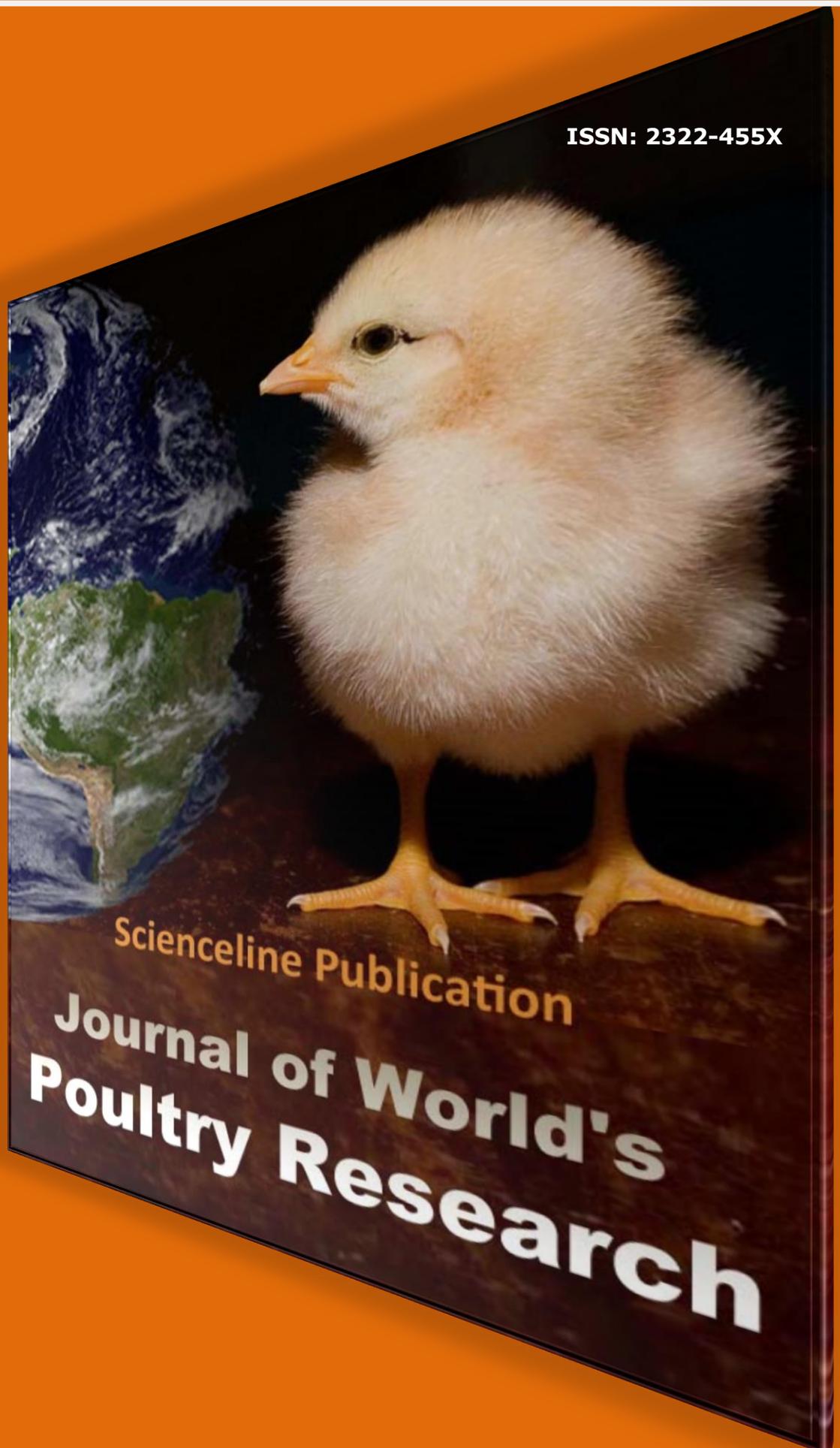
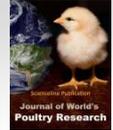




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Volume 15 (3); September 25, 2025

Research Paper

Effects of Nettle (*Urtica dioica*) Supplementation on Productive Performance, Biochemical Parameters, and Gut Microbiota in Broiler Chickens

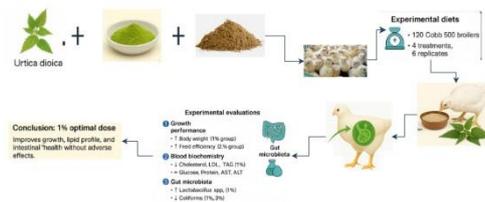
Pedraza EV, P Ferro, Guerrero JAV, Valdivia JC, Flores ASG, Ramos JAC, Chayña ET, and Garcia PPA.

J. World Poult. Res. 15(3): 275-283, 2025; pii: S2322455X2500026-15

DOI: <https://dx.doi.org/10.36380/jwpr.2025.26>

ABSTRACT: The global poultry industry is challenged to meet rising demands for sustainable production, prompting interest in plant-based feed additives like *Urtica dioica* due to their nutritional and functional properties. The objective of this study was to evaluate the effects of dietary inclusion of *Urtica dioica* on growth performance, serum biochemical indicators, and intestinal microbiota composition in broiler chickens. The 42-day feeding trial involved 120 male Cobb 500 broiler chickens with an average initial body weight of 41.7 ± 1.2 g. Broiler chickens were randomly allocated to four experimental groups, each consisting of six replicates with ten chickens. The treatment groups received basal diets supplemented with 1% (T1), 2% (T2), or 3% (T3) *Urtica dioica*, while the control group (T0) was fed the basal diet without additives. Productive performance parameters were recorded weekly, and on day 42, blood profiles and intestinal microbiota composition were evaluated. Results showed that 1% *Urtica dioica* significantly improved live weight and feed conversion ratio (FCR) compared to the control group, with no additional benefits observed at higher inclusion levels. The biochemical assessment showed that broiler chickens supplemented with 1% *Urtica dioica* exhibited significant reductions in total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triacylglycerol levels compared to the control group. Microbial analysis demonstrated a significant increase in *Lactobacillus* spp. populations and a decrease in *coliform* bacteria in the 1% supplementation group, suggesting improved gut health. These findings indicated that 1% *Urtica dioica* supplementation enhances growth, lipid metabolism, and intestinal health in broiler chickens.

Keywords: *Urtica dioica*, Medicinal Plant, Chicken, Productive performance, Gut microbiota



Pedraza EV P Ferro, Guerrero JAV, Valdivia JC, Flores ASG, Ramos JAC, Chayña ET, and Garcia PPA (2025). Effects of Nettle (*Urtica dioica*) Supplementation on Productive Performance, Biochemical Parameters, and Gut Microbiota in Broiler Chickens. *World Poult. Res.* 15(3): 275-283. DOI: <https://dx.doi.org/10.36380/jwpr.2025.26>

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Research Paper

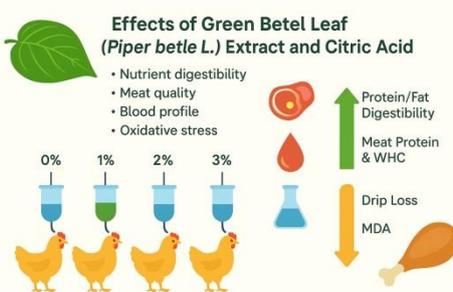
Effects of Green Betel Leaf (*Piper betle* L.) Extract and Citric Acid on Nutrient Utility, Health, and Meat Quality of Broiler Chickens

Nasution ASP, Mahfudz LD, and Utama CS.

J. World Poult. Res. 15(3): 284-290, 2025; pii: S2322455X2500027-15

DOI: <https://dx.doi.org/10.36380/jwpr.2025.27>

ABSTRACT: The broiler chicken industry has rapidly expanded in recent decades due to increasing demand for affordable animal protein and the species' high production efficiency. This study aimed to evaluate the effects of supplementing broiler chickens' drinking water with green betel leaf extract and citric acid on their nutrient utilization, health, and meat quality. A total of 200 unsexed day-old chicks (DOC) of Cobb 500 strain broiler chickens with an average initial weight of 42.17 ± 0.53 g were used. A completely randomized design (CRD) was implemented, comprising four treatment groups with five replications each, and each replicate consisted of 10 broiler chickens. The treatments involved administering drinking water containing a mixture of green betel leaf extract and citric acid at concentrations of 0% (T0, control), 1% of drinking water (10 ml, T1), 2% of drinking water (20 ml, T2), and 3% of drinking water (30 ml, T3). Parameters assessed included nutrient digestibility (protein, fat, and true metabolizable energy), meat quality (protein, fat, cholesterol content, water holding capacity, and drip loss), blood profiles (erythrocyte, leukocyte, hemoglobin, and hematocrit levels), and oxidative stress indicators (superoxide dismutase and malondialdehyde). Results indicated that T3 significantly had higher protein digestibility (77.22%), fat digestibility (76.00%), true metabolizable energy (3,010.09 kcal/kg), and meat protein content (21.26%) compared to T0, while reducing drip loss (27.93%) and MDA levels (7.88 nmol/mL) compared to T0. In



Nasution ASP, Mahfudz LD, and Utama CS (2025). Effects of Green Betel Leaf (*Piper betle* L.) Extract and Citric Acid on Nutrient Utility, Health, and Meat Quality of Broiler Chickens. *J. World Poult. Res.* 15(3): 284-290. DOI: <https://dx.doi.org/10.36380/jwpr.2025.27>

conclusion, supplementing drinking water with 3% feed additive effectively enhances nutrient utility, health, and meat quality in broiler chickens.

Keywords: Betel leaf, Broiler, Citric acid, Meat quality, Nutrient utility

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Research Paper

Influence of Breed, Sex, and Age on Body Composition of Turkeys Reared in Southern Benin

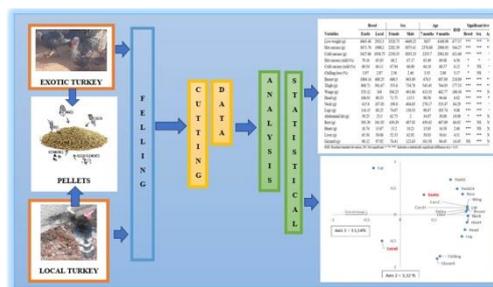
Baba IL, Bonou AG, Adzona PP, Dotche I, Salifou C, and Youssao Aboudou Karim I.

J. World Poult. Res. 15(3): 291-302, 2025; pii: S2322455X2500028-15

DOI: <https://dx.doi.org/10.36380/jwpr.2025.28>

ABSTRACT: Exotic turkey breeds generally exhibit superior growth performance compared to indigenous breeds in Benin. While local turkeys are recognized for their hardiness, they tend to demonstrate comparatively lower growth rates and have less well-documented carcass characteristics. The present study aimed to evaluate the body composition of the local turkey population reared in Southern Benin. Data were collected from 40 turkeys per breed (Exotic and local), totaling 80 turkeys. Each breed included 20 males and 20 females. The turkeys were divided into two age groups, including 7-month-old turkeys (Age-Group 1) and 9-month-old turkeys (Age-Group 2). Following a 24-hour hydrous diet, the turkeys were slaughtered by jugular vein, bled, scalded in water at 75°C, manually plucked, and the hot carcasses were weighed. Each carcass was cut to measure the weight of the wishbone, thigh and shank, wings, head, neck, gizzard, heart, liver, and legs. The current results indicated that the slaughter, hot carcass, and cold carcass weights of the exotic turkey were significantly higher than those of the local turkey. Additionally, male turkeys had notably higher slaughter, hot carcass, and cold carcass weights than females, regardless of breed. Furthermore, the live weight of turkeys at 7 months (3637 g) was significantly lower than at 9 months (4160.98 g). The weights of the breast, thigh, and tail cuts increased significantly as the turkeys aged. However, exceptions were observed in abdominal fat and specific parameters such as cold carcass yield, slaughter weight, and fifth-quarter components, including the heart, gizzard, head, and legs, which showed a positive correlation, especially in the exotic-type breeds. In contrast, no statistically significant correlation was observed between carcass yields at slaughter and abdominal fat in the exotic breed compared to the local breed. It might be beneficial to consider selecting or crossbreeding the local turkey population with more efficient exotic breeds to improve their overall body composition.

Keywords: Body composition, Carcass yield, Exotic turkey, Local turkey, Southern Benin



Baba IL, Bonou AG, Adzona PP, Dotche I, Salifou C, and Youssao Aboudou Karim I (2025). Influence of Breed, Sex, and Age on Body Composition of Turkeys Reared in Southern Benin. *J. World Poult. Res.* 15(3): 291-302. DOI: <https://dx.doi.org/10.36380/jwpr.2025.28>

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Research Paper

Circular Valorization of Acid Silage from Invasive *Pterygoplichthys* Species in Hens' Diets: Impacts on Laying Performance and Egg Quality

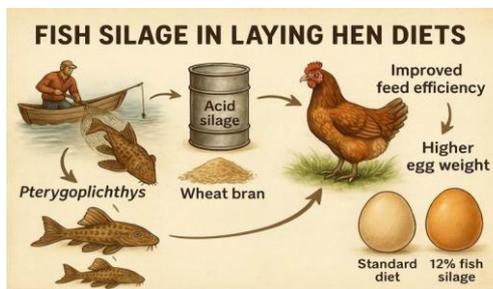
Juárez A, Ordaz G, Cuellar JC, Salas G, and Gutiérrez E.

J. World Poult. Res. 15(3): 303-313, 2025; pii: S2322455X2500029-15

DOI: <https://dx.doi.org/10.36380/jwpr.2025.29>

ABSTRACT: *Pterygoplichthys* species, an invasive fish, offers a sustainable protein alternative in poultry feed, aligning with circular economy goals and reducing environmental impact. This study aimed to assess the impact of incorporating various levels of acid silage derived from *Pterygoplichthys* species (ASP) into laying hen diets on productive performance and egg quality, as part of a biological waste valorization strategy aligned with circular economy principles. Sixty 35-week-old Rhode Island Red hens (BW 1932.1 ± 10.81 g) were randomly assigned to four isoproteic and isoenergetic dietary treatments containing 0%, 6%, 12%, and 18% ASP over 13 weeks. Productive, egg quality, and economic indicators were recorded weekly. The inclusion of 12% ASP resulted in the highest egg production (82.08%), egg mass (49.32 g/hen/day), and number of eggs per hen per week (5.6), along with improved feed conversion ratio (2.59) and the highest economic efficiency index (17.90%) and profitability, in comparison to the other treatments. Egg quality also improved with ASP, regardless of the inclusion level, particularly in egg weight (60.08 g), shell thickness (0.35 mm), and Haugh units (73.83), compared to the control group. Quadratic regression models identified optimal ASP inclusion levels ranging from 11.2% to 12.3%, depending on the variable analyzed. In conclusion, the inclusion of 12% ASP in laying hen diets represents an effective, profitable, and environmentally responsible nutritional strategy that aligns with the principles of the circular economy and sustainable food production.

Keywords: Acid silage, Circular economy, Egg production, Invasive species



Juárez A, Ordaz G, Cuellar JC, Salas G, and Gutiérrez E (2025). Circular Valorization of Acid Silage from Invasive *Pterygoplichthys* Species in Hens' Diets: Impacts on Laying Performance and Egg Quality. *J. World Poult. Res.* 15(3): 303-313. DOI: <https://dx.doi.org/10.36380/jwpr.2025.29>

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Research Paper

A Field Study on Infectious Bronchitis Virus in Broiler Chickens in Southern Iraq

Abdulrasol MA and Abd El-Ghany WA.

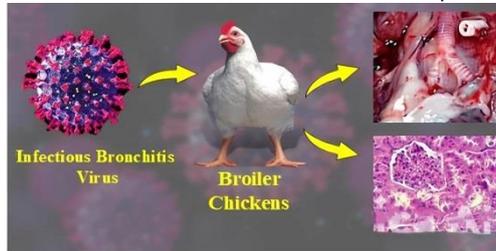
J. World Poult. Res. 15(3): 314-320, 2025; pii: S2322455X2500030-15

DOI: <https://dx.doi.org/10.36380/jwpr.2025.30>

ABSTRACT: Infectious bronchitis virus (IBV) is a highly transmissible avian Gamma-coronavirus that continues to pose a major challenge to poultry health and productivity worldwide, particularly in broiler production systems. The present investigation aimed to detect and characterize IBV infections in broiler flocks located in two districts of Southern Iraq between late 2024 and early 2025. A total of 200 clinically affected broilers (100 broilers from each flock) were sampled, with tracheal and kidney tissues collected for clinical evaluation, histopathological assessment, and viral isolation. Suspected IBV-infected chickens showed respiratory distress, increased mortality, and kidney lesions. The suggestive post-mortem lesions were caseous plug exudates at the tracheal bifurcation, as well as congested and hyperemic kidneys. The inoculation of tracheal and kidney tissue suspension in embryonated chicken eggs (ECEs) resulted in death, stunting, curling, dwarfism, congestion, and subcutaneous hemorrhages. The histopathological findings in tracheal tissues revealed epithelial desquamation, goblet cell depletion, and lymphocytic infiltration, while kidney findings exhibited tubular degeneration, glomerular disruption, and fibrin deposition. These findings emphasize the need for future studies to focus on the molecular identification of circulating strains, vaccine matching, and monitoring of post-vaccination protection levels in Iraq.

Keywords: Broiler chicken, Histopathology, Infectious bronchitis virus, Isolation, Kidney, Trachea

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Abdulrasol MA and Abd El-Ghany WA (2025). A Field Study on Infectious Bronchitis Virus in Broiler Chickens in Southern Iraq. *J. World Poult. Res.*, 15(3): 314-320. DOI: <https://dx.doi.org/10.36380/jwpr.2025.30>

Research Paper

Assessing Performance and Economic Efficiency of Table Eggs Production in Southern Togo

Ihou AFY, Georges AA, Alayi A, and Mansingh PJ.

J. World Poult. Res. 15(3): 321-331, 2025; pii: S2322455X2500031-15

DOI: <https://dx.doi.org/10.36380/jwpr.2025.31>

ABSTRACT: Ensuring better allocation of productive resources necessitates socioeconomic considerations. This study examined the performance of table egg production in southern Togo by determining the breeders' production efficiency level through the stochastic frontier analysis in table egg production. Consequently, identifying the factors that significantly impact technical and allocative efficiency, and explaining the reasons for the technical inefficiency of table egg production. A parametric approach was used to estimate the technical and allocative efficiency levels from a stochastic frontier analysis. Data were collected from primary sources via a structured questionnaire (open-ended) administered to 88 poultry farms in southern Togo (2021) randomly. The parameters measured in this study were table egg production, the feed consumption during the production (each stage separately), veterinary treatment costs (drugs, vitamins), the flock size, the size of the exploitation, and the related costs of production. The finding indicated that 70% of table egg poultry farms in the Maritime Region of southern Togo are moderately technically efficient, although individual efficiency varies. Factors, such as flock size, labor, and veterinary treatments significantly influence the egg production process. Estimating the stochastic production function frontier revealed that inefficiencies in layer production largely stem from technical inefficiency among producers rather than inefficient resource allocation. The present study shows that poultry farms in Southern Togo exhibit medium technical efficiency but demonstrate effective allocation efficiency. Despite high-capacity facilities and financial constraints, the variation in the poultry breeders' production efficiency is explained by both endogenous and exogenous socioeconomic factors revealed through Tobit analysis. These factors are categorized into two groups, including primary (age, education, active membership, density, conflicts, gender), and secondary (credit, type of feed, association membership). Despite moderate technical efficiency, Southern Togo's poultry farms showed effective resource allocation. Financial constraints hinder full facility optimization, and unregulated input markets contribute to fluctuating costs.

Keywords: Allocative efficiency, Poultry farming, Stochastic frontier analysis, Technical efficiency

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Ihou AFY, Georges AA, Alayi A, and Mansingh PJ (2025). Assessing Performance and Economic Efficiency of Table Eggs Production in Southern Togo. *J. World Poult. Res.*, 15(3): 321-331. DOI: <https://dx.doi.org/10.36380/jwpr.2025.31>

Research Paper

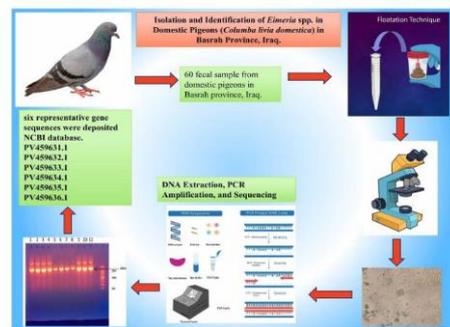
Isolation and Identification of *Eimeria* spp. From Domestic Pigeons (*Columba livia domestica*) in Basrah, Iraq

Saood AI, Khaleefah IA, Aljassim KBN, Mohammad SS, Alkinani AK, and Najem HA.
J. World Poult. Res. 15(3): 332-337, 2025; pii: S2322455X2500032-15
DOI: <https://dx.doi.org/10.36380/jwpr.2025.32>

ABSTRACT: Coccidiosis is a widespread disease among pigeons worldwide, resulting in significant economic losses due to mortality, morbidity, and reduced feed efficiency. The present study aimed to isolate a field strain of *Eimeria* spp. in domestic pigeons (*Columba livia domestica*) in Basra, Iraq. To ensure precise species identification, light microscopy techniques were combined with the advanced molecular method of polymerase chain reaction (PCR). Sixty fresh fecal samples were collected from pigeon lofts, ensuring minimal environmental disturbance and contamination. The flotation method was employed to analyze fecal samples. The present results indicated that 36 out of 60 samples contained coccidian oocysts, which represented an overall prevalence of 60%. The PCR technique was employed to amplify the 18S rRNA genes, which were subsequently utilized for sample detection. Six representative sequences were selected, registered, and deposited in the NCBI database (PV459631.1, PV459632.1, PV459633.1, PV459634.1, PV459635.1, and PV459636.1). The present findings were validated through molecular analysis, and six representative gene sequences were submitted to the National Centre for Biotechnology Information (NCBI) database. The current study revealed a high prevalence of coccidiosis, affecting 60% of domestic pigeons in Basra, highlighting its impact on their health. Moreover, this is the first report of *Eimeria chalcoptereae* in pigeons from Basra, Iraq.

Keywords: *Eimeria chalcoptereae*, Molecular technique, Phylogeny, Pigeon

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Saood AI, Khaleefah IA, Aljassim KBN, Mohammad SS, Alkinani AK, and Najem HA (2025). Isolation and Identification of *Eimeria* spp. From Domestic Pigeons (*Columba livia domestica*) in Basrah, Iraq. *J. World Poult. Res.* 15(3): 332-337. DOI: <https://dx.doi.org/10.36380/jwpr.2025.32>

Research Paper

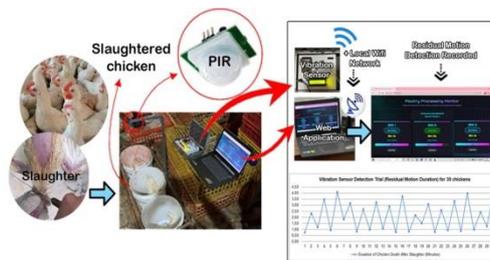
Effects of Vibration Sensor on Mitigation Risk of Halal Chicken Slaughtering System

Rosyidi K, Santoso I, Wibisono Y, and Sucipto S.
J. World Poult. Res. 15(3): 338-349, 2025; pii: S2322455X2500033-15
DOI: <https://dx.doi.org/10.36380/jwpr.2025.33>

ABSTRACT: Ensuring compliance with halal standards in poultry slaughtering involves both following religious principles and implementing strict scientific and technological measures. Integrating Internet of Things (IoT) technology provides opportunities to enhance the reliability and objectivity of halal verification processes, particularly in identifying critical control points, such as animal death, specifically the death of chickens during the slaughter process, before entering the scalding stage. The present study aimed to design a halal risk mitigation system based on IoT, focusing on the critical point of complete chicken death, defined as the total absence of movement in the chicken after slaughter, through critical analysis. It is known that the stage between post-slaughter and pre-burning is the most crucial phase, where the highest risk is that the chicken has not entirely died due to ineffective slaughter. This system was developed using a NodeMCU ESP8266 microcontroller connected to a vibration sensor or passive infrared sensor and was equipped with real-time notifications via the Thingspeak cloud dashboard, indicating the waiting time for complete death and the number of vibrations. Testing on 30 chickens demonstrated a detection accuracy of 92.5% compared to manual observations by halal auditors, with consistent performance across different environmental conditions. This system can detect the movement of chicken remains after slaughter in an average of 15 to 20 seconds, providing an early warning of potential halal violations rules. The current results demonstrated that the vibration sensor effectively facilitated the execution of halal slaughtering principles through an early-warning mechanism designed to prevent chickens from entering the scalding phase while still alive. This ensures the humane death of chickens and the regulation of halal critical control points in line with the Indonesian national standard for halal poultry slaughter.

Keywords: Criticality analysis, Halal slaughter, Risk management, Poultry industry, Vibration sensor

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Rosyidi K, Santoso I, Wibisono Y, and Sucipto S. (2025). Effects of Vibration Sensor on Mitigation Risk of Halal Chicken Slaughtering System. *J. World Poult. Res.* 15(3): 338-349. DOI: <https://dx.doi.org/10.36380/jwpr.2025.33>

Research Paper

Growth Performance, Carcass Traits, and Economic Aspects of Pekin Duck Growing in Dhamrai Area of Bangladesh

Sultana S, Islam S, Islam MA, Khatun R, and Ahmed S.

ABSTRACT: White Pekin ducks are a valuable addition to the poultry industry in Bangladesh with their adaptability, rapid growth rate, and excellent meat quality. This study aimed to evaluate the growth performance, meat quality, and socio-economic aspects of White Pekin ducks in the Dhamrai area of Bangladesh. The study was carried out between January and April 2024 by distributing a total of 250 one-day-old Pekin ducklings among 25 selected farmers. Farmers were chosen based on their willingness, capacity, and adequate housing facilities to observe the growth characteristics, carcass traits, and economic values of Pekin ducks. The farmers were mainly middle-aged (46 years) with an average family size of 4.16 and had farming experience of 9 years. Livestock and poultry rearing were the primary occupation for 72% of farmers, and Pekin duck farming was pursued for both household consumption and extra income. The ducks showed consistent growth, increasing from 53.17 g at day-old to 1812.82 g at 9 weeks, with an average daily gain of 82.97 g. At the marketing age (10 weeks), the average dressing percentage was 74. The carcasses of male and female ducks differed slightly in terms of organ and fat distribution, with males generally had heavier internal organs and giblets, while females had slightly higher fat (2.10%). The nutrient content of breast and thigh muscles showed differences in water and fat content, with males generally having a higher dry matter of 29.13%. The Benefit-Cost ratio of Pekin duck farming was 1.59; however, the majority of farmers faced constraints due to higher feed prices and a lack of quality ducklings for Pekin duck production. Therefore, improving management practices, biosecurity measures, and access to better inputs could enhance the profitability and sustainability of Pekin duck farming.

Keywords: Benefit-cost ratio, Carcass trait, Dressing percentage, Growth performance, Pekin duck



Sahatmana G, Islam S, Islam MA, Ghoshur E, and Ahmed S (2025). Growth Performance, Carcass Traits, and Economic Aspects of Pekin Duck Growing in Dhamrai Area of Bangladesh. *J. World Poult. Res.*, 15(3): 350-365. DOI: <https://dx.doi.org/10.36380/jwpr.2025.34>

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Research Paper

Simulation of Multiple Mediation Variables for Finding the Ideal Model to Improve the Performance of the Chicken Farming Business in Indonesia

Darmawan DP, Arisena GMK, Wiguna PPK, Dewi NLMM, Dewi AAIAP, Sahatmana GW, Krisnandika AAK and Rahayu NNAP.

J. World Poult. Res. 15(3): 366-378, 2025; pii: S2322455X2500035-15
DOI: <https://dx.doi.org/10.36380/jwpr.2025.35>

ABSTRACT: The success of chicken farming can be assessed using performance indicators. The present study aimed to investigate internal and external environmental factors, entrepreneurial skills, innovation, financial management, and the business performance of chicken farms in the Penebel District, Indonesia. A total of 51 chicken farmers meeting the criteria were included as the study sample. Data collection methods included interviews, surveys, documentation, and literature review. The analysis employed quantitative descriptive methods, including simple tabulation and generalized structured component analysis software. The feasibility of the initial model was tested, and if any discrepancies were found, the model was re-specified and retested until it achieved overall goodness-of-fit criteria. The simulation model included 11 paths connecting variables. Five path coefficients demonstrated significant effects, while six did not. Significant effects were found between the internal environment and entrepreneurship, the external environment and entrepreneurship, the internal environment and innovation, the external environment and innovation, and the internal environment and financial management. The present findings indicated that entrepreneurship did not serve as a mediating variable. The internal and external environments significantly impacted farmers' entrepreneurial skills. However, entrepreneurial skills did not significantly enhance business performance. Furthermore, internal and external factors influenced innovation, but innovation did not affect business performance.



Darmawan DP, Arisena GMK, Wiguna PPK, Dewi NLMM, Dewi AAIAP, Sahatmana GW, Krisnandika AAK and Rahayu NNAP (2025). Simulation of Multiple Mediation Variables for Finding the Ideal Model to Improve the Performance of the Chicken Farming Business in Indonesia. *J. World Poult. Res.*, 15(3): 366-378. DOI: <https://dx.doi.org/10.36380/jwpr.2025.35>

Keywords: Business competence, Business environment, Business performance, Chicken farming, Financial management, Innovation

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Research Paper

Integrating Halal Management Systems and Control Points in Poultry Processing: A Transnational Compliance Case from Thailand to Malaysia

Khamnurak H, Taeali A, and Rahman MM.

J. World Poult. Res. 15(3): 379-388, 2025; pii: S2322455X2500036-15
DOI: <https://dx.doi.org/10.36380/jwpr.2025.36>

ABSTRACT: Halal poultry production in Thailand is vital for strengthening its position in the global halal poultry market. The present study aimed to explore the strategic significance of halal management systems (HMS) and halal control points (HCPs) for the global halal poultry industry by investigating their application in Thailand and assessing compliance with Malaysian standards. The present study focused on certified Thai poultry slaughter and processing facilities located in Thailand and approved for export to Malaysia. Additionally, the present study explored the religious, technical, and institutional requirements compatible with poultry processing to fulfill the requirements of the Department of Veterinary Services and the Department of Islamic Development Malaysia. Employing a qualitative case study methodology that encompassed document analysis, in-depth interviews, and field observations, the present study identified eight critical HCPs immersed within a large 59-step operational process, focusing on key issues concerning animal welfare, ritual slaughter, hygiene, and prevention measures contamination. The integration of HMS and HCPs into the operational flow of poultry processing facilities required a structured approach, incorporating halal food-general requirements, the Malaysian protocol for halal meat and poultry production, and the Malaysian halal management. The present findings provided significant contributions to cross-border halal governance, supporting the development of a framework that enhances halal assurance in the international poultry processing industry and comprehensively addresses the halal poultry standards market.

Keywords: Halal control point, Halal management system, Poultry processing, Slaughterhouse



Khannurak H, Taali A, and Rahman MM (2025). Integrating Halal Management Systems and Control Points in Poultry Processing: A Transnational Compliance Case from Thailand to Malaysia. *J. World Poult. Res.*, 15(3): 379-388. DOI: <https://dx.doi.org/10.36380/jwpr.2025.38>

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Research Paper

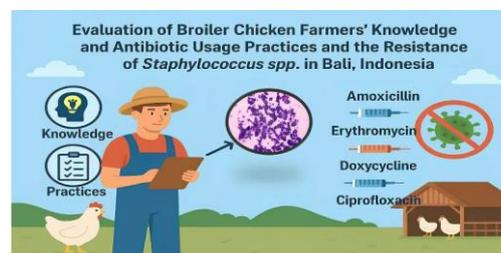
Evaluation of Broiler Chicken Farmers' Knowledge and Antibiotic Usage Practices and the Resistance of *Staphylococcus* spp. in Bali, Indonesia

Putra IGBAK, Agustina KK, Suardana IW, Suwiti NK, I TenayaWM, Sudipa PH, Utami YM, and Besung INK.

J. World Poult. Res. 15(3): 389-395, 2025; pii: S2322455X2500037-15

DOI: <https://dx.doi.org/10.36380/jwpr.2025.37>

ABSTRACT: Antimicrobial resistance (AMR) is a global threat that seriously affects public health, animal welfare, and the sustainability of food production systems. In the poultry sector, irrational use of antibiotics is a key factor contributing to the emergence of resistance. This study investigated the association between broiler farmers' knowledge and their antibiotic usage practices with the resistance of *Staphylococcus* spp. in broiler chickens. The study employed a cross-sectional design and included 20 broiler farms located in Tabanan Regency, Indonesia. Knowledge and practices were assessed using structured questionnaires, while five tracheal swab samples were collected from randomly selected chickens on each farm. These samples were pooled and tested in the laboratory to identify *Staphylococcus* spp.



Putra IGBAK, Agustina KK, Suardana IW, Suwiti NK, I TenayaWM, Sudipa PH, Utami YM, and Besung INK (2025). Evaluation of Broiler Chicken Farmers' Knowledge and Antibiotic Usage Practices and the Resistance of *Staphylococcus* spp. in Bali, Indonesia. *J. World Poult. Res.*, 15(3): 389-395. DOI: <https://dx.doi.org/10.36380/jwpr.2025.37>

and determine their susceptibility to amoxicillin, ciprofloxacin, erythromycin, and doxycycline. The antibiotic susceptibility test followed the guidelines of the Clinical and Laboratory Standards Institute (CLSI), and the associations between variables were examined using cubic regression analysis. Based on the scores obtained from the structured questionnaires, the majority of farmers demonstrated a moderate level of knowledge (65%) and a moderate level of antibiotic usage practices (60%). Laboratory testing revealed that *Staphylococcus* spp. isolates showed the highest resistance to amoxicillin (75%), followed by erythromycin (60%), doxycycline (55%), and ciprofloxacin (30%). The results showed that most farmers had moderate knowledge (65%) and practices (60%), with the highest resistance observed against amoxicillin (75%), followed by erythromycin (60%), doxycycline (55%), and ciprofloxacin (30%). A significant positive correlation was found between farmers' knowledge scores and their antibiotic usage practice scores ($r = 0.683$, $R^2 = 0.467$), indicating that higher knowledge was associated with better practices; however, practices were not significantly associated with inhibition zone diameters. These findings suggested that low antibiotic literacy may lead to improper use, while bacterial resistance is also influenced by external factors such as environmental contamination, horizontal gene transfer, and centralized medication protocols in contract farming.

Keywords: Antimicrobial resistance, Broiler, Knowledge, Practice, *Staphylococcus* spp.

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Research Paper

Effects of Fermented *Sargassum binderi* Meal on Productivity and Egg Quality of Laying Hens

Dewi YL, Sofyan A, and Mahata ME.

