken manure and Eco Farming were also not significant ($p = 0.727$ and $p = 0.935$, respectively). The interaction between both factors was close to the significance threshold but remained non-significant at the 5% level ($F = 2.875$; $p = 0.053$). These results indicate that treatment effects on plant height had not yet become clearly expressed during the early growth stage.

At 25 DAP, the overall model became significant ($F = 2.716$; $p = 0.037$). Chicken manure significantly affected plant height ($F = 3.590$; $p = 0.049$), whereas Eco Farming spray intensity did not show a significant main effect ($F = 0.168$; $p = 0.847$). The interaction between chicken manure and Eco Farming was significant ($F = 3.553$; $p = 0.026$), indicating that plant height at this stage depended on the specific combination of the two treatments (Figure 1). Descriptively, the highest plant height was recorded in A1E2, followed by A2E1, A2E0, and A1E0, whereas A0E0 produced the lowest value. This pattern suggests that plant height during the mid-vegetative stage responded to the synchrony between soil-applied manure and foliar Eco Farming application.

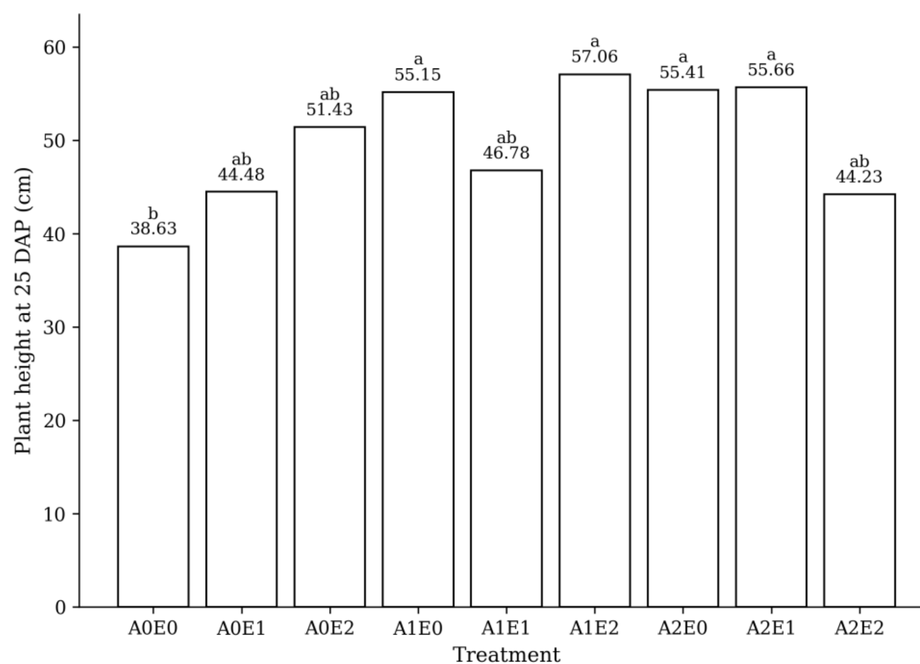

Figure 1. Plant height at 25 days after planting (DAP) under factorial combinations of chicken manure dose and Eco Farming spray intensity. Bars represent treatment means. Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test at $\alpha = 0.05$.

At 35 DAP, the overall model remained significant ($F = 2.560$; $p = 0.047$). Chicken manure continued to have a significant effect on plant height ($F = 4.213$; $p = 0.032$), while Eco Farming remained non-significant ($F = 0.220$; $p = 0.805$). The interaction term was not significant at $\alpha = 0.05$, although it remained close to the threshold ($F = 2.903$; $p = 0.051$). This indicates that, at the later vegetative stage, plant height was influenced mainly by chicken manure rather than by Eco Farming spray intensity or their interaction.

Overall, these findings show that chicken manure was the more consistent factor affecting plant height, particularly at 25 and 35 DAP, whereas Eco Farming did not produce a significant main effect across observation times. The significant interaction observed at 25 DAP indicates that treatment combinations were particularly important during the mid-vegetative stage, when plant growth was likely more sensitive to the synchrony between soil-applied and foliar-applied nutrient inputs. Similar responses have been reported in maize under integrated nutrient management, where organic amendments improve baseline nutrient availability and support vegetative growth (Essilfie et al., 2024).

3.2 Leaf number

Leaf number was evaluated at 15, 25, and 35 DAP to assess canopy establishment during vegetative growth. At 15 DAP, the overall model was significant ($F = 2.720$; $p = 0.037$), although the main effects of chicken manure and Eco Farming were not significant ($p = 0.126$ and $p = 0.837$, respectively). In contrast, the interaction between chicken manure and Eco Farming was significant ($F = 4.187$; $p = 0.014$), indicating that early leaf development depended on the specific combination of both factors rather than on either factor alone (Figure 2). The highest leaf number at this stage was observed in A2E1 and A1E0, whereas the lowest values occurred in A2E2, A0E1, and A0E0.

At 25 DAP, the overall model was not significant at the 5% level ($F = 2.026$; $p = 0.102$).

DOI: 10.51200/jsffs.v2i1.7393

However, the interaction between chicken manure and Eco Farming remained significant (F = 3.288; p = 0.034), whereas the main effects of chicken manure and Eco Farming were not significant (p = 0.313 and p = 0.753, respectively). The highest leaf number was recorded in A2E0 and A2E1, while the lowest values were observed in A0E0 and A2E2. This result indicates that leaf formation at the mid-vegetative stage was affected more by the combined treatment than by either factor individually.

At 35 DAP, no significant effects were detected. The overall model was not significant (F = 1.112; p = 0.401), and neither chicken manure, Eco Farming, nor their interaction showed significant effects (p = 0.821, p = 0.859, and p = 0.131, respectively). This suggests that differences in leaf number among treatment combinations became less apparent at the later vegetative stage (Figure 2).

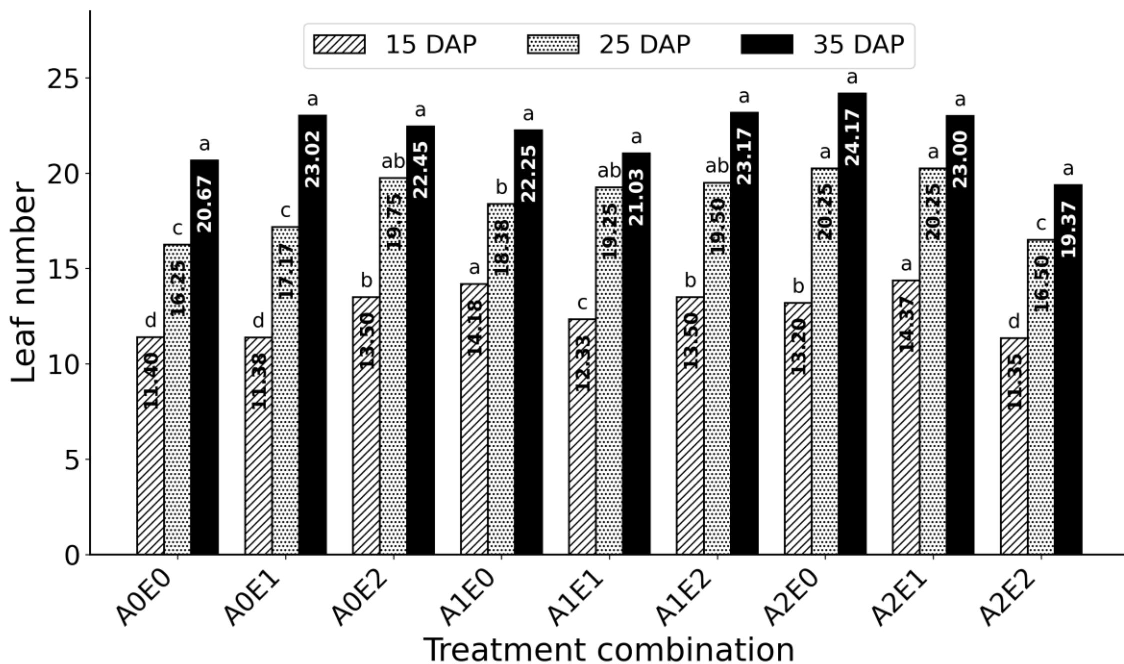

Figure 2. Leaf number at 15, 25, and 35 days after planting (DAP) under factorial combinations of chicken manure dose and Eco Farming spray intensity. Bars represent treatment means. Means followed by the same letter within the same observation time are not significantly different at α = 0.05.

3.3 Days to female flowering

Days to female flowering were significantly influenced by Eco Farming spray intensity, whereas chicken manure and the interaction between both factors were not significant. The two-way ANOVA showed that the overall model was not significant at the 5% level (F = 1.895; p = 0.124), but Eco Farming had a significant main effect on days to flowering (F = 5.147; p = 0.017). In contrast, chicken manure had no significant effect (F = 0.095; p = 0.910), and the interaction between chicken manure and Eco Farming was also not significant (F = 1.168; p = 0.358).

Duncan's multiple range test showed that E2 had the lowest mean value for days to flowering (53.72 days), followed by E1 (54.19 days) and E0 (54.42 days), indicating that the seven-spray treatment accelerated female flowering compared with the lower spray intensities (Figure 3). By contrast, the means for chicken manure levels were very similar and remained within the same homogeneous subset, confirming the absence of a significant manure effect.

Earlier flowering is agronomically important in baby corn because harvest timing is directly linked to silk emergence. The earlier flowering observed under E2 suggests that

repeated Eco Farming application may have improved plant physiological status or nutrient availability during the transition from vegetative to reproductive growth, allowing plants to reach flowering slightly sooner. Similar benefits of timely foliar nutrient supply in maize have been reported previously (Ssemugenze et al., 2025).

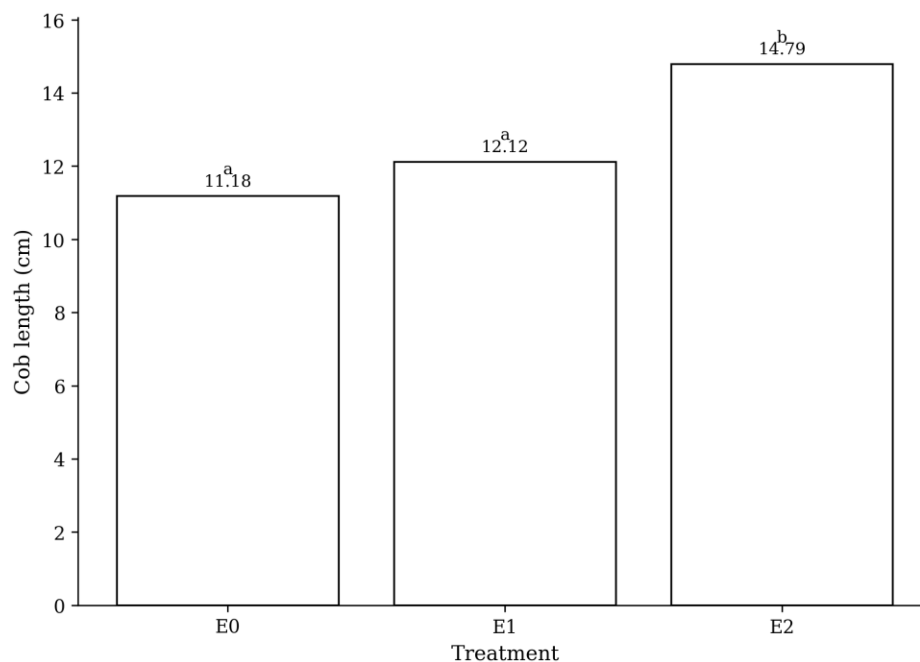

Figure 3. Days to female flowering under Eco Farming treatments. Bars represent treatment means. Lower values indicate earlier flowering. Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test at α = 0.05.

3.4 Husk weight

Husk weight was significantly influenced by Eco Farming spray intensity, whereas chicken manure and the interaction between both factors were not significant. The two-way ANOVA showed that the overall model was marginal at the 5% level ($F = 2.324$; $p = 0.066$). Chicken manure did not significantly affect husk weight ($F = 1.215$; $p = 0.320$), and the interaction between chicken manure and Eco Farming was also not significant ($F = 1.405$; $p = 0.272$). In contrast, Eco Farming had a significant main effect on husk weight ($F = 5.268$; $p = 0.016$), indicating that the frequency of foliar application played an important role in husk development.