J. World Poult. Res. 15(3): 396-406, 2025; pii: S2322455X2500038-15

ABSTRACT: Brown algae, such as *Sargassum binderi* (*S. binderi*), are abundantly present in marine ecosystems and constitute a valuable natural resource. The present study aimed to investigate the effect of fermented *S. binderi* meal on the performance of laying hens and egg quality, as well as to establish the safe limits of its usage in the diet of laying hens. The 200 Isa Brown laying hens were randomly assigned to five different treatment groups based on fermented *S. binderi* levels, including the control group, which received 0% fermented *S. binderi*, while the other treatment groups received 4%, 8%, 12%, and 16% fermented *S. binderi*, respectively, over six weeks. At the end of the experiment, 80 eggs were assessed for quality. The variables included feed intake, hen day egg production, egg weight, egg mass, and feed conversion ratio, as well as external egg quality measures such as eggshell weight, percentage of eggshell, eggshell thickness, eggshell strength, egg length, egg width, and egg shape index and internal egg quality parameters including yolk weight, percentage of yolk, albumin weight, percentage of albumin, yolk color index, and haugh unit (HU). The current results indicated that the inclusion of fermented *S. binderi* at levels of 0, 4, 8, 12, and 16% in laying hens' diets had no significant effect on performance or external and internal egg quality. The utilization of fermented *S. binderi* is considered safe when incorporated at levels up to 16% within diets for laying hens.

Keywords: Egg quality, Fermented *Sargassum binderi*, Laying hen, Performance, *Sargassum binderi*



Dewi YL, Sofyan A, and Mahata ME (2025). Effects of Fermented *Sargassum binderi* Meal on Productivity and Egg Quality of Laying Hens. *J. World Poultry Res.*, 15(3): 396-406. DOI: <https://dx.doi.org/10.36380/jwpr.2025.38>

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Review

Newcastle Disease Virus Infection in Domestic Pigeons: Epidemiology, Pathogenesis, Diagnosis, and Vaccination Strategies with Emphasis on Chitosan Nanoparticles

Abdulrasol MA, Abd El-Ghany WAE-G, and Najem HA.

J. World Poultry Res. 15(3): 407-417, 2025; pii: S2322455X2500039-15

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ABSTRACT: Newcastle disease virus (NDV), also known as avian paramyxovirus-1 (APMV-1), is a highly contagious pathogen that affects most avian species, including domestic pigeons (*Columba livia*), leading to Newcastle disease (ND). The ND in pigeons is attributed to pigeon-specific strains of NDV, predominantly characterized by the emergence of pigeon paramyxovirus-1 (PPMV-1). This viral strain is specifically adapted to affect avian species, particularly within the pigeon population, resulting in distinct pathological features associated with the disease. The ND was correlated with severe respiratory, neurological, and gastrointestinal manifestations, resulting in elevated morbidity and mortality rates, which may reach up to 80%. The present study provided an updated overview of the pathogenesis, clinical and pathological features, and diagnostic approaches related to NDV infection in domestic pigeons worldwide. Conventional and modern vaccination strategies were discussed in the present study, with a focus on mucosal immunization. Chitosan-based nanoparticles (CS-NPs) have emerged as a promising vaccine delivery platform due to their compatibility with biological systems, strong adhesion to mucosal surfaces, and ability to enhance antigen stability and stimulate the immune response. The CS-NPs improved antigen uptake at mucosal surfaces in poultry and stimulated both humoral and cellular immune responses, which included activating cytotoxic T cells, producing cytokines, and secreting immunoglobulins at mucosal sites. The present review may contribute to the advancement of more effective and targeted vaccine strategies against NDV in pigeons and other avian species.

Keywords: Chitosan, Live vaccine, Nanoparticle, Newcastle disease, Paramyxovirus, Pigeon



Abdulrasol MA, Abd El-Ghany WAE-G, and Najem HA (2025). Newcastle Disease Virus Infection in Domestic Pigeons: Epidemiology, Pathogenesis, Diagnosis, and Vaccination Strategies with Emphasis on Chitosan Nanoparticles. *J. World Poultry Res.*, 15(3): 407-417. DOI: <https://dx.doi.org/10.36380/jwpr.2025.39>

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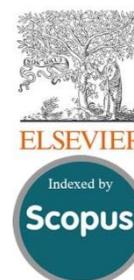
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Effects of Nettle (*Urtica dioica*) Supplementation on Productive Performance, Biochemical Parameters, and Gut Microbiota in Broiler Chickens

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ABSTRACT

The global poultry industry is challenged to meet rising demands for sustainable production, prompting interest in plant-based feed additives like *Urtica dioica* due to their nutritional and functional properties. The objective of this study was to evaluate the effects of dietary inclusion of *Urtica dioica* on growth performance, serum biochemical indicators, and intestinal microbiota composition in broiler chickens. The 42-day feeding trial involved 120 male Cobb 500 broiler chickens with an average initial body weight of 41.7 ± 1.2 g. Broiler chickens were randomly allocated to four experimental groups, each consisting of six replicates with ten chickens. The treatment groups received basal diets supplemented with 1% (T1), 2% (T2), or 3% (T3) *Urtica dioica*, while the control group (T0) was fed the basal diet without additives. Productive performance parameters were recorded weekly, and on day 42, blood profiles and intestinal microbiota composition were evaluated. Results showed that 1% *Urtica dioica* significantly improved live weight and feed conversion ratio (FCR) compared to the control group, with no additional benefits observed at higher inclusion levels. The biochemical assessment showed that broiler chickens supplemented with 1% *Urtica dioica* exhibited significant reductions in total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triacylglycerol levels compared to the control group. Microbial analysis demonstrated a significant increase in *Lactobacillus* spp. populations and a decrease in *coliform* bacteria in the 1% supplementation group, suggesting improved gut health. These findings indicated that 1% *Urtica dioica* supplementation enhances growth, lipid metabolism, and intestinal health in broiler chickens.

Keywords: *Urtica dioica*, Medicinal Plant, Chicken, Productive performance, Gut microbiota

INTRODUCTION

Poultry meat production provides an economical and accessible source of animal protein, essential for meeting the increasing demand driven by global population growth (Bist et al., 2024; Mnisi et al., 2024). However, the intensified nature of modern broiler production poses challenges related to animal health, welfare, and sustainability (Teixeira et al., 2023). In this context, interest has grown in the use of natural feed additives capable of improving productive performance while enhancing the physiological resilience of chickens

(Mitrović et al., 2020). *Urtica dioica* is a perennial herbaceous plant native to temperate regions of Europe, Asia, and North America, and is also found in the Peruvian Amazon, where it has long been used in traditional medicine (Bussmann and Sharon, 2006; Grauso et al., 2020; Rengifo et al., 2020). The antioxidant and anti-inflammatory properties of *Urtica dioica*, derived from its high content of phenolic compounds, flavonoids, vitamins, and essential minerals, support its potential use as a functional additive in poultry diets (Zenão et al., 2017; Kiani et al., 2020).

The nutritional composition of *Urtica dioica* leaves includes a high content of crude protein (33.8%), fiber (9.1%), essential amino acids, vitamins A, C, and K, and trace elements (Devkota et al., 2022; Taheri et al., 2022). These characteristics have led to growing interest in its application as a functional feed additive in poultry production. Several studies have demonstrated that dietary supplementation with *U. dioica* enhances productive performance in broiler chickens, including increased body weight gain and improved FCR (Behboodi et al., 2021; Chehri et al., 2022). Furthermore, several studies have reported positive effects on serum lipid profiles, including reductions in cholesterol and triglyceride levels (Maina et al., 2023; Teixeira et al., 2023), as well as favorable modulation of intestinal microbiota, characterized by increased populations of *Lactobacillus* spp. and reduced coliform bacteria, suggesting a prebiotic effect with implications for nutrient absorption and intestinal mucosal integrity (Abed and Ali, 2022). However, additional studies are required to clarify the underlying mechanisms, define optimal inclusion levels, and evaluate the effectiveness of *Urtica dioica* under varying production conditions. Therefore, the objective of this study was to evaluate the effects of dietary inclusion of *Urtica dioica* on growth performance, serum biochemical indicators, and intestinal microbiota composition in broiler chickens.

MATERIALS AND METHODS

Ethical approval

The experimental protocol was approved by the Institutional Research Ethics Committee of the National University Pedro Ruiz Gallo (UNPRG), located in Lambayeque, Peru. The study was conducted in accordance with the Peruvian Animal Protection and Welfare Law (Law No. 30407) and followed the guidelines established by the World Organization for Animal Health (OIE) to ensure the welfare of animals used for scientific purposes.

Harvesting and preparation of *Urtica dioica* meal

Fresh leaves and stems of *Urtica dioica* L. were collected during their flowering season from the Tsuntsunsa Native Community, Bagua, Amazonas, Peru (709 m.a.s.l.; Latitude 5°23'8.1" S, Longitude 78°28'4"W). Only healthy plant material was selected, ensuring the exclusion of damaged or contaminated parts. The collected plant material was first washed with distilled water to remove contaminants, dust, and foreign particles. The preliminary drying process was conducted at room

temperature (approximately 25°C) for 24 hours under controlled ventilation to prevent microbial growth. Afterward, the material was placed in a Memmert® UN75 forced-air oven (Memmert GmbH, Germany) and dried at 40°C for 24 hours to achieve consistent moisture reduction while maintaining the integrity of bioactive constituents. Once dried, the plant material was subjected to a two-step milling process to produce a uniform fine powder. Initially, it was coarsely ground using a Fritsch® Universal Cutting Mill PULVERISETTE 19 (Fritsch GmbH, Germany) to reduce the particle size. The resulting material was then finely milled and passed through a 1 mm mesh sieve to ensure consistency in particle size distribution. The resulting *Urtica dioica* meal was immediately stored in airtight containers under dry, dark conditions to preserve its nutritional and bioactive properties until further incorporation into the experimental diets. This preparation process followed the procedure described by Devkota et al. (2022).

Animal handling and experimental design

A total of 120 male Cobb 500 broiler chickens, each one-day-old and averaging 41.7 ± 1.2 g in body weight, were used in the experimental trial. Broiler chickens were randomly assigned to four dietary groups, each comprising six replicates with ten chickens per replicate. The treatment groups received basal diets supplemented with 1% (T1), 2% (T2), and 3% (*Urtica dioica*), while the control group (T0) was fed the basal diet without additives. The chickens were placed in 24 separate pens (0.30 m² each) within a facility equipped with controlled temperature and adequate ventilation. The ambient temperature was maintained at 32-34°C during the first week and was gradually decreased by approximately 2-3°C per week to reach 24°C by the end of the experimental period. Throughout the 42-day experimental period, all broiler chickens had *ad libitum* access to feed and water and were maintained under a lighting regimen of 23 hours of light and 1 hour of darkness per day.

Experimental diets

Feed for this study was prepared from conventional components, including corn, soybean meal, wheat by-products, and soybean oil. None of the treatments contained antibiotics, growth-stimulants, or anticoccidial additives. Broiler chickens were fed using an age-specific nutritional plan divided into three distinct phases: starter (days 1-14), grower (days 15-28), and finisher (days 29-42). The diets corresponding to each production phase were formulated to meet the nutritional demands of broiler

chickens, providing approximately 22% crude protein (CP) at the start and decreasing to 18% toward the end, with energy levels ranging between 3,200 and 3,100 kcal/kg. The basal diet was chemically analyzed following standardized procedures outlined by AOAC (1990). Throughout the 42-day growth period, broiler chickens had *ad libitum* access to feed and fresh water. A detailed composition of ingredients and nutritional values is presented in Table 1.

Table 1. Nutritional composition of experimental diets for broiler chickens at different growth phases (Starter, grower, and finisher)

Ingredient	Starter	Grower	Finisher
	(1-14 days) (%)	(15-28 days) (%)	(29-42 days) (%)
Yellow corn	55.00	58.00	61.00
Soybean meal (44% CP)	36.00	30.00	26.00
Wheat by-product	2.00	5.00	6.00
Soybean oil	3.50	3.50	3.50
Dicalcium phosphate	1.80	1.60	1.50
Calcium carbonate	1.00	1.00	0.90
Salt	0.40	0.35	0.30
DL-Methionine	0.30	0.28	0.25
L-Lysine	1.20	1.10	1.00
Choline chloride (60%)	0.10	0.10	0.10
Vitamin-mineral premix ¹	0.50	0.50	0.50
Mycotoxin binder	0.10	0.10	0.10
Calculated Composition (%)			
Metabolizable energy (kcal/kg) ²	3.2	3.15	3.1
Crude protein (%)	22.00	20.00	18.00
Crude fiber (%)	3.50	3.80	4.00
Calcium (%)	1.00	0.90	0.85
Available phosphorus (%)	0.45	0.42	0.40
Sodium (%)	0.18	0.17	0.16
Methionine + Cystine (%)	0.85	0.80	0.75
Lysine (%)	1.30	1.10	1.00

Vitamin and Mineral Premix per kg of diet: Vitamins: Retinol, 10,000,000 IU; Cholecalciferol, 3,000,000 IU; Tocopherol, 15,000 IU; Menadione, 2.5 g; Riboflavin, 6 g; Calcium pantothenate, 6 g; Niacin, 20 g; Pyridoxine, 4 g; Cyanocobalamin, 0.012 g; Biotin, 0.15 g; Folic acid, 0.5 g; Thiamine, 2 g. Minerals: Copper (Cu), 6 g; Zinc (Zn), 60 g; Manganese (Mn), 60 g; Iron (Fe), 40 g; Iodine (I), 1 g; Selenium (Se), 0.3 g; Cobalt (Co), 0.15 g. ²The metabolizable energy (kcal/kg) was estimated using the equation by [Carpenter and Clegg \(1956\)](#).

Production parameters

Production performance was evaluated by measuring body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) during the 42-day experimental period. Measurements were taken in three feeding phases: starter (1-14 days), grower (15-28 days), and finisher (29-42 days), as well as for the overall period (1-42 days).

Body weight gain

BWG was calculated as the difference between the final and initial body weights, recorded on days 0, 21, and 42. All measurements were performed in the morning before feed distribution.

$$\text{BWG (g)} = \text{Final live weight (g)} - \text{Initial live weight (g)} \quad (\text{Formula 1})$$

Feed intake

FI was determined daily by subtracting the weight of the residual feed from the amount of feed offered. Residual feed was weighed each morning before new feed was provided. Values were expressed per chicken per day.

$$\text{FI (g/chicken/day)} = \frac{\text{Feed offered (g)} - \text{Residual feed (g)}}{\text{Number of chickens}} \quad (\text{Formula 2})$$

Feed conversion ratio

FCR was calculated as the ratio of total FI to total body weight gain for each replicate.

$$\text{FCR} = \frac{\text{Total FI (g)}}{\text{Total BWG (g)}} \quad (\text{Formula 3})$$

Blood serum parameters

At day 42, a total of 48 broiler chickens (three per replicate and twelve per treatment) were randomly chosen for blood sampling. Around 6 mL of blood was collected from each chicken during slaughter by exsanguination and placed into sterile centrifuge tubes. The samples were then centrifuged at 3000 rpm for 15 minutes to separate the serum, which was promptly stored at -20 °C for subsequent analysis. Biochemical parameters, including total protein, albumin, globulin, triglycerides, total cholesterol, high-density lipoprotein (HDL) cholesterol, glucose, aspartate aminotransferase (AST), and alanine aminotransferase (ALT), were assessed using commercial diagnostic kits from Wiener Lab® (Rosario, Argentina) and BIOLABO® (Maizy, France), following the manufacturers' instructions. Total protein was measured using the colorimetric method described by [Henry et al. \(1974\)](#), while albumin was determined following the procedure of [Doumas et al. \(1971\)](#). Globulin concentration was obtained by subtracting albumin from total protein.

The activities of AST and ALT were interpreted based on the protocol outlined by the [Center \(2007\)](#).

Ileal and cecal bacterial counts

On day 42 of the trial, ileal and cecal digesta were aseptically collected from 48 broiler chickens (three chickens per replicate, 12 per treatment) immediately post-slaughter to prevent microbial alterations. Approximately one g of fresh intestinal content was homogenized in 9 mL of sterile buffered peptone water (Condalab®, Madrid, Spain) using a vortex mixer for one minute. Serial dilutions were prepared, and 0.1 mL aliquots were plated onto selective culture media. All media were prepared one day prior and poured into sterile Petri dishes under aseptic conditions. Collection tubes were autoclaved at 121°C for 10 minutes and sealed with aluminum foil until use. After homogenization and shaking for 30 minutes to facilitate microbial suspension, 1 mL of each sample was further diluted in 9 mL of phosphate-buffered saline (PBS; Oxoid™, Basingstoke, UK). *Lactobacillus* spp. were cultured on de Man, Rogosa, and Sharpe (MRS) agar (Oxoid™, UK) under anaerobic conditions at 37°C for 72 hours. Total aerobic bacteria and *coliforms* were cultured on MacConkey agar (Oxoid™, UK) under aerobic conditions at 37°C for 48 hours. Colony counts were performed using an automatic colony counter (Scan® 500, Interscience, France), and results were expressed as log₁₀ colony-forming units (CFU) per gram of intestinal content.

Statistical analysis

All collected data were first subjected to a normality check using the Shapiro–Wilk test to ensure appropriate distribution for parametric analysis. Subsequently, one-way analysis of variance (ANOVA) was conducted using the General Linear Model (GLM) procedure in SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA). When significant treatment effects were detected ($P < 0.05$), Tukey's post hoc test was applied to identify differences among group means. Results are reported as mean values \pm standard error (SE), and statistical significance was declared at $P < 0.05$.

RESULTS

Growth and feed efficiency in broiler chickens

There was no significant difference in feed consumption across the treatment groups throughout the experimental period, with values ranging between 3460 and 3475 g/chicken ($p > 0.05$). However, significant differences were observed in live weight at days 21 and

42, with the highest values recorded in chickens supplemented with 1% *Urtica dioica*, showing an improvement of 8.96% and 7.40% compared to the control group, respectively ($p < 0.05$). At Day 21, broilers in the 1% group showed the best growth performance (730 g), followed by the 3% group (720 g). A similar trend was noted at Day 42, where the 1% group achieved the highest live weight (2395 g), significantly higher than the control (2230 g), the 3% group (2250 g), and slightly higher than the 2% group (2360 g; $p < 0.05$). The FCR also exhibited significant differences ($p < 0.05$), with the control group showing the least efficient feed utilization. Significant differences ($p < 0.05$) were observed in the overall FCR, with the most efficient value recorded in the 2% group (1.78), followed by the control (1.79), 3% (1.84), and 1% (1.86; Table 2).

Biochemical parameters in broiler chickens

It is clear from Table 3 that the treatments with *Urtica dioica* inclusion resulted in a significant decrease ($p < 0.05$) in total cholesterol, HDL, and LDL levels, with the 1% inclusion group showing the most notable reductions. The 1% group had significantly reduced total cholesterol (125 mg/dL) compared to the control group, and although triacylglycerol levels were numerically lower (45 mg/dL), this difference was not statistically significant ($p > 0.05$). However, there were no significant differences in glucose, total protein, albumin, globulin, or liver function parameters (AST and ALT) among the treatment groups when compared to the control group ($p > 0.05$; Table 3).

Duodenal and cecal microbial populations in broilers

Feeding broiler chickens with diets containing *Urtica dioica* at different inclusion levels significantly affected the microbial population in both the duodenum and cecum ($p < 0.05$). The count of *Lactobacillus* spp. in the duodenum increased significantly ($p < 0.05$) in the 1% *Urtica dioica* group (4.00 CFU/g) compared to the control group (2.10 CFU/g). Similarly, a significant decrease in *coliform* bacteria was observed with increasing levels of *Urtica dioica*, with the 3% group showing the lowest count (6.90 CFU/g) compared to the control (7.90 CFU/g; $p < 0.05$). In the cecum, the count of *Lactobacillus* spp. also increased significantly ($p < 0.05$) with 1% inclusion, reaching 3.60 CFU/g compared to 2.20 CFU/g in the control group. The *coliform* bacteria count in the cecum significantly decreased in the 1% group (6.10 CFU/g) compared to the control group (9.10 CFU/g; $p < 0.05$; Table 4).

Table 2. Effects of *Urtica dioica* inclusion levels on feed intake, live weight, and feed conversion in broiler chickens aged 1 to 42 days.

Parameter	Control	Percentage of dietary inclusion of <i>Urtica dioica</i> (%)			SEM	P-value
		1%	2%	3%		
Feed consumption g/chicken						
1-21 days	0.60 ^a ± 0.03	0.61 ^a ± 0.03	0.62 ^a ± 0.03	0.60 ^a ± 0.03	0.03	0.311
22-42 days	1,120 ^a ± 12.0	1,115 ^a ± 12.0	1,122 ^a ± 12.0	1,118 ^a ± 12.0	12.0	0.352
1-42 days	3,460 ^a ± 25.0	3,450 ^a ± 25.0	3,475 ^a ± 25.0	3,468 ^a ± 25.0	25.0	0.622
Live weight (g)						
Initial	43.00 ^a ± 1.40	43.30 ^a ± 1.40	43.50 ^a ± 1.40	42.90 ^a ± 1.40	1.40	0.939
Day 21	670.00 ^c ± 15.0	730.00 ^a ± 15.0	698.00 ^b ± 15.0	720.00 ^a ± 15.0	15.0	< 0.01
Day 42	2,230.00 ^c ± 20.0	2,395.00 ^a ± 20.0	2,360.00 ^b ± 20.0	2,250.00 ^c ± 20.0	20.0	< 0.01
Feed conversion ratio						
0-21 days	1.23 ^c ± 0.01	1.27 ^a ± 0.01	1.26 ^b ± 0.01	1.26 ^b ± 0.01	0.01	< 0.01
22-42 days	1.88 ^c ± 0.04	2.05 ^a ± 0.04	1.92 ^b ± 0.04	1.94 ^b ± 0.04	0.04	< 0.01
1-42 days	1.79 ^c ± 0.03	1.86 ^a ± 0.03	1.78 ^b ± 0.03	1.84 ^b ± 0.03	0.03	< 0.01

SEM: Standard error of mean. ^{a,b,c} Values in a row with different superscripts are significantly different (p < 0.05).

Table 3. Effects of *Urtica dioica* inclusion levels on serum biochemical parameters and liver function in broiler chickens at 42 days of age

Parameter	Control	Dietary inclusion of <i>Urtica dioica</i> (%)			SEM	P-value
		1%	2%	3%		
Total cholesterol (mg/dL)	150.00 ^a ± 1.31	125.00 ^c ± 1.31	145.00 ^b ± 1.31	143.00 ^b ± 1.31	1.31	< 0.01
HDL (mg/dL)	97.00 ^a ± 0.07	90.00 ^c ± 0.07	94.00 ^b ± 0.07	93.50 ^b ± 0.07	0.07	< 0.01
LDL (mg/dL)	54.00 ^a ± 0.07	48.00 ^c ± 0.07	52.00 ^b ± 0.07	51.80 ^b ± 0.07	0.07	< 0.01
Triacylglycerols (mg/dL)	60.00 ^a ± 1.28	45.00 ^a ± 1.28	58.00 ^a ± 1.28	56.50 ^a ± 1.28	1.28	0.424
Glucose (mg/dL)	184.32 ^a ± 3.55	191.44 ^a ± 3.55	185.02 ^a ± 3.55	186.62 ^a ± 3.55	3.55	0.504
Total protein (g/dL)	2.80 ^a ± 0.17	3.06 ^a ± 0.17	2.95 ^a ± 0.17	2.99 ^a ± 0.17	0.17	0.720
Albumin (g/dL)	1.30 ^a ± 0.14	1.39 ^a ± 0.14	1.42 ^a ± 0.14	1.56 ^a ± 0.14	0.14	0.638
Globulin (g/dL)	0.04 ^a ± 0.01	0.06 ^a ± 0.01	0.05 ^a ± 0.01	0.05 ^a ± 0.01	0.01	0.537
Liver function						
AST (UI/L)	308.70 ^a ± 20.54	353.38 ^a ± 20.54	360.38 ^a ± 20.54	355.94 ^a ± 20.54	20.54	0.285
ALT (UI/L)	2.90 ^a ± 0.37	3.20 ^a ± 0.37	3.10 ^a ± 0.37	2.98 ^a ± 0.37	0.37	0.940

SEM: Standard error of mean. ^{a,b,c} Values in a row with different superscripts are significantly different (p < 0.05). HDL: High-density lipoprotein; LDL: Low-density lipoprotein; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase.

Table 4. Microbial counts in the duodenum and cecum (CFU/g) of broiler chickens at 42 days of age fed diets with different *Urtica dioica* inclusion levels

Parameter	Control	Dietary inclusion of <i>Urtica dioica</i> (%)			SEM	P-value
		1%	2%	3%		
Duodenum						
<i>Lactobacillus</i> spp.	2.10 ^c ± 0.14	4.00 ^a ± 0.14	3.70 ^b ± 0.14	3.40 ^b ± 0.14	0.14	0.020
<i>Coliform</i> bacteria	7.90 ^a ± 0.18	7.20 ^b ± 0.18	7.00 ^b ± 0.18	6.90 ^c ± 0.18	0.18	0.030
Cecum						
<i>Lactobacillus</i> spp. (Cecum)	2.20 ^b ± 0.16	3.60 ^a ± 0.16	3.30 ^{ab} ± 0.16	3.10 ^b ± 0.16	0.16	< 0.01
<i>Coliform</i> bacteria	9.10 ^a ± 0.23	6.10 ^c ± 0.23	7.40 ^b ± 0.23	7.00 ^b ± 0.23	0.23	0.020

* SEM: Standard error of mean. ^{a,b,c} Values in a row with different superscripts are significantly different (p < 0.05).

DISCUSSION

In the present study, the inclusion of 1% *Urtica dioica* in the diet was associated with improvements in BW and FCR in Cobb 500 broiler chickens from 1 to 42 days of age. These results are consistent with the findings of [Teixeira et al. \(2023\)](#), who reported that dietary supplementation with 1% *Urtica dioica* significantly improved growth performance and feed efficiency in Ross 308 broilers, attributing these effects to the presence of bioactive compounds with antioxidant, immunomodulatory, and digestive properties. It is worth noting that, although the 3% inclusion of *Urtica dioica* resulted in higher BW compared to the 2% group, no improvement in FCR was observed. This phenomenon may be related to the presence of antinutritional factors, such as tannins and alkaloids, which are found in higher concentrations of *Urtica dioica* and can interfere with nutrient absorption and utilization ([Keshavarz et al., 2014](#)). However, in the present study, differences in FCR between treatments indicate variations in feed utilization efficiency. While the control and 2% *Urtica dioica* groups exhibited the best FCR, the 1% and 3% inclusion groups demonstrated higher body weights. These findings align with those reported by [Maina et al. \(2023\)](#), who observed that supplementing Cobb 500 broiler diets with 1.5% *Urtica dioica* enhanced weight gain, while a 2% inclusion level led to improved FCR, indicating a dose-dependent effect. Similarly, [Keshavarz et al. \(2014\)](#) observed that *Urtica dioica* supplementation influenced lipid metabolism in broiler chickens, which may explain the observed differences in BWG and FCR among treatments. Higher dietary inclusion levels of *Urtica dioica* may introduce antinutritional factors, such as tannins and alkaloids, which could impair nutrient absorption and utilization ([Gadde et al., 2017](#)).

The biochemical blood evaluation is a key tool for the continuous monitoring of poultry health, as it allows for the early detection of physiological alterations and facilitates the timely diagnosis of various diseases ([Franciosini et al., 2023](#)). In the present study, the dietary inclusion of *Urtica dioica* significantly reduced total cholesterol and LDL levels in broilers, with the most pronounced effect observed in the 1% treatment. However, as the supplementation rate increased, cholesterol levels also increased, suggesting a dose-dependent response. Despite higher cholesterol levels in the 2% and 3% groups, other biochemical parameters remained stable across treatments, indicating that the main effect of *Urtica*

dioica was on cholesterol modulation. These findings aligned with previous studies suggesting that nettle's bioactive compounds, such as phytosterols and flavonoids, contribute to lowering cholesterol by inhibiting its intestinal absorption and promoting its excretion ([Righi et al., 2021](#)). The reduction in LDL levels observed in the 1% and 3% treatments further supports the role of *Urtica dioica* in modulating lipid metabolism ([Hashem and Salem, 2022](#)). Interestingly, the present study showed significantly lower HDL concentrations in all *Urtica dioica* groups compared to the control, with the most pronounced reduction in the 1% group (90.00 mg/dL vs. 97.00 mg/dL). These findings are consistent with those of [Teixeira et al. \(2023\)](#), who also reported reduced HDL levels in broilers fed *Urtica urens*, whereas higher values were observed in the control group. In concordance with the findings of [Safamehr et al. \(2012\)](#), the inclusion of *Urtica dioica* in broiler diets did not significantly alter other biochemical parameters, including triacylglycerols, glucose, total protein, albumin, and globulin. Similarly, [Adam et al. \(2020\)](#), who conducted their study with Arbor Acres broiler chickens, found no significant changes in blood metabolites with nettle supplementation, further supporting its safety as a dietary additive. Additionally, the stability of AST and ALT levels across treatments in the present study suggests that *Urtica dioica* does not negatively impact liver function, confirming previous reports that its supplementation did not induce hepatic stress or toxicity in broilers ([Özen and Korkmaz, 2003](#)).

In the present study, a significant decrease in the logarithmic total number of *coliforms* was observed, along with an increase in *Lactobacillus* spp. (cfu/g) in the duodenal and cecal contents of broiler chickens, with the most notable effect seen in the 1% *Urtica dioica* supplementation. These results align with those of [Abed and Ali \(2022\)](#), who studied Ross 380 broiler chickens and found that *Urtica dioica* can promote intestinal microbial balance by boosting beneficial bacteria and reducing pathogens. Specifically, supplementation with *Urtica dioica* significantly enhanced the growth of beneficial bacteria such as *Lactobacillus* spp., which are vital for maintaining a healthy gut environment. This effect may be due to bioactive compounds in *Urtica dioica*, like flavonoids and phenols, which have antimicrobial properties capable of disrupting pathogenic bacterial cell membranes and interfering with their metabolism ([Kupnik et al., 2021](#)). The reduction in *coliform* bacteria supports previous studies highlighting *Urtica dioica's* ability to inhibit both Gram-positive and Gram-negative bacteria

because of its phenolic compounds with antibacterial activity (Tabari et al., 2016). However, increasing the supplementation to 2% and 3% did not produce significant differences between these levels, indicating that higher doses of *Urtica dioica* do not offer additional benefits. This aligns with findings by Kiani et al. (2020), who reported that *Lactobacillus* spp. produces bacteriocins with stronger antibacterial effects than *Urtica dioica* extracts against antibiotic-resistant bacteria. In this context, the microbial changes caused by *Urtica dioica* may depend on interactions between its bioactive compounds and the gut microbiota, underscoring the importance of identifying the optimal dosage to maximize benefits for broiler gut health without causing adverse effects.

CONCLUSION

Dietary inclusion of 1% *Urtica dioica* significantly enhanced growth performance, modulated lipid metabolism by reducing serum total cholesterol and LDL levels, and improved gut microbial balance by increasing *Lactobacillus* counts and reducing coliform populations in broiler chickens. No adverse effects were observed on liver enzymes or other biochemical markers, supporting its safety as a feed additive. Future studies should investigate other *Urtica* species, explore their potential as a natural growth promoter, and evaluate their effects on gut microbiota dynamics, immune responses, and carcass traits under commercial conditions.

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Availability of data and materials

Data generated or analyzed during this study are available upon reasonable request from the corresponding author.

Authors' contributions

Edwaldo Villanueva Pedraza contributed to the conceptualization of the study, drafted the original manuscript, and managed the overall project. Pompeyo Ferro participated in the conceptualization and contributed to the review and editing of the manuscript. Jeiner Alexander Villanueva Guerrero was responsible for the execution of the experiment and the collection of experimental data. Johnny Cueva Valdivia performed the formal data analysis and statistical evaluations. Anthony Smith Guevara Flores assisted with data collection and fieldwork. José Alberto Carlos Ramos supervised the study process and validated the results. Euclides Ticona Chayña provided supervision and contributed to the critical review and editing of the manuscript. Papa Pio Ascona García supported the development of the methodology. All authors have read and approved the final version of the manuscript before publication in the present journal.

Competing interests

The authors declare that they have no competing interests.

Ethical considerations

The authors affirm that this manuscript is original and has not been submitted for publication elsewhere. Furthermore, they assure that the data included in this manuscript is truthful and has not been manipulated.

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Effects of Green Betel Leaf (*Piper betle* L.) Extract and Citric Acid on Nutrient Utility, Health, and Meat Quality of Broiler Chickens

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ABSTRACT

The broiler chicken industry has rapidly expanded in recent decades due to increasing demand for affordable animal protein and the species' high production efficiency. This study aimed to evaluate the effects of supplementing broiler chickens' drinking water with green betel leaf extract and citric acid on their nutrient utilization, health, and meat quality. A total of 200 unsexed day-old chicks (DOC) of Cobb 500 strain broiler chickens with an average initial weight of 42.17 ± 0.53 g were used. A completely randomized design (CRD) was implemented, comprising four treatment groups with five replications each, and each replicate consisted of 10 broiler chickens. The treatments involved administering drinking water containing a mixture of green betel leaf extract and citric acid at concentrations of 0% (T0, control), 1% of drinking water (10 ml, T1), 2% of drinking water (20 ml, T2), and 3% of drinking water (30 ml, T3). Parameters assessed included nutrient digestibility (protein, fat, and true metabolizable energy), meat quality (protein, fat, cholesterol content, water holding capacity, and drip loss), blood profiles (erythrocyte, leukocyte, hemoglobin, and hematocrit levels), and oxidative stress indicators (superoxide dismutase and malondialdehyde). Results indicated that T3 significantly had higher protein digestibility (77.22%), fat digestibility (76.00%), true metabolizable energy (3,010.09 kcal/kg), and meat protein content (21.26%) compared to T0, while reducing drip loss (27.93%) and MDA levels (7.88 nmol/mL) compared to T0. In conclusion, supplementing drinking water with 3% feed additive effectively enhances nutrient utility, health, and meat quality in broiler chickens.

Keywords: Betel leaf, Broiler, Citric acid, Meat quality, Nutrient utility

INTRODUCTION

Broiler chickens are among the most commonly raised poultry due to their rapid growth rate, short rearing period, and efficient feed conversion, making them a key commodity in the livestock industry. However, despite these advantages, broiler chickens are highly vulnerable to oxidative stress, particularly due to the overproduction of reactive oxygen species (ROS). Under normal physiological conditions, including superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-), are natural by-products of cellular metabolism and play essential roles in cell signaling and immune defense (Chen et al., 2021). When present in excess, however, ROS overwhelm the body's antioxidant defense systems, resulting in oxidative stress. This condition leads to lipid peroxidation, protein and DNA damage, impaired immune

responses, reduced feed efficiency, slower growth, and deteriorated meat quality in broiler chickens (Surai et al., 2019; Mishra and Jha, 2019; Oke et al., 2024).

Diet plays a crucial role in modulating oxidative stress, as nutritional interventions can either exacerbate or mitigate ROS production. Diets lacking in antioxidants or containing oxidized fats can increase ROS levels, whereas supplementation with natural antioxidants has been shown to reduce oxidative damage and improve overall performance. In this context, herbal additives rich in bioactive compounds have gained attention as natural alternatives to synthetic antioxidants. One such plant is green betel leaf, known for its high content of phenolic compounds, flavonoids, and tannins, which exhibit potent antioxidant, antimicrobial, and immunostimulatory effects (Lodang et al., 2020; Setyabudi et al., 2020). Active constituents such as hydroxychavicol, eugenol, and

kavikol have been reported to scavenge free radicals, inhibit oxidative enzymes, and enhance the activity of endogenous antioxidants like superoxide dismutase (SOD, Sumarya et al., 2016; Nguyen et al., 2020).

In addition, citric acid commonly used organic acid in poultry production, supports gut health by lowering the pH of drinking water and the gastrointestinal tract, thereby suppressing pathogenic bacteria such as *Escherichia coli* and *Salmonella* while enhancing mineral absorption and nutrient digestibility (Vieira et al., 2017; Liao et al., 2018). The combination of green betel leaf extract and citric acid in broiler chickens' drinking water may provide a synergistic approach to mitigating oxidative stress and improving broiler chickens' performance through their combined antioxidant and antimicrobial effects. Therefore, evaluating the integrated use of these additives is important for enhancing nutrient utility, health, and meat quality in broiler chicken production.

This study aimed to evaluate the effects of supplementing drinking water with green betel leaf extract and citric acid on nutrient utility, health, and meat quality in broiler chickens.

MATERIALS AND METHODS

Ethical approval

All procedures involving animals were approved by the Faculty of Animal and Agricultural Science, Diponegoro University, Semarang, Indonesia (Approval No. 61-05/A-12/KEP-FPP).

Experimental design

The experiment was carried out from December 2024 to January 2025 at the Poultry House of the Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang. A total of 200 unsexed day-old chicks (DOC) of Cobb 500 strain broiler chickens with an average initial weight of 42.17 ± 0.53 g were used. A completely randomized design (CRD) was implemented, comprising four treatment groups with five replications each, and each replicate consisted of 10 broiler chickens. The treatments involved administering drinking water containing a mixture of green betel leaf extract and citric acid at concentrations of 0% (T0, control), 1% of drinking water (10 ml, T1), 2% of drinking water (20 ml, T2), and 3% of drinking water (30 ml, T3). A proximate analysis was conducted on the commercial feed (BR1 for starter and BR2 for finisher, produced by PT Sreeya Sewu Indonesia Tbk). Both feed and water were provided daily at 7 a.m. The broilers were housed in 1×1 m² pens, maintaining a stocking density of 10 broiler chickens/m². The ambient

temperature within the pens was kept between 24°C and 32°C, and each pen was illuminated using a 50-watt bulb to ensure adequate lighting. Data on the nutrient composition of commercial BR1 and BR2 feed are listed in Table 1. Feed was analyzed for proximate and mineral content at the Laboratory of Feed and Nutrition Science, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Indonesia.

Table 1. Nutrient composition of commercial starter (BR1) and finisher (BR2) feed in the present study

Nutrient composition	Starter	Finisher
Metabolizable energy (kcal/kg)	3,082.56	3,116.27
Crude protein (%)	21.88	19.63
Crude fat (%)	4.59	4.74
Crude fiber (%)	4.41	4.32
Calcium (%)	1.05	1.08
Phosphorus (%)	0.76	0.81

Feed additive preparation

The feed additive was prepared using a decoction method based on Kaneria et al. (2012). Fresh green betel leaves obtained from local farms. Fresh green betel leaves were oven-dried at 50°C to reduce moisture, ground into powder, and 50 g of the powder was dissolved in 1 liter of distilled water. The mixture was homogenized, incubated in a water bath at 100°C for 30 minutes, then allowed to cool for 10-15 minutes. It was filtered to separate the solid residue, resulting in a green betel leaf extract. This extract was then mixed with 1 g of citric acid to produce the final feed additive solution. Citric acid is used to optimize the work of green betel leaf extract, so the authors do not differentiate the dosage of citric acid used. The additive was administered in volumes of 10 ml (T1), 20 ml (T2), and 30 ml (T3), depending on the treatment group. Data on the content of green betel leaf extract and citric acid are listed in Tables 2 and 3.

Table 2. Green betel leaf extract content used in the present study

Composition	Percentage
Proanthocyanidin	9.75%
Catechin	6.84%
Gallic acid	4.72%
Rutin	3.93%
Hydroxychavicol	2.87%
Quercetin glycoside	2.42%
Eugenol	2.41%
Ellagic acid	1.86%

Source: Analysis results from the Phytochemistry Laboratory, Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, Indonesia.

Table 3. Citric acid content used in the present study

Content	Percentage
Oxygen	58.33%
Carbon	37.50%
Hydrogen	4.17%

Source: Analysis results from the Phytochemistry Laboratory, Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, Indonesia.

Nutrient digestibility

Data on protein digestibility, fat digestibility, and true metabolizable energy were obtained using the total excreta collection method based on Hossain *et al.* (2013). One broiler chicken per replicate was placed in a battery cage. After a 24-hour fasting period (water only), chickens were fed diets containing Fe₂O₃ and treatment water. Fe₂O₃ is used as an indicator to differentiate the excreta, where excreta without the indicator appear green, while excreta containing the indicator turn reddish. Excreta marked red were collected and sprayed hourly with 0.2 N HCl to prevent nitrogen loss. This was repeated over 3 days. Excreta from 20 endogenous chickens (fasted for 2 days) were also collected.

Blood profile and oxidative stress

Blood samples (3 mL) were collected from the brachial vein using a syringe and stored in EDTA-containing vacutainers. Samples were kept in a cooler box and analyzed in the laboratory.

Meat protein, fat, and cholesterol levels

Meat samples from the thigh and breast (50 g) were homogenized and analyzed in the laboratory to determine the levels of protein, fat, and cholesterol.

Water holding capacity

The method used to measure water holding capacity is based on Qiao *et al.* (2001). A 0.3 g meat sample was pressed between Whatman filter papers under a 35 kg load for 5 minutes, and the wet area was traced to determine water holding capacity.

Drip loss

The method used to measure drip loss is based on Sarkar *et al.* (2024). A 100 g meat sample was sealed in a plastic bag, boiled in an 80°C water bath for 1 hour, then

cooled in 10°C water for 15 minutes. After drying, the sample was weighed to determine drip loss.

Data analysis

Data were analyzed using SPSS version 26.0. Data were analyzed using one-way ANOVA. If significant differences were found, Duncan's multiple range test was used to compare treatments (at a 5% significance level).

RESULT AND DISCUSSION

Nutrient digestibility

The addition of green betel leaf extract and citric acid significantly increased protein digestibility in broiler chickens compared to T0 ($p < 0.05$, Table 4). The highest digestibility was observed in T3 (3% additive), which did not differ significantly from T2 but was significantly higher than T1 and T0. T0 had the lowest value among the groups. The improvement is attributed to the synergistic action of betel leaf phytochemicals and citric acid, which enhance protease activity, optimize gut pH, suppress pathogenic bacteria, and support gut health (Lodang *et al.*, 2020; Melaku *et al.*, 2021).

The combination of green betel leaf extract and citric acid significantly improved fat digestibility in broiler chickens compared to T0 ($p < 0.05$, Table 4). The highest digestibility was observed in T3 (76.00%), followed by T2 and T1, while the lowest was in the control group T0 (73.53%). The improvement is attributed to enhanced lipase activity, better fat emulsification, and suppression of digestive-inhibiting bacteria. Bioactive compounds in betel leaf, such as flavonoids, tannins, and saponins, stimulate bile secretion and aid fat breakdown (Singh *et al.*, 2023). Citric acid supports an optimal pH for lipase function and improves fatty acid solubility, enhancing absorption efficiency (Hosseini *et al.*, 2017).

Supplementation of green betel leaf extract and citric acid significantly increased true metabolizable energy in broiler chickens ($p < 0.05$), with the highest value in T3 (3,010.09 kcal/kg) compared to T0 (2,826.34 kcal/kg) (Table 4). The enhancement is linked to improved protein and fat digestibility, increased ATP production via the Krebs cycle, and better mitochondrial efficiency (Bottje *et al.*, 2017).

Table 4. Effects of green betel leaf extract and citric acid supplementation on nutrient digestibility in broiler chickens

Parameters	Treatments			
	T0	T1	T2	T3
Protein digestibility (%)	74.87 ^c	75.74 ^b	76.44 ^{ab}	77.22 ^a
Fat digestibility (%)	73.53 ^c	74.27 ^{bc}	75.10 ^{ab}	76.00 ^a
True metabolizable energy (kcal/kg)	2,826.34 ^c	2,890.25 ^{bc}	2,949.91 ^{ab}	3,010.09 ^a

^{a-c} Different superscripts in the same column indicate a significant difference ($p < 0.05$). T0: Drinking water without feed additive (control), T1: Drinking water + 1% feed additive, T2: Drinking water + 2% feed additive, T3: Drinking water + 3% feed additive.

Meat quality

The combination of green betel leaf extract and citric acid significantly increased broiler chickens' meat protein content ($p < 0.05$), with the highest value in T3 (21.26%) compared to T0 (18.95%) (Table 5). Bioactive compounds in betel leaf support gut health and reduce oxidative stress (Hossain et al., 2017), while citric acid optimizes proteolytic enzyme activity and mineral absorption (Hosseini et al., 2017).

The combination of green betel leaf extract and citric acid significantly increased broiler chickens' meat fat content ($p < 0.05$), with the highest in T3 (7.21%) compared to T0 (5.19%) (Table 5). Bioactive compounds in betel leaf promote bile secretion and lipid absorption, while citric acid optimizes lipase activity and energy metabolism via the citric acid cycle (Han et al., 2016; Ding et al., 2022).

The combination of green betel leaf extract and citric acid significantly increased broiler chickens' meat cholesterol levels ($p < 0.05$), with the highest value in T3 (87.62 mg/100g) compared to T0 (84.66 mg/100g, Table 5). This increase may result from enhanced cholesterol absorption, elevated acetyl-CoA availability, and stimulated endogenous synthesis (Ge et al., 2019). Although betel leaf has hypocholesterolemic properties, dosage or interaction effects may have reversed its function. Despite contradicting earlier studies, cholesterol levels remained within the normal range (80-120 mg/100g; Giampietro-Ganeco et al., 2020).

The addition of green betel leaf extract and citric acid significantly improved the water-holding capacity (WHC) of broiler chicken meat ($p < 0.05$), with the highest value in T3 (62.04%) compared to T0 (59.73%) (Table 5). Bioactive compounds in betel leaf protect muscle structure, while citric acid helps regulate pH and glycolysis, enhancing protein stability (Panpipat and Chaijan, 2016; Chodkowska et al., 2022). Their synergistic effect leads to more compact muscle tissue with improved water retention, indicating better meat quality.

The supplementation of green betel leaf extract and citric acid significantly reduced drip loss in broiler chicken meat ($p < 0.05$), with the lowest value in T3 (27.93%) compared to T0 (30.20%) (Table 5). Bioactive compounds protect against heat-induced protein denaturation, while citric acid stabilizes pH and enhances protein-water interactions (Lodang et al., 2020; Unal et al., 2022). Their synergistic effects result in juicier, higher-quality meat with less moisture loss during cooking.

Blood profile and oxidative stress

The combination of green betel leaf extract and citric acid significantly increased erythrocyte levels in broiler chickens ($p < 0.05$), with the highest count observed in T3 ($2.82 \times 10^6/\mu\text{L}$) compared to T0 ($2.34 \times 10^6/\text{mm}^3$, Table 6). The synergistic interaction of bioactive compounds found in betel leaf and citric acid contributes to improved hematological parameters by increasing iron bioavailability, promoting erythropoietin synthesis, and safeguarding bone marrow from oxidative damage, thereby supporting red blood cell formation.

The combination of green betel leaf extract and citric acid significantly increased leukocyte levels in broiler chickens ($p < 0.05$), with the highest count in T3 ($22.95 \times 10^3/\text{mm}^3$) compared to T0 ($20.41 \times 10^3/\text{mm}^3$, Table 6). Flavonoids and alkaloids stimulate immune cell production and enhance pathogen resistance (Rahayu et al., 2023), while citric acid supports leukocyte function through antioxidant protection and better metabolic activity (Krauze et al., 2021).

The combination of green betel leaf extract and citric acid significantly increased hemoglobin levels in broiler chickens ($p < 0.05$), with the highest value in T3 (11.91 g/dL) compared to T0 (9.89 g/dL, Table 6). The bioactive properties of citric acid contribute to hemoglobin synthesis by improving the absorption of essential minerals, shielding red blood cells from oxidative damage, and enhancing both the quality and functionality of erythrocytes (Elnaggar and El-Kelawy, 2024).

Supplementation with green betel leaf extract and citric acid significantly increased hematocrit levels in broiler chickens ($p < 0.05$), with T3 (27.20%) showing the highest value compared to T0 (23.99%, Table 6). Bioactive compounds in citric acid promote iron absorption, protect erythrocyte membranes from oxidative stress, and stimulate erythropoietin production, thus improving both quantity and quality of red blood cells (Islam et al., 2021).

Dietary supplementation with green betel leaf extract and citric acid significantly increased blood SOD levels in broiler chickens ($p < 0.05$), with the highest activity observed in T3 (2.717 U/gHb) compared to T0 (2.460 U/gHb, Table 6). This enhancement is attributed to the synergistic effects of flavonoids and citric acid in inducing SOD gene expression and stabilizing the enzyme (Tang et al., 2019; Ebeid and Al-Homidan, 2022).

Supplementation with green betel leaf extract and citric acid significantly reduced blood MDA levels in broiler chickens ($p < 0.05$), with the lowest value in T3 (7.88 nmol/mL) compared to T0 (9.91 nmol/mL, Table 6). The effect is linked to enhanced antioxidant capacity from flavonoids, tannins, and citric acid, which act as free radical scavengers, metal chelators, and membrane stabilizers, improving cellular integrity and metabolic health (Sahin et al., 2016; Magied, 2019).

Table 5. Effects of green betel leaf extract and citric acid supplementation on broiler chickens' meat quality

Parameters	T0	T1	T2	T3
Meat protein content (%)	18.95 ^c	20.17 ^b	20.65 ^{ab}	21.26 ^a
Meat fat content (%)	5.19 ^a	5.83 ^{ab}	6.50 ^{bc}	7.21 ^c
Meat cholesterol content (mg/100g)	84.66 ^a	85.66 ^b	86.44 ^{bc}	87.62 ^c
Water holding capacity (%)	59.73 ^c	60.27 ^{bc}	60.78 ^b	62.04 ^a
Drip loss (%)	30.20 ^c	29.27 ^{bc}	28.42 ^{ab}	27.93 ^a

^{a-c} Different superscripts in the same column indicate a significant difference ($p < 0.05$). T0: Drinking water without feed additive (control), T1: Drinking water + 1% feed additive, T2: Drinking water + 2% feed additive, T3: Drinking water + 3% feed additive.

Table 6. Effects of green betel leaf extract and citric acid supplementation on blood profile and oxidative stress in broiler chickens

Parameters	T0	T1	T2	T3
Erythrocyte ($10^6/\text{mm}^3$)	2.34 ^b	2.48 ^b	2.70 ^a	2.82 ^a
Leukocyte ($10^3/\text{mm}^3$)	20.41 ^c	21.30 ^{bc}	22.23 ^{ab}	22.95 ^a
Hemoglobin (g%)	9.89 ^c	10.51 ^{bc}	11.18 ^{ab}	11.91 ^a
Hematocrit (%)	23.99 ^c	25.08 ^{bc}	26.15 ^{ab}	27.20 ^a
SOD (U/gHb)	2,460 ^c	2,549 ^{bc}	2,636 ^{ab}	2,717 ^a
MDA (nmol/mL)	9.91 ^c	9.13 ^{bc}	8.28 ^{ab}	7.88 ^a

^{a-c} Different superscripts in the same column indicate a significant difference ($p < 0.05$). T0: Drinking water without feed additive (control), T1: Drinking water + 1% feed additive, T2: Drinking water + 2% feed additive, T3: Drinking water + 3% feed additive. SOD: Superoxide dismutase, MDA: Malondialdehyde.

CONCLUSION

The addition of 3% green betel leaf extract and citric acid as a feed additive can improve nutrient utility, health, and meat quality in broiler chickens. Further research on molecular mechanisms behind the synergistic effects of green betel leaf extract and citric acid, such as their impact on gut microbiota, antioxidant enzyme expression, and nutrient absorption pathways, is warranted.

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Author's contributions

Adam Satria Pratama Nasution conducted the research, collected and analyzed the data, and drafted the manuscript. Luthfi Djauhari Mahfudz and Cahya Setya Utama reviewed and edited the manuscript. All authors

have read and approved the final version of the manuscript.

Competing interests

The authors have declared no conflict of interest.

Ethical considerations

Plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been checked by all authors.

Availability of data and materials

All data generated during the study are included in this article. Any additional information is available upon reasonable request from the authors.

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Influence of Breed, Sex, and Age on Body Composition of Turkeys Reared in Southern Benin

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ABSTRACT

Exotic turkey breeds generally exhibit superior growth performance compared to indigenous breeds in Benin. While local turkeys are recognized for their hardiness, they tend to demonstrate comparatively lower growth rates and have less well-documented carcass characteristics. The present study aimed to evaluate the body composition of the local turkey population reared in Southern Benin. Data were collected from 40 turkeys per breed (Exotic and local), totaling 80 turkeys. Each breed included 20 males and 20 females. The turkeys were divided into two age groups, including 7-month-old turkeys (Age-Group 1) and 9-month-old turkeys (Age-Group 2). Following a 24-hour hydrous diet, the turkeys were slaughtered by jugular vein, bled, scalded in water at 75°C, manually plucked, and the hot carcasses were weighed. Each carcass was cut to measure the weight of the wishbone, thigh and shank, wings, head, neck, gizzard, heart, liver, and legs. The current results indicated that the slaughter, hot carcass, and cold carcass weights of the exotic turkey were significantly higher than those of the local turkey. Additionally, male turkeys had notably higher slaughter, hot carcass, and cold carcass weights than females, regardless of breed. Furthermore, the live weight of turkeys at 7 months (3637 g) was significantly lower than at 9 months (4160.98 g). The weights of the breast, thigh, and tail cuts increased significantly as the turkeys aged. However, exceptions were observed in abdominal fat and specific parameters such as cold carcass yield, slaughter weight, and fifth-quarter components, including the heart, gizzard, head, and legs, which showed a positive correlation, especially in the exotic-type breeds. In contrast, no statistically significant correlation was observed between carcass yields at slaughter and abdominal fat in the exotic breed compared to the local breed. It might be beneficial to consider selecting or crossbreeding the local turkey population with more efficient exotic breeds to improve their overall body composition.

Keywords: Body composition, Carcass yield, Exotic turkey, Local turkey, Southern Benin

INTRODUCTION

Livestock production is a significant industry in West Africa, employing over 80% of the working population as either a primary or secondary activity (FAOSTAT, 2023). The livestock sub-sector has been thriving for nearly ten years, with a variety of animal species being raised, especially poultry. In Benin, poultry farming is resurging, particularly in the Southern region, where breeders raise different species, including local chickens, guinea fowl, ducks, pigeons, quails, and turkeys (Dotché et al., 2021). Numerous studies have been conducted on the zootechnical characteristics, carcass traits, and meat quality of these species. Studies on local chickens have

concentrated on chicken feed, growth performance, carcass quality, and meat quality (Youssao et al., 2009; Tougan et al., 2013; Gangbédjé et al., 2023). Additionally, zootechnical performance and meat quality of duck meat have been studied in Benin (Aboh et al., 2011; Houéssionon et al., 2020). In guinea fowl raised in Benin, studies have primarily focused on the technical and sanitary challenges of traditional guinea fowl production (Boko et al., 2012) as well as their zootechnical characteristics (Dahouda et al., 2008, 2009; Boko et al., 2012; Houndonougbo, 2017). Although the zootechnical performance and slaughter characteristics of many species have been documented in recent years, there is limited information on turkeys, which are currently classified as

neglected breeds (Dotché *et al.*, 2021). The current body of literature on turkey farming in Benin is mainly limited to the findings of Dèdèhou *et al.* (2019) in Northwestern Benin and Dotché *et al.* (2021) in Southern Benin, which primarily focused on the classification of turkey farming practices. The existing studies do not consider the influence of zootechnical factors, including genetic variation and age, on animal performance, body composition, and carcass quality. Similarly, there are no published scientific data on the potential relationships between body components and carcass quality in turkeys in Benin. These different characteristics vary according to the turkey breed or strain, and for the same strain, they vary according to sex and age at the time of slaughter. To enhance the contribution of turkeys to food security, the present study aimed to investigate the impact of breed, sex, and age at slaughter on the body composition of turkeys raised in Southern Benin.

MATERIAL AND METHODS

Ethical approval

The study protocol has been approved by the ethics committee of the Laboratory of Animal Biotechnology and Meat Technology of Benin (N°210 DPSA/LBATV/D).

Study area

The study was conducted at the Agricultural College of Sékou in Allada and the Laboratory of Animal Biotechnology and Meat Technology at the University of Abomey-Calavi, Benin. Animals were reared and slaughtered at the Agricultural College of Sékou, while their body composition was analyzed at the Abomey-Calavi laboratory.

Both townships are situated on the Atlantic coast. The department has a sub-equatorial climate, characterized by two distinct rainy seasons. The main rainy season occurs from April to July, while a secondary, shorter rainy period happens between September and November. The region receives approximately 1,200 millimeters of rainfall annually. The average monthly temperatures range from 27°C to 31°C, while the relative humidity varies from 65% and 97% throughout the year. Allada township spans 381 km², representing 11.78% of the Atlantic department's total area. It is located between 6°32' and 6°48' N latitude and 2°0' and 2°16' E longitude and bordered by the townships of Toffo to the north, Tori-Bossito to the south, Zè to the east, and Bopa to the west (Nangbe, 2006). Abomey-Calavi covers 539 km² and shares borders with Zè to the north, the Atlantic Ocean to the south, So-Ava

and Cotonou to the east, and Tori-Bossito and Ouidah to the west.

Study design

Eighty turkeys were used in the present study, including 40 exotic (Figure 1) and 40 local turkeys (Figure 2), with an equal number of males and females (20 males and 20 females). The turkeys were divided into two age groups, including 7-month-old turkeys (Age-Group 1) and 9-month-old turkeys (Age-Group 2). The local turkeys were produced from a selected breeding group of two males and four females, and the exotic one-day-old turkeys were imported from Ghana. Each turkey was marked with a number on a cloth strip around the leg. The local turkeys' reproducers were purchased from local poultry breeders in Southern Benin. The turkeys were reared within a traditional breeding system characterized by a well-ventilated henhouse spanning 300 m², constructed from locally sourced materials, which was located at the Agricultural College of Sékou, Benin. The feeding regimen consisted of commercial feeds, including a starter feed, a growing feed, and a finishing feed, which were systematically designed to meet the nutritional needs of the turkeys at different stages of growth (Table 1). The starter feed was administered during the first 8 weeks, followed by grower feed from 8 to 20 weeks, and finishing feed from 20 weeks until the end of the experiment at 28 or 36 weeks. Additionally, the animals had *ad libitum* access to water and a forage-based feed supplement, which included *Panicum CI*, *Moringa oleifera* leaves, *Amaranthus* species leaves, or *Allium* species leaves. The turkeys were exposed to natural light and benefited from the ambient temperature, which ranged from 24°C to 29°C.

To reduce turkey mortality and ensure their health during the experiment, they were vaccinated against Newcastle disease at 26 days old (AVI ND LASOTA®, France), against infectious bronchitis at 3 days old (AVI IB H120®, in France), and fowl pox (Diftosec®, France) at 35 days of age, with a booster administered at 105 days age. In addition to vaccination efforts, several preventive measures were implemented, including ALFACERYL®, a water-soluble formulation comprising a mixture of antibiotics and vitamins, which was administered at one day of age for five days. This intervention aimed to mitigate and address a range of potential bacterial infections. Furthermore, at 21 days of age, Alfamisol® (Alfasan, Netherlands) was utilized as a deworming agent for the turkeys, thereby contributing to their overall health management strategy.



Figure 1. A 9-month-old exotic turkey reared in Allada, Benin.



Figure 2. A 9-month-old local turkey reared in Allada, Benin

Table 1. Chemical composition of the commercial diets given to turkeys at different physiological stages

Nutrient constituents	Starter (0 to 8 weeks)	Grower (8 to 20 weeks)	Laying (20 to 28/36 weeks)
Energy (kcal/kg feed)	2900	2800	2500
Crude protein (%)	21	19	18.5
Lysine (%)	1.1	1	0.9
Methionine (%)	1	0.44	0.44
Calcium (%)	1.08	1.01	3.5
Total phosphorus (%)	0.55	0.5	0.5
Crude ash (%)	7.37	7.12	13
Crude cellulose (%)	2.5	3.32	-
Sodium (%)	0.2	-	-
Crude fat (%)	5.54	5	4.5
Flavomycin (%)	0.007	0.007	0.005
Chloride (%)	0.19	-	-

Body composition and carcass quality

Before slaughter, turkeys were fasted for 24 hours. Two groups of turkeys, each with 40 turkeys, were slaughtered and bled sequentially by cutting the jugular vein. Following the bleeding process, the turkeys were subjected to a scalding procedure in water at a temperature of 75°C, after which they were manually plucked. The legs were severed at the tarsometatarsal joint, and the head was separated from the neck at the skull-atlas junction. The abdominal and thoracic cavity organs were then removed. The hot carcasses were weighed using a scale with a 5000 g capacity. The cold carcass weight was taken 24 hours later after refrigeration. The weight of each carcass cut was determined, including the breast, thigh-drumstick, wings, head, neck, gizzard, heart, liver, and legs.

Statistical analysis

The data on slaughter, hot carcass, cold carcass, and cut weights were recorded in Microsoft Excel and analyzed using SAS software (SAS Institute Inc., Cary, NC, USA, 2013). Carcass yields were calculated based on turkey live weights, cut weights (breast, thigh-drumstick, and wing), and cold carcass weights. The analysis of

variance was performed using the GLM procedure of SAS. The significant effects of the breed, sex, and age on each carcass variable were determined using an F-test. Means were calculated using the Proc Means procedure and compared using a T-test. Correlations between variables were determined using the Proc CORR procedure. Principal component analysis of carcass characteristics was performed with the Proc PRINCOMP procedure. A significance level of $p < 0.05$ was used for all comparisons in both tests.

RESULTS

Body composition

Breed

The body composition and the yields of carcasses and cuts are presented in Table 2. Breed-specific variations in turkey body composition were notable. The weights of exotic turkeys at slaughter, hot carcass, and cold carcass were all greater than those of native turkeys ($p < 0.05$), and the same significant difference ($p < 0.05$) was

observed for hot carcass yield, cold carcass yield, chilling loss, and abdominal fat weight. Exotic turkeys were notably heavier than local turkeys in terms of breast, thigh-drumstick, wings, head, neck, legs, heart, liver, abdominal fat, and carcass rest ($p < 0.05$). However, there was no significant difference between the breeds in gizzard weights ($p > 0.05$).

Sex

The body composition of turkeys varied significantly by sex (Table 2). The male animals exhibited significantly higher slaughter, hot carcass, and cold carcass weights than the females ($p < 0.05$). Additionally, this trend was observed for carcass cuts, including breast, thigh-drumstick, wings, head, legs, liver, gizzard, and abdominal fat. Furthermore, the hot carcass yield of males was found to be significantly higher than that of females, while cold carcass yield and chilling loss were similar for both sexes ($p < 0.05$). Additionally, carcass rest and heart weights exhibited comparable patterns in both breeds.

Age

The age of the animal significantly affected the carcass characteristics (Tables 2). The live, hot carcass, cold carcass, and breast weight of 7-month-old exotic turkeys (Age-Group 1) were found to be significantly ($p < 0.05$) higher than those of local turkeys at 7 months, and the same trend was observed in turkeys aged 9 months

(Age-Group 2). Conversely, the weight of the fifth-quarter components, namely thigh-drumstick, wings, head, neck, legs, abdominal fat, rest, heart, and gizzard, did not differ significantly with age, regardless of breed. A comparison of the two age groups indicated that the older turkeys (9 months) had better carcass composition, except for the fifth-quarter components.

Interaction between sex and breed

Carcass traits for each breed by sex are shown in Table 3. In male exotic turkeys, apart from hot carcass and cold carcass yields, body composition was significantly higher than in females ($p < 0.05$). In the local breed, chilling loss, thigh-drumstick, and head measurements were significantly higher in males than in females ($p < 0.05$). Conversely, females of the local breed had a greater hot carcass weight than males ($p < 0.05$).

Interaction between breed and slaughter age

The carcass characteristics of breeds by age are presented in Table 4. Apart from the fifth quarter, carcass characteristic components of exotic turkeys were significantly lower ($p < 0.05$) at 7 months compared to those slaughtered at 9 months of age. The components of body composition in local turkeys did not vary significantly by slaughter age.

Table 2. Turkey body composition by breed, sex, and age at slaughter time

Variables	Breed		Sex		Age		RSD	Significant level		
	Exotic	Local	Female	Male	7 months	9 months		Breed	Sex	Age
Live weight (g)	4865.48	2932.5	3328.73	4469.25	3637	4160.98	477.37	***	***	***
Hot carcass (g)	3671.70	1980.2	2282.39	3075.41	2376.88	2980.93	544.27	***	***	***
Cold carcass (g)	3427.06	1930.75	2250.53	3035.25	2335.7	2962.85	421.60	***	***	*
Hot carcass yield (%)	70.16	65.83	68.2	67.17	65.49	69.88	6.56	*	*	*
Cold carcass yield (%)	69.54	64.11	67.94	66.80	64.10	68.37	6.12	*	NS	*
Chilling loss (%)	3.97	2.87	2.56	2.46	3.35	2.80	5.17	*	NS	*
Breast (g)	1084.14	480.25	600.5	963.89	676.5	887.89	218.89	***	***	***
Thigh (g)	808.71	501.67	555.6	754.78	545.45	764.93	177.53	***	***	***
Wings (g)	533.12	343	384.25	491.86	413.35	462.77	100.36	***	***	NS
Head (g)	106.91	80.33	71.75	115.5	90.58	96.66	6.82	***	***	**
Neck (g)	415.8	187.83	198.8	404.83	270.17	333.47	84.29	***	***	*
Legs (g)	116.15	88.25	74.07	130.33	98.67	105.74	9.08	***	***	*
Abdominal fat (g)	39.25	25.5	62.75	2	34.67	30.08	19.98	*	***	NS
Rest (g)	585.39	341.92	439.39	487.92	459.42	467.89	64.92	***	NS	NS
Heart (g)	18.74	13.67	13.2	19.21	15.83	16.58	2.68	***	NS	NS
Liver (g)	65.36	50.08	52.53	62.92	58.83	56.61	4.51	***	***	NS
Gizzard (g)	98.12	97.92	74.41	121.63	101.58	94.45	14.45	NS	***	NS

RSD: Residual standard deviation, NS: Not significant. */**/**: Indicates a statistically significant difference at $p < 0.05$.

Table 3. Variation in turkey body composition by breed and sex

Variables	Exotic		Local		RSD	Significant level
	Female	Male	Female	Male		
Live weight (g)	3774.96 ^b	5955.99 ^a	2882.50 ^c	2982.50 ^c	477.37	***
Hot carcass (g)	2616.79 ^b	4880.00 ^a	2037.67 ^b	1913.50 ^c	544.27	***
Cold carcass (g)	2463.40 ^b	4237.33 ^a	1948.00 ^c	1850.30 ^c	421.6	**
Hot carcass yield (%)	68.81 ^a	70.28 ^a	67.59 ^a	64.06 ^b	6.56	NS
Cold carcass yield (%)	68.23 ^a	72.09 ^a	67.65 ^a	63.8	6.12	NS
Chilling loss (%)	0.67 ^b	7.28 ^a	0.46 ^b	7.01 ^a	5.17	*
Breast (g)	706.25 ^b	1462.04 ^a	494.75 ^c	465.75 ^c	218.89	***
Thigh-drumstick (g)	628.04 ^b	989.39 ^a	483.17 ^c	520.17 ^b	177.53	**
Wings (g)	418.51 ^b	647.73 ^a	350.00 ^c	336.00 ^c	100.36	***
Head (g)	72.66 ^c	141.17 ^a	70.83 ^c	89.83 ^b	6.82	***
Neck (g)	228.77 ^b	602.83 ^a	168.83 ^b	206.83 ^b	84.29	***
Legs (g)	79.39 ^c	152.92 ^a	68.75 ^d	107.75 ^b	9.08	***
Abdominal fat (g)	78.49 ^a	0.00 ^c	47.00 ^b	4.00 ^c	19.98	*
Rest (g)	491.37 ^b	679.42 ^a	387.42 ^c	296.42 ^c	64.92	***
Heart (g)	13.73 ^b	23.75 ^a	12.67 ^b	14.67 ^b	2.68	***
Liver (g)	57.97 ^b	72.75 ^a	47.08 ^c	53.08 ^b	4.51	*
Gizzard (g)	72.90 ^c	123.33 ^a	75.92 ^b	119.92 ^a	14.45	NS

RSD: Residual standard deviation. ^{a,b,c} Means of the same row followed by different superscript letters differ significantly at $p < 0.05$, NS: Not significant. */ **/ ***: $p < 0.05$.

Table 4. Variation in turkey body compositions by breed and slaughter at ages seven months and nine months

Variables	Exotic		Local		RSD	Significant level
	Age 1	Age 2	Age 1	Age 2		
Live weight (g)	4474.00 ^b	5256.95 ^a	2800.00 ^c	3065.00 ^c	477.37	*
Hot carcass (g)	2921.50 ^b	3932.61 ^a	1832.25 ^c	2029.25 ^c	544.27	**
Cold carcass (g)	2750.20 ^b	3671.70 ^a	1745 ^c	1925 ^c	421.6	**
Hot carcass yield (%)	65.53 ^b	73.56 ^a	65.45 ^b	66.20 ^b	6.56	*
Cold carcass yield (%)	62.47	70.16	62.47	70.16	6.12	*
Chilling loss (%)	3.3	3.97	3	3.57	5.17	*
Breast (g)	908.00 ^b	1260.28 ^a	445.00 ^c	515.50 ^c	218.89	*
Thigh-drumstick (g)	681.40 ^b	936.03 ^a	409.50 ^c	593.83 ^b	177.53	NS
Wings (g)	499.20 ^b	567.04 ^a	327.50 ^c	358.50 ^c	100.36	NS
Head (g)	105.67	108.16	75.5	85.17	6.82	NS
Neck (g)	359.33	472.27	181	194.67	84.29	NS
Legs (g)	114.83	117.48	82.5	94	9.08	NS
Abdominal fat (g)	47.83	30.66	21.5	29.5	19.98	NS
Rest (g)	574.33	596.45	344.5	339.33	64.92	NS
Heart (g)	17.67	19.82	14	13.33	2.68	NS
Liver (g)	63.67	67.06	54	46.17	4.51	***
Gizzard (g)	101.17	95.07	102	93.83	14.45	NS

Age 1: 7 Months, Age 2: 9 Months, RSD: Residual standard deviation, NS: Not significant. */ **/ *** ^{a,b,c} Means of the same row followed by different superscript letters differ significantly at $p < 0.05$.