Duncan's multiple range test showed that E2 produced the highest husk weight (137.39 g), whereas E0 and E1 produced lower values of 98.00 g and 110.64 g, respectively (Figure 4). This result indicates that the seven-spray treatment was superior to the lower spray intensities in promoting husk development. By contrast, the chicken manure means remained within the same homogeneous subset, confirming that manure dose did not significantly affect husk weight.

These findings suggest that husk development in baby corn was more responsive to repeated Eco Farming application than to chicken manure dose. Because baby corn is harvested at an immature stage, husk weight may reflect the effectiveness of nutrient supply during the early reproductive period, when ear-associated tissues are still actively developing. This interpretation is in line with previous studies showing that nutrient management can improve baby corn ear traits and quality-related characteristics (Haque et al., 2024; Yeasmin et al., 2024).

DOI: 10.51200/jsffs.v2i1.7393

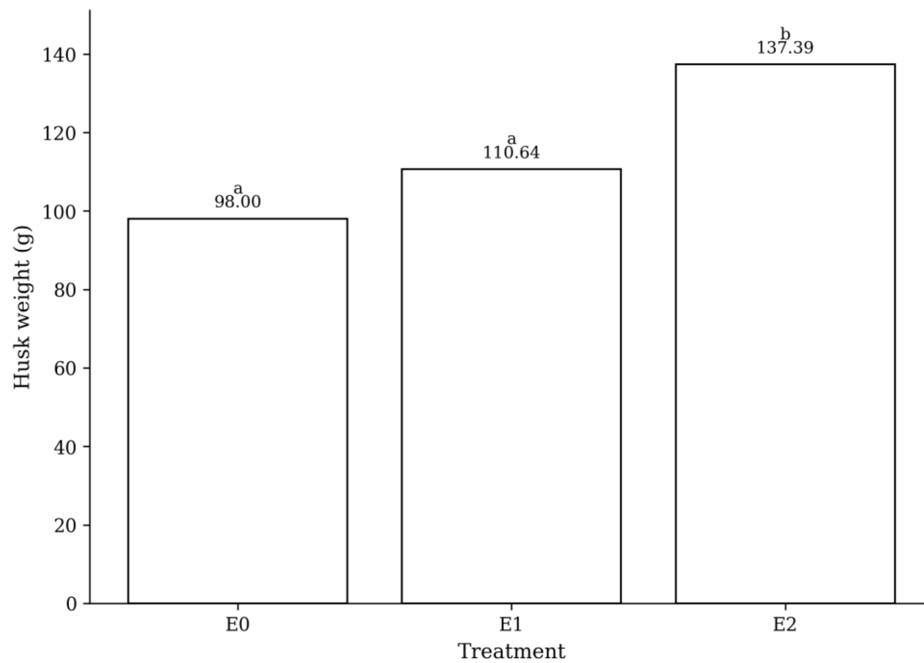

Figure 4. Husk weight under Eco Farming treatments. Bars represent treatment means. Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test at α = 0.05.

3.5 Cob weight

Cob weight did not show a significant response to chicken manure, Eco Farming, or their interaction. The two-way ANOVA showed that the overall model was not significant (F = 1.473; p = 0.235). The main effect of chicken manure was not significant (F = 0.861; p = 0.439), and the interaction between chicken manure and Eco Farming was also not significant (F = 0.849; p = 0.513). Eco Farming showed a tendency toward significance (F = 3.333; p = 0.059), but this effect did not reach the 5% significance threshold (Figure 5).

These results indicate that cob weight was relatively stable across treatment combinations under the conditions of the present study. One plausible explanation is that baby corn is harvested at a very early developmental stage, namely 1–3 days after silk emergence, before substantial kernel filling and dry matter accumulation can occur. As a result, cob weight may be less sensitive to nutrient management treatments than traits such as cob length or husk weight, which can respond earlier during ear formation. Similar observations have been noted in baby corn studies where some ear-quality traits responded more clearly than fresh cob mass under short harvest windows (Haque et al., 2024; Jena et al., 2025).

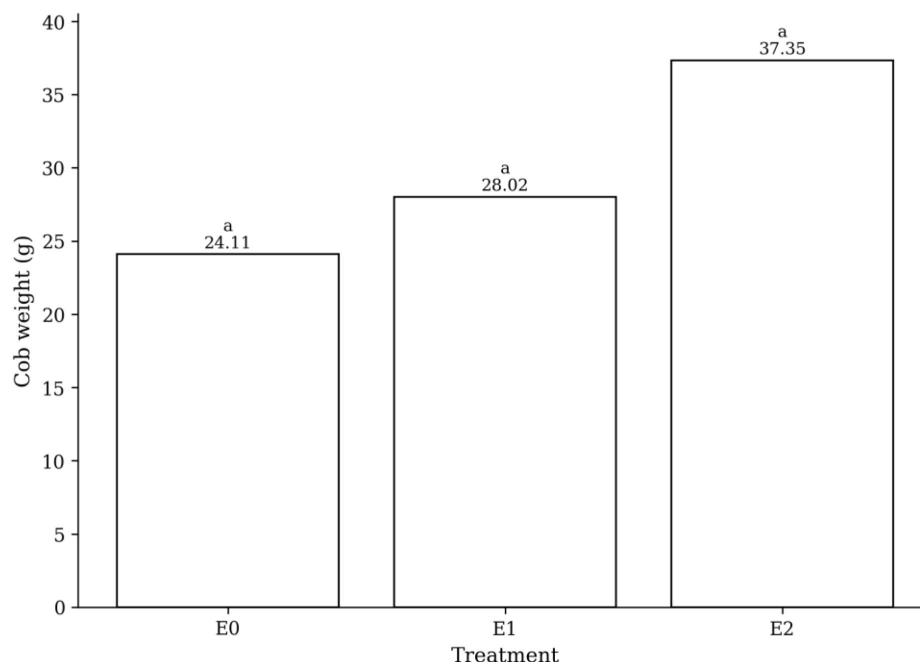

Figure 5. Cob weight under Eco Farming treatments. Bars represent treatment means. No significant difference among treatments was detected by analysis of variance at $\alpha = 0.05$.

3.6 Cob length

Cob length was significantly affected by Eco Farming spray intensity, whereas chicken manure and the interaction between both factors were not significant at the 5% level. The two-way ANOVA showed that the overall model was significant ($F = 3.594$; $p = 0.012$). Eco Farming had a significant main effect on cob length ($F = 7.034$; $p = 0.006$), indicating that differences in spray intensity contributed substantially to variation in cob elongation. Chicken manure showed a near-significant tendency ($F = 3.332$; $p = 0.059$), but the effect did not reach the 5% significance threshold. The interaction between chicken manure and Eco Farming was not significant ($F = 2.005$; $p = 0.137$), suggesting that the response of cob length to Eco Farming was relatively consistent across manure levels (Figure 6).

Duncan’s multiple range test supported this pattern. For Eco Farming, E2 produced the greatest cob length (14.79 cm), whereas E0 and E1 produced shorter cobs of 11.18 and 12.12 cm, respectively. This confirms that the seven-spray treatment was associated with the longest cobs. Although chicken manure showed a tendency to increase cob length, its main effect was not statistically significant at $\alpha = 0.05$.

These findings indicate that cob elongation in baby corn was more responsive to Eco Farming intensity than to chicken manure dose. Because cob length is a key market-related quality trait in baby corn, the significant response under E2 suggests that more frequent foliar application improved conditions for ear development during the early reproductive stage. Comparable improvements in baby corn yield and quality traits under enhanced nutrient management have been reported previously (Jena et al., 2025; Yeasmin et al., 2024).

Taken together, the results show trait-specific responses. Chicken manure more consistently supported vegetative growth, especially plant height, whereas Eco Farming spray intensity had clearer effects on flowering time and market-relevant ear traits such as husk weight and cob length. The significant early-stage interactions for plant height and leaf number further indicate that the effectiveness of one input depended on the level of the other, particularly during crop establishment. Overall, these results support the use of integrated nutrient management to optimize different stages of baby corn development (Adeniyan et al., 2016; Essilfie et al., 2024).

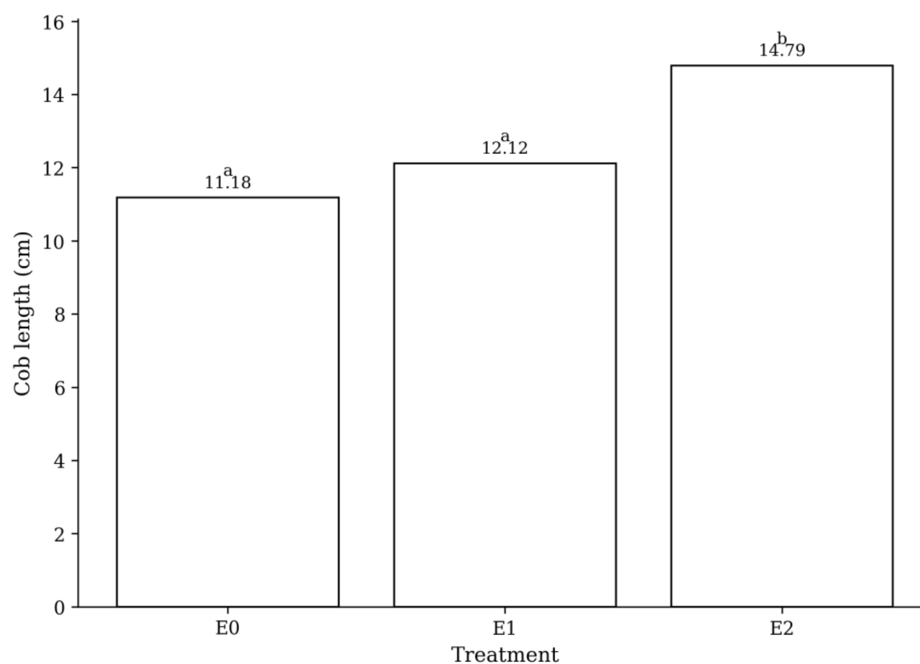

Figure 6. Cob length under Eco Farming treatments. Bars represent treatment means. Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test at α = 0.05.

4. Conclusion

Chicken manure and Eco Farming affected baby corn performance in different ways. Chicken manure more consistently influenced vegetative growth, particularly plant height at 25 and 35 DAP. Eco Farming spray intensity did not significantly affect plant height, but it significantly accelerated female flowering and increased husk weight and cob length, with seven sprays producing the best response. Interaction effects were important during the early to mid-vegetative stage, as shown by significant interactions on plant height at 25 DAP and on leaf number at 15 and 25 DAP. Cob weight did not respond significantly to either factor or their interaction, likely because baby corn was harvested at an early stage before substantial biomass accumulation occurred. Overall, the results indicate that chicken manure was more important for supporting vegetative growth, whereas intensified Eco Farming spraying was more effective for improving flowering earliness and market-relevant ear traits. These findings provide practical support for integrating soil-applied chicken manure with repeated foliar Eco Farming application in baby corn cultivation.

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Journal of Smart Farming and Food Security
Universiti Malaysia Sabah Press
e-ISSN: 3122-7090
Volume 2, Issue 1, Pages 49–59 (30 June 2026)
DOI: https://doi.org/10.51200/jsffs.v2i1.7399

Research Article
OPEN ACCESS

Effect of extenders supplemented with varying levels of royal jelly on caprine semen quality at different chilling storage times

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Manuscript received: 22 February 2026 | Accepted: 18 May 2026 | Published online: 8 June 2026

Abstract: This study investigated the impact of royal jelly (RJ) supplementation on the quality of caprine semen during chilled storage. Twelve semen samples were collected from three mature bucks and divided into four treatment groups. Each group was diluted using a tris–citric acid–fructose–egg yolk (TCFY) extender supplemented with different concentrations of RJ (0%, 0.05%, 0.10%, and 0.15%). Semen quality parameters, including total motility, progressive motility, viability, morphology, sperm concentration, semen volume, and pH, were evaluated at 0, 24, and 48 hours of storage at 4 °C. The results demonstrated that RJ supplementation influenced semen quality in a concentration-dependent manner. Low to moderate concentrations of RJ (0.05% and 0.10%) showed a partial protective effect by maintaining higher sperm membrane viability at extended storage (48 h) compared to the highest concentration, but did not consistently improve motility parameters. In contrast, the highest concentration (0.15%) resulted in a significant reduction ($p < 0.05$) in sperm motility and viability, particularly after 48 hours of storage. Sperm morphology was generally unaffected by RJ supplementation, while semen volume remained stable and pH showed a slight decline with increased storage duration. Overall, RJ did not improve total or progressive motility compared to the T0, but exhibited limited concentration-dependent effects on sperm viability. These findings indicate that RJ supplementation cannot be considered a general enhancer of caprine semen quality during chilled storage, as its effects vary depending on concentration and parameter measured. Therefore, its application as a semen extender additive requires careful optimisation, particularly to avoid potential inhibitory effects at higher concentrations. Further studies are recommended to evaluate its effects on fertilisation success and reproductive performance under field conditions.