Correlations among carcass parameters of exotic and local turkeys

Table 5 displays the correlation coefficients between the turkey's body components and slaughter weight. Except for abdominal fat and cold carcass yield, the slaughter weight of the exotic breeds was favorably associated with the carcass and fifth-quarter components. Carcass and fifth-quarter components indicated a negative correlation with abdominal fat ($p < 0.05$) in the exotic turkeys. Moreover, correlations between carcass yields at

slaughter time and abdominal fat were non-significant in the exotic breed ($p > 0.05$). Similarly, to the exotic breed, live weight at slaughter showed a significant correlation with the weights of the hot carcass, cold carcass, cuts, and fifth-quarter components, such as the neck and the remaining parts of the carcass in the local breed. Conversely, live weight at slaughter time exhibited a negative correlation with both cold carcass yield and hot carcass yield ($p < 0.05$).

Table 5. Correlations among the proportions of carcass cuts from exotic (above the diagonal) and local (below the diagonal) turkeys

Variable	W Breast	W ThDru	W Wing	W head	W neck	W leg	W Rest	W Heart	W Liver	W Gizzard	W Fat
Breast	1	-0.13 ^{NS}	-0.53**	0.35 ^{NS}	0.81***	0.55**	-0.54**	0.17 ^{NS}	0.65***	-0.14 ^{NS}	0.54**
Thigh-Dru	0.19 ^{NS}	1	0.77***	0.53**	-0.1 ^{NS}	0.54**	0.59***	0.56**	0.44*	0.41 ^{NS}	0.22 ^{NS}
Wing	-0.86***	-0.01 ^{NS}	1	0.09 ^{NS}	-0.58***	0.19 ^{NS}	0.69***	0.45*	0.72***	0.29 ^{NS}	0.32 ^{NS}
Head	-0.96***	-0.05 ^{NS}	0.78***	1	0.31 ^{NS}	0.66***	-0.06 ^{NS}	0.47*	0.05 ^{NS}	-0.04 ^{NS}	-0.23 ^{NS}
Neck	-0.72**	-0.41 ^{NS}	0.3 ^{NS}	0.78***	1	0.47*	-0.6***	0.3 ^{NS}	-0.78***	-0.04 ^{NS}	-0.71***
Leg	-0.86***	-0.14 ^{NS}	0.64*	0.95***	0.87***	1	0.16 ^{NS}	0.6***	-0.03 ^{NS}	0.29 ^{NS}	-0.33 ^{NS}
WRest	-0.67*	-0.75***	0.55 ^{NS}	0.47 ^{NS}	0.45 ^{NS}	0.38 ^{NS}	1	-0.01 ^{NS}	0.76***	0.51*	0.71***
WHeart	-0.53 ^{NS}	0.17 ^{NS}	0.83***	0.37 ^{NS}	-0.20 ^{NS}	0.12 ^{NS}	0.42 ^{NS}	1	0.19 ^{NS}	0.15 ^{NS}	-0.44*
WLiver	-0.94***	-0.43 ^{NS}	0.71**	0.85***	0.75**	0.74**	0.83***	0.44 ^{NS}	1	0.23 ^{NS}	0.56**
WGizzard	-0.92***	-0.10 ^{NS}	0.9***	0.89***	0.59*	0.78***	0.55 ^{NS}	0.63*	0.83***	1	0.4 ^{NS}
WFat	-0.36 ^{NS}	-0.43 ^{NS}	0.63*	0.13 ^{NS}	-0.18 ^{NS}	-0.01 ^{NS}	0.73**	0.74**	0.41 ^{NS}	0.37 ^{NS}	1

LW: Live weight, CarH: Hot carcass, CarcC: Cold carcass, Yield1: Hot carcass yield, Yield24: Cold carcass yield, Thigh-drum/ThDru: Thigh-drumstick, W: Weight, NS: Not significant. * / **/ ***: p < 0.05: Indicates a statistically significant difference at p < 0.05.

Table 6. Correlations between proportions of carcass cuts and body components in local turkeys

Variable	W Breast	W ThDru	W Wing	W head	W neck	W leg	W Rest	W Heart	W Liver	W Gizzard	W Fat
LW	0.83***	-0.18 ^{NS}	-0.82***	-0.82***	-0.46 ^{NS}	-0.65*	-0.34 ^{NS}	-0.67*	-0.72*	-0.94***	-0.21 ^{NS}
CarcH	0.86***	-0.04 ^{NS}	-0.88***	-0.82***	-0.46 ^{NS}	-0.67*	-0.45 ^{NS}	-0.71*	-0.76***	-0.98***	-0.36 ^{NS}
CarcC	0.86***	-0.03 ^{NS}	-0.88***	-0.82***	-0.46 ^{NS}	-0.67*	-0.46 ^{NS}	-0.71*	-0.76***	-0.98***	-0.36 ^{NS}
Yield1	0.02 ^{NS}	0.65*	-0.26 ^{NS}	0.1 ^{NS}	0.11 ^{NS}	0.04 ^{NS}	-0.47 ^{NS}	-0.20 ^{NS}	-0.06 ^{NS}	-0.13 ^{NS}	-0.70*
Yield24	0.05 ^{NS}	0.67*	-0.28 ^{NS}	0.09 ^{NS}	0.1 ^{NS}	0.04 ^{NS}	-0.50 ^{NS}	-0.23 ^{NS}	-0.10 ^{NS}	-0.16 ^{NS}	-0.72*
Breast	0.93***	0.04 ^{NS}	-0.91***	-0.88***	-0.54 ^{NS}	-0.74*	-0.55 ^{NS}	-0.69*	-0.85***	-0.99***	-0.39 ^{NS}
Thigh-Dru	0.88***	0.2 ^{NS}	-0.87***	-0.82***	-0.55 ^{NS}	-0.69*	-0.62*	-0.66*	-0.84***	-0.98***	-0.45 ^{NS}
Wing	0.82***	-0.03 ^{NS}	-0.81***	-0.79***	-0.49 ^{NS}	-0.65*	-0.41 ^{NS}	-0.64*	-0.74**	-0.96***	-0.26 ^{NS}
Head	-0.50 ^{NS}	-0.12 ^{NS}	0.16 ^{NS}	0.6*	0.69**	0.7**	0.22 ^{NS}	-0.27 ^{NS}	0.45 ^{NS}	0.19 ^{NS}	-0.21 ^{NS}
Neck	0.43 ^{NS}	-0.39 ^{NS}	-0.77***	-0.36 ^{NS}	0.22 ^{NS}	-0.14 ^{NS}	-0.13 ^{NS}	-0.92***	-0.27 ^{NS}	-0.65*	-0.49 ^{NS}
Leg	0.05 ^{NS}	-0.25 ^{NS}	-0.34 ^{NS}	0.1 ^{NS}	0.44 ^{NS}	0.34 ^{NS}	-0.09 ^{NS}	-0.73**	-0.06 ^{NS}	-0.30 ^{NS}	-0.42 ^{NS}
Fat	0.34 ^{NS}	-0.46 ^{NS}	-0.15 ^{NS}	-0.52 ^{NS}	-0.49 ^{NS}	-0.51 ^{NS}	0.32 ^{NS}	0.05 ^{NS}	-0.22 ^{NS}	-0.46 ^{NS}	0.61*
Rest	0.61*	-0.44 ^{NS}	-0.71**	-0.68*	-0.28 ^{NS}	-0.55 ^{NS}	0.02 ^{NS}	-0.58 ^{NS}	-0.42 ^{NS}	-0.81***	-0.02 ^{NS}
Heart	0.61*	0.19 ^{NS}	-0.36 ^{NS}	-0.71**	-0.82***	-0.76***	-0.21 ^{NS}	0.04 ^{NS}	-0.58*	-0.72**	0.23 ^{NS}
Liver	-0.22 ^{NS}	-0.69**	-0.14 ^{NS}	0.1 ^{NS}	0.43 ^{NS}	0.12 ^{NS}	0.65*	-0.25 ^{NS}	0.47 ^{NS}	-0.09 ^{NS}	0.17 ^{NS}
Gizzard	-0.99***	-0.26 ^{NS}	0.87***	0.94***	0.7**	0.85***	0.72**	0.54 ^{NS}	0.94***	0.92***	0.44 ^{NS}
Chilling	-0.37 ^{NS}	-0.39 ^{NS}	0.4 ^{NS}	0.17 ^{NS}	0.09 ^{NS}	-0.02 ^{NS}	0.62*	0.57*	0.53 ^{NS}	0.52 ^{NS}	0.55 ^{NS}

LW: Live weight, CarH: Hot carcass, CarcC: Cold carcass, Yield1: Hot carcass yield, Yield24: Cold carcass yield, Thigh-drum/ThDru: Thigh-drumstick, W: Weight, NS: Not significant. * / **/ ***: $p < 0.05$. Indicates a statistically significant difference at $p < 0.05$

Table 7. Correlations between proportions of carcass cuts and body components in exotic turkeys

Variable	W Breast	W ThDru	W Wing	W head	W neck	W leg	W Rest	W Heart	W Liver	W Gizzard	W Fat
LW	0.82***	-0.33 ^{NS}	-0.62**	0.15 ^{NS}	0.92***	0.37 ^{NS}	-0.77***	0.22 ^{NS}	-0.86***	-0.23 ^{NS}	-0.82***
CarcH	0.83***	-0.36 ^{NS}	-0.69***	0.11 ^{NS}	0.92***	0.28 ^{NS}	-0.79***	0.1 ^{NS}	-0.91***	-0.22 ^{NS}	-0.74***
CarcC	0.7***	-0.56**	-0.77***	-0.04 ^{NS}	0.82***	0.07 ^{NS}	-0.87***	-0.03 ^{NS}	-0.92***	-0.3 ^{NS}	-0.72***
Yield1	0.58***	-0.34 ^{NS}	-0.69***	-0.04 ^{NS}	0.59***	-0.1 ^{NS}	-0.57**	-0.42*	-0.8***	-0.03 ^{NS}	-0.17***
Yield24	-0.04 ^{NS}	-0.68***	-0.63***	-0.45*	0.02 ^{NS}	-0.69***	-0.47*	-0.76***	-0.49*	-0.16 ^{NS}	0.11 ^{NS}
Breast	0.89***	-0.42*	-0.72***	0.1 ^{NS}	0.87***	0.27 ^{NS}	-0.8***	0.05*	-0.88***	-0.27 ^{NS}	-0.71***
Thigh-Dru	0.78***	-0.27 ^{NS}	-0.62***	0.18 ^{NS}	0.92***	0.3 ^{NS}	-0.79***	0.17 ^{NS}	-0.9***	-0.2 ^{NS}	-0.75***
Wing	0.7***	-0.25 ^{NS}	-0.46*	0.09 ^{NS}	0.81***	0.29 ^{NS}	-0.77***	0.24 ^{NS}	-0.84***	-0.21 ^{NS}	-0.77***
Head	0.78***	-0.33 ^{NS}	-0.69***	0.32 ^{NS}	0.88***	0.29 ^{NS}	-0.84***	0.12 ^{NS}	-0.88***	-0.29 ^{NS}	-0.76***
Neck	0.77***	-0.36 ^{NS}	-0.72***	0.09 ^{NS}	0.95***	0.25 ^{NS}	-0.75***	0.12 ^{NS}	-0.88***	-0.17 ^{NS}	-0.73***
Leg	0.84***	-0.28 ^{NS}	-0.61***	0.22 ^{NS}	0.92***	0.45*	-0.71***	0.19 ^{NS}	-0.84***	-0.14 ^{NS}	-0.76***
Fat	-0.54**	0.22 ^{NS}	0.32 ^{NS}	0.25 ^{NS}	-0.7***	-0.33 ^{NS}	0.7***	-0.46*	0.52**	0.4 ^{NS}	1***
Rest	0.68***	-0.19 ^{NS}	-0.51*	-0.01 ^{NS}	0.84***	0.48*	-0.36 ^{NS}	0.08 ^{NS}	-0.75***	0.15 ^{NS}	-0.52**
Heart	0.73***	-0.12 ^{NS}	-0.42*	0.25 ^{NS}	0.9***	0.43*	-0.72***	0.47*	-0.73***	-0.12 ^{NS}	-0.81***
Liver	0.64***	-0.39 ^{NS}	-0.48*	0.16 ^{NS}	0.67***	0.35 ^{NS}	-0.73***	0.31 ^{NS}	-0.58***	-0.2 ^{NS}	-0.78***
Gizzard	0.69***	-0.41 ^{NS}	-0.69***	-0.03 ^{NS}	0.87***	0.23 ^{NS}	0.7***	0.07 ^{NS}	-0.87***	0.03 ^{NS}	-0.65***
Chilling	0.79***	0.48*	-0.03 ^{NS}	0.55**	0.7***	0.81***	-0.08 ^{NS}	0.48*	-0.34 ^{NS}	0.18 ^{NS}	-0.36 ^{NS}

LW: Live weight, CarH: Hot carcass, CarcC: Cold carcass, Yield1: Hot carcass yield, Yield24: Cold carcass yield, Thigh-drum/ThDru: Thigh-drumstick, W: Weight, NS: Not significant. * / **/ ***: $p < 0.05$.
Indicates a statistically significant difference at $p < 0.05$.

The correlations between hot and cold carcass weight and the weights of the breast, thigh-drumstick, neck, and carcass rest were statistically significant ($p < 0.05$) for the local breed. Conversely, cold carcass weight was inversely correlated with gizzard weight and had no significant effects on the yields of leg, fat, and liver weights ($p < 0.05$). Unlike the current observations in exotic breeds, the correlation between abdominal fat and cold carcass yield, as well as gizzard weight, was found to be negative ($p < 0.05$) in local turkeys. However, abdominal fat was positively correlated with breast weight ($p < 0.05$). The correlation coefficients between carcass cut proportions in exotic and local turkeys are presented in Table 6. In exotic turkeys, the breast proportion was positively associated with abdominal fat, liver, neck, and leg proportions ($p < 0.05$). Conversely, the breast proportion was negatively associated with wing size ($p < 0.05$). In local turkeys, the abdominal fat proportion was positively correlated with that of the heart ($p < 0.05$). Breast proportion was negatively correlated with the fifth-quarter components of the animal and cuts, except for the thigh-drumstick ($p < 0.05$).

The correlations between the proportions of carcass cuts and body components of local turkeys are presented in Table 6. A statistically significant correlation was observed between live weight and breast proportions, while there was a negative correlation between live weight and the other carcass cuts and fifth-quarter components ($p < 0.05$). The correlation between breast proportion and body components was notably positive ($p < 0.05$), in

comparison to the correlations observed between these proportions and those of other carcass cuts and fifth-quarter components.

The correlations between the proportions of carcass cuts and body components of exotic turkey are presented in Table 7. Live weight had positive correlation coefficients with breast proportions and negative correlation with those of other carcass cuts and fifth-quarter components ($p < 0.05$). The proportions of breast, neck, and heart indicated a significantly positive correlation with body components compared to the other cuts and fifth-quarter components, which exhibited more negative correlations with the different body components ($p < 0.05$). Additionally, the fat percentage was negatively correlated with all body components ($p < 0.05$).

Principal component analysis of turkey carcass characteristics

Figure 3 shows the principal component analysis of turkey carcass characteristics. The current results were interpreted using two axes. The initial two axes together account for 16.65% of the total inertia, with 13.14% attributed to the first axis and 3.32% to the second (Figure 3). Regardless of the axis considered, the two local and exotic breeds are in opposition to each other. Most quantitative variables, including live weight, hot carcass weight, cold carcass weight, and weight of certain cuts, were linked to the exotic breed through axis 1. The fat weight, in addition to the chilling loss and gizzard, served to differentiate the local breed from exotic breeds.

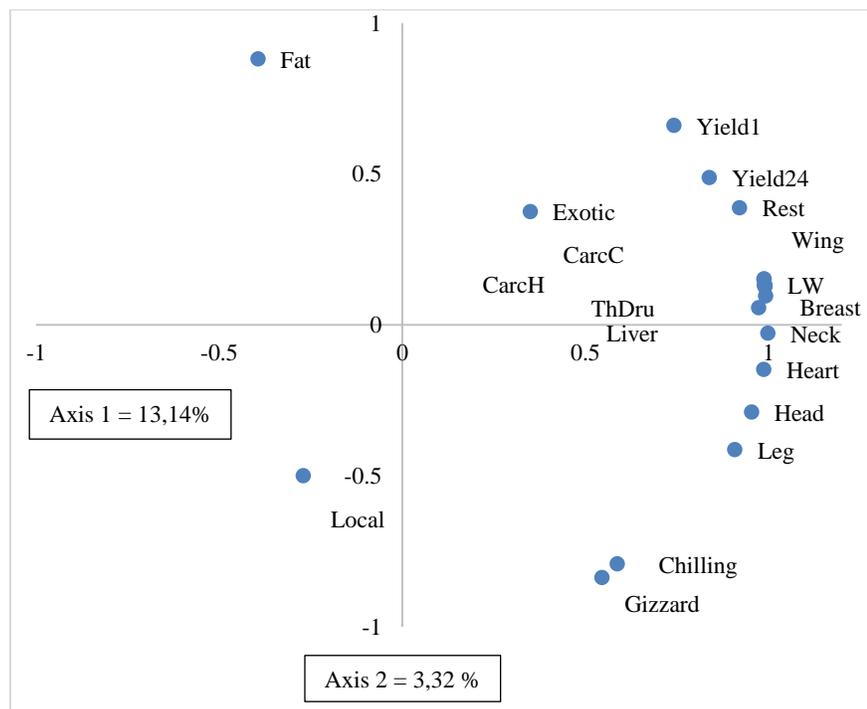


Figure 3. Principal component analysis of exotic and local turkey carcass characteristics. LW: Live weight, CarcH: Hot carcass, CarcC: Cold carcass, Yield1: Hot carcass yield, Yield24: Cold carcass yield, ThDru: Thigh-drumstick.

DISCUSSION

Effects of breed on body composition

The present study indicated that exotic turkey breeds had higher slaughter weights and better carcass characteristics compared to local turkeys when reared under the same conditions and slaughtered at the same age. This difference in body composition is due to genetic factors. The exotic turkey is a genetic type with eumetrical and mediolateral features (Dotché et al., 2021), while the local turkey is relatively brevilinear with an ellipometric shape (Dédèhou et al., 2019). The impact of genetic variation on slaughter weight and body composition has been observed in different established and reproductive turkey lines (Chartrin et al., 2018), as well as in Isa Brown, Harco, and Lohman chickens reared in Benin (Tossou et al., 2019), lean or force-fed Barbary and Mulard ducks (Houessionon et al., 2019), Djallonké and Sahelian sheep within traditional farming systems (Djenontin et al., 2017), and Borgou, Lagunaire, and Zebu Peulh cattle grazing on natural pastures (Salifou et al., 2013). Due to the genetic diversity among local and exotic turkey breeds, it is essential to establish specific age parameters for slaughter to optimize both their genetic characteristics and economic viability. Each breed possesses unique attributes that can be better leveraged through tailored management practices in terms of their harvesting age. This age is defined by the growth curve of each breed and usually occurs at the inflection point. Therefore, studying the growth curves of these two genetic types is essential.

The correlation between slaughter weight and carcass and fifth-quarter components was found to be positive in exotic and local breeds. A similar finding was reported by Laudadio et al. (2009) for turkeys in Italy. Therefore, slaughter weight acts as an indicator of turkey carcass conformation. It is reasonable to infer that those higher weights lead to better carcass conformation. The principal component analysis demonstrated that exotic turkeys have a more favorable spatial distribution of carcass components, indicating better carcass conformation than local turkeys.

Abdominal fat was negatively correlated with carcass and fifth-quarter components in exotic breeds. The relationship between abdominal fat and carcass composition revealed significant differences between exotic and local turkey breeds. It has been observed that an increase in abdominal fat correlates with a decrease in the proportion of lean meat within the carcass, ultimately leading to a reduction in carcass quality for exotic breeds. Conversely, no such correlation was identified in local turkey breeds, suggesting a distinct metabolic or genetic profile. It may be hypothesized that the notable absence of abdominal fat in local turkeys was linked to their relatively lower slaughter weights, indicating potential implications

for breeding and management strategies in poultry production.

Effects of sex on body composition

The present study demonstrated that the primary characteristics of turkey carcasses, including live weight, hot carcass weight, and body component weight, were significantly higher in males compared to females. This discrepancy in performance between males and females can be attributed to the pronounced sexual dimorphism observed in this species, with females exhibiting a lower average body weight than males. This dimorphism was observed in a multitude of species and breeds of the same species (Damaziak et al., 2013; Portillo-Salgado et al., 2022). In breeding turkeys, males have higher slaughter weight, breast, and thigh-drumstick yields compared to females, which is consistent with the pattern observed in standard animals (Chartrin et al., 2018). In Muscovy ducks, the mean live weight at slaughter, hot carcass weight, cold carcass weight, hot carcass yield, and cold carcass yield were found to be greater in males than in females (Houessionon et al., 2019). Additionally, the weight of specific body components differed between the sexes. The same trends were obtained in ecotypes of the local *Gallus gallus* poultry population in Benin (Tougan et al., 2013; Edénakpo et al., 2020). Similarly, the male turkeys in the present study exhibited higher weights and carcass characteristics than the females (Damaziak et al., 2013; Portillo-Salgado et al., 2022).

Since the growth curves of males and females vary in most species, the age at slaughter for local and crossbred turkeys should be determined based on sex, which was not done in the current study. The slaughter age can be chosen according to the male or female's tendency to deposit carcass or abdominal fat. In standard turkeys from typical breeding farms, males are slaughtered at 16 weeks of age, weighing 14-15 kg, while females are slaughtered at 12 weeks, weighing 6-7 kg (Chartrin et al., 2018).

Effects of age on body composition

The current study revealed that 9-month-old turkeys exhibit more advanced development of body components compared to their 7-month-old counterparts. A comprehensive analysis indicated that there was a positive correlation between age and different metrics, including live weight, hot carcass weight, cold carcass weight, and the weights of individual cuts, indicating that physiological growth and development continue to progress significantly during this period. The chosen slaughter ages in the present study correspond to the ages at which turkeys are typically processed in Benin. The 7- to 9-month period marks the end of the accelerated growth phase or the start of the slow growth phase. During the accelerated growth phase, cells multiply and grow larger. In the slow-growth phase, animals' weight increases due to an increase in cell size (Pérez-Lara et al., 2013). This increase in animal weight is accompanied by a change in

meat texture, with an impact on meat sensory quality (Salgado Pardo et al., 2024). The growth in live weight at slaughter, hot carcass weight, cold carcass weight, and the weight of distinct carcass components with age has been documented by Chartrin et al. (2018) in turkeys and Houessionon et al. (2019) in Barbary ducks in Benin.

CONCLUSION

The present findings demonstrated that exotic turkey breeds exhibit greater slaughter weights and carcass characteristics compared to local turkey breeds, when both are raised and processed under identical environmental conditions and at the same age. Both exotic and local breeds exhibited a positive correlation among slaughter weight, carcass weight, and fifth-quarter components. Notably, the exotic breed revealed a negative correlation between abdominal fat and both carcass and fifth-quarter components. In contrast, the local breed did not display a significant correlation between abdominal fat and carcass components. Regardless of breed, male turkeys consistently demonstrated higher live weights, hot carcass weights, and body component weights compared to their female counterparts. Additionally, 9-month-old turkeys presented more developed body components than those at 7 months old, suggesting a developmental advantage with age. Overall, exotic turkeys have better carcass characteristics than the local breed. It is recommended that future studies evaluate the technological and organoleptic characteristics of meat from these two genetic types of turkey. Focusing on attributes such as tenderness, juiciness, and flavor could deepen the understanding of the meat quality associated with these genetic variants, benefiting breeding practices and consumer choices.

DECLARATIONS

Finding

This study received no funding or financial support.

Competing interests

The authors declared no conflicts of interest.

Authors' contributions

Baba Issimouha Loukyatou, Dotche Ignace, and Youssao Aboudou Karim Issaka contributed to the study design and planning. Baba Issimouha Loukyatou and Bonou Assouan Gabriel were responsible for data collection and drafting the first version of the manuscript. Data analysis and interpretation were carried out by BABA Issimouha Loukyatou, Adzona Pitchou Prudence, Dotche Ignace, and Youssao Aboudou Karim Issaka. Salifou Chakirath and Youssao Aboudou Karim Issaka contributed to the critical revision and editing of the manuscript. All authors read and approved the final version of the manuscript.

Ethical considerations

The authors hereby affirm this manuscript has been prepared and submitted in accordance with the highest standards of scientific integrity. All authors have been checked the text of the manuscript for plagiarism, dual publication and/or submission, falsification and/or fabrication, and redundancy.

Availability of data and materials

The data will be available upon a reasonable request from the corresponding author.

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Circular Valorization of Acid Silage from Invasive *Pterygoplichthys* Species in Hens' Diets: Impacts on Laying Performance and Egg Quality

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ABSTRACT

Pterygoplichthys species, an invasive fish, offers a sustainable protein alternative in poultry feed, aligning with circular economy goals and reducing environmental impact. This study aimed to assess the impact of incorporating various levels of acid silage derived from *Pterygoplichthys* species (ASP) into laying hen diets on productive performance and egg quality, as part of a biological waste valorization strategy aligned with circular economy principles. Sixty 35-week-old Rhode Island Red hens (BW 1932.1 ± 10.81 g) were randomly assigned to four isoproteic and isoenergetic dietary treatments containing 0%, 6%, 12%, and 18% ASP over 13 weeks. Productive, egg quality, and economic indicators were recorded weekly. The inclusion of 12% ASP resulted in the highest egg production (82.08%), egg mass (49.32 g/hen/day), and number of eggs per hen per week (5.6), along with improved feed conversion ratio (2.59) and the highest economic efficiency index (17.90%) and profitability, in comparison to the other treatments. Egg quality also improved with ASP, regardless of the inclusion level, particularly in egg weight (60.08 g), shell thickness (0.35 mm), and Haugh units (73.83), compared to the control group. Quadratic regression models identified optimal ASP inclusion levels ranging from 11.2% to 12.3%, depending on the variable analyzed. In conclusion, the inclusion of 12% ASP in laying hen diets represents an effective, profitable, and environmentally responsible nutritional strategy that aligns with the principles of the circular economy and sustainable food production.

Keywords: Acid silage, Circular economy, Egg production, Invasive species

INTRODUCTION

The introduction of exotic fish species into freshwater ecosystems represents an increasing global challenge, with negative impacts on biodiversity and the local economies of fishing communities (Britton et al., 2023). Among exotic fish species, *Pterygoplichthys* species native to South America stands out as one of the most concerning invasive species due to its remarkable adaptability and high reproductive capacity (Hussan et al., 2021). Its proliferation has contributed to the displacement of native species of commercial value, the disruption of trophic chains, and the degradation of aquatic habitats in tropical and subtropical regions of the Americas, Asia, and Africa (Sarkar et al., 2023; Marr et al., 2024). Within this context,

the circular economy emerges as a strategic tool to mitigate the ecological impact of invasive species by promoting the utilization of undesirable biomass through its transformation into value-added products (Duan et al., 2022). The use of acid silage techniques to process *Pterygoplichthys* species enables the transformation of a potential environmental contaminant into a valuable resource for animal nutrition (Raa et al., 1982; Raeesi et al., 2023). This type of silage is produced from fishery by-products viscera, scales, heads, and discarded whole fish, and has proven to be a viable alternative to fishmeal due to its high protein value and low production cost (Bianchi et al., 2014; Madage et al., 2015; Raeesi et al., 2023).

Several studies have demonstrated the potential of fish silage as a functional ingredient in animal feeding. The inclusion of fish silage in diets for monogastric animals such as pigs (Parrini *et al.*, 2023), broiler chickens (Garcés *et al.*, 2015; Shabani *et al.*, 2021), and laying hens (Gaviria *et al.*, 2022) has shown improvements in productive parameters, feed efficiency, and product quality, comparable to those achieved with conventional ingredients. Beyond its nutritional benefits, the use of fish silage supports the sustainability of agri-food systems by decreasing reliance on traditional protein feedstuffs such as soybean meal or fishmeal, whose production is linked to significant environmental costs (Islam and Peñarubia, 2021; Libonatti *et al.*, 2023). In poultry production, the use of non-conventional feed ingredients is a key strategy to enhance the resilience of production systems in the face of market fluctuations and ecological constraints (Malenica *et al.*, 2023; Edenakpo *et al.*, 2025). Incorporating alternative ingredients derived from underutilized biomass, such as fish by-products or invasive species, enables the formulation of sustainable diets without compromising productivity, while improving resource efficiency and reducing the environmental impact of the sector (Libonatti *et al.*, 2023). This strategy is consistent with the United Nations Sustainable Development Goals, especially those focused on responsible production, waste minimization, and the protection of aquatic ecosystems (Neale *et al.*, 2025).

Considering the abundance and ecological threat of *Pterygoplichthys* species in aquatic ecosystems worldwide, as well as its suitable nutritional composition, its use as an ingredient in poultry diets represents an innovative and sustainable strategy in response to its uncontrolled expansion (Marr *et al.*, 2024), which has negatively affected biodiversity and small-scale fisheries (Hussan *et al.*, 2021). This invasive species, characterized by its high reproductive potential and ecological adaptability, has demonstrated a remarkable nutritional profile, with more than 50% crude protein content, comparable to that of conventional protein sources such as fishmeal (Hasrianti *et al.*, 2022). Utilizing its biomass through acid silage not only enables the valorization of a problematic biological residue but also contributes to reducing pressure on traditional inputs, in alignment with circular economy principles and environmental sustainability. Accordingly, the objective of this study was to assess the impact of varying inclusion levels of acid silage derived from *Pterygoplichthys* species in laying hen diets on productive performance and egg quality, as a sustainable alternative in balanced feed formulation.

MATERIALS AND METHODS

Ethical approval

The procedures for animal care and handling followed the Mexican Official Standard 062-ZOO (NOM-062-ZOO, 1999), which outlines technical specifications for the production, care, and use of laboratory animals, as well as the International Guiding Principles for Biomedical Research Involving Animals (CIOMS, 2012).

Production of acid fish silage

To produce the acid silage from *Pterygoplichthys* species (ASP), whole fish were ground to obtain a homogeneous paste. The fish were collected from the daily catches of local fishermen, who incidentally trapped them in their nets, as this species has become a pest in the region's aquatic ecosystems. The resulting paste was then treated with 85% formic acid (Sigma-Aldrich, Mexico) at a ratio of 2.35 L per 100 kg of fresh fish to promote controlled fermentation (Raa *et al.*, 1982). The resulting mixture was stirred manually once per day for 15 consecutive days under ambient temperature conditions (25-30°C) to facilitate enzymatic activity and the hydrolysis of muscle proteins. To ensure the consistency and reproducibility of the fermentation process, the stirring protocol was standardized. Although no active temperature control was applied, the process was conducted under stable ambient conditions, with temperatures ranging between 25 and 30°C, as verified through daily monitoring. This passive control was sufficient to maintain enzymatic activity and ensure the effectiveness of the fermentation process. Once the pH stabilized between 4.0 and 4.5, indicative of bacterial inhibition and material preservation, the silage was stored in airtight plastic containers until its use in the formulation of experimental diets.

To reduce the moisture content of the silage (64%), an adsorption technique was employed using wheat bran as a drying agent. The liquid silage was blended with wheat bran in specific ratios, determined based on the planned inclusion level for each experimental diet. The resulting mixture was left to dry at ambient temperature in shaded conditions for seven days, allowing gradual moisture reduction without thermal denaturation of the proteins. Once dehydration was complete, the material was processed using a manual blade mill to break down clumps formed during drying and to facilitate its homogeneous incorporation into the rest of the diet ingredients. This methodology for producing and conditioning acid fish silage helped preserve the nutritional quality of the fish

(Table 1), reduce the risk of undesirable microbial growth, and improve the material’s physical handling during feed formulation (Raa et al., 1982; Chalamaiah et al., 2012).

Table 1. Nutritional composition and amino acid profile of acid silage from *Pterygoplichthys* species.

Chemical composition	Value on a dry matter basis
Metabolizable energy (Mcal/kg)	2.95
Crude protein (%)	51.20
Dry matter (%)	34.33
Calcium (%)	7.75
Total phosphorus (%)	5.36
Essential amino acids (g/100 g of protein)	
Lysine	8.2
Methionine	2.6
Threonine	4.5
Tryptophan	1.2
Valine	5.2
Isoleucine	4.2
Leucine	7.9
Phenylalanine	4.0
Arginine	6.1
Histidine	2.4

Study design

The research was conducted at the poultry facilities of the Faculty of Veterinary Medicine and Animal Science at the Universidad Michoacana de San Nicolás de Hidalgo, located at kilometer 9.5 on the Morelia–Zinapécuaro highway, in the municipality of Tarímbaro, Michoacán,

Mexico. A total of 60 dual-purpose Rhode Island Red hens, 35 weeks of age, were used in the study. Although this breed is classified as dual-purpose, the present study focused exclusively on evaluating parameters related to egg production and egg quality. The hens were housed in conventional individual battery cages measuring 40 × 40 × 45 cm (length, width, and height, respectively) and randomly assigned to four dietary treatments in a completely randomized design (n = 15 hens/treatment), to evaluate the effects of increasing levels of ASP in laying hen diets. The hens were housed under conventional open-sided sheds. The temperature ranged from 20 to 30°C, with relative humidity between 50% and 70%. A photoperiod of 15 hours of light per day was maintained, with a minimum light intensity of 20 lux over 80% of the area. Ventilation was natural, ensuring adequate air exchange and thermal comfort. Environmental parameters were monitored daily to ensure consistency throughout the trial.

Before the experimental period, all hens underwent a 7-day adaptation phase to the experimental diets. During this period, the hens were gradually transitioned from the basal diet to the assigned treatment diets by increasing the proportion of the experimental feed daily. This strategy was implemented to minimize feed refusal and ensure physiological adaptation, thereby stabilizing intake patterns before data collection began. The treatments included a control diet (0% ASP inclusion) and three experimental diets containing 6%, 12%, and 18% ASP, respectively, as a partial replacement for conventional protein sources. All diets were formulated to be isoproteic and isoenergetic, and were adjusted to meet the nutritional requirements of laying hens according to NRC (1994) guidelines (Table 2).

Table 2. Ingredients and estimated nutritional composition of the experimental diets

Ingredient composition (%)	Inclusion level of <i>Pterygoplichthys</i> species silage (%)			
	0 (control)	6	12	18
Wheat bran	26.0	25.5	25.0	24.5
Ground sorghum	47.0	46.5	46.0	45.5
Soybean meal (44% CP)	19.0	17.5	14.0	10.0
Fish silage (52% CP)	0.0	6.0	12.0	18.0
Calcium carbonate	7.0	6.5	6.0	5.5
Microminerals [†]	1.0	1.0	1.0	1.0
Estimated chemical composition				
Metabolizable energy (Mcal/kg)	2.88	2.87	2.86	2.85
Crude protein (%)	17.6	17.8	17.7	17.5
Calcium (%)	3.7	3.6	3.6	3.5
Total phosphorus (%)	0.85	1.05	1.30	1.50
Lysine (%)	0.82	0.83	0.85	0.86
Methionine (%)	0.31	0.33	0.35	0.37
Threonine (%)	0.65	0.67	0.66	0.64
Tryptophan (%)	0.21	0.22	0.22	0.21

[†]Per kilogram of diet: Vitamin A: 8,000 IU; Vitamin D₃: 2,000 IU; Vitamin E: 50 mg; Vitamin K: 3 mg; Vitamin B₁: 1.5 mg; Vitamin B₂: 4 mg; Vitamin B₆: 0.12 mg; Vitamin B₁₂: 15 mg; Folic acid: 0.6 mg; Pantothenic acid: 10 mg; Niacin: 30 mg; Biotin: 0.1 mg; Choline: 300 mg; Iron: 50 mg; Copper: 10 mg; Zinc: 70 mg; Manganese: 100 mg; Iodine: 1 mg; Selenium: 0.3 mg; Antioxidants: 50 mg.

Dry matter and crude protein levels in the raw materials, experimental diets, and ASP were analyzed following AOAC official methods 934.01 and 976.05 (AOAC, 1990). Gross energy was determined using an adiabatic bomb calorimeter (Model 1281, Parr Instrument Company, Moline, IL, USA). Sample preparation for the quantification of amino acids in the ASP was conducted following the AOAC method 994.12 (AOAC, 1990). This procedure involved hydrolyzing the samples with 6M HCl at 110°C for 24 hours. Methionine determination involved a preliminary oxidation with performic acid, followed by amino acid profiling through reverse-phase high-performance liquid chromatography (HPLC), as outlined by Henderson et al. (2000), using a Hewlett-Packard HPLC system (Model 1100).

Productive performance

The body weight of each hen was monitored weekly throughout the experimental period using a digital scale (Torrey L-EQ®, capacity: 0.001-5.0 kg). Daily feed intake (g) was calculated as the difference between the amount of feed offered and the feed refused, using the same weighing equipment. Weekly weight gain (WWG) was determined using the following formula. $WWG = F_w - I_w$

Where F_w is the final weight and I_w is the initial weight. Feed conversion ratio (FCR) was calculated by dividing the total feed intake (g) by body weight gain.

To assess diet profitability by treatment, the cost per kilogram of eggs produced (Y_i) was calculated using a modified version of the equation proposed by Bellaver et al. (1985).

$$Y_i = \frac{P_i * Q_i}{E_i}$$

Where Y_i is the feed cost per kg of eggs produced in the i -th treatment (0%, 6%, 12%, and 18% ASP inclusion), P_i is the price per kg of the diet used in the i -th treatment, Q_i is the total amount of feed consumed by the i -th treatment, and E_i is the total kg of eggs produced. Additionally, the economic efficiency index (EEI) was calculated using the following expression.

$$EEI = \frac{EP_{kg} \times EC_{\$}}{FI_{kg} \times FC_{\$}}$$

Where EP is total egg production (kg), EC is egg cost (\$/kg), FI is feed intake (kg), and FC is feed cost (\$/kg).

Egg quality

A total of 13 eggs from each treatment were evaluated. Each egg was considered an individual sample, and the mean values for each treatment were calculated based on the measurements obtained. The whole egg

weight (g), as well as the weights of the yolk (g), albumen (g), and shell (g), were recorded. Shell thickness (mm) was also measured. Egg shape index (%) was calculated as the ratio between egg height and width using a digital caliper (model 1114-300a, Georgia, USA). Egg surface area (cm²) was estimated using the following equation.

$$S_a = 4.835 \times EW^{0.662}$$

Where P_s is surface area and EW is egg weight (g) (Paganelli et al., 1974). Albumen height (mm) was measured using a digital caliper, and the Haugh Unit (HU) was calculated using the following equation.

$$HU = 100 \times \text{Log}(AH + 7.7 - 1.7 \times EW^{0.37})$$

Where AH is albumen height (mm) and EW is egg weight (g; Williams, 1992).

Statistical analysis

All statistical analyses were performed using SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA). Before data analysis, the normality of the distribution and homogeneity of residual variance were assessed using the PROC UNIVARIATE procedure. The Shapiro-Wilk test was used to verify normality, and Bartlett's test was applied to assess variance homogeneity.

All statistical analyses were carried out with SAS version 9.4 (SAS Institute Inc., Cary, North Carolina, USA). Data were analyzed by repeated measures ANOVA using the PROC MIXED procedure (Littell et al., 1998). In the model, the individual hen was considered the experimental unit. The effects of treatment, week, and their interaction were evaluated on feed intake, body weight, weight gain, and egg production. The following statistical model was used in this study.

$$Y_{ijkl} = \mu + T_i + G(T)_{j(i)} + W_k + T \times W_{ik} + e_{ijkl}$$

Where, Y_{ijkl} is response variable; μ is overall population mean; T is fixed effect of the i -ésimo treatment (i : 0%, 6%, 12%, and 18% ASP inclusion); $G(T)_{j(i)}$ is random effect of the j -ésima hen nested within the i -ésimo treatment; W_k is fixed effect of the k -ésima week (k : 1, 2, 3, ..., 13); $T \times W_{ik}$ is the fixed interaction effect between treatment and week. e_{ijkl} is a random error associated with each observation, assumed to be normally and independently distributed ($\sim NID = 0, \sigma_e^2$).

Egg quality indicators were analyzed using ANOVA with the PROC GLM procedure. The effects of treatment, week, and their interaction were evaluated. The following statistical model was used.

$$Y_{ijk} = \mu + T_i + W_j + T \times W_{ij} + e_{ijk}$$

Where, Y_{ijk} is the response variable; μ is the overall population mean; T is the fixed effect of the i -ésimo

treatment ($i = 0\%, 6\%, 12\%$, and 18% ASP inclusion); W_j is the fixed effect of the j -ésima week ($k: 1, 2, 3, \dots, 13$); $T \times W_{ij}$ is the fixed interaction effect between treatment and week. ε_{ijkl} is a random error associated with each observation, assumed to be normally and independently distributed ($\sim NID = 0, \sigma_e^2$).

Statistical differences among means were evaluated using the least squares means (LsMeans) method, with a significance threshold set at $\alpha \leq 0.05$. Data are reported as LsMeans \pm standard error of the mean (SE).

To determine the optimal inclusion level of ASP in the diets of laying hens, a quadratic regression analysis was performed using SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA). The regression was based on six key performance indicators: Weekly feed intake (g/hen), feed conversion ratio, laying rate (%), eggs per hen per week, egg weight (g), and the economic efficiency index. For each dependent variable, a quadratic regression model of the following form was fitted.

$$Y = \beta_0 + \beta_1 X + \beta_2 X^2 + \varepsilon$$

Where Y is the response variable, X is the inclusion level of fish silage in the diet (%); $\beta_0, \beta_1, \beta_2$ are coefficients to be estimated; ε is a random error term associated with each observation ($\sim NID = 0, \sigma_e^2$). The model was fitted using the PROC REG procedure, and associations were considered statistically significant at $p < 0.05$. The optimal inclusion level was determined by solving for the vertex of the parabola generated by the quadratic model using the following expression.

$$X_{\text{optimal}} = -\frac{\beta_1}{2\beta_2}$$

Where X_{optimal} is the percentage of ASP inclusion that maximizes or minimizes the evaluated variable, depending on the direction of the curve's concavity (β_2).

RESULTS

Productive performance

During the 13-week evaluation period, the inclusion of ASP in laying hen diets significantly influenced ($p < 0.05$) various indicators of productive performance, growth, and economic efficiency (Table 3). Feed intake increased progressively with higher inclusion levels, reaching its highest value ($p < 0.05$) in the 12% ASP treatment (127.67 g/hen/day), in comparison to the other treatments. In contrast, final body weight also showed a significant increase ($p < 0.05$), with the 12% and 18% ASP inclusion treatments resulting in higher weights (1914.5 g and 1904.0 g, respectively) compared to the control group (1819.2 g).

Body weight change was negative across all treatments; however, the 12% ASP treatment exhibited the smallest reduction of body weight ($p < 0.05$), with respect to the other treatments, suggesting better weight maintenance (Table 3). The number of eggs per hen per week showed significant differences ($p < 0.05$), reaching its peak in the 12% ASP group (5.6 eggs), followed by the 18% and 6% groups (5.1 and 5.0 eggs, respectively), while the control group had the lowest value (4.7 eggs; Table 3). The laying rate was highest in the 12% inclusion group (82.08%), showing significant differences ($p < 0.05$) compared to the control group (66.94%). Egg mass was also significantly greater in the 12% ASP treatment (49.32 g/hen/day) compared to the other treatments ($p < 0.05$, Table 3).

In terms of feed efficiency, the most favorable feed conversion ratio (FCR) was observed in the group receiving 6% ASP (2.53), whereas the control group exhibited the highest FCR value (i.e., lowest efficiency; $p < 0.05$, Table 3). The economic efficiency index (EEI) increased progressively with higher levels of ASP inclusion, reaching its highest value ($p < 0.05$) in the 12% group (17.90%), while the control group registered the lowest EEI (16.82%; $p < 0.05$, Table 3).

Table 3. Effect of acid fish silage inclusion level on productive performance, growth variables, and economic efficiency in laying hens

Indicator	Inclusion level of <i>Pterygoplichthys</i> species silage (%)				SEM	p-values		
	0 (control)	6	12	18		T	W*	T × W
Feed intake (g)	105.46	113.81	127.67	116.38	0.116	<.0001	0.3511	0.2268
Initial body weight (g)	1940.2	1937.5	1916.2	1934.0	0.514	0.3275	<.0001	<.0001
Final body weight (g)	1819.2	1865.4	1914.5	1904.0	0.486	0.0046	<.0001	<.0001
Body weight change (g)	-121.2	-68.7	-1.6	-33.3	0.443	<.0001	0.5833	0.9237
Eggs/hen/week	4.7 ^a	5.0 ^{ab}	5.6 ^d	5.1 ^b	0.007	<.0001	<.0001	0.2418
Egg production (%)	66.94 ^a	72.72 ^b	82.08 ^c	73.88 ^b	0.108	<.0001	<.0001	0.3324
Egg mass (g/hen/day)	38.09 ^a	44.97 ^b	49.32 ^c	44.37 ^b	0.073	<.0001	<.0001	0.0864
FCR	2.73 ^c	2.53 ^a	2.59 ^a	2.63 ^b	0.006	<.0001	<.0001	0.0197
EEI (%)	16.82	16.99	17.90	17.76	0.020	<.0001	0.8723	0.2521

SEM: Standard error of the mean; T: Treatment; W: Week; FCR: Feed conversion ratio; EEI: Economic efficiency index. *13 weeks. ^{a,b,c} Different superscript letters indicate differences within a row ($p < 0.05$).

Egg quality

Over the 13-week experimental period, dietary inclusion of ASP in laying hen diets led to significant variations in multiple internal and external egg quality traits ($p < 0.05$; Table 4). Egg weight increased significantly with ASP inclusion, rising from 56.93 g in the control group to 61.82 g in the 6% ASP treatment, and remaining around 60 g in the 12% (60.08 g) and 18% (60.05 g) ASP groups ($p < 0.05$, Table 4). A similar trend was observed for shell weight, which was significantly greater in the 6% and 12% ASP groups compared to the control ($p < 0.05$; Table 4). Likewise, eggshell thickness was higher in the 6% ASP group (0.35 mm) than in the control group (0.33 mm; $p < 0.05$). No significant interaction effects between treatment and week were detected for these variables ($p > 0.05$; Table 4). Regarding estimated egg surface area, statistically significant differences were also found ($p < 0.05$), showing a rising trend with increasing ASP inclusion, peaking at 72.70 cm² in the 18% treatment, in comparison to the other treatments (Table 4). Egg shape index increased ($p < 0.05$) in the 12% and 18% ASP groups, rising from 74.03% in the control group to 74.52% (Table 4).

Regarding internal egg quality, albumen weight increased significantly ($p < 0.05$) in the treatments with ASP, particularly in the 6% group (35.69 g) compared to the control (31.03 g; Table 4). Yolk weight was significantly influenced by dietary treatment ($p < 0.05$), with the highest value recorded in the 12% ASP group (15.92 g), and the lowest in the 18% group (15.28 g). The control group showed an intermediate value (15.71 g), differing significantly from the 12% and 18% treatments (Table 4). Albumen height increased significantly with increasing levels of ASP inclusion ($p < 0.05$), from 7.30 mm in the control group to 7.60 mm, 7.80 mm, and

8.00 mm in the 6%, 12%, and 18% ASP groups, respectively (Table 4). In contrast, yolk height differed significantly only in the 18% ASP group, which showed a lower value (16.13 mm) compared to the other treatments ($p < 0.05$; Table 4). No significant treatment per week interaction effects were observed for albumen weight, yolk weight, albumen height, or yolk height ($p > 0.05$).

Haugh unit values were significantly higher ($p < 0.05$) in the ASP treatments, peaking at 73.83 in the 6% group compared to 68.54 in the control group (Table 4). The week of evaluation also had a significant effect ($p < 0.05$), but no treatment \times week interaction was detected ($p > 0.05$).

Estimation of optimal inclusion levels of acid silage from *Pterygoplichthys* species

The relationship between the inclusion level of ASP in laying hen diets and productive and economic variables was analyzed using quadratic regression models. Among the evaluated indicators, the economic efficiency index (EEI; percentage profitability) reached its optimal value at an inclusion level of 12.19%. For feed conversion ratio and egg production rate, the estimated optimal inclusion levels were approximately 12.3% and 11.79%, respectively, suggesting a physiological convergence of the positive effects of ASP within a range close to 12% (Table 5).

Figure 1 shows the fitted quadratic regression curves for each of the evaluated indicators. In all cases, the curves exhibit an initial increase followed by a stabilization or slight decrease, reflecting the nonlinear response of productive and economic variables to increasing levels of ASP inclusion. The inflection points of the curves indicate the estimated levels of maximum response for each variable.

Table 4. Effect of acid fish silage inclusion level on internal and external egg quality parameters in laying hens

Egg quality	Inclusion level of <i>Pterygoplichthys</i> species silage (%)				SEM	p-values		
	0 (control)	6	12	18		T	W [*]	T \times W
Egg weight (g)	56.93 ^a	61.82 ^c	60.08 ^b	60.05 ^b	0.055	<.0001	<.0001	0.5654
Shell weight (g)	4.6 ^{ab}	5.0 ^{bc}	5.0 ^{bc}	4.8 ^b	0.005	<.0001	0.7361	0.4170
Shell thickness (mm)	0.33 ^a	0.35 ^b	0.34 ^{ab}	0.34 ^{ab}	0.001	<.0001	0.7409	0.5302
Egg surface area (cm ²)	68.8 ^a	71.65 ^b	71.54 ^b	72.70 ^c	0.043	<.0001	0.225	0.132
Shape index (%)	74.03 ^a	74.09 ^a	74.52 ^b	74.40 ^b	0.032	<.0001	0.5252	0.0914
Albumen weight (g)	31.03 ^a	35.69 ^d	33.70 ^c	33.29 ^b	0.047	<.0001	0.5850	0.1780
Albumen height (mm)	7.30 ^a	7.60 ^b	7.80 ^c	8.00 ^d	0.001	<.0001	0.3753	0.1391
Yolk weight (g)	15.71 ^b	15.79 ^{bc}	15.92 ^c	15.28 ^a	0.013	<.0001	0.0239	0.2742
Yolk height (mm)	15.00 ^a	15.01 ^a	14.98 ^a	16.02 ^b	0.018	<.0001	0.4667	0.8243
Haugh units	68.54 ^a	73.83 ^c	71.35 ^b	70.20 ^b	0.062	<.0001	<.0001	0.1897

SEM: Standard error of the mean; T: Treatment; S: Week. *13 weeks. ^{a,b,c} Different superscript letters indicate differences within a row ($p < 0.05$).

Table 5. Regression estimates and optimal inclusion levels of acid fish silage on productive performance and economic efficiency variables in Rhode Island Red hens

Indicator	Regression estimates*			Critical point		
	Intercept	β_1	β_2	OI (%)	OV	R ²
Feed intake (g)	104.2965 (<.0001)	2.9294 (<.0001)	-0.1206 (<.0001)	12.14	122.08	0.830
FCR	3.4296 (<.0001)	-0.1205 (<.0001)	0.0049 (0.0040)	12.30	2.69	0.942
Egg production rate (%)	64.2053 (<.0001)	2.1861 (0.0010)	-0.0927 (0.0018)	11.79	77.09	0.799
Eggs/hen/week	4.5558 (<.0001)	0.1239 (<.0001)	-0.0055 (<.0001)	11.25	5.25	0.626
Egg weight (g)	55.6823 (<.0001)	0.7521 (0.0190)	-0.0336 (0.0321)	11.18	59.89	0.781
EEI	16.86681 (<.0001)	5.34776 (0.0013)	0.0017 (0.05012)	12.19	17.91	0.653

FCR: Feed conversion ratio; EEI: Economic efficiency index; OI: Optimal inclusion level of silage; OV: Optimal value of the indicator. *Estimator (p-value)

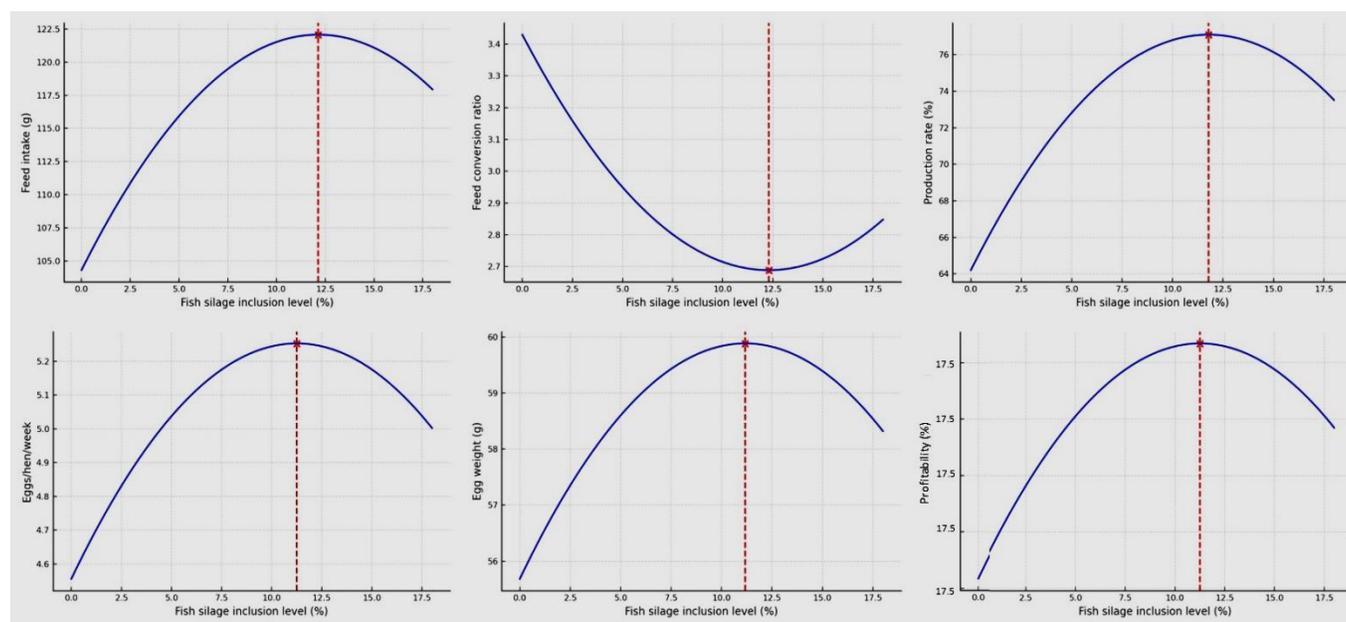


Figure 1. Regression curves for productive and economic indicators as a function of fish silage inclusion level in Rhode Island Red hens

DISCUSSION

The inclusion of ASP in laying hen diets was evaluated as a protein alternative within a circular economy framework, aiming to reduce dependence on conventional ingredients such as soybean meal and enhance production sustainability. The results demonstrated that inclusion levels between 12% and 13% led to significant improvements in productive, economic, and egg quality indicators, suggesting efficient utilization of this by-product.

From a productive standpoint, a significant increase was observed in the number of eggs per hen, egg mass,

and laying rate in the treatments that included ASP, particularly in the 12% group. This effect could be associated with enhanced bio-accessibility of key essential amino acids, including lysine, leucine, and valine, provided by the ASP (Chalamaiah et al., 2012). These amino acids play a central role in protein synthesis for reproductive tissues, yolk formation, and ovarian follicle development processes that are critical for sustained egg laying and egg quality in hens (Ji et al., 2014; Macelline et al., 2021).

Acid silage, being subjected to controlled hydrolysis during the fermentation process with formic acid,

produces a shorter peptide fraction that is highly digestible (Mayta-Apaza et al., 2022). This characteristic facilitates the transcellular transport of peptides and amino acids across the intestinal epithelium via specific transporters such as PepT1, thereby optimizing absorption and reducing metabolic competition among nutrients (Cruz-Casas et al., 2021). This enhanced digestive efficiency may partly explain the improved protein utilization in diets containing acid silage, particularly in adult hens, whose intestinal enzymatic activity and absorptive capacity may be moderately diminished due to age. Additionally, bioactive peptides derived from fermented fish have been reported to possess immunomodulatory and antioxidant properties, which help maintain intestinal integrity and reduce oxidative stress, an important factor associated with productivity decline during prolonged laying periods (Alizadeh-Ghamsari et al., 2023; Chaklader et al., 2023). In this context, improved gut health may lead to more efficient energy redistribution toward reproductive functions, such as yolk production and egg formation. The nutritional behavior of silage in the gastrointestinal tract of hens also includes modulation of gastric pH, which enhances the activity of proteolytic enzymes such as pepsin, thereby improving the solubilization of structural proteins and the release of amino acids in the proventriculus and gizzard (Olukosi and Dono, 2014). The modulation of pH, combined with a reduction in pathogenic microorganisms due to the acidity of the silage, contributes to a more stable and metabolically efficient intestinal environment (Olukosi and Dono, 2014; Alizadeh-Ghamsari et al., 2023).

The slight reduction in body weight observed during the experimental period is consistent with physiological adaptations that occur in laying hens as they transition from peak to post-peak production (Khatibi et al., 2021). During this stage, metabolic priorities shift toward maintaining egg production efficiency rather than supporting further somatic growth or weight gain. This reallocation of nutrients, along with the natural aging process, may contribute to a gradual loss of body mass (Noetzold and Zuidhof, 2025). Importantly, the observed change was not related to dietary restriction or health issues, as hens had *ad libitum* access to feed, exhibited normal behavior, and remained clinically healthy throughout the study.

Egg quality also showed positive responses in hens fed with ASP. Shell thickness was greater in the silage-supplemented treatments, which may be attributed to improved intestinal absorption of minerals, particularly calcium, phosphorus, and zinc elements essential for the

mineralization of the eggshell's organic matrix (Li et al., 2017). This improvement could be linked to the acidic environment generated by residual formic acid in the silage, which has been associated with increased solubility and availability of calcium in the digestive tract of poultry (Guinotte et al., 1995; Gordon and Roland, 1997; Ricke et al., 2020). Eggshell formation relies on the activity of calcium channels and transport pumps regulated by calcitriol, the active form of vitamin D₃. The expression and efficiency of these mechanisms are influenced by the bioavailability of dietary minerals (Nys and Guyot, 2011). Therefore, the improved eggshell structure observed in hens supplemented with ASP may be explained by enhanced calcium availability, promoted by the acidifying effect of the silage and the reduction in mineral competition.

Haugh units also increased in the treatments supplemented with ASP. This parameter primarily depends on the viscosity and density of the albumen, which can deteriorate under conditions of oxidative stress, chronic inflammation, or low protein efficiency (Williams, 1992; Obianwuna et al., 2022). The presence of antioxidant peptides derived from fish collagen and muscle proteins may help reduce systemic oxidative damage, preserving the three-dimensional structure of ovalbumen and other functional egg components (Walayat et al., 2022). Additionally, fish fermentation generates short peptides with high digestibility, which can be rapidly absorbed and utilized by the liver for the synthesis of plasma and storage proteins such as ovalbumen and ovotransferrin, which are subsequently secreted into the egg albumen (Obianwuna et al., 2022). This efficient metabolic pathway supports the stability of the protein gel in the egg white, resulting in higher Haugh unit values. In contrast, yolk pigmentation did not differ among treatments, which can be attributed to the low carotenoid content of the silage. Unlike ingredients such as yellow corn, marigold, or alfalfa, which are rich in lutein and zeaxanthin, acid silage lacks these compounds and therefore does not alter yolk coloration (Karadas et al., 2006). With respect to the higher values of albumen weight and Haugh units observed at the 6% ASP inclusion level, these outcomes may be attributed to the greater egg weight recorded in this group, supporting a positive correlation between albumen mass and structural integrity (Chang et al., 2024). In contrast, at higher inclusion levels of ASP (12-18%), the decline in these parameters could be partially explained by reduced albumen weight and potentially lower protein quality possibly due to amino acid imbalances, elevated non-protein nitrogen content, or

decreased nutrient digestibility which may have limited albumen height and Haugh unit expression despite continued silage inclusion (Alagawany et al., 2020).

From a productive efficiency perspective, both the economic efficiency index (EEI) and percentage profitability reached their highest values in the treatment with 12% inclusion of ASP. The use of quadratic regression models allowed this level to be identified as the point of maximum economic return, indicating a favorable balance between feed cost and hen productivity. This finding aligns with the report by Boumans et al. (2022), who stated that the use of animal-derived by-products can reduce feed formulation costs without compromising zootechnical performance. The improvement in EEI observed in this study may be attributed to the relatively low cost of the silage (6.00/kg USD), combined with its high protein density and digestive functionality, which supports the maintenance or even enhancement of feed conversion efficiency and egg production. Moreover, the quadratic regression analysis revealed that the optimal inclusion level of ASP (12%) aligned closely with the inflection points of key productive and economic indicators, including egg production (11.79%), feed conversion ratio (12.3%), and the economic efficiency index (12.19%). This convergence suggests that, at this level, the diet achieves a biologically efficient balance between nutrient utilization and productive output, which is further reflected in the improved cost-effectiveness of egg production.

Additionally, from an environmental and sustainability perspective, the use of ASP as a feed ingredient represents an innovative strategy for the valorization of an invasive species, contributing to its population control while simultaneously reducing pressure on conventional protein sources, such as soybean meal or wild-caught fish. This approach not only offers zootechnical and economic benefits but also aligns with the principles of the circular economy promoted by the Bianchi et al. (2014) and the United Nations Sustainable Development Goals (Neale et al., 2025), particularly those related to the responsible use of natural resources, sustainable food production, and the mitigation of environmental impacts on aquatic ecosystems.

CONCLUSION

The use of acid silage produced from *Pterygoplichthys* species represents a nutritionally viable and functional alternative in laying hen nutrition. Isoproteic and isoenergetic diets does not compromise productive

performance or egg quality and provides benefits in terms of feed efficiency and profitability. These attributes support its potential role as a sustainable input aligned with the principles of the circular economy and environmentally responsible production systems. A 12% inclusion level provides optimal performance across multiple zootechnical and economic parameters, including egg production, egg mass, feed conversion ratio, economic efficiency index, and profit margin. Future research could explore its interaction with functional additives, its long-term effects on reproductive health, or its integration into sustainable production certification systems.

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Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Aureliano Juárez and Juan Carlos Cuellara. The first draft of the manuscript was written by Aureliano Juárez, Guillermo Salasa, Ernestina Gutiérrez, Gerardo Ordaz, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated for the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no conflicts of interest.

Ethical considerations

Ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been checked by all the authors.

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A Field Study on Infectious Bronchitis Virus in Broiler Chickens in Southern Iraq

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ABSTRACT

Infectious bronchitis virus (IBV) is a highly transmissible avian Gamma-coronavirus that continues to pose a major challenge to poultry health and productivity worldwide, particularly in broiler production systems. The present investigation aimed to detect and characterize IBV infections in broiler flocks located in two districts of Southern Iraq between late 2024 and early 2025. A total of 200 clinically affected broilers (100 broilers from each flock) were sampled, with tracheal and kidney tissues collected for clinical evaluation, histopathological assessment, and viral isolation. Suspected IBV-infected chickens showed respiratory distress, increased mortality, and kidney lesions. The suggestive post-mortem lesions were caseous plug exudates at the tracheal bifurcation, as well as congested and hyperemic kidneys. The inoculation of tracheal and kidney tissue suspension in embryonated chicken eggs (ECEs) resulted in death, stunting, curling, dwarfism, congestion, and subcutaneous hemorrhages. The histopathological findings in tracheal tissues revealed epithelial desquamation, goblet cell depletion, and lymphocytic infiltration, while kidney findings exhibited tubular degeneration, glomerular disruption, and fibrin deposition. These findings emphasize the need for future studies to focus on the molecular identification of circulating strains, vaccine matching, and monitoring of post-vaccination protection levels in Iraq.

Keywords: Broiler chicken, Histopathology, Infectious bronchitis virus, Isolation, Kidney, Trachea

INTRODUCTION

The major difficulties threatening poultry production worldwide are viral diseases. Globally, infectious bronchitis (IB) is considered the second most economically damaging viral disease affecting the poultry sector, following the highly pathogenic avian influenza (De Wit and Cook, 2019). Infectious bronchitis is responsible for substantial economic losses to the poultry industry, particularly in broiler flocks where it reduces weight gain, feed efficiency, and survival rates. Losses may range from \$0.03 to \$0.10 per broiler, depending on strain virulence and flock age (Jackwood and de Wit, 2020; Rafique et al., 2024).

As reported by the International Committee on Taxonomy of Viruses (ICTV, 2024), the infectious bronchitis virus (IBV) belongs to the genus *Gamma-coronavirus*, subgenus *Igacovirus*, within the subfamily *Orthocoronavirinae* of the family *Coronaviridae*. The

virus possesses a pleomorphic, enveloped structure and contains a positive-sense, single-stranded, non-segmented RNA genome of approximately 27.6 kb. This genome encodes both non-structural proteins and the major structural proteins, namely the nucleocapsid (N), membrane (M), envelope (E), and spike (S) proteins (Dimitrov et al., 2019; Quinteros et al., 2022).

The IBV is commonly considered a respiratory pathogen that replicates in the tracheal mucosa (Amarasinghe et al., 2018), while some strains of the virus show broad tissue tropism to kidneys, reproductive tract, bursa of Fabricius, gastrointestinal tract (proventriculus and cecal tonsils), and spleen (Rafique et al., 2024). In laying hens, variant strains of IBV may cause damage to the reproductive tract (Cook et al., 2012; Ramsubeik et al., 2023). This broad tissue tropism emphasizes the complexity of IBV pathogenesis (Bande et al., 2016; Rafique et al., 2024).

The IBV mainly spreads systemically through tracheal macrophages and blood monocytes, leading to deep respiratory infections (De Wit and Cook, 2019). In some cases, the virus has also been detected in cloacal swabs and cecal tonsils, suggesting the possibility of retrograde viral ascent from the lower gastrointestinal tract to the kidneys via the ureters, particularly with nephropathogenic strains (Quinteros et al., 2022). Infection of the nasal passages and tracheal lining with IBV rapidly destroys the ciliated epithelium, leading to impaired mucociliary clearance and thereby increasing susceptibility to secondary bacterial infections (Cook et al., 2012). In addition, nephropathogenic IBV strains are capable of inducing marked renal pathology, including tubular epithelial cell necrosis, inflammatory infiltration, and renal dysfunction progressing to failure (Hoerr, 2021). The severity of IBV is influenced by the management practices, live virus vaccines, immunosuppressive conditions, and coexisting pathogens (Hoerr, 2021). Consequently, this study aimed to detect the presence of IBV in broiler chicken flocks in two districts of Southern Iraq. The investigation included viral isolation and histopathological examination of the trachea and kidneys to evaluate dual viral tropism, providing a comprehensive understanding of the pathological behavior of IBV under field conditions.

MATERIALS AND METHODS

Ethical approval

This study was conducted following the ethical guidelines for animal research approved by the Institutional Animal Ethics Committee of the College of Veterinary Medicine, University of Basrah, Basrah, Iraq.

Sampling

According to farm records and veterinary interviews, two infected commercial broiler farms were included in this study: one located in Abu Khosib (approximately 3,000 chickens) and the other in Shat Al-Arab (approximately 7,000 chickens), Basra Governorate, Southern Iraq. The affected flocks were 14 and 20 days of age, respectively.

Both farms had received a single combined live vaccine via drinking water at 5 days of age, consisting of the La Sota strain of Newcastle disease virus (NDV) and the H120 strain of infectious bronchitis virus (IBV), provided by Boehringer Ingelheim, Germany. However, no booster IBV vaccinations or additional vaccines and drugs were administered before the outbreak, mainly due

to poor management practices and limited awareness of proper vaccination protocols among the farmers. From each farm, 100 clinically affected broilers were selected (200 broiler chickens in total) for pathological examination and virus detection.

This field-based pathological investigation was conducted between December 2024 and March 2025 in Basra Governorate, Southern Iraq. The study included two commercial broiler farms: one in Abu Khosib with approximately 3,000 broilers aged 14 days, and another in Shat Al-Arab with approximately 7,000 broilers aged 20 days. An active outbreak in the previous flocks was suggestive of IBV infection and was characterized by severe respiratory distress with gasping and a high mortality rate of average of about 40% both farms.

A total of 200 chickens (100 from each flock) showing severe respiratory manifestations were selected and delivered to the pathology laboratory at the College of Veterinary Medicine, University of Basrah, for further examination. A systematic necropsy procedure was performed, and the gross lesions of the respiratory and renal systems were recorded.

Viral isolation

The inoculation of IBV was performed using the allantoic cavity of 9-11-day-old specific pathogen-free (SPF) embryonated chicken eggs (ECEs) obtained from Nile S.P.F. Company, Giza, Egypt. Tracheal and renal tissues from clinically affected broilers showing typical gross lesions were aseptically collected, pooled, and homogenized in sterile phosphate-buffered saline (PBS) supplemented with antibiotics (1000 IU/mL penicillin and 1000 µg/mL streptomycin) to prepare a 10% (w/v) suspension. The homogenate was clarified by centrifugation at 3000 rpm for 15 minutes at 4°C using a refrigerated high-speed centrifuge (Sigma 3-30KS, Sigma Laborzentrifugen GmbH, Germany), and the supernatant was collected. Approximately 0.2 mL of the filtrate was then inoculated into the allantoic cavity through a 0.22 µm syringe filter under sterile conditions. The inoculated eggs were incubated at 37°C and monitored daily for embryo viability and lesions. Embryos that died within the first 24 hours were discarded as non-specific. After 4-7 days, embryos were chilled at 4°C for 6 hours, and allantoic fluid and embryo lesions were examined. Gross pathological changes, such as stunting, curling, subcutaneous hemorrhages, and dwarfing, were considered indicative of IBV replication. The inoculation and isolation procedures were designed according to the methods described by Hoan et al. (2023).