Keywords: caprine semen, chilled storage, royal jelly, semen extender, sperm motility

1. Introduction

Artificial insemination (AI) is a key technique in goat breeding, allowing for the effective use of high-quality male genetics to boost productivity and genetic diversity across the livestock industry. The effectiveness of AI largely depends on how well semen quality is maintained during storage, particularly in terms of motility, structural integrity, and fertilising ability. In

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Citation: Mojuwil, W. G., Abdullah, N. H. S., Janong, N. S., Rahman, M. M., Kobun, R., & Utamy, R. F. (2026). Effect of extenders supplemented with varying levels of royal jelly on caprine semen quality at different chilling storage times. Journal of Smart Farming and Food Security, 2(1), 49–59. https://doi.org/10.51200/jsffs.v2i1.7399

tropical climates like Malaysia, especially in states like Sabah, improving semen preservation methods is crucial to prevent heat-related damage to sperm and to ensure ongoing improvements in goat production and food security (Batool et al., 2024). Semen extenders are essential solutions designed to maintain sperm viability during storage by shielding cells from cold shock, microbial contamination, and oxidative damage. However, caprine sperm are uniquely sensitive to preservation stresses compared to other species. Goat semen contains bulbourethral gland secretions, specifically phospholipase A, which interacts with egg-yolk-based extenders to produce toxic fatty acids that compromise sperm membranes (Pintus & Ros-Santaella, 2021).

During chilled storage at 4°C to 5°C, sperm cells undergo "cold shock," which triggers an overproduction of Reactive Oxygen Species (ROS). This biochemical shift leads to lipid peroxidation, which damages the polyunsaturated fatty acids in the sperm plasma membrane, resulting in a decline in motility and DNA stability (Mocé et al., 2020). To counter these issues, natural additives with antioxidant properties have gained interest as sustainable alternatives to synthetic supplements. Royal Jelly (RJ), a substance secreted by nurse bees, is well known for its rich content of proteins, vitamins, and strong antioxidants such as flavonoids and phenolic compounds (Peykova-Shapkova et al., 2024). These bioactive components, particularly the unique fatty acid 10-hydroxy-2-decenoic acid (10-HDA), provide RJ with antimicrobial and anti-inflammatory properties that are thought to protect sperm from oxidative damage during storage (Moradi et al., 2013).

Despite these benefits, excessive use of synthetic additives has caused concerns regarding chemical residues and long-term sperm toxicity. Moreover, maintaining the quality of goat semen during chilled storage continues to be a major challenge because the quality still declines sharply after 24 to 48 hours, limiting the success of AI programs. Previous research on RJ use in caprine species has been inconsistent, with limited scientific guidance on optimal concentrations. Specifically, the optimal amount of RJ and its impact across different chilling durations remain uncertain; while low doses may protect the cell, high concentrations may alter the osmotic balance or cause cytotoxic effects that accelerate cell death (Coskun Cetin & Karaca, 2023). This lack of precise protocol has reduced breeders' confidence in using RJ as a reliable and sustainable biological additive.

Therefore, this study aimed to evaluate the effects of varying rates of RJ supplementation in extenders on the quality and functional integrity of caprine semen. The study sought to determine the most effective concentration for improving sperm motility, viability, and membrane integrity across different chilled storage times. Specifically, the objectives were: (i) to determine the effects of different concentrations of RJ (0.05%, 0.10%, and 0.15%) on caprine sperm motility, morphology, and viability; and (ii) to assess the impact of these levels on sperm membrane integrity over 0, 24, and 48 hours of storage.

2. Materials and Methods

2.1 Study location

This research was conducted at the Faculty of Sustainable Agriculture, University Malaysia Sabah, Sandakan, Sabah.

2.2 Semen collection

Three sexually mature bucks (approximate 2 years old) under intensive management at FPL were used. Semen was collected biweekly for 8 weeks, then pooled and divided into four treatments (T0–T3). The artificial vagina (AV) was used with the temperature of approximately 45 °C before being closed with a rubber stopper. The open side of the AV was connected with a 15 mL graduated semen collecting tube. The lubricant was placed at the AV before collection took place.

2.3 Preparation of extender

The T0 extender was prepared using the tris-citric acid-fructose-egg yolk (TCFY) formula, which consisted of 3.028 g of tris, 1.675 g of citric acid, 1.250 g of fructose, 20 mL of egg yolk, and 0.1 g of penicillin (Ng et al., 2022). First, the tris, citric acid, fructose, and penicillin were dissolved in approximately 70 mL of distilled water and stirred on a hot plate for 10 minutes. The egg yolk was then added to the same beaker, and the volume was adjusted to 100 mL with distilled water. The mixture was stirred again on a hot plate for another 10 minutes. The resulting TCFY solution was filtered first using filter paper and then using a 0.45 µm syringe filter. After filtration, the extender was stored in a chiller at 4°C until further use.

Royal jelly group (TCFYRJ): To prepare the RJ extender, 3.028 g of tris, 1.675 g of citric acid, 1.250 g of fructose, and 0.1 g of penicillin were dissolved in approximately 70 mL of distilled water. The solution was stirred on a hot plate for 10 minutes. Royal jelly was added at the appropriate concentrations (0.05 g, 0.10 g, and 0.15 g) to achieve final concentrations of 0.05% (T1), 0.1% (T2), and 0.15% (T3), respectively. The mixture was stirred thoroughly to ensure the RJ is fully dispersed or dissolved. Afterward, 20 mL of egg yolk was added to the same beaker, and the final volume was adjusted to 100 mL using distilled water. The solution was stirred again on a hot plate for another 10 minutes using gentle heat to avoid protein denaturation. The final extender was first filtered using filter paper and subsequently passed through a 0.45 µm syringe filter. While some loss of larger bioactive components in RJ (e.g., certain proteins and lipid-associated fractions) may occur during filtration, the selected pore size was used to ensure sterility while retaining most low–molecular-weight bioactive compounds. The prepared extender was stored at 4°C until use.

2.4 Semen dilution

Six percent glycerol was prepared by mixing 0.6 mL glycerol with 9.4 mL distilled water. For each treatment, 10 mL of semen extender was mixed with 5 mL of 6% glycerol, 4 mL of TCFY (T0) or TCFYRJ (T1–T3), and 1 mL of semen, resulting in a final glycerol concentration of approximately 2%. This concentration was selected based on previous study (Sabri et al., 2024), indicating that lower glycerol levels can reduce osmotic and toxic effects on sperm while still providing adequate cryoprotection.

2.5 Experimental design

All semen samples were diluted with either TCFY (T0) or TCFY supplemented with RJ (TCFYRJ: T1, T2, and T3). The diluted semen samples were equilibrated in a water bath at 25°C for 30 minutes before being transferred to a refrigerator at 4°C for storage. The samples were stored for 0, 24, and 48 hours prior to semen analysis. For evaluation, chilled semen samples were rewarmed in a water bath at approximately 37 °C for 30 seconds before sperm quality assessments were conducted.

The experiment was arranged in a 4×3 factorial experiment under a Completely Randomized Design (CRD), consisting of four extender treatments (T0: 0.0% RJ; T1: 0.05% RJ; T2: 0.1% RJ, and T3: 0.15% RJ) and three storage durations (0, 24 h, and 48 h). Each treatment combination was considered an experimental unit and was replicated three times, resulting in a total of 36 experimental units.

2.6 Physical evaluation

Semen volume was measured using a 1 mL syringe, where the gel mass was removed for more accurate results (El-Hanoun et al., 2014). Semen pH was measured using universal pH paper, where the colour of the paper indicated the pH of the semen (Khadr et al., 2015).

2.7 Sperm evaluation

2.7.1 Assessment of sperm count

Sperm concentration was calculated using a hemacytometer (WHO, 2010). The fresh semen was mixed with semen dilution fluid. Semen dilution fluid consists of 500 mL of distilled water, 5 mL of 35–40% formaldehyde, and 25g of sodium bicarbonate. About 380 µL of semen dilution fluid and 20 µL of diluted semen was mixed using a pipette. To enable capillary action to draw the cell suspension between the chambers, the mixture was gradually loaded underneath the coverslip. Ten times objective on a compound microscope was set on centre of the hemacytometer grid lines. Sperm cells were counted manually in one set of 16 squares, and counting was continued until all four sets of 16 corner squares had been evaluated. The measurement analysis was according to the following formula: Average of sperm count in all 4 sets × semen dilution × 10^6 = Sperm cell × 10^6 mL^{-1}.

2.7.2 Assessment of sperm viability

Semen viability was analysed using the eosin-nigrosine stain. An eosin-nigrosine stain was performed to differentiate the live and dead sperm. Live sperm do not take up the eosin stain and appear white in colour, whereas dead sperm absorb the stain and appear reddish due to the loss of membrane integrity (El-Hanoun et al., 2014). About 50 mL of distilled water and 0.5g of eosin powder was mixed in a beaker to make 1% of the eosin stain. To ensure that all the eosin powder will thoroughly be dissolved in the distilled water, the mixture was mixed uniformly using a glass rod. A drop of diluted semen was placed on a glass slide. Next, two drops (100µl) of 10% nigrosine were added along with a drop (50µl) of 1% eosin to the same slide. Another clean slide was taken and smeared. The smear was left to dry naturally. The slide was examined under a compound microscope (Biological Compound Microscope, View Solution Inc) with a 40× objective lens, the slide. Three measurements were made, each with a count of 200 sperm (Ng et al., 2022).

2.7.3 Assessment of sperm motility

About 3 µL of diluted semen was put on the warm glass slide. A coverslip was put on the warm slide. The glass slide was observed under a compound microscope (Biological Compound Microscope, View Solution Inc) with a 40X objective. Three measurements were made, each with a count of 200 sperm (Ng et al., 2022). The sperm was graded based on its movement. There were four grades of sperm motility as shown in Table 1.

Table 1. Grades of sperm motility (WHO, 2010).

Grade	Description
A	Rapid progressive motility, which sperm can swim fast in a straight line.
B	Slow or sluggish or non-linear progressive motility (the sperm move forward but in a curved or crooked motion).
C	Non-progressive motility or vibrate, (the sperm move their tail but not moving forward).
D	Immotile (fail to move at all).

2.7.4 Assessment of sperm morphology

Sperm abnormalities were observed by using the eosin-nigrosine stain. Eosin-nigrosine staining was performed to differentiate the sperm morphology. About 50 mL of distilled water and 0.5g of eosin powder was mixed in a beaker to make 1% of the eosin stain. To ensure that all the eosin powder will thoroughly be dissolved in distilled water, the mixture was mixed uniformly using a glass rod. A drop of diluted semen was placed on a glass slide. Next, add two drops of 10% nigrosine along with a drop of 1% eosin to the same slide. They then were mixed and

DOI: 10.51200/jsffs.v2i1.7399

waited for 10 seconds. Another clean slide was taken and smeared. The smear was left to air-dry naturally. The slide was examined under a compound microscope using a 40× objective lens. Three measurements were made, each involving a count of 200 sperm (Ng et al., 2022).

2.8 Statistical analysis

Data were analysed by using two-way analysis of variance (ANOVA) in SPSS software to evaluate the main effects of extender treatment and storage duration. The interaction effect between extender treatment and storage duration was not included in the statistical model. Differences among means were compared using Tukey's test at $p < 0.05$.