Histopathological examination

For histopathological examination, tissue samples from the tracheal bifurcation region and the posterior lobes of the kidneys, including both cortex and medulla, were collected. All collected tissues were promptly immersed in 10% neutral-buffered formalin and fixed for 48-72 hours. Following fixation, samples were processed through a standard histological protocol, which included dehydration in graded ethanol, clearing in xylene, and embedding in paraffin wax. Tissue blocks were sectioned at a thickness of 4-5 μm using a rotary microtome (Leica RM2125 RTS, Germany) and placed onto glass slides. Sections were subsequently stained with hematoxylin and eosin (H&E) according to established procedures to enable microscopic evaluation (Bancroft and Layton, 2019; Layton et al., 2019). A compound light microscope (Olympus CX23, Japan) was used for histopathological examination at magnifications of $\times 40$ and $\times 100$.

RESULTS

Broiler chickens from both examined flocks exhibited severe respiratory signs, including sneezing, tracheal rales, open-mouth breathing, and dyspnea (Figure 1a). Affected chickens had died on their backs after a period of labored breathing and agitation, suggesting acute anoxia (Figure 1b).

The post-mortem gross examination of the trachea in the suffering and dead chickens revealed severe mucosal congestion with the presence of diffuse catarrhal to fibrinous diphtheritic cheesy exudates or caseous plugs at the tracheal bifurcation region (Figure 2a). The kidneys were enlarged and congested (Figure 2b).

Viral isolation using SPF ECEs revealed embryonic mortality by the 5th day post-inoculation. Out of 30 inoculated embryos (15 from each flock), 24 embryos developed characteristic IBV-induced lesions, including growth dysplasia and dwarfism, curling and stunting of the body, congestion of internal organs, and subcutaneous hemorrhages (Figure 3).

The histopathological examination of the trachea is shown in Figure 4. Extensive epithelial damage, desquamation, and sloughing of epithelial cells were observed in 160 out of 200 examined samples (80%), accompanied by marked mucosal hyperplasia and irregular thickened epithelial zones. A markedly reduced number of goblet cells with vacuolated cytoplasm, along with submucosal edema, heterophilic infiltration, and vascular congestion, was detected in 140 samples (70%).

The histopathological findings in kidney tissue are illustrated in Figure 5. The tubule epithelium revealed multifocal to diffuse degenerative and necrotic changes in 150 samples (75%), with luminal obstruction by cellular debris and proteinaceous casts. Vacuolar degeneration, pyknosis, and karyorrhexis were also common. Atrophy of glomerular tufts, accompanied by infiltration of inflammatory cells such as neutrophils and mononuclear cells within the interstitium, was observed in approximately 100 out of 200 examined kidney samples (50%). In 60 out of 200 samples (30%), tubular epithelium was replaced by fibrinous material and necrotic debris, leading to narrowing of the lumina due to epithelial swelling. These gross and microscopic findings confirm the presence of IBV-induced pathological changes in both respiratory and renal tissues.

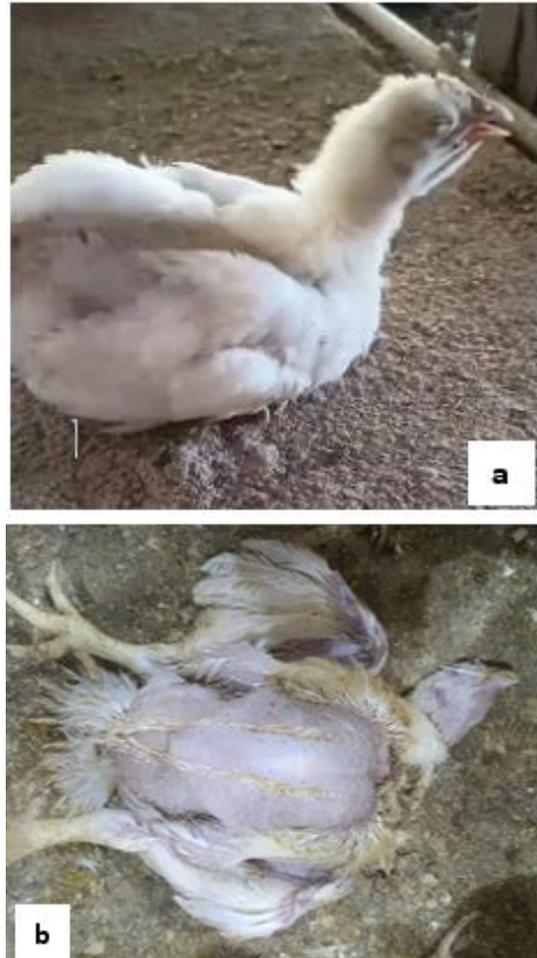


Figure 1. Broiler chickens (*Gallus gallus domesticus*) naturally infected with infectious bronchitis virus in Basrah, Southern Iraq, during 2024-2025. **a:** 14-day-old chickens show open mouth breathing, **b:** 20-day-old broiler chickens found dead and lying on their backs.

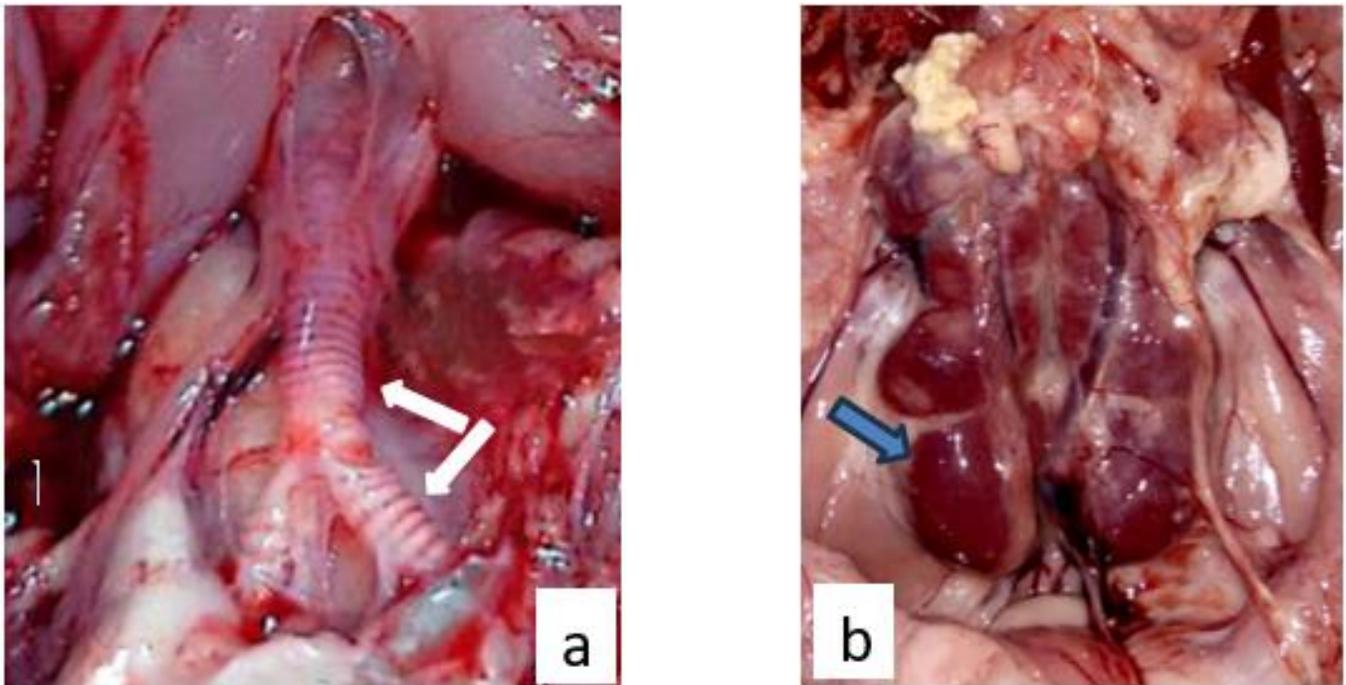


Figure 2. Gross lesions of 14-day-old broiler chickens (*Gallus gallus domesticus*) naturally infected with infectious bronchitis virus in Basrah, Southern Iraq, during 2024-2025. **a:** Yellow caseous material in trachea and tracheal bifurcation (white arrows). **b:** Swollen and congested kidney lobes (blue arrow).

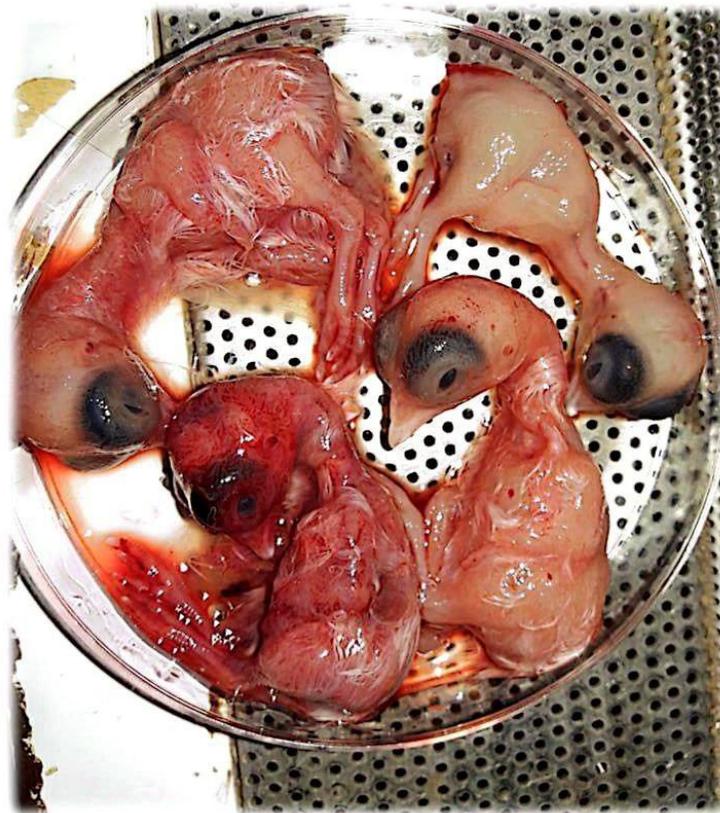


Figure 3. Specific pathogen-free chicken embryos at day 5 post-inoculation with infectious bronchitis virus suspected tissue suspension. Embryos show stunting, curling, and hemorrhages (typical lesions of IBV infection).

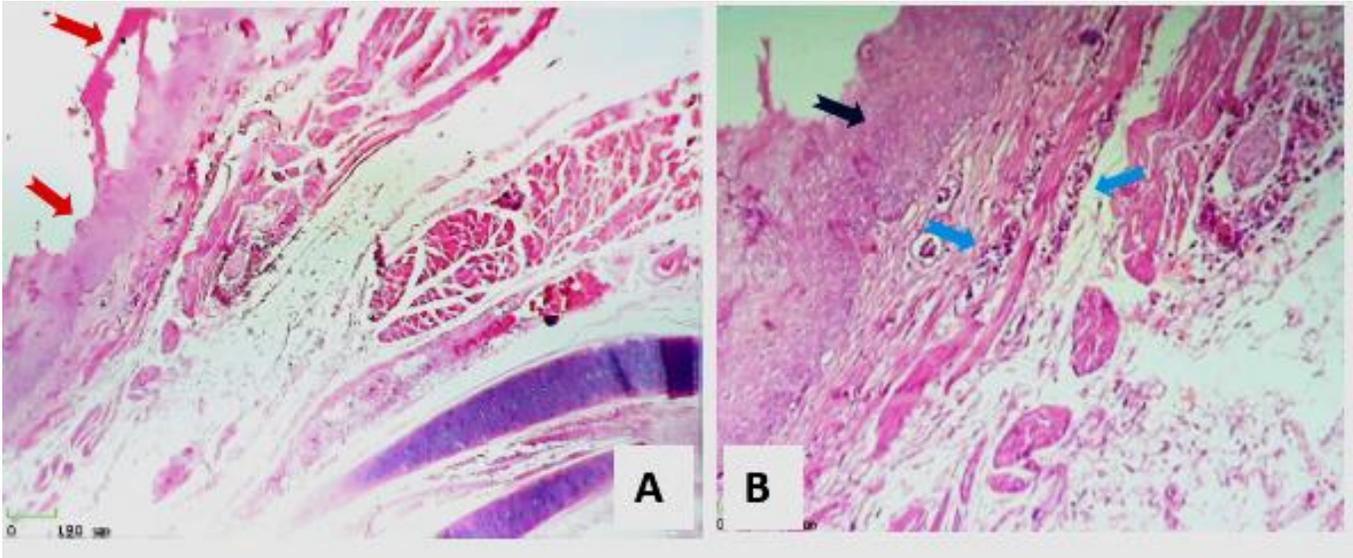


Figure 4. Tracheal tissue of broiler chickens (*Gallus gallus domesticus*) naturally infected with infectious bronchitis virus in Basrah, Southern Iraq, during 2024-2025. **A:** Extensive epithelial damage, sloughing, and desquamation (red arrow). **B:** Thickened epithelial areas (black arrow) with infiltration of inflammatory cells (blue arrows; H&E stain, $\times 100$).

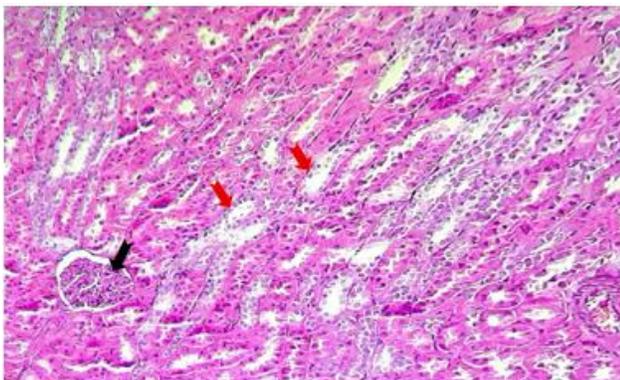


Figure 5. Histological alterations of the kidney of broiler chickens (*Gallus gallus domesticus*) naturally infected with infectious bronchitis virus in Basrah, Southern Iraq, during 2024-2025. There is epithelial cell degeneration, necrosis, and vacuolation of glomerular endothelial cells (black arrow), as well as dilatation of renal tubules (red arrow; H&E stain, $\times 40$).

DISCUSSION

Infectious bronchitis virus (IBV), a highly transmissible avian Gammacoronavirus, continues to represent a major global threat to poultry production, especially in broiler flocks. The infection is characterized by a notably short incubation period of only 18-36 hours, with the upper respiratory tract serving as the primary site of viral replication (Falchieri et al., 2024). IBV infection causes acute respiratory distress, renal damage, reproductive failure, and high mortality with considerable economic losses (Hoerr, 2021; Rafique et al., 2024).

The history of the affected broiler chicken flocks revealed up to 40% mortality rate by day 14 and 20 of age in both examined flocks, reflecting the high virulence and renal tropism (nephron-pathogenic potential) of the circulating field strains.

The mentioned respiratory signs and lesions, including gasping, rales, dyspnea, and the caseous plug at the tracheal bifurcation, are characteristic of IBV infections. These findings are aligned with those reported by Falchieri et al. (2024) in the United Kingdom and El Nembr et al. (2025) in Egypt. Moreover, the enlargement and congestion of the renal lobes are consistent with the pathogenesis of nephro-pathogenic IBV strains that are reported previously (Grgić et al., 2008; Hasan et al., 2020; Quinteros et al., 2022). The observed mortalities, stunting, curling, dwarfism, and hemorrhages of the inoculated embryos validate the diagnostic utility of ECEs for IBV isolation (Hoan et al., 2023; Berhanu et al., 2025).

The microscopic tracheal lesions reflect both direct viral cytopathic effects and the host's immune response. The combinations of deciliation, epithelial desquamation, goblet cell loss, and hyperplasia suggest both destructive and reparative tissue dynamics. These findings, together with epithelial apoptosis and regenerative hyperplasia in IBV-infected tracheal tissue, are described by Han et al. (2017) and El Nembr et al. (2025). Moreover, fibrin exudation within the lamina propria may reflect vascular leakage due to local cytokine storm induced by the viral replication (El Nembr et al., 2025).

The renal tubular necrosis, epithelial desquamation, and glomerular alterations represent hallmark lesions of nephron-pathogenic IBV strains and are consistent with their established pathogenic mechanisms (Hoerr, 2021). Moreover, previous studies have emphasized the role of pro-inflammatory cytokines in exacerbating tissue injury during systemic IBV infections (Quinteros et al., 2022; El Nemr et al., 2025).

In this field outbreak, the frequency of tracheal pathology occurrence, along with the renal damage, confirmed the dual tropism of IBV in the affected flocks. This pattern is consistent with the circulation of nephropathogenic IBV variants, which have been known to induce both respiratory and renal diseases (Rafique et al., 2024). The marked histopathological lesions and associated mortality confirmed the importance of timely diagnosis, effective vaccination programs, and tailored immune prophylaxis in endemic regions such as Southern Iraq.

CONCLUSION

Under field conditions, the concurrent respiratory and renal lesions, along with elevated mortality rates, suggest the circulation of a highly virulent nephron-pathogenic IBV strain in broiler farms in Southern Iraq. Such findings correlate with the observed clinical signs and histopathological damage in tracheal and renal tissues. In addition, the severe histopathological changes in the trachea and kidney tissues provide a clear explanation for the clinical signs and mortality observed during the outbreak. These results emphasized the importance of early diagnosis and continuous monitoring of IBV strains, especially in regions with intense poultry production and variable biosecurity standards. Therefore, vaccination strategies based on the use of the local virus strains, along with the application of high biosecurity levels, are crucial to limit the spread and consequences of IBV. Overall, these findings reinforce the urgent need for ongoing surveillance, genotype-based vaccine selection, and strict biosecurity in regions endemic with virulent IBV strains. Future studies should focus on the molecular identification of circulating IBV strains, vaccine matching, and monitoring of post-vaccination conditions, especially in regions facing mixed infection pressures and variable management practices.

DECLARATIONS

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Authors' contributions

Muhammadtaher Abdulrazaq Abdulrasol contributed to field sampling, histopathological examination, data collection, and writing the initial draft. Wafaa A. Abd El-Ghany participated in conceptualization, supervision, critical review, and editing. All authors read and approved the final manuscript.

Ethical considerations

Ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been checked by all the authors. All procedures involving animals were performed according to internationally accepted welfare standards, and samples were collected only from clinically diseased broiler chickens suspected of IBV infection.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors have not declared any competing interests.

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Assessing Performance and Economic Efficiency of Table Eggs Production in Southern Togo

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ABSTRACT

Ensuring better allocation of productive resources necessitates socioeconomic considerations. This study examined the performance of table egg production in southern Togo by determining the breeders' production efficiency level through the stochastic frontier analysis in table egg production. Consequently, identifying the factors that significantly impact technical and allocative efficiency, and explaining the reasons for the technical inefficiency of table egg production. A parametric approach was used to estimate the technical and allocative efficiency levels from a stochastic frontier analysis. Data were collected from primary sources via a structured questionnaire (open-ended) administered to 88 poultry farms in southern Togo (2021) randomly. The parameters measured in this study were table egg production, the feed consumption during the production (each stage separately), veterinary treatment costs (drugs, vitamins), the flock size, the size of the exploitation, and the related costs of production. The finding indicated that 70% of table egg poultry farms in the Maritime Region of southern Togo are moderately technically efficient, although individual efficiency varies. Factors, such as flock size, labor, and veterinary treatments significantly influence the egg production process. Estimating the stochastic production function frontier revealed that inefficiencies in layer production largely stem from technical inefficiency among producers rather than inefficient resource allocation. The present study shows that poultry farms in Southern Togo exhibit medium technical efficiency but demonstrate effective allocation efficiency. Despite high-capacity facilities and financial constraints, the variation in the poultry breeders' production efficiency is explained by both endogenous and exogenous socioeconomic factors revealed through Tobit analysis. These factors are categorized into two groups, including primary (age, education, active membership, density, conflicts, gender), and secondary (credit, type of feed, association membership). Despite moderate technical efficiency, Southern Togo's poultry farms showed effective resource allocation. Financial constraints hinder full facility optimization, and unregulated input markets contribute to fluctuating costs.

Keywords: Allocative efficiency, Poultry farming, Stochastic frontier analysis, Technical efficiency

INTRODUCTION

The socioeconomic development of any nation, particularly in West Africa, hinges on the efficient utilization and effective management of its resources (Okorn and Egbe, 2023). Agriculture, with a special focus on livestock farming, plays a crucial role in this dynamic. The rapidly growing population in this region has significantly heightened the demand for animal protein (Tubb and Seba, 2021). The developing countries would need to produce over 100 billion tons of meat by 2030,

emphasizing the critical role of animal protein in meeting nutritional needs. Poultry products, such as broiler chickens and eggs, constitute a substantial portion of the global food supply (Tubb and Seba, 2021). Every year, the world produces over 850 billion eggs, equivalent to over 50 million tons of eggs (Moustafa et al., 2018). The Food and Agriculture Organization emphasizes the need to increase food production and make it more accessible to significantly improve nutritional status (Herforth et al., 2020). The developing countries expect to produce over

300 billion tons of meat by 2025 to meet the rising demand for animal protein. Globally, egg consumption is staggering, with 67,349 eggs consumed every second, translating to 1,250 billion eggs annually and an average per capita consumption of 145 eggs in 2009 (Herforth *et al.*, 2020). Given this urgency, poultry farming is indispensable for meeting the population's needs for animal protein, particularly for egg consumption (Tubb and Seba, 2021).

In West Africa, and specifically in Togo, poultry farming holds significant economic and nutritional importance. Among various livestock sectors, egg production stands out as the most vital source of high-quality animal protein and income (Houndjo *et al.*, 2018). Eggs are one of the richest sources of animal-derived protein, containing all essential amino acids in balanced proportions (Gbaguidi, 2001). In Togo, poultry farming contributes substantially to the Gross Domestic Product (GDP), accounting for 14%. The sector is highly dynamic, with broiler and egg production contributing 4.6% and 2.7%, respectively, to agricultural revenues (Lamboni, 2017). The short production cycle of chickens, the high quality of poultry products, and the relative ease of investment make modern poultry farming a central player in meeting the demand for these products (Tubb and Seba, 2021). Togo ranks as the third-leading country in poultry production within the West African Economic and Monetary Union (WAEMU), following Burkina Faso and Senegal. The creation of the Regional Center of Excellence on Avian Sciences (CERSA) has further bolstered the sector's growth through the professional training offered to the breeders and scientific improvement in the poultry field.

Despite this growth, the production of table eggs has fallen short of expectations in recent years due to several challenges (Soviadan *et al.*, 2022). High feed costs, caused by fluctuating raw material prices and questionable feed quality, as well as a lack of training among poultry farmers, significantly contribute to inefficiency in poultry production across sub-Saharan Africa, particularly in Togo (Balehegn *et al.*, 2020). This has led to an insufficient supply of table eggs, necessitating massive imports (Balehegn *et al.*, 2020). Understanding the factors contributing to inefficiency in modern poultry farming is crucial for making useful recommendations. Many poultry farmers lack technical knowledge and management skills and do not seek expert advice, limiting their profit-maximizing capacity and resulting in significant capital losses (Candemir *et al.*, 2021). The poultry industry in Sub-Saharan Africa, including Togo, faces significant

challenges such as disease outbreaks, insufficient grain production, and limited purchasing power in certain regions. These issues must be addressed to support the sector's growth. The need to strengthen poultry farming is further emphasized by supply-demand imbalances in animal protein sources, particularly as Togo works to enhance its domestic production and reduce dependency on imports (Erdaw and Beyene, 2022; Abadula *et al.*, 2022). The COVID-19 pandemic has further highlighted the importance of local production due to disruptions in global supply chains and pandemic control measures (Pujawan *et al.*, 2022). To address these challenges, it is imperative to examine and improve the current productivity and technical efficiency levels in poultry units. The research aimed to explore the factors influencing the economic efficiency of table egg production and assess the current level of efficiency. By identifying these factors, the authors of the current study can provide recommendations to enhance productivity and sustainability in the poultry sector in Togo and West Africa. This Study aimed to determine the level of efficiency in table egg production, identify the factors that have a significant impact on technical and allocative efficiency, and explain the reasons for the technical inefficiency of table egg production.

MATERIAL AND METHODS

Ethical approval

This study was conducted according to the guidelines of the University of Lome, Lomé, Togo.

Study area

The Maritime Region was selected for this study due to its high density of layer hen farming, attributed to favorable climatic conditions and the routine consumption of table eggs in households, restaurants, and hotels. The region spans 6,667 km² and includes seven prefectures, including Golfe (capital: Lomé), Lacs (capital: Aného), Bas-Mono (capital: Afagnan), Vo (capital: Vogan), Yoto (capital: Tabligbo), Zio (capital: Tsévié), and Avé (capital: Kévé). This survey focused on five prefectures with the densest layer of farming activities: Golfe, Vo, Avé, Yoto, and Zio. The region is bordered by the Atlantic Ocean to the south, the Aflao-Ghana border and Avé Prefecture to the west, Zio Prefecture to the north, and Lacs Prefecture to the east. It is characterized by a lagoon system and uneven urban distribution, with population densities ranging from 1,307 inhabitants/km² to 357 inhabitants/km². The climate is sub-equatorial, with an

average annual rainfall of 864 mm and an average temperature of 27.4°C.

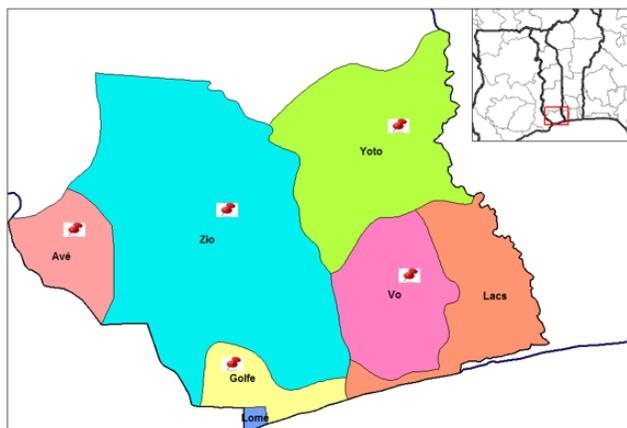


Figure 1. The map of the Delimitations of the 7 prefectures of Lome-Togo and the survey locations.

Source: Authors' compilation (2024)

Data collection method

Data were collected from both primary and secondary sources. Primary data were obtained through surveys of layer hen farmers using a two-part structural questionnaire (open-ended) on farm characteristics, production factors, management competencies, and health aspects. Stratified non-probability sampling (snowball sampling) was used, focusing on five prefectures, including Avé, Zio, Yoto, Vo, and Golfe as displayed in Figure 1. The survey, conducted in 2021, had formal authorization from CERSA/UL. The sample size, determined using Slovin's formula, was 88 table egg producers, with input from the Institute for Technical Support and Advice (ICAT/Togo) and the National Association of Poultry Producers of Togo (ANPAT/Togo). Primary data were collected using structured questionnaires through scheduled interviews with poultry farmers. Data collected included production, inputs, output, and input prices, major socio-economic characteristics, constraints faced by farmers, and the impact of COVID-19 on the input prices.

The survey form was designed using Sphinx software version 5.0, selected for its user-friendly interface. Physical forms were utilized for field data collection, enabling the gathering of additional on-site information and the expansion of the database with unforeseen variables relevant to the research topic. Upon completion of the survey, SPSS V23 and Stata 14 were used for data analysis. The stochastic frontier production was applied, followed by the prediction of efficiency scores, concluding with the Tobit analysis to fulfill all the study objectives.

Model for analyzing technical and allocative efficiency

There were two primary methods for analyzing economic efficiency, including the deterministic method using Data Envelopment Analysis (DEA) and the parametric stochastic method using Stochastic Frontier Analysis (SFA) estimation. Based on a review of previous studies, the parametric method was chosen for its robustness, consideration of unobserved variables, and realistic results. Empirical studies have applied stochastic frontier analysis to assess farm-level efficiencies, using these functions to predict performance levels (Coelli, 1995). Subsequently, predicted efficiency scores were regressed against the regressed specific farm variables, such as management experience and property characteristics, to determine factors contributing to efficiency variations (Coelli, 1995). The model can be specified by follows formula.

$$Y = f(X, \beta) + (v - u) \tag{1.1}$$

$$u \geq 0; -\infty \leq v \leq +\infty$$

In detail, the equation can be written as:

$$Y_i = \beta_0 + \sum \beta_j X_{ij} + \varepsilon_i \tag{1.2}$$

$$\text{Où } \varepsilon_i = v_i - u_i$$

In matrix form:

$$Y = X\beta + \varepsilon \tag{1.3}$$

Where:

Y represents production output, while X denotes production inputs. The parameter β consists of fixed values to estimate, representing the elasticity of production with respect to input X. The term u_i corresponds to positive values of a random variable linked to producers' technical inefficiency. Additionally, v_i represents the error term associated with measurement errors and other random factors, such as climate, theft, luck, and neighborhood conflicts, all of which can influence production.

According to Panda (1996), v_i has a normal distribution with mean $\mu_v=0$ and constant variance σ_v^2 ; they are independent of u_i , assumed to have a semi-normal distribution with mean $\mu_u=0$ and constant variance σ_u^2 .

Under these conditions, the ratio of the observed output of the i^{th} producer to the potentially defined output by the production frontier, given the production inputs X_i , is used to calculate technical efficiency (TE_i).

$$TE_i = \frac{Y_i}{\exp(\beta \sum \ln x_i)} = \frac{\exp((\beta \sum \ln x_i) - u_i)}{\exp(\beta \sum \ln x_i)} = \exp(-u_i) \tag{1.4}$$

Where $\exp(\cdot)$ represents the exponential function.

Specification of the technical efficiency estimation model

In this study, the Cobb-Douglas production function is used, commonly employed in economics and econometrics as a model of production function. It represents the effects of technology on two or more production factors and on production itself. The function is presented as follows.

$$Y_i = X_i^{\beta_{i1}} \tag{1.5}$$

For the estimation, the natural logarithm is used to estimate the parameters β , which represent elasticities. This is commonly known in econometrics as a log-log model. The dependent variable, egg production, was specified as a function of five independent variables (i.e., building area, flock size, feed served until laying starts, hen weight at laying start, and expenditure per laying hen). The stochastic production frontier for poultry egg production is calculated as follows.

$$Y_i = X_1^{\beta_1} X_2^{\beta_2} X_3^{\beta_3} X_4^{\beta_4} X_5^{\beta_5} \varepsilon_i \tag{1.6}$$

By linearizing the function for robustness, the formula becomes

$$\ln Y_i = \ln(\beta_0) + \beta_1 \ln(X_{1i}) + \beta_2 \ln(X_{2i}) + \beta_3 \ln(X_{3i}) + \beta_4 \ln(X_{4i}) + \beta_5 \ln(X_{5i}) + \varepsilon_i \tag{1.7}$$

Where Y_i represents production output, measured as the quantity of eggs produced per hen per cycle. X_1 corresponds to the building area in square meters, while X_2 denotes flock size, referring to the number of chicks. X_3 represents the quantity of feed served before laying, specifically between the 16th and 19th weeks. X_4 indicates the hen's weight at the start of laying, and X_5 accounts for expenditure per laying hen, measured in FCFA. The parameter β consists of coefficients of the variables, representing their respective elasticities. v_i corresponds to random error, whereas u_i reflects technical inefficiency effects, indicating the gap a farmer must close to reach the production frontier. The $u_i =$ technical inefficiency effects, representing what remains for the farmer to reach the production frontier.

Allocative efficiency is estimated by the cost frontier, similar to the technical efficiency specification. The cost frontier, derived from the dual relationship with the production frontier, measures allocative efficiency by assessing how effectively a farmer utilizes inputs in proportion to their respective costs (Khai et al., 2011). The model includes input prices ($P_{x_{ik}}$) and total egg production $\ln(Y_i^*)$, adjusted for statistical noise. The model is specified as follows.

$$\ln(C_i) = \sigma_0 + \sigma_1 \ln P_{X_{1i}} + \sigma_2 \ln P_{X_{2i}} + \sigma_3 \ln P_{X_{3i}} + \sigma_4 \ln P_{X_{4i}} + \sigma_5 \ln P_{X_{5i}} + \sigma_6 \ln P_{X_{6i}} + \ln Y_i + \varepsilon_i \tag{1.8}$$

Where,

C_i represents the total cost of egg production, measured in FCFA. $P_{X_{1i}}$ corresponds to the cost of the building area per square meter, while $P_{X_{2i}}$ refers to the price of chicks per unit. $P_{X_{3i}}$ accounts for labor costs per subject, whereas $P_{X_{4i}}$ captures the expenditure on feed served before laying, specifically between the 16th and 23rd weeks. $P_{X_{5i}}$ represents the cost of veterinary treatments per subject, including the number of vaccines administered. $P_{X_{6i}}$ denotes the cost of medication and vitamins, measured in FCFA per kilogram. $\ln(Y_i^*)$ signifies table egg production adjusted for statistical noise. Finally, ε_i represents the composite error term, and σ corresponds to the parameter to be estimated.

Verification of the existence of inefficiencies

The estimation procedure follows (Coelli, 1995), which involves maximizing the natural logarithm of the likelihood function and calculating the likelihood ratio (LR). The natural logarithm of the likelihood function can be mathematically expressed as follows.

$$Y = \sigma_v^2 / \sigma_u^2 \tag{1.9}$$

To test for the existence of technical and allocative inefficiency, Coelli (1995) suggested using the generalized likelihood ratio test.

According to the literature, the frequently used method to explain inefficiencies is a two-step process (Labiya et al., 2012), including the first step estimates inefficiencies from a production function, and the second step involves regressing efficiency scores to determine factors influencing the performance of the Decision-Making Units (DMUs) considered. These models are also known as "censored regression models" or "truncated regression models." The choice of the Tobit model is justified by the continuous nature of efficiency indices, which take values between 0 and 1 (Labiya et al. 2012). The model of inefficiency is as follows.

$$U = \delta_0 + \delta_1 Z_{1i} + \delta_2 Z_{2i} + \delta_3 Z_{3i} + \delta_4 Z_{4i} + \delta_5 Z_{5i} + \delta_6 Z_{6i} + \delta_7 Z_{7i} + \delta_8 Z_{8i} + \delta_9 Z_{9i} + e \tag{2}$$

Where U represents inefficiency, while Z_1 denotes the education level of the farmer, measured in years. Z_2 corresponds to the presence of a disinfection footbath, recorded as a binary variable. Z_3 indicates whether the farm is fenced, also represented as a binary variable. Z_4 reflects cooperative membership status, Z_5 captures access to credit, and Z_6 measures the farm's proximity to the farmer's residence, all recorded as binary variables. Z_7 accounts for veterinary support availability, Z_8 represents sales blockage, and Z_9 corresponds to the mortality rate, expressed as a percentage. Finally, δ denotes the vector of parameters to be estimated.

Statistical analysis

The study used STATA 15 software to perform the stochastic frontier analysis, examine the production frontier of table eggs, and predict both technical and allocative efficiency. Economic efficiency is then calculated from these predicted efficiency measures. Subsequently, Tobit analysis is conducted to identify the factors influencing breeders' technical efficiency beyond the effects of input usage in production.

RESULTS AND DISCUSSIONS

Technical efficiency of table egg farms

According to Table 1, the overall productivity of layer hens in the surveyed farms was 235.92 eggs. Given the low standard deviation (35.87), it can be concluded that the egg yields across different farms were close to the

average value, which was low compared to yields in Cameroon, where the required productivity was 270 eggs. Farms owned by women have a relatively lower egg yield (192 eggs per hen) compared to those owned by men (238 eggs per hen). This finding indicated that women in Togo had not fully mastered poultry farming, unlike women in Benin, who achieved a yield of 236 eggs per hen, as reported by [Siéwé et al. \(2019\)](#).

The average cost of veterinary treatment was 218 FCFA per subject, which was relatively high compared to the standard set in projects, which was 100 to 150 FCFA per subject. The maximum cost of veterinary treatment (including vaccines, medication, and vitamins) was much higher than the report established by [Traore \(2014\)](#). This cost can be explained by the relatively small flock sizes, which still allocate the means to undergo all treatments.

Table 1. The stochastic frontier production variables' descriptive statistics of the table egg production

Variable	Average	Std. dt.	Min	Max
Laying cycle productivity per hen	235.92	35.87	120	285
Male producer	238.01	34.26	230	245
Female producer	192	46.21	228.31	243.52
Poultry house floor area	242.63	219.61	20	1000
Pre-laying feed consumption	9.63	1.90	3.54	13
Weight at the point of lay	1.51	0.19	1.3	2.2
Expenditure per laying hen	2286.38	471.72	1532	5103.75

Std. dt: Standard deviation, Min: Minimum, and Max: Maximum

Table 2. The stochastic frontier analysis for predicting technical efficiency in table egg production

Variables	Coef.	Std Err	Z	P> Z
LX ₁ Poultry house floor area	-0.08394	0.0001366	-614.58	0.000***
LX ₂ Number of birds	0.006573	0.0001236	53.17	0.000***
LX ₃ Pre-laying feed consumption	-0.24665	0.0005997	-411.31	0.000***
LX ₄ Weight at the point of lay	0.130336	0.0002364	551.26	0.000***
LX ₅ Expenditure per laying hen	0.019259	0.0001015	189.77	0.000***
Inefficiency parameter				
σ_v^2	1.27E ⁻¹⁷	0.0001		
σ_u^2	0.3328	0.02508		
$\sigma_s^2 = \sigma_u^2 + \sigma_v^2$	0.11078	0.02518		
$\gamma = \frac{\sigma_u^2}{\sigma_v^2}$	0.985	0.025089		
Logarithme de vraisemblance			41.43	

*** Significance at 1%, Coef: Coefficient, Std Err: Standard error, Z: Z-score

Estimation of technical efficiency

Table 2 shows that all variables in the function were significant at the 1% threshold, although two variables negatively impacted the production function. The

estimated coefficients represented the partial elasticities concerning each corresponding variable. An increase of 10% in the poultry house area (LX1) without a corresponding increase in flock size will result in an 8%

decrease in egg production per hen. This finding can be explained by the energy wastage among laying hens due to the additional space, which distracted them from laying, as the hens spend more time running around and playing (Khan et al., 2022).

The feed given to laying hens (LX2) from the chick stage until the laying age was above normal, which explained the negative coefficient. A 1% increase in feed consumption before laying age will lead to a 20% reduction in egg production per hen. The variables, including flock size, weight at the onset of laying, and expenditures on a hen ready to lay, were positively significant at the 1% threshold. This means that a 1% increase in each of these variables will increase egg production per hen per cycle by 0.6% (LX3), 13% (LX4), and 1% (LX5), respectively.

The results of the stochastic model estimation show that all variables are significant, although some are negative, indicating a negative relationship between these variables and the production output. These results differ from those of Siéwé et al. (2019), who found a perfectly

positive reaction. However, the three other variables show a positive relationship with the production output, confirming the findings of Dhehibi and Chemak (2010).

The maximum likelihood estimator (γ) of the stochastic frontier production model (LR) was statistically significant at the 1% threshold. Therefore, the null hypothesis of the absence of technical inefficiency was rejected. The variance value γ (0.985), significantly different from zero at the 1% threshold, indicated the presence of productive inefficiencies. Approximately 98% of the difference between observed productivity and potential productivity of laying hens within the studied farms is partly due to the inefficiency of the poultry farmers. Indeed, in this study, 2% of the differences were due to random effects, including measurement errors, which can arise from the nature of the data being averages at the farm level. The closer the value of (γ) is to 1, the smaller the difference between the results from a stochastic estimation and those from a deterministic estimation (Briec et al., 2005).

Table 3. Cross-table of technical efficiency score of farmers' table egg production in the Maritime region of Togo (2021)

	Average	Std. dt.	Min	Max
Technical efficiency of poultry farms	72%	0.144	40%	90%
Gender type				
Man as managers	75%	0.0153	70%	76%
Women as managers	57%	0.0853	30%	84%
Poultry farming training				
Yes	73%	0.0164	70%	76%
No	68%	0.041	60%	77%
Membership in a cooperative				
Yes	71%	0.020	66%	75%
No	74%	0.023	69%	79%

Std. dt: Standard deviation, Min: Minimum, and Max: Maximum

The surveyed farms have an average technical efficiency of 72%, which was relatively low compared to the average efficiency of poultry farms in Benin, standing at 92.38% (Siéwé et al., 2019).

Table 3 illustrates the technical efficiency of layer poultry farms in the Maritime region, along with a comparison based on gender, participation in training, and membership in a cooperative. It can be inferred that men exhibit higher technical efficiency at 75% compared to 57% for women. Furthermore, individuals who had received training demonstrated higher efficiency at 73% compared to 68% for those who entered the sector without

formal training. However, regarding membership in a cooperative, there was an observed effect, with a technical efficiency of 71% for cooperative members and 74% for non-cooperative members. This disparity may be attributed to inter-cooperative conflicts.

Analysis of technical inefficiency

Table 4 presents the descriptive analysis of the variables used in the Tobit model. It shows how the table egg poultry farms are presented in the region. In Table 5, three variables are responsible for the technical inefficiency of layer poultry farms. The variable "level of

education" indicated that individuals with higher levels of education struggle to undergo training and do not contribute to the technical efficiency of the operation. The chick price hurts efficiency, meaning that for every 1% increase in chick price, technical efficiency decreased by 22%. This may be attributed to a lack of resources to adequately feed and treat the layers once acquired. The mortality rate reflected farmer discouragement; an increase in chick mortality not only initiates technical inefficiency but also raises concerns and anxiety about resource scarcity. A 1% increase in mortality rate led to a drop in technical efficiency of over 50%. This variable was just at the 1% significance threshold, making it significant at the 5% default threshold.

A new variable in the model estimation was farm closure. The initial assumption for this variable was that it would be significant and positive, which was indeed the case; a fenced poultry farm increases technical efficiency by 5% at the 10% threshold.

The other variables were also significant and positive, and thus did not negatively impact technical efficiency.

Table 4. The statistics describing the tobit model variable that influences the technical efficiency of the table egg production

Qualitative variables	Percentage
Membership in a Cooperative	
Yes	56%
No	44%
Presence Of Footbath	
Yes	66%
No	34%
Credit Access	
Yes	37%
No	63%
Closed Farm	
Yes	32%
No	68%
Near Residences	
Yes	33%
No	66%
Training	
Yes	80%
No	20%

Table 5. The Tobit model output of the factors influencing the technical efficiency of table egg production of Togo (2021)

Variables	Coef.	Std. Dt.	Z	P> Z
Level of education	-0.0270234	0.0154466	1.75	0.084*
Number of personnel	0.0162859	0.0077546	2.10	0.039**
Presence of a footbath	0.070108	0.0261678	2.68	0.009**
Increase in chick price	-0.2236006	0.1037354	-2.16	0.034**
Mortality rate	-0.599072	0.1736052	-3.45	0.001**
Vaccine reminders	0.014632	2.79e-06	5243.75	0.000***
Closed farm	0.0532819	0.0275267	1.94	0.057**
Log-likelihood = 76.831277		LR chi2(14) = 67.08		
Probability > chi-square = 0.0001				

***Significance à 1%, ** Significance à 5% et * significance à 10%, Z: Z-score

Allocative efficiency of table egg production farms

The descriptive output of Table 6 shows that the average cost of the poultry house per square meter was quite high (10111.23 FCFA). The minimum cost registers as zero because some poultry houses were very old, while others were constructed by farmers over the years, with most materials sourced locally. The price of day-old layer chicks varied significantly, as evidenced by the standard deviation. This variance stems from the lack of regulation in chick imports, with numerous suppliers operating in the

field and importing chicks from various sources. The standard cost of veterinary treatments typically falls within the range of 100 to 110 FCFA. However, the actual minimum observed is 82.2 FCFA, primarily influenced by product sales. Regarding vaccines, only doses of 500 and 1000 were available, leading smaller-scale operations with fewer than 500 layers to forego some vaccinations. Conversely, those undertaking all treatments, despite having smaller flocks, incurred higher costs (555.55 FCFA).

Table 6. The stochastic frontier cost variables' descriptive statistics

Variables	Moyenne	Std. dt.	Min	Max
Cost_floor_area /m ² (FCFA/m ²)	10111,23	8384	50	31133,25
Price_Chicks FCFA	928.2443	118.1047	600	1100
Cost_labor/D/L FCFA	1.385094	2.56641	0	20.83333
Veterinary cost FCFA	218.0989	66.14408	82.26496	555.5556
Cost_hitness/L FCFA	73.39631	49.1308	3.555556	300
Cost_feed/L FCFA	38254,92	41204	2100	243000

FCFA: Franc communauté financière Africaine, D/P: Day per chicken layer, Std. dt: Means standard deviation, Min: Minimum, Max: Maximum

Table 7. Stochastic frontier cost analysis for the allocative efficiency prediction of table egg production

Variables	Coef.	Std. Err.	Z	P> Z
LPX ₁ COUT_floor_area/m ² (FCFA/m ²)	-0.038484	0.02231	-1.72	0.085*
LPX ₂ Price_Chicks FCFA	0.736589	0.01213	60.7	0.000***
LPX ₃ Cost_labor	0.2154071	0.0058	37.02	0.000***
LPX ₄ Cost_Medecine	0.000595	0.0009	0.64	0.521
LPX ₅ Cost_Vaccination	0.141527	0.2179	6.49	0.000***
LPX ₆ Cost_hitness	0.013449	0.0081	1.65	0.098*
LPX ₇ Cost_Feed	0.5633576	0.0072	77.34	0.000***
LY Quantity of eggs produced	-0.00882	0.01172	-0.76	0.449
Inefficiency parameter				
σ_v^2	1.6129E ⁻¹⁵	6.43E ⁻⁷		
σ_u^2	0.000	0.011		
$\sigma_s^2 = \sigma_u^2 - \sigma_v^2$	0.0081	0.0017		
$\gamma = \frac{\sigma_u^2}{\sigma_v^2}$	0	0.0110723		
Log-likelihood			55.528	

*** Significance à 1%; **Significance à 5%; *Significance à 10% Coef : Coefficient, Std Err: Standard error, Z : Z-score

Estimation of the allocative efficiency

Table 7 shows that the maximum likelihood estimator (γ) of the stochastic production cost frontier model (LR) was statistically significant at the 1% level. However, its value is equal to zero (0). Thus, the null hypothesis (H0) of the absence of allocative inefficiency was accepted. The variance value of $\gamma = 0.000$ indicated that the inefficiency was technical, and it was also linked to random effects, including measurement errors. Based on the analysis of the stochastic production cost frontier, the last variable represents production cost, and its expected negative sign confirms the model's accuracy and robustness. This sign demonstrates the logical relationship between production and cost, as production costs increase, production naturally declines. The following variables, including chick cost, labor cost per layer, vaccine cost per layer, and pre-laying feed consumption, were highly significant at the 1% level. This implied that a 1% increase in the cost of each of the

mentioned variables would significantly increase production costs by 73%, 21%, 14%, and 56%, respectively. The cost of the poultry house per square meter was negative; this variable was not included in the direct production cost estimation.

The analysis of technical efficiency among table egg farms revealed an average efficiency level of 72%, indicating substantial variations in effectiveness across different farms. While some farms demonstrated commendable efficiency levels, reaching up to 90%, others operate at comparatively lower rates, such as 40% and 50% (Table 3). Notably, factors such as poultry flock size and pre-laying feed consumption emerge as significant determinants of technical efficiency, with larger poultry houses and excessive feed consumption negatively impacting productivity. However, Khan et al. (2022) found the opposite effect, suggesting that optimal management of flock size and feed can improve the

technical efficiency of enterprises. On the other hand, factors such as weight at the start of laying and cost per laying are favorably linked to efficiency. This highlights the critical role of strategic resource allocation and management in enhancing productivity (Dogan et al., 2018; Khan et al., 2022). The Tobit analysis of the factors influencing the technical inefficiency revealed a notable influence linked to socio-economic factors. In particular, the level of education among farm owners, the price of day-old chicks, and mortality rates within production farms all had significant impacts on technical efficiency. Farms operated by individuals with higher education levels tended to exhibit lower efficiency levels, suggesting potential barriers to effective management and resource utilization (Siéwé et al., 2019). Moreover, increases in day-old chick prices have a negative impact on efficiency, possibly due to constraints on investment in critical resources, such as feed and healthcare. Ezeano and Ohaemesi (2007) confirmed the present result, stating that the high price of day-old chicks negatively impacts the technical efficiency of poultry production. Additionally, higher mortality rates contributed to reduced efficiency, reflecting underlying challenges in disease management and overall farm health, as confirmed by Dogan et al. (2018). However, the allocative efficiency shows significant cost and efficiency dynamics in poultry farming. The average cost of poultry houses was high (10,111.23 FCFA/m²), while some older, locally constructed poultry houses incurred zero costs. Day-old layer chick prices vary due to unregulated imports, affecting cost stability. Veterinary treatment costs range widely, with smaller farms either skipping vaccinations or facing high costs due to limited vaccine dose sizes (Siéwé et al., 2019).

The stochastic production cost frontier analysis showed that there was little allocative inefficiency. At the 1% level, it is statistically significant but equal to zero, which means that technical inefficiencies are caused by random effects. The stochastic frontier production model confirmed an inverse relationship between production costs and output. Key cost drivers include chick cost, labor, vaccines, and pre-laying feed, all of which had a significant impact on overall production costs. Notably, direct cost estimations excluded the cost per square meter of the poultry house, indicating additional influencing factors. These insights offer a framework to tackle cost and efficiency issues in the sector, affirming the high efficiency in poultry production, evident in both technical and allocative efficiency (Dogan et al., 2018).

These findings underscore the multifaceted nature of farm efficiency, influenced by a complex interplay of technical, socio-economic, and economic factors. To enhance overall performance and sustainability in table egg production, targeted interventions addressing both technical and socio-economic challenges are essential. Strategies aimed at optimizing resource allocation, improving management practices, and supporting education and training initiatives can help mitigate inefficiencies and promote long-term viability within the sector.

CONCLUSION

The results revealed that, on average, the surveyed farms exhibit lower technical efficiency levels, despite demonstrating effectiveness in resource allocation. The mean economic efficiency index of 0.72, ranging from 0.40 (minimum) to 0.90 (maximum), reflects the varying degrees of efficiency within the poultry farming sector in Southern Togo. This region's poultry industry operates with high intensity and is characterized by abundant labor resources. However, despite having high-capacity poultry houses, farmers often lack the financial means to fully optimize their facilities. Furthermore, the absence of government regulation in the poultry input market contributes to fluctuating prices of feed ingredients and medical supplies, as well as shortages in stock availability. Further in-depth studies are necessary to comprehensively understand the economic efficiency of farms and devise effective solutions to address inefficiency factors. These studies should encompass a thorough examination of all facets of the poultry sector and allow for sufficient time to develop stochastic predictive models for allocative efficiency. By gaining a comprehensive understanding of the sector dynamics and implementing targeted interventions, stakeholders can work towards enhancing the overall efficiency and sustainability of poultry farming practices in the study region. Moreover, by addressing these underlying factors, stakeholders can work towards maximizing productivity and profitability while ensuring the long-term sustainability of table egg farming operations.

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Availability of data and materials

The data is available upon request from the corresponding author.

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Authors' contributions

Atsu Frank Yayra Ihou contributed to data collection, data analysis, and the write-up of the manuscript. Abbey Abbévi Georges conducted the design of the study and supervision. Aime Alayi conceptualized data collection and analysis. Paul Mansingh Jeyabalasingh contributed to the manuscript revision and formatting in English. All authors read and approved the final version of the manuscript.

Competing interests

All authors declare no conflict of interest.

Ethical considerations

Ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been checked by all authors.

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Isolation and Identification of *Eimeria* spp. From Domestic Pigeons (*Columba livia domestica*) in Basrah, Iraq

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ABSTRACT

Coccidiosis is a widespread disease among pigeons worldwide, resulting in significant economic losses due to mortality, morbidity, and reduced feed efficiency. The present study aimed to isolate a field strain of *Eimeria* spp. in domestic pigeons (*Columba livia domestica*) in Basra, Iraq. To ensure precise species identification, light microscopy techniques were combined with the advanced molecular method of polymerase chain reaction (PCR). Sixty fresh fecal samples were collected from pigeon lofts, ensuring minimal environmental disturbance and contamination. The flotation method was employed to analyze fecal samples. The present results indicated that 36 out of 60 samples contained coccidian oocysts, which represented an overall prevalence of 60%. The PCR technique was employed to amplify the *18S rRNA* genes, which were subsequently utilized for sample detection. Six representative sequences were selected, registered, and deposited in the NCBI database (PV459631.1, PV459632.1, PV459633.1, PV459634.1, PV459635.1, and PV459636.1). The present findings were validated through molecular analysis, and six representative gene sequences were submitted to the National Centre for Biotechnology Information (NCBI) database. The current study revealed a high prevalence of coccidiosis, affecting 60% of domestic pigeons in Basra, highlighting its impact on their health. Moreover, this is the first report of *Eimeria chalcopterae* in pigeons from Basra, Iraq.

Keywords: *Eimeria chalcopterae*, Molecular technique, Phylogeny, Pigeon

INTRODUCTION

Coccidiosis is a prevalent disease among pigeons globally and results in considerable economic losses due to mortality, illness, and diminished feed efficiency (Santos et al., 2020; Aboelhadid et al., 2021). The performance of poultry is adversely affected by this condition as it induces acute disease and impairs nutritional utilization (Al-Agouri et al., 2021). Coccidiosis is a significant veterinary

disease with a notable economic impact on the pigeon industry (He et al., 2024).

The signs of coccidiosis include watery diarrhoea accompanied by mucus (Mohammed et al., 2017). Watery diarrhea with mucus is a common sign of mucoid or bloody diarrhea, often associated with clinical features such as dysentery, enteritis, emaciation, decreased feed conversion, drooping wings, poor growth, and even death. This condition serves as one of the early diagnostic

indicators for avian coccidiosis (Ola-Fadunsin et al., 2017). Pigeons are infected by 21 species of intracellular apicomplexan protozoan parasites from the genus *Eimeria* (Albasyouni et al., 2024). To reduce the risk of parasite spread, regularly remove feces and clean cages and floors. Since coccidiosis spreads rapidly in crowded environments, preventing overcrowding in barns is essential, along with ensuring proper ventilation and humidity control in breeding areas. Additionally, a balanced diet supplemented with vitamins and immune-boosting nutrients such as A, E, and C is recommended (Alsayeqh and Abbas, 2023). Although management and biosecurity measures may potentially prevent the introduction of *Eimeria* spp. to a farm, in practice, these measures are insufficient to prevent outbreaks of coccidiosis. However, extensive and prolonged use of anticoccidial medicine has resulted in the development of resistance worldwide against all such medications (Peek and Landman, 2011). In Basra, Iraq, where pigeon breeding holds considerable cultural and economic importance, *Eimeria* spp. presents both health and economic difficulties. The present study aimed to isolate a field strain of *Eimeria* spp. in domestic pigeons (*Columba livia domestica*) of Basra, Iraq.

MATERIALS AND METHODS

Ethical approval

The Research Ethics Committee approved the experimental procedures and animal care. Research Ethics Committee No. 83/37/2025 from the University of Basrah-College of Veterinary Medicine confirms that all protocols were followed, and appropriate measures were taken to minimise discomfort.

Study area and sample collection

The present study was conducted from April to December 2024. A total of 60 adult domestic pigeons (*Columba livia domestica*) from different lofts located in several areas of Basrah, Iraq, were included. All of the pigeons exhibited clinical signs of coccidiosis, including weight loss (Emaciation) and the presence of bloody droppings. There were no pigeons found without signs of coccidiosis. The number of pigeons per loft differed from 20 to 80, with some lofts experiencing substandard living conditions and inadequate provisions and sanitation.

Fecal examination

Fecal samples of approximately 1 g were collected from each pigeon and placed in individual screw-capped plastic containers labelled appropriately. These samples

were subsequently transported to the Department of Veterinary Parasitology at the College of Veterinary Medicine, University of Basrah, Basrah, Iraq. Initially, the samples were examined to determine color, consistency, and the presence of blood, mucus, or other contaminants. The presence or absence of coccidia oocysts was checked using an Olympus microscope (Japan) with Sheather's sucrose solution. In positive samples, oocysts were concentrated via the flotation technique described by Alasadiy et al. (2022). Oocyst morphology and sporulation time were used to identify the species of *Eimeria* spp., following the guidelines outlined by Silva et al. (2022).

Polymerase chain reaction amplification, DNA extraction, and sequencing

The DNA was obtained from the purified oocysts for molecular identification using a commercial DNA extraction kit. The *Eimeria* spp. *18S rRNA* gene was targeted by a polymerase chain reaction (PCR). The QIAamp PowerFecal Pro DNA Kit (Cat. No/ID: 51804, QIAGEN, Australia) was used to extract total DNA from 350 mg of each fecal sample, following the manufacturer's instructions. A nested PCR was conducted using the methods outlined by Yang et al. (2016a;b). An expected PCR product of approximately 1510 bp was anticipated. The primers EiGTF1 5'-TTCACAGGACCCTCCGATC and EIGTR1 5'- AACCATGGTAATTCTATGG were employed for the external amplification of the *18S rRNA* gene.

Sequence and bioinformatics analysis

The positive PCR results were sequenced forward at the MacroGen® sequencing facility (Seoul, South Korea). The obtained sequences were verified using a BLAST search and a sequence identity for further analysis. Sequences were aligned with relevant reference sequences using the CLUSTAL-X approach (Thompson et al., 1997). The Tamura-Nei model was used for a comprehensive evaluation of taxonomic relationships based on nucleotide analysis, and phylogenetic trees were constructed using the maximum likelihood approach (Tamura et al., 2011).

RESULTS

The total population of domestic pigeons was estimated at 60, with a 60% prevalence of coccidian infection, which was detected using the flotation technique. The oocyst was sub-spherical to spherical in shape and lacked a micropyle. The wall was thick, smooth, and consisted of two layers. Sporulation occurred within 24 to 48 hours. An example of a non-sporulated oocyst is shown in Figure 1.

Detection of DNA by the polymerase chain reaction

Polymerase chain reaction was employed to amplify the DNA extracted from *E. chalcoptereae* samples utilizing both forward and reverse primers. Subsequently, eleven samples were identified using PCR (Figure 2). The DNA bands, approximately 1510 base pairs in length, were observed after examination on an agarose gel.

Phylogenetic analysis

According to the sequence analysis results, all six positive strains exhibited a proximate relationship. Six representative sequences were selected and uploaded to the NCBI database. The new records are labelled as PV459631.1, PV459632.1, PV459633.1, PV459634.1, PV459635.1, and PV459636.1. Figure 3 presents the phylogenetic trees, which depict the evolutionary links between pigeon *Eimeria* spp. from the NCBI database and strains isolated from pigeons in Basra, Iraq, marked by the red square.



Figure 1. The *Eimeria chalcoptereae* oocyst that is not sporulated (black arrow, $\times 40$) isolated from a pigeon in Basrah, Iraq.

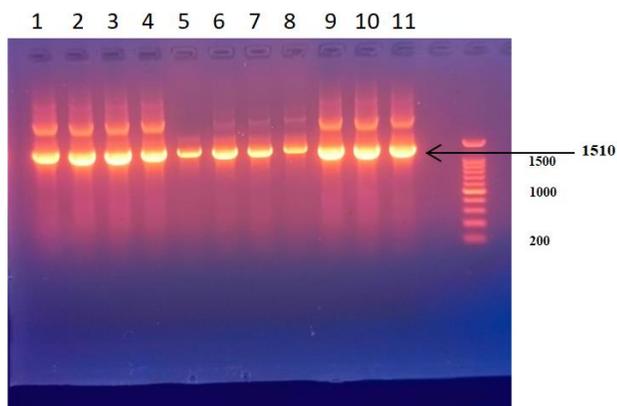


Figure 2. Polymerase chain reaction analysis of *Eimeria chalcoptereae* isolated from a pigeon in Basrah, Iraq, using agarose gel electrophoresis. M: 1510 bp molecular Ladder, Lane: Samples 1-11 were examined.

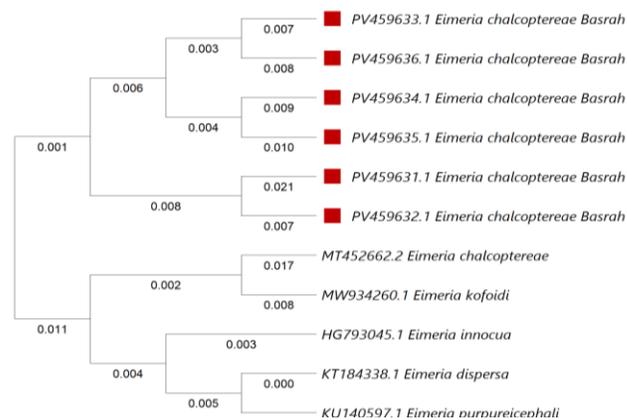


Figure 3. Phylogenetic tree analysis of six *Eimeria chalcoptereae* isolated from pigeons in Basrah, Iraq. It is constructed based on the *Eimeria chalcoptereae* small subunit ribosomal RNA gene, partial sequence, displaying the evolutionary relationships between closely related pigeon *Eimeria* spp. from the NCBI database and strains isolated from pigeons (red square). Utilizing MEGAX11 software, the neighbor-joining approach was used to create the tree and perform evolutionary studies.

DISCUSSION

Based on the present results, numerous breeds of pigeons were bred in Basra, Iraq, for different purposes. According to the present study, 36 out of 60 samples tested positive for coccidian oocysts, indicating an overall incidence of 60%. This prevalence aligns with data from other countries, including 67.58% as documented by Gül et al. (2009) in Turkey, 56.2% as noted by Joseph et al. (2017) in Nigeria, 75% as reported by Ramesh et al. (2018) in India, 58.2% as observed by Elseify et al. (2018) in Egypt, and 52% as reported by Huang et al. (2018) in China. A reduction in food consumption, weakness, and greenish watery diarrhoea were the clinical manifestations of pigeon intestinal coccidiosis. Several studies have reported that coccidiosis causes significant losses in domestic pigeons (Dalloul and Lillehoj, 2005; Bandyopadhyay et al., 2006; Quiroz-Castañeda et al., 2015; Gadelhaq and Abdelaty, 2019). One of the limitations of microscopy is its inability to reliably discriminate across species. For increased sensitivity and species identification, previous studies have emphasized the importance of incorporating molecular diagnostics, such as PCR (Haug et al., 2008). In Al-Diwaniyah province, Iraq, Jawad and Jasim (2025) demonstrated that PCR-based diagnostics significantly enhanced the accuracy of identifying *E. tenella*, indicating its prevalence in broiler farms. The current findings highlighted the crucial role of molecular surveillance in poultry operations, particularly considering that

microscopic detection may underestimate the true level of infection, especially in subclinical cases. In the present study, PCR was employed to validate the initial clinical diagnosis, representing the initial effort to identify and isolate *Eimeria* spp. in Basra, Iraq. Phylogenetic trees are frequently employed to analyze *Eimeria* spp. in domestic pigeons in Basra, Iraq. Six representative sequences were selected, documented, and uploaded to the NCBI database (PV459631.1, PV459632.1, PV459633.1, PV459634.1, PV459635.1, and PV459636.1). In the current investigations, extremely small genetic differences were observed between the *E. chalcoptereae* isolates from Basra, Iraq, ranging from 0.000 to 0.004%, indicating a shared origin or local evolution, along with advantageous genetic conservation. The isolates from Basra and the reference strain of *E. chalcoptereae* (MT452662.2), as documented by Yang et al. (2020), exhibited pairwise genetic distances ranging from 0.007% to 0.009%, indicating a moderate level of divergence, likely due to regional or host-specific factors. It is inferred that the sequences identified in the present study and those documented by Yang et al. (2020) most likely correspond to the same species, as the present study and Yang et al. (2020) are currently the only sources of molecular data available. This is attributed to the role of Basra as a central hub for the illicit transportation of pigeons originating from Persian Gulf countries, despite these pigeons emanating from two distinct and geographically isolated regions (Al-Hasnawy and Rabee, 2023; Jaafar, 2023). Pairwise distances were notably greater, ranging from 0.008% to 0.021%, compared to other *Eimeria* spp., including *E. kofoidi* (MW934260.1), *E. innocua* (HG793045.1), *E. dispersa* (KT184338.1), and *E. purpureicephali* (KU140597.1). The current results confirmed the uniqueness of the *E. chalcoptereae* isolates found in Basra, Iraq, highlighting their differentiation at the species level. This phylogenetic analysis revealed that the isolates from Basra, Iraq, are genetically consistent and clearly distinct from other *Eimeria* spp. These findings have deepened the understanding of local parasite evolution and may guide future studies on host-pathogen interactions and control strategies.

CONCLUSION

The current study revealed a high prevalence of coccidiosis, exceeding 60%, among domestic pigeons in Basra, Iraq. Molecular analysis identified 11 samples that amplified the *18S rRNA* genes. *Eimeria chalcoptereae* was detected in *Columba livia domestica* pigeons, which

presented the first record of *Eimeria chalcoptereae* in Basra, Iraq. Six representative sequences were selected, registered, and deposited in the NCBI database (PV459631.1, PV459632.1, PV459633.1, PV459634.1, PV459635.1, and PV459636.1). To understand the distribution and genetic diversity of *Eimeria* spp., future studies should concentrate on seasonal infection patterns, their effects on productivity, and possible medication resistance. Additionally, molecular surveillance should be expanded throughout bird flocks in Iraq.

DECLARATIONS

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Authors' contributions

The study's conception and design, data collection, and analysis were all conducted by Alaa Ismail Saood, Isam Azeez Khaleefah, Sara Salim Mohammad, Abduljabbar Khadim Alkinani, Khawla Bedan Nassir Aljassim, and Harith Abdulla Najem. Abduljabbar Khadim Alkinani and Khawla Bedan Nassir Aljassim took part in sample processing and laboratory procedures. Harith Abdulla Najem and Sara Salim Mohammad assisted with the manuscript's development and critical review. Alaa Ismail Saood oversaw the study, created the graphical abstract, and completed the manuscript. The final draft of the manuscript was authorized for publication by all authors.

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Conflict of interests

The authors declared that they have no conflicts of interest.

Ethical considerations

The authors are the original authors of this paper, which has not been published anywhere. The authors verified that their original scientific findings served as the basis for the article's writing by running it through a plagiarism index.

Availability of data and materials

The corresponding author can provide the data supporting these findings upon reasonable request.

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Effects of Vibration Sensor on Mitigation Risk of Halal Chicken Slaughtering System

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ABSTRACT

Ensuring compliance with halal standards in poultry slaughtering involves both following religious principles and implementing strict scientific and technological measures. Integrating Internet of Things (IoT) technology provides opportunities to enhance the reliability and objectivity of halal verification processes, particularly in identifying critical control points, such as animal death, specifically the death of chickens during the slaughter process, before entering the scalding stage. The present study aimed to design a halal risk mitigation system based on IoT, focusing on the critical point of complete chicken death, defined as the total absence of movement in the chicken after slaughter, through critical analysis. It is known that the stage between post-slaughter and pre-burning is the most crucial phase, where the highest risk is that the chicken has not entirely died due to ineffective slaughter. This system was developed using a NodeMCU ESP8266 microcontroller connected to a vibration sensor or passive infrared sensor and was equipped with real-time notifications via the Thingspeak cloud dashboard, indicating the waiting time for complete death and the number of vibrations. Testing on 30 chickens demonstrated a detection accuracy of 92.5% compared to manual observations by halal auditors, with consistent performance across different environmental conditions. This system can detect the movement of chicken remains after slaughter in an average of 15 to 20 seconds, providing an early warning of potential halal violations rules. The current results demonstrated that the vibration sensor effectively facilitated the execution of halal slaughtering principles through an early-warning mechanism designed to prevent chickens from entering the scalding phase while still alive. This ensures the humane death of chickens and the regulation of halal critical control points in line with the Indonesian national standard for halal poultry slaughter.

Keywords: Criticality analysis, Halal slaughter, Risk management, Poultry industry, Vibration sensor

INTRODUCTION

The global demand for halal products continues to grow significantly worldwide. This increase is driven not only by the growing Muslim population but also by consumers from different backgrounds who are increasingly valuing food integrity, safety, and quality (Herdiana et al., 2024). In Indonesia, which has the largest Muslim population, halal certification for animal food products goes beyond a religious obligation; it has also become an ethical and regulatory standard within the national food system (Pradana et al., 2024). As a result, the halal aspect has become a primary focus in supervising and managing the food supply chain, particularly in processes directly related to sharia requirements, such as slaughtering in

slaughterhouses.

One of the most crucial issues in the halal poultry slaughter process is to ensure that each chicken experiences complete death as a direct result of slaughter, defined as the complete absence of movement in the chicken after slaughter (Nielsen et al., 2019). This complete death is one of the main requirements for the validity of slaughter according to Islamic law (Jelan et al., 2024). According to the Indonesian National Standard (SNI) 99002:2016 on halal slaughter of poultry (BSN, 2016), the chickens should be entirely dead due to the neck incision before entering the scalding stage (Asih and Sopha, 2024), the animal must be fully deceased after the three main tubes, throat, respiratory tract, and major blood

vessels are cut, prior to entering the scalding stage (Guinebretière et al., 2024). The SNI, developed by the National Standardization Agency of Indonesia (BSN), acts as a national benchmark to ensure product quality, safety, and compliance with regulations (Rosiawan et al., 2018). However, field practice indicated that many chickens are not completely dead from slaughter and instead die from the soaking process, which then became a critical point in the halal assurance system.