3. Results and Discussion

3.1 Sperm quality

Semen volume and pH are presented in Table 2. The mean semen volume was 0.4 mL ejaculation^{-1}, indicating moderate variability among samples. The semen pH remained constant at 7.0, suggesting a stable and neutral environment across ejaculates. Sperm concentration during chilled storage is shown in Table 3. At 0 hours, the mean concentration was 521.7 × 10^6 mL^{-1}. After 24 hours of storage, the concentration decreased to 325.7 × 10^6 mL^{-1}, representing approximately a 37% reduction. A further decline was observed at 48 hours, reaching 164.7 × 10^6 mL^{-1}. Overall, sperm concentration progressively decreased with increasing storage time. The relatively high standard deviations at each time point indicate considerable variability among samples.

Table 2. The semen volume and semen pH of caprine.

Parameter	Mean ± standard deviation
Volume (mL ejaculation^{-1})	0.4 ± 0.3
pH	7.0 ± 0.0

Table 3. The sperm concentration (×10^6 mL^{-1}).

Chilling times	Mean ± standard deviation
0 hour	521.7 ± 200.1
24 hours	325.7 ± 101.1
48 hours	164.7 ± 91.5

Table 4. Percentage (mean ± SD) of the total sperm motility parameters in a different concentration of royal jelly extender regardless of chilled storage hour.

Chilling times	Royal jelly concentration				p-value
	T0	T1	T2	T3	
0 hour	$51.0^{a} \pm 8.1$	$40.1^{ab} \pm 11.8$	$26.0^{bc} \pm 5.3$	$21.9^{c} \pm 0.3$	0.006
24 hours	23.5 ± 1.6	20.1 ± 3.2	29.7 ± 1.2	13.5 ± 14.2	0.134
48 hours	$17.5^{a} \pm 0.8$	$14.7^{b} \pm 0.6$	$6.8^{c} \pm 0.3$	$4.8^{d} \pm 0.2$	<0.001

SD, Standard deviation; T0, Tris-citrate-fructose-yolk, T1, Tris-citrate-fructose-yolk-royal jelly 0.05%; T2, Tris-citrate-fructose-yolk-royal jelly 0.10%; T3, Tris-citrate-fructose-yolk-royal jelly 0.15%. Means with different superscripts in a same row differ significantly ($p < 0.05$).

3.2 Total sperm motility (%)

Contrary to the expected beneficial effects, RJ supplementation did not consistently improve total sperm motility compared to the control (Table 4). At 0 and 48 hours of storage, the control (TCFY) exhibited significantly higher motility than all RJ-treated groups ($p < 0.05$), indicating a detrimental or non-beneficial effect of RJ at the tested concentrations. Although T2 showed a

numerically higher value at 24 hours, the difference was not statistically significant ($P > 0.05$), suggesting no clear advantage of RJ supplementation during storage.

Motility is utilised as an initial and reliable indicator of sperm damage during storage, particularly under conditions of cold or extended storage. The reduced motility observed in RJ-treated groups may be associated with suboptimal concentrations or possible interactions between RJ components and the extender, which could negatively affect sperm membrane stability or metabolic activity.

Sperm are highly vulnerable to oxidative stress due to their limited cytoplasmic antioxidant defences and membranes rich in polyunsaturated fatty acids (Agarwal et al., 2019; Pintus & Ros-Santaella, 2021). Excessive reactive oxygen species generated during semen handling and storage attack membrane lipids and axonemal proteins, leading to impaired flagellar motion. Royal jelly contains flavonoids, phenolic acids, peptides, vitamins, and unique fatty acids such as 10-hydroxy-2-decenoic acid, all of which possess strong free radical–scavenging activity (El-Guendouz et al., 2020; Peykova-Shapkova et al., 2024).

Despite these known antioxidant properties, the present findings suggest that the inclusion levels of RJ used in this study were not optimal to confer protective effects on sperm motility. It is possible that higher concentrations may exert pro-oxidant effects or alter osmotic balance, thereby impairing sperm function. Previous studies have reported improved motility with RJ supplementation in ram, goat, and boar semen (Moradi et al., 2013; Iljenkaite et al., 2020; Coskun Cetin & Karaca, 2023); however, the discrepancy with the current results may be attributed to differences in species, extender composition, dosage, or storage conditions. Therefore, the present findings do not support a positive effect of RJ on sperm motility under the conditions tested and highlight the need for further optimisation of its inclusion level.

3.3 Progressive motility (%)

Progressive motility was not consistently improved by RJ supplementation, and in most cases showed comparable or lower values than the control, particularly at extended storage periods (Table 5). Although a significant difference was observed at 0 hour ($p < 0.05$), this effect was not sustained during storage, and overall, RJ supplementation did not demonstrate a stable beneficial effect on progressive motility.

Progressive motility indicates successful forward propulsion, a crucial factor for sperm mobility within the female reproductive system and subsequent fertilisation (Villani et al., 2022). Therefore, it is considered a key indicator of semen quality. The sperm midpiece's mitochondrial ATP production is essential for progressive movement. Oxidative stress damages mitochondrial membranes, impedes electron transport, and diminishes ATP availability, thereby causing sluggish or non-progressive sperm movement (Carrageta et al., 2023).

Although RJ is known for its antioxidant properties, the inconsistent and generally reduced progressive motility observed in RJ-treated groups suggests that it did not effectively support mitochondrial function under the present conditions. Instead, the decline in motility at higher concentrations and longer storage time may indicate possible osmotic imbalance or metabolic stress induced by RJ supplementation.

The marked reduction in progressive motility, particularly at higher RJ concentration (T3), further suggests that excessive supplementation may negatively affect sperm function rather than provide protection. This indicates that RJ may have disrupted the extender's physiological balance, affecting sperm energy metabolism and motility.

While previous studies have reported beneficial effects of antioxidant supplementation on sperm motility, such effects are strongly dose-dependent and highly species- and extender-specific. Over-supplementation may disturb redox homeostasis and impair mitochondrial activity. Similar dose-dependent responses have been reported for RJ, green tea extract, quercetin, and crocin in semen extenders (Amini et al., 2019; Susilowati et al., 2022; Batool et

al., 2024). However, in the present study, RJ supplementation did not provide consistent protective effects and instead showed reduced performance at higher levels and longer storage duration.

Table 5. Percentage (mean ± SD) of the progressive motility in a different concentration of royal jelly extender.

Chilling times	Royal jelly concentration				p-value
	T0	T1	T2	T3	
0 hour	$6.3^{b} \pm 1.4$	$13.2^{a} \pm 1.8$	$6.1^{b} \pm 2.6$	$4.3^{b} \pm 0.1$	0.005
24 hours	$5.9^{a} \pm 0.2$	$3.9^{a} \pm 0.4$	$4.1^{a} \pm 0.1$	$1.3^{b} \pm 2.3$	0.010
48 hours	$3.9^{a} \pm 0.1$	$3.0^{b} \pm 0.1$	$1.1^{c} \pm 0.1$	$0.0^{d} \pm 0.0$	<0.001

SD, Standard deviation; T0, Tris-citrate-fructose-yolk, T1, Tris-citrate-fructose-yolk-royal jelly 0.05%; T2, Tris-citrate-fructose-yolk-royal jelly 0.10%; T3, Tris-citrate-fructose-yolk-royal jelly 0.15%. Means with different superscripts in a same row differ significantly ($p < 0.05$).

3.4 Sperm viability

Royal jelly supplementation influenced sperm viability in a concentration-dependent manner (Table 6). At 48 hours of chilled storage, semen supplemented with low to moderate concentrations of RJ (T1 and T2) showed significantly higher percentages of live sperm and lower percentages of dead sperm compared with the control and the highest RJ concentration (T3) ($p < 0.05$). In contrast, the highest concentration of RJ (0.15%) resulted in the lowest percentage of live sperm and the highest percentage of dead sperm, indicating a detrimental effect at excessive concentrations. Sperm viability, which reflects plasma membrane integrity, is negatively affected during storage due to oxidative stress, osmotic imbalance, and cold shock (Aitken et al., 2022). The plasma membrane plays an essential role in maintaining ion balance, enzyme activity, and fertilising capacity. The improved viability observed in T1 and T2 may be attributed to the antioxidant properties of RJ, which can stabilise membrane lipids and neutralise ROS, thereby reducing membrane damage and sperm death during storage.

Table 6. Percentage (mean ± SD) of the sperm viability in a different concentration of royal jelly extender.

Sperm viability	Chilling times	Royal jelly concentration				p-value
		T0	T1	T2	T3	
Live	0 hour	71.0 ± 11.0	72.7 ± 9.5	67.4 ± 5.5	77.0 ± 6.5	0.590
	24 hours	65.4 ± 2.0	69.0 ± 33.4	83.7 ± 7.6	73.0 ± 6.0	0.622
	48 hours	$73.0^{c} \pm 5.5$	$92.0^{a} \pm 0.0$	$90.0^{b} \pm 1.7$	$45.6^{d} \pm 1.5$	<0.001
Dead	0 hour	29.0 ± 11.0	27.3 ± 9.5	32.6 ± 5.5	23.0 ± 6.5	0.590
	24 hours	34.6 ± 2.0	31.0 ± 33.4	16.3 ± 7.6	27.0 ± 6.0	0.622
	48 hours	$27.0^{b} \pm 5.5$	$8.0^{c} \pm 5.7$	$10.0^{c} \pm 4.0$	$54.4^{a} \pm 1.5$	<0.001

SD, Standard deviation; T0, Tris-citrate-fructose-yolk, T1, Tris-citrate-fructose-yolk-royal jelly 0.05%; T2, Tris-citrate-fructose-yolk-royal jelly 0.10%; T3, Tris-citrate-fructose-yolk-royal jelly 0.15%. Means with different superscripts in a same row differ significantly ($p < 0.05$).

However, the reduced viability observed in T3 suggests that excessive RJ supplementation may induce osmotic or metabolic stress, leading to impaired membrane stability and increased sperm mortality. Similar concentration-dependent effects of antioxidant supplementation have been reported in previous semen preservation studies (Coskun Cetin & Karaca, 2023). Therefore, the present findings indicate that low to moderate concentrations of RJ may provide partial protection to sperm membrane integrity during chilled storage, whereas higher concentrations may exert detrimental effects.

3.5 Morphology

Royal jelly supplementation did not significantly affect the percentage of bent tail defects during chilled storage (Table 7), although numerically lower values were observed in some RJ treated groups at 0 hour compared with the control. Tail abnormalities commonly arise from osmotic imbalance, membrane destabilisation, and oxidative stress during semen preservation (Pelzman & Sandlow, 2024). These abnormalities may impair sperm motility and fertilising ability. Although RJ contains antioxidant compounds that may help stabilize membrane structure and protect cytoskeletal proteins from oxidative damage, the present findings suggest that the concentrations used were insufficient to produce statistically significant improvements in bent tail morphology. Similar variability and non-significant responses in sperm morphological traits have been reported in studies evaluating antioxidant supplementation in semen extenders (Longobardi et al., 2020). Therefore, under the present experimental conditions, RJ supplementation did not demonstrate a significant protective effect against bent tail abnormalities during chilled storage.

Table 7. Percentage (mean ± SD) of the sperm morphology (Bent Tail) parameters in a different concentration of royal jelly extender.

Chilling times	Royal jelly concentration				p-value
	T0	T1	T2	T3	
0 hour	14.3 ± 2.5	10.6 ± 4.9	11.3 ± 6.1	11.6 ± 0.5	0.721
24 hours	11.3 ± 2.8	13.3 ± 3.7	15.3 ± 2.5	13.0 ± 1.0	0.412
48 hours	13.3 ± 1.5	16.0 ± 4.0	17.0 ± 1.0	15.6 ± 0.5	0.294

SD, Standard deviation; T0, Tris-citrate-fructose-yolk; T1, Tris-citrate-fructose-yolk-royal jelly 0.05%; T2, Tris-citrate-fructose-yolk-royal jelly 0.10%; T3, Tris-citrate-fructose-yolk-royal jelly 0.15%.

Table 8. Percentage (mean ± SD) of the sperm morphology (midpiece defects) parameters in a different concentration of royal jelly extender.

Chilling times	Royal jelly concentration				p-value
	T0	T1	T2	T3	
0 hour	18.0 ± 2.6	14.3 ± 6.1	12.6 ± 1.5	12.6 ± 1.5	0.276
24 hours	15.0 ± 2.6	17.6 ± 4.5	21.6 ± 0.5	17.3 ± 1.5	0.091
48 hours	17.3 ± 3.5	20.3 ± 1.5	20.0 ± 1.0	18.3 ± 5.5	0.679

SD, Standard deviation; T0, Tris-citrate-fructose-yolk; T1, Tris-citrate-fructose-yolk-royal jelly 0.05%; T2, Tris-citrate-fructose-yolk-royal jelly 0.10%; T3, Tris-citrate-fructose-yolk-royal jelly 0.15%.

Midpiece abnormalities were not significantly affected by RJ supplementation during chilled storage (Table 8), although numerically lower values were observed in some RJ-treated groups at 0 hour compared with the control. The sperm midpiece contains mitochondria responsible for ATP production required for sperm motility; therefore, damage to this area may impair sperm function and fertilising ability (Carrageta et al., 2023). Oxidative stress is known to contribute to midpiece defects during semen storage through mitochondrial membrane damage and disruption of cellular metabolism. Although RJ possesses antioxidant properties that may help protect mitochondrial membranes against oxidative injury, the present findings suggest that the concentrations used in this study did not produce statistically significant improvements in midpiece morphology. Similar non-significant responses in sperm morphological characteristics have also been reported in studies evaluating antioxidant supplementation in semen extenders (Moradi et al., 2013; Amini et al., 2019; Coskun Cetin &

DOI: 10.51200/jsffs.v2i1.7399

Karaca, 2023). Therefore, under the present storage conditions, RJ supplementation did not demonstrate a significant protective effect against midpiece defects.

Head defects were less frequent in semen supplemented with RJ, although the statistical significance of this finding differed across the treatment groups (Table 9). The morphology of sperm heads is largely defined throughout spermatogenesis within the testes, and is significantly shaped by genetic determinants, testicular health, and hormonal control factors rather than by conditions encountered after ejaculation (Pelzman & Sandlow, 2024). Therefore, the efficacy of extender supplementation in rectifying pre-existing head abnormalities is typically limited. Sperm head abnormalities, encompassing irregular morphology, detached heads, and acrosomal damage, frequently correlate with compromised chromatin packaging, DNA fragmentation, and impaired acrosomal development during spermatogenesis (WHO, 2010). Given that these defects appear before ejaculation, the lack of robust statistical significance observed in certain treatment comparisons is biologically plausible. Likewise, non-significant or modest impacts on head morphology have been identified in experiments assessing antioxidant supplementation within goat and ram semen extenders (Moradi et al., 2013; Susilowati et al., 2022).

Table 9. Percentage (mean ± SD) of the sperm morphology (head defect) parameters in a different concentration of royal jelly extender.

Chilling times	Royal jelly concentration				p-value
	T0	T1	T2	T3	
0 hour	66.7 ± 10.7	67.3 ± 11.0	68.6 ± 6.6	67.6 ± 3.0	0.993
24 hours	67.0^{a} ± 6.0	60.6^{b} ± 4.1	54.0^{b} ± 5.0	63.6^{ab} ± 3.0	0.048
48 hours	62.6 ± 4.9	56.6 ± 4.5	52.3 ± 3.5	57.3 ± 1.5	0.064

SD, Standard deviation; T0, Tris-citrate-fructose-yolk, T1, Tris-citrate-fructose-yolk-royal jelly 0.05%; T2, Tris-citrate-fructose-yolk-royal jelly 0.10%; T3, Tris-citrate-fructose-yolk-royal jelly 0.15%. Means with different superscripts in a same row differ significantly ($p < 0.05$).

4. Conclusion

In conclusion, the present study demonstrated that RJ supplementation does not consistently enhance semen quality during chilled storage, but exerts a differential effect depending on concentration and parameter evaluated. The data indicate that low to moderate concentrations of RJ (0.05–0.10%) were able to significantly preserve sperm membrane viability at 48 hours of storage compared with higher concentration (0.15%), suggesting a partial protective effect on cell membrane integrity. However, in contrast to this protective effect on viability, RJ supplementation did not improve sperm motility parameters. Both total and progressive motility were generally lower in RJ-treated groups compared to the T0, indicating an inhibitory effect of RJ on sperm kinetic activity during storage. Conversely, elevated concentrations, specifically 0.15%, proved more detrimental to sperm quality, particularly with respect to motility and overall preservation of sperm function. The experimental results further indicate that, while RJ may help maintain certain structural aspects such as membrane integrity under chilled conditions, its effect on sperm movement is negative or non-beneficial under the tested conditions. Overall, the findings suggest that RJ cannot be considered a universal enhancer of caprine semen quality during liquid storage. Its effects are concentration-dependent, with limited membrane-protective activity at lower doses but inhibitory effects on sperm motility compared with TCFY alone.

Data Availability

The data generated during the study are included in the manuscript.

Author Contributions

Wilsa Gly Mojuwil: Conceptualization, Methodology, Writing- Original draft preparation. **Nur Hafizah Syafiah Abdullah:** Data curation. **Mohamad Mijanur Rahman:** Investigation, Supervision. **Rovina Kobun:** Visualization, Validation. **Nur Syafeezah Janong** and **Renny Fatmyah Utamy:** Writing- Reviewing and Editing.

Acknowledgement

The authors acknowledge the Faculty of Sustainable Agriculture, Universiti Malaysia Sabah, Malaysia, for providing funding and research facilities. The authors also acknowledge the limited use of a generative artificial intelligence (AI) tool solely for language refinement, sentence structuring, and enhancement of clarity in this article. Full responsibility for the content, interpretation, and conclusions presented herein rests entirely with the authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Ethics Statement

All animal procedures were conducted in accordance with the guidelines approved by the Animal Ethics Committee of Universiti Malaysia Sabah (JEHUMS), Malaysia (Approval No. AEC 0016/2025).

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Journal of Smart Farming and Food Security
Universiti Malaysia Sabah Press
e-ISSN: 3122-7090
Volume 2, Issue 1, Pages 60–74 (30 June 2026)
DOI: https://doi.org/10.51200/jsffs.v2i1.7351

Research Article
OPEN ACCESS

Data-driven assessment of gastrointestinal parasitism in zero-grazed goats: Influence of age, gender, and body weight

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Manuscript received: 2 February 2026 | Accepted: 29 June 2026 | Published online: 30 June 2026

Abstract: Gastrointestinal parasite (GIP) infections are a primary constraint on health and productivity in intensive caprine production, yet their impact in zero-grazing systems is frequently underestimated. This study aimed to quantify the prevalence and intensity of GIP infections in a research herd and to identify host-specific demographic drivers of parasite shedding. A cross-sectional study was conducted on 50 Katjang goats at the Livestock Research Unit, Universiti Malaysia Sabah. Faecal egg counts (FEC) were quantified using standardised methods, and the resulting data were analysed using Negative Binomial (NB) regression to account for high overdispersion. Results revealed an exceptionally high herd prevalence of 98%. Parasite intensity was highly aggregated, characterised by a small percentage of "super-shedders," including one extreme high-leverage individual exceeding 26,000 eggs per gram (EPG). The NB model ($\alpha = 0.656$, $p < 0.001$) identified age and gender as the most significant predictors of infection intensity. Younger goats (<1 year) exhibited the highest mean FEC (2,640 EPG), suggesting increased susceptibility due to physiological immaturity. Furthermore, gender was a primary driver of variation ($p = 0.034$), with males exhibiting significantly higher mean FEC and greater shedding variance than females. While body weight showed high variability among mid-weight individuals, lower body weight often coincided with peak shedding in younger cohorts. These findings demonstrate that within confined, zero-grazing systems, infection intensity is heavily influenced by host-specific factors rather than environmental exposure alone. Ultimately, identifying these host-specific demographic drivers provides a vital framework for transitioning from traditional, non-selective herd-wide treatments to precision-based, data-driven interventions.

Keywords: data-driven risk assessment, faecal egg count, gastrointestinal parasite, goat host demographic, katjang goats

1. Introduction

Goat production is an important segment of the global livestock industry, making significant contributions to rural livelihoods, particularly in developing and tropical regions (Lu, 2023). As demand for animal protein rises and human and animal populations grow rapidly, small ruminants are attracting renewed interest due to their adaptability to various agro-ecological conditions, efficient feed conversion, and low production costs (FAO, 2024). Goats, in particular, can utilise unproductive land and low-quality forages to produce meat and dairy

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Citation: Bhuiyan, M. S. A., Rusly, R. F., Along, F. N., Candyrine, S. C. L., Kalam, M. A., Abdul Rasid, R., Kobun, R., Rahman, M. M., & Abdul Rahman, N. (2026). Data-driven assessment of gastrointestinal parasitism in zero-grazed goats: Influence of age, gender, and body weight. Journal of Smart Farming and Food Security, 2(1), 60–74. https://doi.org/10.51200/jsffs.v2i1.7351

products rich in key micronutrients such as iron, zinc, and vitamin B12 (Ripoll *et al*., 2020). In Southeast Asia, including Malaysia, goat production has gained increased emphasis in national agro-food strategies to reduce reliance on imported red meat and improve national self-sufficiency. However, despite their biological adaptability, goats are highly susceptible to parasitic infections, which negatively impact productivity and profitability (Sontigun *et al.,* 2025).

One of the most widespread biological challenges to sustainable goat production systems is infection by gastrointestinal parasites (GIP). GIP shown in Figure 1, primarily caused by strongylid nematodes such as *Haemonchus contortus*, *Teladorsagia circumcincta*, and *Trichostrongylus* spp., continues to have significant economic and welfare impacts on small ruminant production systems worldwide (Bautista-Garfias *et al*., 2022; Maurizio *et al*., 2023). GIP infection impairs nutrient utilisation efficiency, causes chronic inflammation, and, in the case of *H. contortus*, leads to significant blood loss and potentially anaemia, reduced growth rates, and mortality, particularly in young or physiologically stressed animals (Mpofu *et al*., 2022). The impact of GIP infection is often exacerbated in tropical climates, characterised by warm temperatures and humidity, which favour the survival and transmission of these parasites throughout the year.

Controlling GI parasitism is the subclinical nature of the infection. Infected goats may appear healthy while experiencing increasing physiological impairment. As a result, parasite infection is often not detected until clinical signs appear or there is a marked decline in animal performance (Saha *et al*., 2026). This subclinical nature of GI parasitism underscores the need for surveillance approaches that quantify parasite infection rather than merely identify its presence. In this context, faecal egg count (FEC), expressed as eggs per gram (EPG) of faeces, has been established as the basis of parasitological surveys in small ruminants.

Faecal egg counting is an objective method for quantifying worm burden in adults and assessing the risk of environmental contamination, thereby supporting evidence-based decision-making in herd health management. Unlike qualitative diagnostic methods, faecal egg counting enables the detection of individual differences in parasite shedding, which is important given the well-documented phenomenon of parasite over-dispersion. Identifying these "super-shedders" is therefore critical for implementing Targeted Selective Treatment (TST), a precision-based approach to worm control that reduces chemical use, maintains refugia, and delays the onset of anthelmintic resistance (Besier *et al.,* 2010).

The McMaster technique remains the most widely used method for FEC determination due to its simplicity, reproducibility, and cost-effectiveness (Whitlock, 2009; Boareki *et al*., 2021). The method has been extensively validated and refined, becoming common method in determining EPG in many research in veterinary pathology. Recent research continues to highlight the value of McMaster-derived FEC data in monitoring infection dynamics, evaluating treatment efficacy, and informing sustainable parasite control programmes, particularly in resource-limited settings (Playford & Besier, 2025).

Despite extensive global research on gastrointestinal parasitism in goats, knowledge gaps remain, particularly at regional and production system levels. In Malaysia, there is limited published information on the prevalence and infection intensity of gastrointestinal parasites, especially under zero-grazing management systems. It is assumed that zero-grazing management can minimise parasite infection in goats. However, emerging evidence indicates that various host factors, such as age, body weight, and gender, can cause significant variation in parasite infection, even in controlled environments (Saha *et al*., 2026). Additionally, indiscriminate deworming practices are common, leading to the emergence of anthelmintic resistance, particularly in Southeast Asian countries (Maurizio *et al*., 2023). For this reason, the present study aims to address this knowledge gap by investigating the prevalence of gastrointestinal parasitic eggs in zero-grazing goats and exploring the relationship between host

demographic characteristics and infection intensity. By generating high-resolution individual-level EPG data, this study seeks to provide a foundation for evidence-based precision livestock parasite management. Ultimately, improved knowledge of GIP epidemiology in zero-grazing goat production will be instrumental in enhancing animal welfare, sustaining anthelmintic efficacy, and increasing the productivity of small ruminants.

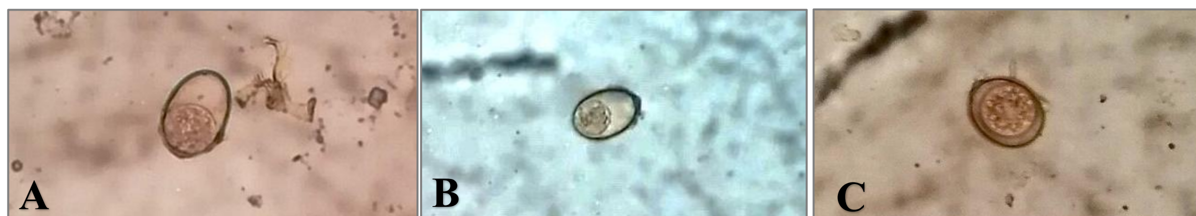

Figure 1. Morphological appearance of a gastrointestinal parasite in goats as observed under low-light microscopy at 10× magnification. Presumptive diagnosed based on their structural shape as *Nematodirus spp.* (A, B) and *Haemonchus contortus* (C).

2. Materials and Methods

2.1 Faecal samples collection and preparation

This faecal samples were collected from Katjang goats in a zero-grazing production system at the Livestock Research Unit, Faculty of Sustainable Agriculture (FSA), Universiti Malaysia Sabah (UMS). A cross-sectional sampling approach was adopted to collect the faeces sample of the goats to ensure that the prevalence rates derived reflect the true position of the herd. A total of 50 faecal samples was collected directly from the rectum of 50 goats to avoid any environmental contamination. The aseptically collected sample was taken by restraining the animal before taking about 10-20 grams of the fresh material using a lubricated disposable glove. For every animal that was sampled, the relevant information of age, sex, pen number, and body weight was documented. Every stool sample was placed in a new, labelled, airtight plastic container. The faecal samples were packed in a cooler box with ice blocks (at 4 °C) to prevent the further development of eggs and larvae of the parasite organisms, and to maintain their morphologic characteristics intact. These samples would then be transported to the Parasitology Laboratory in FSA for further analysis within 24 hours post-sampling time. Potential confounders such as anthelmintic history and physiological status were not controlled in this study due to the recording limitation.

2.2 Faecal analysis

The level of gastrointestinal parasite infection was estimated by using the modified McMaster counting technique (Basripuzi *et al*., 2013). Such a well-established technique was chosen for the calculation of eggs per gram (EPG) of faeces. EPG of faeces was a highly significant quantitative estimate for the helminthic load present in the hosts. Two grams of each 50 faecal samples were processed within 24 hours of sampling timeframe. Fresh faeces were accurately weighed (2 g) and homogenised with 28 mL of saturated sodium chloride solution. Saturated sodium chloride was selected as the flotation medium due to its high specific gravity (approximately 1.20), which facilitates the buoyancy and flotation of common nematode eggs. The resulting mixture provided a 1:15 dilution ratio (2 g of faeces in a total suspension of 30 mL). The mixture was then agitated to ensure a uniform suspension and strained through a fine sieve (or double-layered gauze) to remove coarse debris while allowing parasite eggs to pass freely into the filtrate for further analysis.

2.3 Quantitative analysis and EPG

Faecal suspension was promptly aspirated into a pipette and used to inoculate the chambers of

DOI: 10.51200/jsffs.v2i1.7351

a McMaster counting slide. The slide was allowed to sit for 5 to 10 minutes to allow the less dense parasitic eggs to float to the surface of the coverslip. Eggs in the grid chambers of the counting slide were counted under a low-light microscope at a 10× power magnification. Counting was performed based on the total number of eggs of the parasites present in the grid chambers of the two counting chambers and was not specifically in regard to morphological types of parasitic eggs, to obtain a total infection intensity.

Calculation of the EPG for each sample was performed using the following formula, with consideration of the 1:15 dilution and the counted eggs observed on the slide (Basripuzi *et al*., 2013):

$$\text{EPG} = \frac{\text{Total number of eggs counted}}{\text{Volume counted (mL)}} \times \frac{\text{Total volume of suspension (mL)}}{\text{Weight of faeces (g)}}$$

For a standard McMaster slide, the counted sample volume will be 0.30 mL for the two grids (often referring to two chambers of 0.15 mL each). With the standardised values (counted sample: 0.30 mL, total suspension: 30 mL, sample of faeces: 2 g).

These EPG counts give the necessary values for the assessment of parasite load to form the basis of statistical analysis concerning the influence of the host factors (age, gender, weight).

2.4 Experimental design and statistical analysis

Descriptive statistics were performed for all variables, with parasite prevalence calculated as the proportion of positive samples to the total number of goats sampled. Measures of central tendency for FEC, including mean, standard deviation (SD), and range, were used to characterise the parasite burden. The statistical analysis followed a two-stage approach using Minitab version 2026. Initially, a General Linear Model (GLM) and standard Ordinary Least Squares (OLS) Regression were performed to determine the effects of gender, age, and body weight on FEC. However, diagnostic checks of the initial model, including the Test for Equal Variances and residual analysis, indicated significant heteroscedasticity and non-normal distribution of residuals. The FEC data exhibited high aggregation and overdispersion, a common trait in parasitological counts where a few "super-shedders" skew the distribution.

To address this, a Negative Binomial (NB) Regression was implemented as a more suitable alternative to the linear and Poisson models. The NB model utilised a logarithmic link function to account for the count-based nature of the data and incorporated a dispersion parameter (α) to handle the variance-to-mean relationship. The final model evaluated the influence of host demographic factors (age, gender, and weight) on infection intensity, with the level of significance set at $p < 0.05$. This data-driven modelling approach ensured a more precise risk assessment by capturing the exponential nature of parasite shedding patterns within the intensive zero-grazing system.

3. Results and Discussion

Data from 50 sampled goats revealed substantial inter-herd variation in parasite shedding. To ensure the statistical stability of the final regression model, Observation 39 (ID 086; Female, 26,550 EPG) was analysed independently and excluded from the main regression computation. This extreme value - exceeding the average male EPG by more than 11-fold; exerted an excessively high leverage that would mathematically distort the regression coefficients and mask the subtle demographic trends of the remaining 98% of the herd (Appendix - Table A1). However, from an epidemiological standpoint, this individual represents a critically meaningful biological phenomenon rather than statistical noise: a classic "super-shedder" contributing

disproportionately to potential environmental contamination within a zero-grazing system. Separating this extreme point allowed for a more stable interpretation of baseline herd dynamics while highlighting the urgent practical need for Targeted Selective Treatment (TST) strategies on the farm. Within the remaining model population, observations 15, 18, and 49 were flagged as having large residuals, indicating instances where actual faecal egg counts (FEC) deviated notably from model predictions. For instance, mature animals such as ID 001 (8 years old) and ID 160 (5 years old) both reached peak burdens of 3,750 EPG, demonstrating that high-intensity shedding can still manifest in older cohorts under intensive management conditions.

The EPG counts showed utmost variation ranging from as low as 150 EPG to as high as 3462.5 (Table 1). The parasite distribution is highly skewed (1.13 for females, -1.05 for males), where a few individuals (the high-risk shedders) contributed to most of the eggs.

Table 1: Descriptive Analysis of Gastrointestinal Parasite Infection (N=49).

Variable	Gender	N	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum	Skewness
EPG	F	41	1182.93	142.095	909.850	150	500	1000	1550	3750	1.13
	M	8	2412.5	477.507	1350.60	0	1137.5	2875	3462.5	3750	-1.05

Note: EPG: Faecal egg count expressed in egg per gram; SE: Standard Error; StDev: Standard deviation.

3.1 Effect of gender on parasite count

The present findings indicated that male goats exhibited a higher mean faecal (EPG = 2,412.50) than female goats (EPG = 1,182.93) as shown in the Table 1. The regression model was used to evaluate the impact of gender on parasite shedding. Highly significant gender influence ($p = 0.007$), where on average, male goats had an EPG that was 1,010 units higher than females when other factors were constant. This suggests that under this zero-grazing conditions, males may be more susceptible to high parasite burdens or may act as primary contributors to environmental contamination. Male goats are frequently reported to harbour greater parasite burdens, a pattern commonly attributed to the immunosuppressive effects of androgens, particularly testosterone (Grear *et al*., 2009; Sellau *et al*., 2024). Elevated testosterone levels are known to modulate immune function, directing physiological resources toward growth and reproductive investment at the expense of parasite resistance (Hellard *et al*., 2013). In the present study, most male goat have maintained EPG values exceeding 2,000, indicating moderate to high infection intensity. Such variability is characteristic of gastrointestinal parasite (GIP) infections in small ruminants and reflects the inherently over-dispersed distribution of helminth populations rather than uniform host susceptibility (Maurizio *et al*., 2023).

Notably, one male goat (Tag 196) was recorded zero EPG, suggesting potential genetic resistance or enhanced immunocompetence or could be an outlier result, provided if there is no anthelmintic treatment or prevalence in place. Such inter-individual variation has been widely reported and supports the role of host genetics in regulating parasite susceptibility, offering opportunities for selective breeding for parasite resistance (Notter, 2013).

In contrast, one female goats (ID 086) exhibited extreme variation in parasite burden could be a critical ‘super-shedder’ (the highest EPG; 26,550). Even this data was excluded from the statistical analysis as deemed to be outlier, in most population, even a small percentage of ‘high-risk shedders’ could be responsible for the vast majority of environmental contamination and the exclusion may bias epidemiological interpretation. In zero-grazing environment, this single individual could potentially contaminate the entire pen area far more than the rest of the herd combined. This animal represents a classic “super-shedder,” contributing disproportionately to environmental contamination.

In females, such elevated egg output is frequently associated with the periparturient rise

DOI: 10.51200/jsffs.v2i1.7351

(PPR), during which immunological relaxation happened during late pregnancy and early lactation. This can result in increased worm fecundity and egg shedding. In contrast with study by Hassanen *et al*, (2020), GIT parasites prevalent was higher in male (75.4%) than female (62.4%) goat reared in Egypt. This study observed that the infection pattern strongly supports the 80/20 rule, whereby approximately 20% of hosts account for 80% of parasite transmission (Cooper *et al*., 2019). These findings highlight the limitations of blanket anthelmintic treatment and strongly support the adoption of faecal egg count–based TST. Targeting high-shedding individuals such as Tags 086 and 100 would substantially reduce overall parasite pressure while preserving refugia and mitigating the development of anthelmintic resistance (Besier, 2012).

Table 2. Analysis of variance (ANOVA) of faecal egg count (EPG) distribution in intra-population variation and gender influences under zero grazing conditions.

Source	DF	Adj SS	Adj MS	F-Value	p-Value
Regression	3	19387034	6462345	7.94	0.000
Weight, Kg	1	97053	97053	0.12	0.731
Age	1	4923960	4923960	6.05	**0.018***
Gender	1	6601221	6601221	8.11	**0.007***
Error	**45**	**36614905**	**813665**		

* p < 0.05

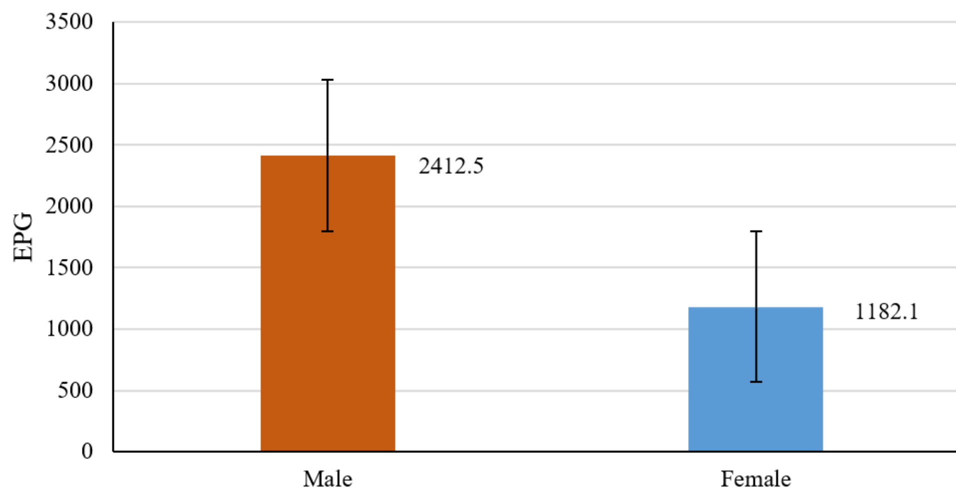

Figure 2. Comparative distribution of mean faecal egg count (counted in egg per gram; EPG) between male and female goats, revealing pronounced intra-population variation and elevated parasite shedding patterns among male goats in a zero-grazing system ($p < 0.05$). Error bars represent standard deviation.

3.2 Effect of body weight on parasite count

The regression indicates that weight and age are non-linear predictors. While young, low-weight goats (like IDs 104, 106, 100) were vulnerable, the presence of high-EPG adults suggests that age-conferred immunity may be incomplete or compromised by other stressors in this zero-grazing system. The sampled population was stratified into six body weight categories to evaluate the relationship between body mass and gastrointestinal parasite burden (Table 3). The analysis revealed marked variation in faecal egg counts (FEC) across the weight ranges,

with the highest number of animals in the 22.1-27.0 kg category (n = 13). Figure 3 further highlights the substantial intra-group variability, showing a wide spread of EPG values across all weight categories.

Interestingly, the lowest weight category (1.0-7.0 kg) also recorded a high mean EPG of 1,800, reflecting the increased susceptibility of young goats. This study aligns with established findings that younger and lighter goats are generally more susceptible to gastrointestinal nematode infections, largely due to immature immune systems and limited exposure. Conversely, the heaviest goats (35.1-62.0 kg) showed the lowest coefficient of variation (44.82%), indicating more consistent parasite burdens likely due to acquired immunity and physiological robustness. Despite these trends, statistical analysis confirmed no significant association between body weight and parasite burden ($p > 0.05$), emphasising that body weight alone is an unreliable predictor of infection intensity.

These findings reinforce the importance of individual-level monitoring using faecal egg counts rather than relying on weight or general group averages. Weight can be affected by several factors such as genetics, feed, rear practices and feed conversion ratio (Lim *et al.,* 2022; Davison *et al.,* 2023; Ojo *et al.*, 2024). The presence of "super-shedders" underscores the necessity of TST, which focuses interventions on high-risk individuals, reducing pasture contamination while preserving refugia and mitigating anthelmintic resistance (Arthur *et al.,* 2010; Tan *et al*., 2017). Collectively, Table 3 and Figure 3 demonstrate that infection dynamics in goats are highly heterogeneous and influenced by multiple interacting factors, including age, genetics, and immune status, rather than body weight alone.

Table 3. Influence of body weight on parasite load as measured by faecal egg count (in egg per gram, EPG).

Weight Range (kg)	Sample Size (n)	Mean EPG±SD	CV (%)
1.0 - 7.0	5	1,800.00 ±1,496.99	83.17
7.1 - 18.0	8	571.43 ± 419.18	73.36
18.1 - 22.0	12	850.00 ± 482.89	56.81
22.1 - 27.0	11	3,470.83 ±1,326.15	211.08
27.1 - 35.0	6	1,825.00 ±1,230.75	67.44
35.1 - 62.0	7	2,392.86 ±1,072.55	44.82

Note: CV: coefficient of variantion.

DOI: 10.51200/jsffs.v2i1.7351

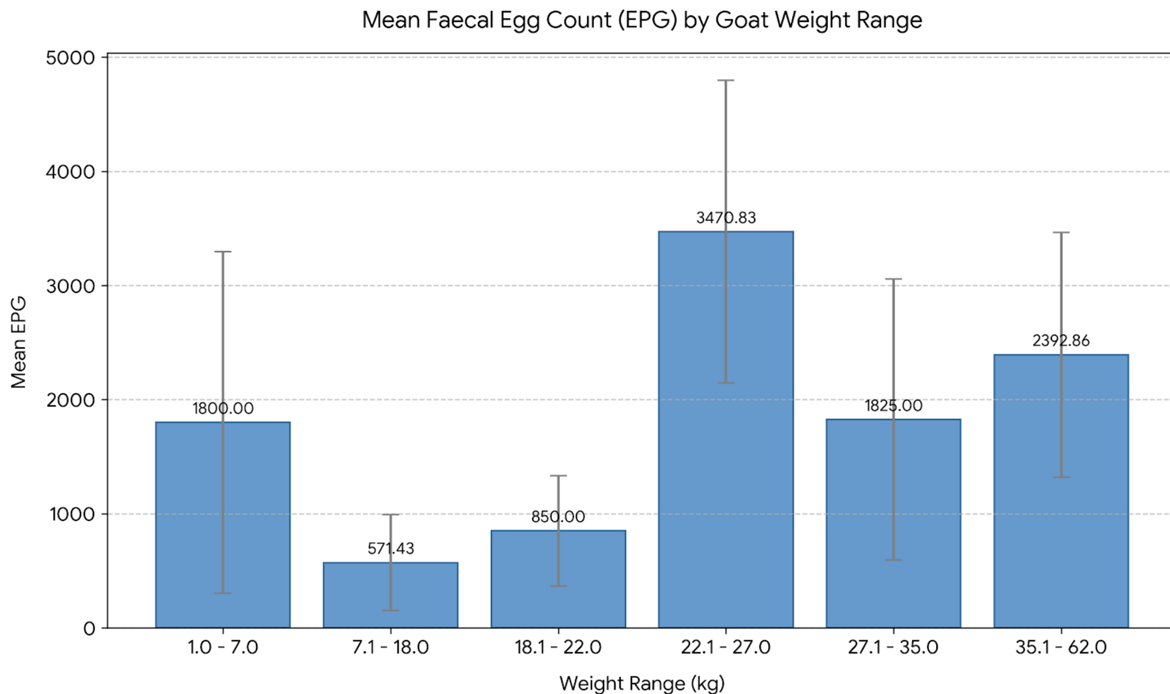

Figure 3. Influence of body weight on mean faecal egg count (in egg per gram; EPG) and associated variations in infection intensity. P value was more than 0.05, thus there was no significant difference among the weight groups. Error bars represent standard deviation.

3.3 Effect of age on parasite count

The influence of host age on gastrointestinal parasite (GIP) burden demonstrated distinct patterns across the four study cohorts: Group 1 (<1 year), Group 2 (1–2 years), Group 3 (3–4 years), and Group 4 (≥5 years). Regression analysis confirmed that age significantly impacts EPG ($p = 0.018$), with the highest mean parasite count observed in Group 1 (Mean EPG = 2,640) (Table 4 and Figure 4). This peak suggests that young kids are most susceptible to infection due to immunological immaturity and a lack of prior exposure to GIP.

Interestingly, the lowest burden was recorded in Group 2 (Mean EPG = 977.6), which may represent a transient "immunity honeymoon" period where initial exposure has triggered a protective response, but long-term environmental accumulation hasn't yet peaked. In contrast, the oldest cohort (Group 4) exhibited the least variability in shedding. This stability suggests a state of host-parasite equilibrium, where mature goats have developed a consistent, albeit not necessarily "zero," level of resilience that keeps shedding within a predictable range. However, the high overall counts across all groups, frequently exceeding the recommended clinical threshold of 1,000 EPG, suggest that the current deworming program is either ineffective or inconsistently applied. To mitigate this, frequent monitoring and TST are essential, particularly for Group 3.

Identifying and treating "high-risk" breeders before they enter the reproductive cycle is critical; if an animal consistently fails to manage its GIP burden, it should be culled to prevent the propagation of susceptible genetics within the herd.

To accurately model these patterns, a Negative Binomial (NB) Regression was utilised to account for the over dispersed nature of the count data Figure 5). The model identified gender as a primary driver of shedding ($p = 0.034$), while the significant overdispersion parameter ($\alpha = 0.656$, $p < 0.001$) validated the NB model over standard linear methods. Unlike linear models that assume constant change, the NB model captures the exponential relationships and "super-shedder" dynamics (e.g., ID 001 and ID 160) typical of biological systems. By focusing management efforts on these high-intensity shedders and protecting the vulnerable young kids

in Group 1, the farm can effectively reduce pasture contamination and enhance overall herd immunity.

Table 4. Influence of age on parasite load as measured by faecal egg count (counted in egg per gram, EPG)

Variable	Age Group	N	Mean	SE Mean	StdDev	Minimum	Q1	Median	Q3	Maximum
EPG	1 (<1y)	6	2640.00	633.92	1417.48	200	1525	2900	3625	3750
	2 (1-2y)	29	977.59	127.49	686.55	150	475	750	1300	2750
	3 (3-4y)	6	1433.33	459.29	1125.02	300	450	1125	2588	3150
	4 (>4y)	8	2206.25	387.81	1096.89	800	1113	2125	3275	3750

Note: SE: Standard error; StdDev: Standard Deviation.

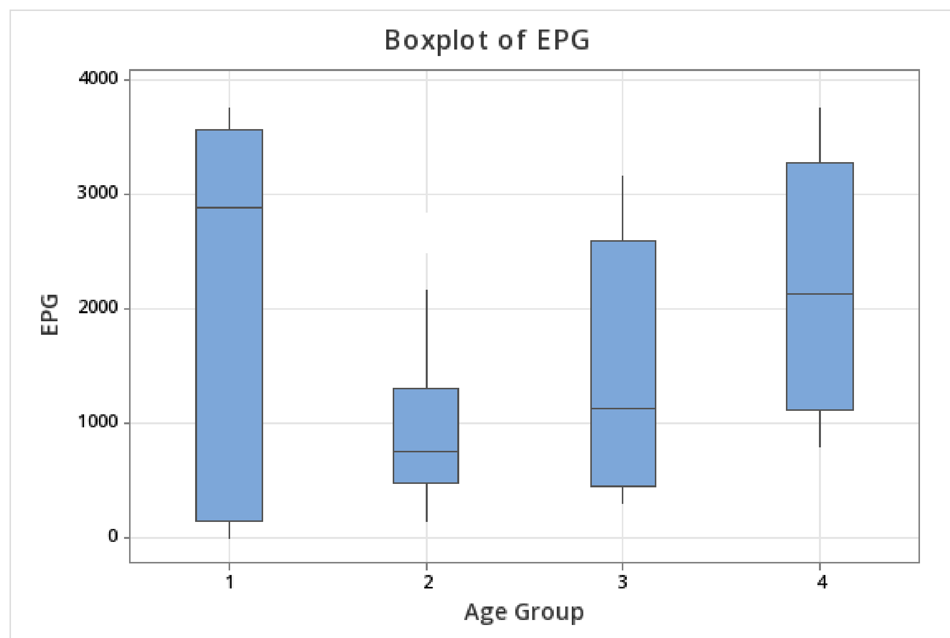

Figure 4. Influence of age on parasite burden - evidenced by variations in mean egg per gram (EPG) ($p < 0.05$). Bars represent data range.
Note: Age group: 1 (< 1 year); 2 (1-2 years); 3 (3-4 years); 4 (> 4 years).

DOI: 10.51200/jsffs.v2i1.7351

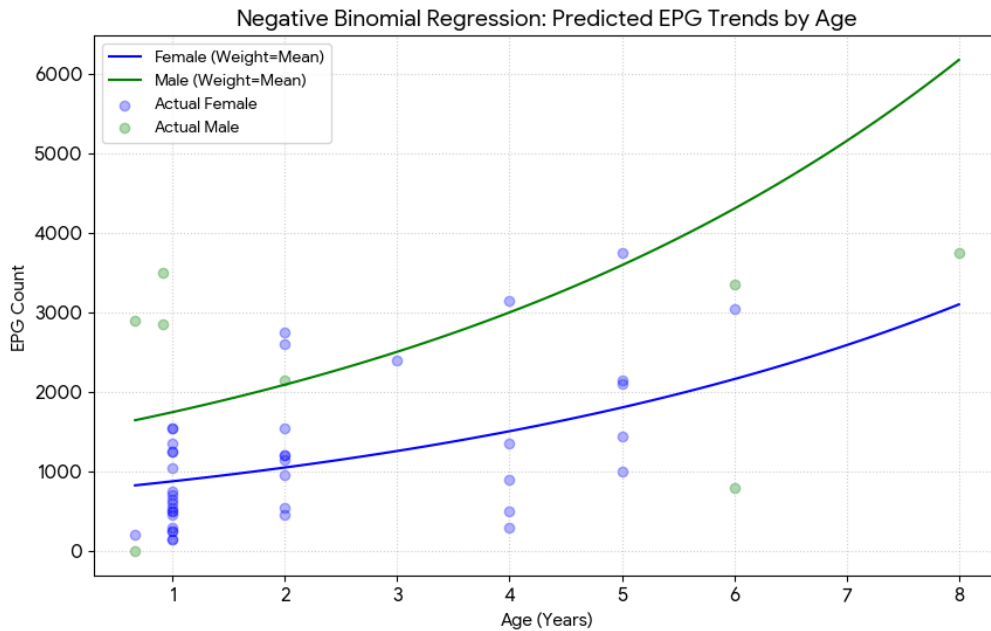

Figure 5. Negative binomial regression: Predicted faecal egg (measured in egg per gram; EPG) trends by age - illustrated an increasing trend.

3.4 Linking age and gender dynamics

Building upon the age-related trends, the interaction between host age and gender further defines the susceptibility landscape of the herd. The Test for Equal Variances (Figure 6) confirms that while age dictates the average burden, gender significantly influences the spread and predictability of that burden. Specifically, male goats not only exhibited higher mean EPG counts but also displayed a significantly larger variance compared to females. This indicates that while female goats tend to cluster around a moderate infection level, the male population is more prone to harbouring "super-shedders" with extreme EPG values. By identifying these high-variance groups through ANOVA, it becomes clear that gender-specific physiological or behavioural factors likely exacerbate the age-related vulnerabilities previously discussed.

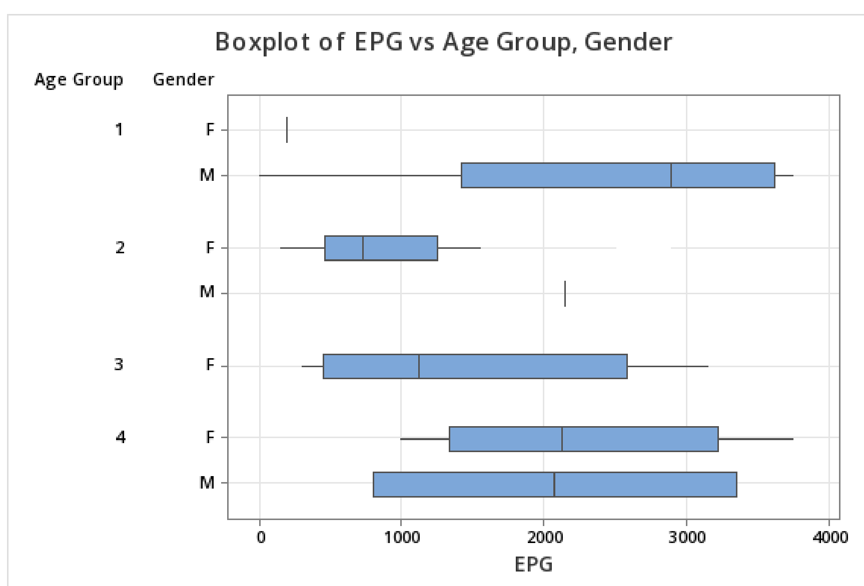

Figure 6. Effect of age group and gender to parasite burden (mean egg per gram (EPG)) using ANOVA -Test for Equal Variance. Bars represent data range.

Note: Age group: 1 (< 1 year); 2 (1-2 years); 3 (3-4 years); 4 (> 5 years); Gender: F: female; M: male goat.

3.5 Prevalence of gastrointestinal parasites (GIP)

The overall prevalence of GIP in the study population was found to be exceptionally high, with 98% of the goats (49 out of 50) testing positive for parasite eggs. This near-universal prevalence is characteristic of tropical goat farming systems where the warm, humid climate facilitates year-round larval development. In this zero-grazing environment, the high prevalence suggests that despite the lack of traditional pasture grazing, the animals are consistently exposed to infective larvae, likely through contaminated bedding, floor slats, or shared feeding troughs. The prevalence did not significantly vary between age groups, indicating that while the intensity of shedding changes as the animal matures, the risk of infection remains constant across the entire herd. However, the findings are limited to a single herd and may not represent broader production systems in Malaysia.

3.6 Dynamics of infection intensity and parasite overdispersion

In Malaysian smallholder and commercial livestock enterprises, existing literature demonstrated that herd prevalence frequently reaches 100%, with mean FECs often exceeding 2,000 EPG in untreated populations. Due to sustained high temperatures and humidity in tropical environments, *Haemonchus contortus* can progress from egg to infective third-stage larvae (L3) in as little as 4 days. Consequently, even within zero-grazing systems, the risk of reinfection remains substantial if environmental factors, such as un-aged manure application on adjacent fodder crops, allow for larval survival.

In contrast, parasite burdens in temperate climates are highly seasonal, characteristically dropping to near-zero values during winter periods. Small ruminant parasitism in those regions is heavily influenced by the "spring rise," a sudden elevation in FEC among peri-parturient does driven by the reactivation of hypobiotic larvae. Malaysian caprine production faces an entirely different epidemiological paradigm: a "constant rise" facilitated by a perennial tropical climate that lacks a cold season capable of reducing environmental larval populations. This constant exposure places local herds within a perpetual high-risk threshold, where parasite-induced production losses, such as depressed weight gain and annual mortality rates between 10% and 40%, significantly escalate.

While zero-grazing housing limits direct pasture contact, it does not fully eliminate parasite transmission. Infective larvae of gastrointestinal strongyles can survive within moist manure on slotted pen floors for up to 22–23 days. Given that a single female H. contortus can deposit 5,000 to 10,000 eggs per day under optimal tropical conditions, environmental contamination accumulates rapidly (Basripuzi *et al.,* 2013; Carson *et al*., 2023). This reproductive capacity directly contextualises the extreme shedding intensity observed in this study, exemplified by a single female "super-shedder" (ID 086) with an FEC of 26,550 EPG.

While prevalence outlines the epidemiological distribution of infection, the intensity of shedding reveals the severe underlying physiological burden within the flock. The overall mean FEC for this research herd stood at 1,418 EPG, surpassing the standard clinical threshold at 1,000 EPG established for severe infection in tropical regions. The individual-level data from this study strongly illustrate the phenomenon of biological overdispersion, wherein a highly skewed minority of the population, predominantly young kids and breeding males, is responsible for the vast majority of the total environmental egg output.

Identifying these high-intensity demographic cohorts provides a clear alternative to traditional, non-selective blanket anthelmintic treatments, which are known to accelerate the development of drug resistance. Furthermore, because parasite resistance is a heritable trait, quantifying individual variations in FEC offers a reliable phenotypic metric for selective breeding programmes aimed at progressively improving herd-level resilience over subsequent generations (Notter, 2013). However, certain microenvironmental factors inherent to intensive zero-grazing practices, including slotted floor designs, localised manure accumulation rates,

and humidity levels at the pen floor interface, were not evaluated in the present study due to operational limitations.

4. Conclusion

This study highlights the significant impact of host age and gender on the shedding patterns of gastrointestinal parasites within a Malaysian zero-grazing goat farm. The results demonstrate that younger goats (<1 year) and male goats represent the most vulnerable demographic cohorts, exhibiting both the highest mean FEC and the greatest variability in shedding intensity. The statistical validation provided by the Negative Binomial Regression and ANOVA Test for Equal Variances underscores that parasite distribution across the herd is highly non-uniform and heavily driven by specific high-risk individuals. Ultimately, these findings demonstrate that quantifying infection intensity and identifying host-specific demographic drivers are essential for transitioning from traditional, non-selective herd-wide treatments to precision-based, data-driven interventions. The marked overdispersion observed confirms that controlling internal parasites in intensive tropical systems relies on identifying highly susceptible cohorts rather than assuming uniform environmental exposure. Focusing monitoring efforts on these high-risk demographics, specifically through the strategic management of persistent super-shedders and the rigorous surveillance of breeding males, provides a robust framework for reducing environmental parasite loads, optimising herd health, and mitigating the rising threat of anthelmintic resistance within the tropical livestock industry.

Data Availability

Data partially presented in this paper. For further data information, available upon request.
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

Md Safiul Alam Bhuiyan: Conceptualisation, Methodology, Supervision. **Rayhan Firdaus Rusly:** Data curation, Original draft preparation. **Fatin Naazira Along:** Editing, Laboratory work. **Su Chui Len Candyrine:** Writing, Reviewing. **Mohamad Asrol Kalam:** Writing, Reviewing. **Rohaida Abdul Rasid:** Writing, Reviewing. **Rovina Kobun:** Writing, Reviewing. **Mohammad Mijanur Rahman:** Writing, Validating. **Norafizah Abdul Rahman:** Software, Writing, Reviewing, Editing, Corresponding author.

Acknowledgement

The authors extend their sincere appreciation to all researchers who contributed, directly or indirectly, to the study on parasitic infestation. Thank you to Universiti Malaysia Sabah for providing place, chemicals and farm assistance through out this research. The authors acknowledge the limited use of a generative artificial intelligence (AI) tool, Gemini and ChatGPT 5.0, solely for language refinement, sentence structuring, and enhancement of clarity in this article. Full responsibility for the content, research design, interpretation, and conclusions presented herein rests with the authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Ethics Statement

Only minimal faecal sampling involving animals and the procedures were reviewed and conducted in accordance with relevant institutional and national guidelines. The care and use of farm animals were with present of university's veterinarian. No ethical approval is required.

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Appendix

Table A1. Comprehensive Parasitological Data for the Study Population (N=50).

No	ID	Age (month)	Pen	Weight, kg	Gender	Parasite	EPG
1	006	12	1	25.5	F	10	500
2	043	12	1	23	F	21	1050
3	054	12	1	30	F	11	550
4	104	03	1	3.5	F	4	200
5	106	03	1	3.5	F	27	1350
6	016	12	2	22	F	31	1550
7	027	12	2	27	F	31	1550
8	051	12	2	26	F	25	1250
9	057	12	2	13	F	25	1250
10	009	12	3	19	F	13	650
11	013	12	3	22	F	9	450
12	014	12	3	22	F	5	250
13	030	12	3	25	F	12	600
14	039	12	3	21	F	15	750
15	001	90	4	62	M	75	3750
16	022	48	5	38	F	63	3150
17	082	72	5	24.5	F	61	3050
18	160	60	5	35	F	75	3750
19	174	60	5	32	F	43	2150
20	189	36	5	27	F	48	2400
21	002	72	6	44.5	M	67	3350
22	080	48	6	27	F	10	500
23	100	11	6	11	M	70	3500
24	138	48	6	22	F	6	300
25	044	12	7	18	F	6	300
26	059	12	7	10	F	3	150
27	105	03	7	3.5	M	58	2900
28	024	60	8	37	F	29	1450
29	102	60	8	42	F	42	2100
30	164	48	8	28	F	18	900
31	194	48	8	21	F	27	1350
32	196	08	8	7	M	0	0
33	012	12	9	14	F	10	500
34	045	12	9	25	F	5	250
35	053	12	9	10.5	F	3	150
36	066	12	9	12	F	14	700
37	136	24	10	25.5	F	55	2750
38	148	24	10	24	F	24	1200
39	086	48	10	27	F	531	26550
40	088	60	10	28	F	20	1000
41	021	24	11	18	F	19	950
42	031	24	11	20	F	11	550
43	047	24	11	22	F	23	1150
44	050	24	11	20	F	9	450
45	110	03	11	3.5	M	57	2850
46	200	24	11	40	M	43	2150
47	003	24	12	18.5	F	31	1550
48	011	24	12	27.5	F	52	2600
49	040	24	12	22	F	24	1200
50	171	72	12	53	M	16	800

Note: F = Female M = Male