In the context of halal chicken slaughter, it is essential to distinguish between complete death and brain stem death (Shahdan et al., 2017). Complete death refers to the total halt of all biological functions, including reflexes and voluntary movements, after slaughter, indicating that the animal has died solely as a result of the cut (Espinosa, 2024). In contrast, brain stem death is a more complex condition characterized by the irreversible loss of brain stem function, which may still permit residual reflexes or spasmodic movements even if consciousness is lost (Fuseini, 2019; Friedman et al., 2021). Determining brain stem death through histopathological examination is the gold standard; however, in practical slaughterhouse settings, such examination is not feasible. Instead, observable behavioral indicators such as persistent residual motion, muscle spasms, or reflexive leg movements are widely used as non-invasive indicators of incomplete brain stem shutdown (Sazili et al., 2023; Ibrahim et al., 2024).

Incomplete death in poultry during slaughter is especially problematic because, under Islamic law, the chickens are required to die directly after slaughter (Samoylov et al., 2023). If the chicken dies due to other conditions, such as hot water during the scalding process, the meat is assumed not halal (Thaha et al., 2023). In modern industry, detection of complete death is often only done visually by operators, which is prone to human error, especially in large-scale and process-intensive production (Vieira et al., 2024). These findings highlighted a critical need for a real-time and objective monitoring system to verify complete death before the scalding stage.

To ensure a complete death according to the sharia, the monitoring system must detect not only the absence of spasmodic movements, but also ensure that all physiological functions have stopped, including both reflex responses and heartbeats. This will reduce the risk of the chicken dying from scalding instead of slaughter and maintain the halal status of the products.

Conventional chicken slaughtering systems in many slaughterhouses, especially on an industrial scale, still rely on manual supervision and visual sampling (Agrawal et al., 2025). In this model, staff observe whether the chicken

has stopped moving and then allow the chicken to enter the hot water immersion tank (Astruc and Terlouw, 2023). However, not all movements (or lack thereof) are valid indicators of complete death. Spasmodic or convulsive movements may occur as a result of nervous or muscular system activity that has not entirely shut down, even though the chicken appears unconscious.

This weakness is worsened by production pressures in modern industrial systems, which prioritize speed and efficiency over accuracy regarding halal standards. In systems operating at an automated conveyor pace, every second matters, and a thorough examination of the chicken's health after slaughter is often neglected (Voogt et al., 2023). As a result, many chickens still have brain stem activity during the scalding stage and die from heat, which automatically invalidates the halal status of the product.

In response to these challenges, Internet of Things (IoT)-based approaches offer great potential in halal control systems (Alkahtani et al., 2024). One solution being developed was the use of vibration sensors or passive infrared motion sensors (PIR sensors) to detect residual motion post-slaughter (Verma et al., 2021). These sensors can be installed on conveyor lines or inspection stations before the chickens enter the scalding tank. When movement or convulsions are detected, indicating that the chicken is not fully dead, the system will give an alarm or stop the conveyor automatically.

To identify the most critical failure points in this process, the failure mode, effect, and criticality analysis method was used (Chennoufi and Chakhrit, 2024). This method maps all stages of the slaughter process and detects potential failures such as incomplete slaughter, delayed slaughter, or operator error (La Fata et al., 2022). These evaluations inform the development of an IoT-enabled automated control system designed to perform preventive, corrective, and predictive functions.

The IoT-based complete death detection system addresses technical challenges in chicken slaughtering and ensures adherence to national halal standards. By automating the documentation of slaughter compliance, the system enhances operational transparency and supports integration into wider halal certification frameworks (Maryuliano and Andarwulan, 2024). The system, integrated with a real-time dashboard and cloud storage, allows for transparent monitoring and enhances public trust in the halal industry.

It has been stated in several studies conducted by Islam et al. (2023), Ibrahim et al. (2024), and Suliman et al. (2024) that there is still a significant gap in integrating

technological systems that address the core of the halal issue, namely, deaths due to slaughter. Many studies have focused on post-harvest traceability, the use of blockchain in the supply chain, or managerial approaches to halal risk (Rahim et al., 2020). As demonstrated in a study of Sari et al. (2024), sustainable strategic planning and management driven by green management, digital transformation, and halal business management significantly enhances the ecological (Ahmad et al., 2024), social, and economic performance of halal-oriented micro, small, and medium enterprises (MSMEs; Fischer and Nisa, 2025). However, no approach automatically detects the life-and-death status of chickens immediately after slaughter.

By integrating the failure mode, effects, and criticality analysis (FMECA) method for risk identification and IoT technology for residual motion detection, the proposed system specifically addresses the most critical issue in the slaughter process (Ghiaci and Ghouschi, 2023). This goes beyond production efficiency; it is about preserving the integrity of Shariah, a non-negotiable value in the halal

food system (Amijaya et al., 2024). Integrating technology into the halal assurance system should be part of the national strategy to develop a halal industry 4.0 ecosystem, featuring IoT, automation, and digital traceability tools (Ellahi et al., 2025). By utilizing IoT, sensor data, and automatic alarm systems, slaughterhouses can enhance the effectiveness and efficiency of their production processes while fostering stronger relationships with consumers domestically and internationally, ultimately leading to increased market acceptance.

In the context of increasing global competition and rising demands for halal meat, Indonesia has strategic potential to lead in the technological advancement of the halal industry (Sucipto et al., 2020). The present study aimed to develop a halal risk mitigation system utilizing the IoT, supported by FMECA analysis, focusing on the critical aspect of ensuring proper chicken death, which is characterized by the complete lack of movement in the chicken after slaughter, as determined through criticality analysis.

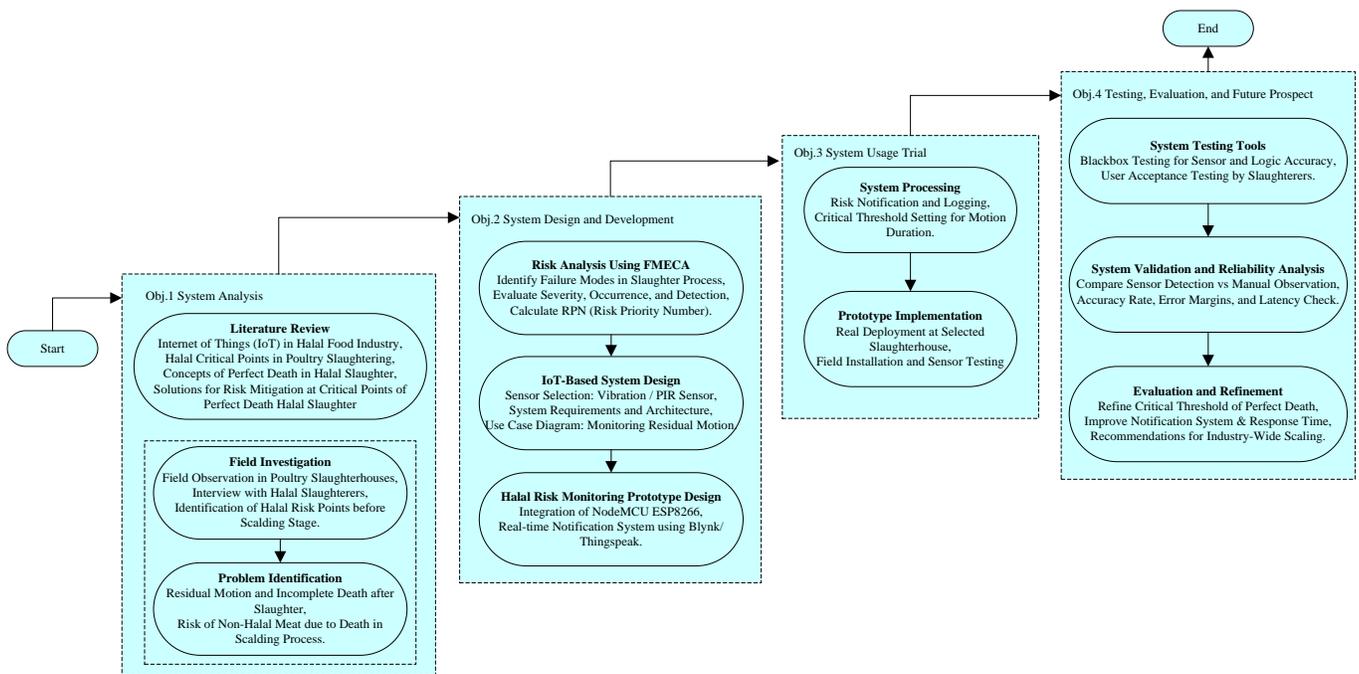


Figure 1. Flowchart of IoT-based halal risk mitigation system design (Designed by authors).

MATERIALS AND METHODS

Ethical approval

The present study received ethical approval from the Ethics Committee of the Halal Product Process Assistance Institution (LP3H) of the Indonesian Islamic Boarding School Association under approval code LP3H.IPI/EA/027/VII/2025.

Materials and tools

The main components of the IoT system included a NodeMCU ESP8266 microcontroller, which served as the main data processing unit and IoT signal transmitter, a vibration sensor module or PIR HC-SR501 to detect residual chicken movement after slaughter, an LED with 520-525 lux as a visual indicator, and a Wi-Fi module to

transmit data to an IoT platform-based monitoring server such as Blynk or Thingspeak.

Technology approaches

The present study utilized an applied engineering approach to develop a technology-driven system for halal risk mitigation. The method involves four stages. First, system analysis identified halal risks, especially incomplete death before scalding, through literature review and field observations. Second, system design and development applied FMECA to map critical failure points and developed an IoT-based prototype using vibration/PIR sensors with a NodeMCU ESP8266 microcontroller. Third, a field trial tested the prototype in a slaughterhouse to detect residual motion and assess real-time performance. Finally, the system evaluation validated accuracy against halal auditor observations and examined stability under operational conditions.

System analysis

This stage focused on identifying halal risks in the chicken slaughtering process, particularly the risk of incomplete death before scalding. The analysis identified potential failure modes in the slaughtering process and ranked them using the FMECA framework to find the most important control points that need technological intervention. The criticality value was used as a composite risk indicator, derived from severity, occurrence, and detectability metrics. In the present study, the calculation was based on $\alpha = 0.45$ (Contribution to total failures), $\beta = 0.95$ (Severity in terms of Sharia impact), and $\lambda_p = 0.035$ failures per hour (Occurrence frequency). Higher C values indicated greater potential impact on system integrity and halal compliance. The value of λ_p represented the estimated failure rate, derived from field observations or historical production records.

Table 1. List of materials for the design of an IoT-based halal risk mitigation system

Component name	Specification	Function
ESP8266 microcontroller	NodeMCU ESP8266 (Wi-Fi enabled, GPIO ≥ 6)	Main unit of the data processor and IoT signal sender
Breadboard	Mini breadboard (400/830 dots)	Place for electronic circuit assembly
Jumper Cable	Male-to-Male and Male-to-Female wires	Connecting components on the breadboard
Relay Module	1 Channel 5V Relay Module	Controls output devices such as lights or buzzers
Buzzer (optional)	5V Active Buzzer Module	Alternative sound alarm when the chicken is not completely dead
Wi- Fi/ Communication module (internal)	Embedded in ESP8266	Send data to the dashboard or server
Enclosure/Box	ABS plastic box	Protects the device from dust and moisture
IoT Server/Dashboard	Platforms such as Blynk, Thingspeak, or Firebase	Displays sensor data in residual motion
USB cable	Micro USB to USB-A	Connects ESP8266 to a computer/power source
Android/iOS Smartphone (Optional)	Android 8.0+ / iOS 13+ RAM: ≥ 2 GB Connection: Wi-Fi Storage: ≥ 16 GB	Access real-time monitoring dashboard (Blynk/Thingspeak) Receive notifications/alarms if the chicken has not died completely Perform remote control of the system (on/off relay) if the system supports it
Supporting Application (Optional)	Blynk IoT Thingspeak Viewer MQTT Dashboard Firebase App	Display sensor status and data logs Provides manual/emergency control

System design and development

In the system design stage, FMECA was applied to identify critical failure points in the slaughtering process.

An IoT-based system utilizing vibration or PIR sensors and NodeMCU ESP8266 has been developed to detect post-slaughter movement and send real-time alerts via

platforms such as Blynk or Thingspeak. This enables early warnings and objective halal compliance monitoring, comprising several key components, as detailed in Table 1.

System usage trial

The system was implemented in a real slaughterhouse to assess effectiveness and gather user feedback. The system detected residual movements within a time threshold and alerted operators if chickens were not entirely dead, as demonstrated through trials conducted on 30 chickens. The methodological framework of the present study (Figure 1) comprised four sequential stages: system analysis, design and development, field testing, and evaluation. Figure 2 illustrates the system workflow. The IoT-based vibration sensor system prototype was then tested under actual slaughterhouse conditions to assess its

reliability, focusing on sensor performance and real-time data consistency (Figure 3).

Testing and evaluation

System validation involved black-box testing and user acceptance testing to confirm that the system consistently detects residual motion in real operational conditions and complies with halal assessments. Accuracy was assessed by comparing the system output with manual observations, as outlined in the methodological framework (Figure 1) and demonstrated in the system workflow (Figure 2). The comparative results between the sensor readings and manual observations were presented in Figure 3, which was created by the authors using Microsoft Visio and was not adapted from any external source. Improvements were implemented based on the results, focusing on sensor sensitivity and logic refinement.

Table 2. Risk factors associated with halal chicken meat production process based on SNI 99002:2016 in Pasuruan Chicken Slaughter Center, Indonesia (2025)

Process stages	Risk factor	Risk code	Risk priority level	α	β	λ_p (per hour)	t (hour)	C	Recommendation action
Chicken Arrival	Chicken from non-halal certified sources	R1	Acceptable risk	0.30	0.80	0.015	100	0.36	Supplier halal certificate verification and IoT tracking
Chicken Transport	The chicken died during transportation	R2	Acceptable risk	0.25	0.75	0.020	100	0.375	Use ventilation containers and an on/off monitoring system
Ante-mortem Inspection	Sick/unfit chicken not detected	R3	Acceptable risk	0.20	0.85	0.018	100	0.306	Chicken health audit and inspection officer certification
Slaughter	The slaughterer is not halal certified	R5	High-priority risk	0.40	0.90	0.025	100	0.9	Require halal slaughterer certification and periodic sharia audit
Slaughter	Improper cutting point	R7	High-priority risk	0.35	0.85	0.030	100	0.893	Sharia cutting point training and slaughter verification
Slaughter	Chicken is not completely dead	R8	Extreme priority risk	0.45	0.95	0.035	100	1.496	Post-slaughter Vibration Sensor for residual motion detection, and perfect time of death monitoring
Blood Removal	Blood does not come out completely	R9	Acceptable risk	0.25	0.70	0.012	100	0.21	Blood flow monitoring and slaughter SOP training
Hot Water Immersion	Water contaminated with impurities	R10	Acceptable risk	0.20	0.65	0.010	100	0.13	Separate the temperature sensor and water circulation system
Offal Handling	Contact with unclean internal parts	R11	Acceptable risk	0.15	0.60	0.010	100	0.09	Separate offal area and equipment cleanliness audit
Packaging and Storage	No halal label or improper storage temp	R13	Acceptable risk	0.10	0.50	0.008	100	0.04	Digital labeling and automatic temperature monitoring

Note: α is the proportional weight of risk severity to overall process; β is the proportional weight of sharia criticality level; λ_p (per hour) is the estimated failure rate, derived from field observations or historical production records; t (hour) is the exposure time considered in the risk evaluation; and C is criticality index, calculated by combining α , β , λ_p , and t; higher values indicate higher priority for mitigation.

RESULT AND DISCUSSION

Mapping critical points of halal risk in the slaughter process

Based on the data obtained in Table 2, as well as the results of observations and analysis using the FMECA method, this analysis identified the post-slaughter to pre-scalding stages as the most critical halal risk points, accounting for 45% of the overall risk distribution ($\alpha = 0.45$), with an extreme priority risk category ($C = 1.496$). This stage was crucial in ensuring that the chicken experiences complete death, which is a necessary condition for slaughter according to Islamic law and in accordance with the SNI 99002: 2016 guideline (Musawa et al., 2024). The SNI 99002:2016 standard specifies the halal slaughtering procedures for poultry in Indonesia, highlighting that the animal should be completely dead after slaughter to be considered halal. One of the most significant risks occurs when the chicken does not die instantly during slaughter but instead dies from hot water scalding, which renders the meat non-halal according to Sharia (Ramli et al., 2024).

Within the FMECA analysis framework, the highest risk was identified in R8 (Chickens did not die completely), which was classified as an extreme priority risk category. This risk indicated that the failure has a direct and profound impact on the halal status of the products. The analysis indicated that the C value reached 1.496, the highest among all identified risks (Table 2). This finding highlighted that failure in R8 (chickens did not die completely) had the most significant impact on halal compliance and overall system integrity, which indicated that incomplete chicken mortality is a significant and critical issue that needs technological solutions.

As illustrated in Table 2, the values of α and β were assigned according to each risk factor's proportional impact and the level of Sharia criticality within the overall process, with assumed values derived from the literature. The highest C index was recorded at R8 (Chickens were not completely dead, $C = 1.496$), indicating the most critical risk and the highest priority for mitigation with an IoT-based death detection system.

The present study demonstrated that the IoT-based vibration sensor system successfully detected residual movements in slaughtered chickens with an accuracy of 92.5% compared to halal auditor observations. These findings indicated that the system could objectively monitor incomplete death within 15-20 seconds post-slaughter, thereby reducing the risk of chickens entering

the scalding tank while still alive. Similar to the present study, Neil et al. (2024) reported that spasms and reflexes are reliable indicators of incomplete death, as historically confirmed through manual observation. In contrast, the IoT-based system provided automated and real-time detection, overcoming the subjectivity of human judgment. Furthermore, while Ismail and Huda (2024) highlighted the urgent need for innovation in post-slaughter monitoring, the present results provided empirical evidence that such an innovation was technically feasible and effective under real slaughterhouse conditions.

The use of this innovative vibration sensor technology aligns with the findings of Hidayati et al. (2024), who emphasize that certified slaughtering practices and technological interventions are crucial for ensuring halal compliance, particularly in industrial settings prone to inconsistencies. To address related risks, the present study applied an IoT-based real-time monitoring system using vibration or PIR sensors to detect post-slaughter movement. The system alerted when signs of life lasted more than 3 minutes, in line with manual slaughter standards that require complete blood loss. Additionally, it featured visual alarms and digital audits to support halal verification.

Figure 2 illustrates the workflow of an IoT-based halal risk mitigation system designed to detect the perfect death of chickens after slaughter, addressing critical risks identified through FMECA analysis, particularly the failure of chickens to die from the slaughter cut, which may result in death during the hot water scalding process and thus invalidate the halal status of the meat. The system begins with activating a power supply to the ESP8266 microcontroller, which was connected to a vibration or PIR sensor that detected post-slaughter residual motion. When movement was detected, the ESP8266 processed the signal and activated a relay module to trigger a visual indicator such as an LED light, serving as a warning to operators. In parallel, the data is transmitted via Wi-Fi to a cloud-based dashboard for real-time monitoring and digital traceability. The system also included a maintenance feature that enabled users to monitor operational indicators and perform recalibration as needed to ensure ongoing accuracy and compliance with halal standards.

The system included cloud dashboard integration (Thingspeak or Blynk), enabling remote monitoring, live data visualization, and event history recording, essential for traceability and auditability as mandated by SNI 99002:2016. The integration of these features supported data-driven halal assurance by reducing reliance on manual inspection. According to Fuseini et al. (2021), visual inspection alone cannot guarantee the complete loss

of brainstem function, making objective sensor-based detection essential for ensuring slaughter compliance. Additionally, Nusran *et al.* (2023) highlighted the potential of IoT to enhance transparency and automation in halal verification, aligning with trends in industrial digitalization. The wiring schematic indicated that the system's architecture facilitated parallel computing, enabling the simultaneous monitoring of multiple

components of chickens. With adjustable sensitivity settings and routine maintenance, the system demonstrated excellent adaptability to diverse operational conditions. This compact design enhanced halal control points, facilitated digital transformation, and signified a strategic innovation for developing a modern, dependable, and technologically advanced halal framework industry.

Table 3. Residual motion detection test results, including vibration sensor versus manual observation for detecting complete death in halal chicken slaughter

Chicken number	Vibration sensor detection (residual motion duration)	Sensor status	Halal auditor's visual observation	Auditor's status
1	45 seconds	Perfectly Dead	No Motion	Perfectly Dead
2	2 min 20 sec	Not Dead	Slight Spasms	Not Dead
3	1 min 10 sec	Perfectly Dead	No Motion	Perfectly Dead
4	3 min 30 sec	Not Dead	Neck Movement Detected	Not Dead
5	55 seconds	Perfectly Dead	No Motion	Perfectly Dead
6	4 min 05 sec	Not Dead	Leg Vibration Detected	Not Dead
7	1 min 50 sec	Perfectly Dead	No Motion	Perfectly Dead
8	3 min 10 sec	Not Dead	Minor Movements Observed	Not Dead
9	50 seconds	Perfectly Dead	No Motion	Perfectly Dead
10	2 min 40 sec	Not Dead	Delayed Minor Spasms	Not Dead
11	58 seconds	Perfectly Dead	No Motion	Perfectly Dead
12	3 min 15 sec	Not Dead	Neck Spasms	Not Dead
13	1 min 05 sec	Perfectly Dead	No Motion	Perfectly Dead
14	2 min 55 sec	Not Dead	Neck Twitches	Not Dead
15	46 seconds	Perfectly Dead	No Motion	Perfectly Dead
16	3 min 45 sec	Not Dead	Leg Movement Detected	Not Dead
17	49 seconds	Perfectly Dead	No Motion	Perfectly Dead
18	2 min 10 sec	Not Dead	Slight Reflexes	Not Dead
19	1 min 30 sec	Perfectly Dead	No Motion	Perfectly Dead
20	3 min 05 sec	Not Dead	Subtle Movements	Not Dead
21	47 seconds	Perfectly Dead	No Motion	Perfectly Dead
22	2 min 35 sec	Not Dead	Weak Spasms	Not Dead
23	55 seconds	Perfectly Dead	No Motion	Perfectly Dead
24	3 min 20 sec	Not Dead	Limb Reflexes	Not Dead
25	52 seconds	Perfectly Dead	No Motion	Perfectly Dead
26	4 min 00 sec	Not Dead	Persistent Movement	Not Dead
27	59 seconds	Perfectly Dead	No Motion	Perfectly Dead
28	2 min 25 sec	Not Dead	Reflex Detected	Not Dead
29	1 min 15 sec	Perfectly Dead	No Motion	Perfectly Dead
30	3 min 50 sec	Not Dead	Leg Twitching	Not Dead

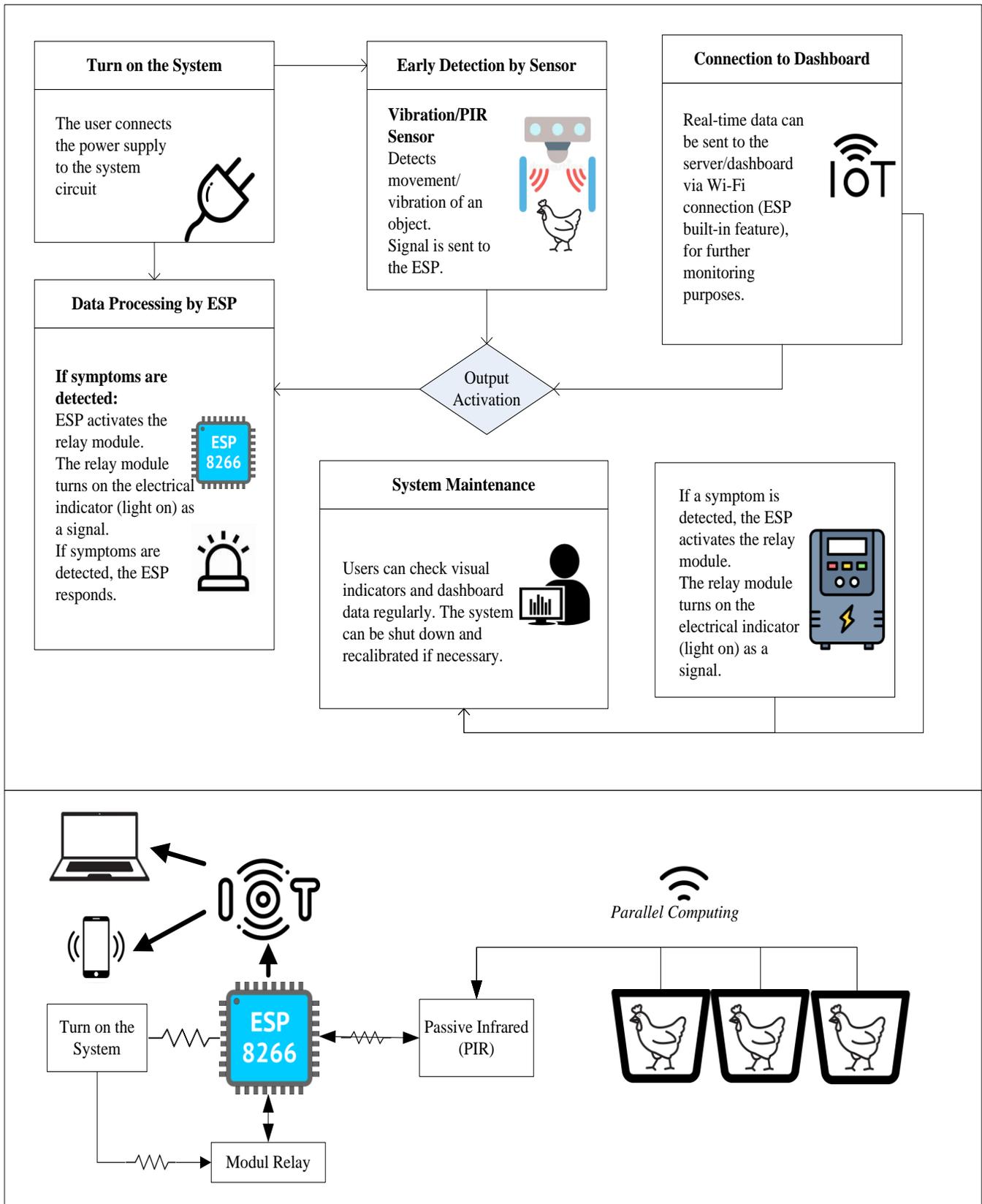


Figure 2. IoT-based system workflow for detecting complete death in halal chicken slaughter (Designed by authors).

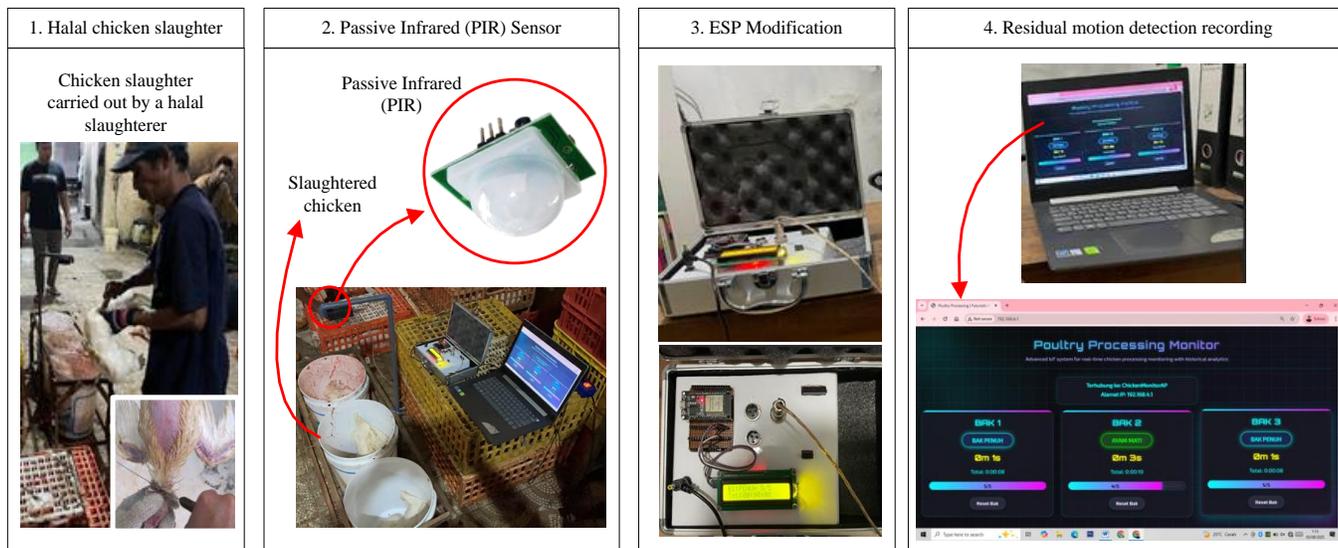


Figure 3. Residual motion detector trial after halal chicken slaughter in Pasuruan Chicken Slaughter Center, Indonesia

System usage trial

The prototype of the IoT-based halal risk mitigation system was tested on 30 chickens at a slaughterhouse to evaluate its accuracy and reliability in real operational settings. The system, equipped with an ESP8266 microcontroller and vibration sensor, successfully detected residual post-slaughter movements within 0 to 5 minutes, achieving 92.5% accuracy compared to manual halal auditor assessments. In six cases, the system provided early warnings, which were later verified as accurate through manual confirmation observation. The system detected movement within 15-20 seconds of activation, demonstrating real-time responsiveness, which is essential for high-speed slaughter processes. It accurately identified subtle indicators such as muscle spasms, establishing itself as a dependable early warning instrument for potential halal violations.

Apart from accuracy and sensitivity, the system demonstrated stable performance under different lighting and temperature conditions (22 to 30°C) during brief trials conducted in a commercial poultry slaughterhouse in Pasuruan, Indonesia. Although the test consistently yielded reliable results without requiring recalibration, it was conducted at only one location and within a limited scope timeframe. These findings supported the feasibility of integrating the system into industrial slaughter lines as a decision-support tool for verifying complete chicken death. The system plays a vital role in enhancing halal assurance by providing rapid response, environmental resilience, and high-precision technology.

Figure 3 shows the system usage trial of the IoT-based residual motion detector after halal chicken slaughter. The process started with halal slaughtering performed by a certified slaughterer, followed by the use of a PIR sensor to detect residual motion in the slaughtered chickens. The ESP8266 microcontroller was then customized and connected to the sensor to collect signals and trigger the relay module. Finally, the remaining motion data were recorded and displayed on a digital dashboard for monitoring and traceability. Table 3 presents the results of this system trial, comparing sensor detection with manual observations by halal auditors to evaluate accuracy.

Testing and prospects

The present study introduced a vibration sensor-based system designed to detect residual motion in chickens after slaughter, in accordance with Islamic law and SNI 99002:2016. Residual movements such as spasms or reflexes indicate incomplete physiological death.

The present study utilized manual observation by certified halal auditors as the reference standard for validating the sensor system. However, considering the potential for human error, the sensor-based system provided a more objective and consistent way to detect residual motion, especially in high-volume operational settings. The average detection time for non-compliant deaths was three minutes and three seconds, matching critical thresholds. In real-time, automated alerts improve decision-making and prevent non-halal processing,

making it a valuable tool for strengthening halal assurance in poultry slaughterhouses.

The current findings are in line with the study of [Tang et al. \(2024\)](#), which confirmed that sensor-based monitoring can enhance halal compliance by decreasing reliance on human intervention and improving traceability aspects. [Fletcher et al. \(2025\)](#) stated that post-slaughter residue movement can cause ambiguity in determining death status, so an objective and data-driven approach is needed. Additionally, [Ibrahim et al. \(2024\)](#) demonstrated that the integration of IoT technology into halal critical control points improves monitoring transparency and minimizes the risk of non-compliance, particularly in high-throughput slaughtering facilities. Similarly, [Alkahtani et al. \(2024\)](#) reported that the use of automated sensor systems in smart slaughterhouses significantly enhances the consistency of compliance detection and enables remote auditing within digital halal certification frameworks. These findings highlight the importance of the proposed system for large-scale field trials and real-time integration with cloud-based halal assurance platforms.

CONCLUSION

The present study demonstrated that the IoT-based vibration sensor system successfully detected incomplete death in chickens after slaughter with an accuracy of 92.5% compared to halal auditor observations. These crucial findings indicated that IoT technology had strong potential to strengthen objective and real-time monitoring of halal compliance during the critical post-slaughter to pre-scalding stage. For further studies, it is suggested to evaluate the long-term reliability and stability of the IoT-based vibration sensor system in different slaughterhouse environments, analyze its economic feasibility and scalability for large-scale poultry industries, and investigate integration with blockchain or AI-driven platforms to improve traceability, certification integrity, and consumer trust in halal assurance systems.

DECLARATIONS

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Authors' contributions

Khafizh Rosyidi conducted the research, collected and analyzed the data, and drafted the manuscript. Imam Santoso, Yusuf Wibisono, and Sucipto Sucipto reviewed and edited the manuscript. All authors have read and approved the final edition of the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest related to this study.

Ethical considerations

All authors confirmed that ethical issues such as plagiarism, publication consent, misconduct, data fabrication or falsification, duplicate submission, and redundancy have been thoroughly checked and found to be compliant.

Availability of data and materials

All data generated during the present study are included in this article. Additional information is available from the authors upon reasonable request.

Competing interests

The authors declared no conflict of interest in this article.

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Growth Performance, Carcass Traits, and Economic Aspects of Pekin Duck Growing in Dhamrai Area of Bangladesh

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ABSTRACT

White Pekin ducks are a valuable addition to the poultry industry in Bangladesh with their adaptability, rapid growth rate, and excellent meat quality. This study aimed to evaluate the growth performance, meat quality, and socio-economic aspects of White Pekin ducks in the Dhamrai area of Bangladesh. The study was carried out between January and April 2024 by distributing a total of 250 one-day-old Pekin ducklings among 25 selected farmers. Farmers were chosen based on their willingness, capacity, and adequate housing facilities to observe the growth characteristics, carcass traits, and economic values of Pekin ducks. The farmers were mainly middle-aged (46 years) with an average family size of 4.16 and had farming experience of 9 years. Livestock and poultry rearing were the primary occupation for 72% of farmers, and Pekin duck farming was pursued for both household consumption and extra income. The ducks showed consistent growth, increasing from 53.17 g at day-old to 1812.82 g at 9 weeks, with an average daily gain of 82.97 g. At the marketing age (10 weeks), the average dressing percentage was 74. The carcasses of male and female ducks differed slightly in terms of organ and fat distribution, with males generally had heavier internal organs and giblets, while females had slightly higher fat (2.10%). The nutrient content of breast and thigh muscles showed differences in water and fat content, with males generally having a higher dry matter of 29.13%. The Benefit-Cost ratio of Pekin duck farming was 1.59; however, the majority of farmers faced constraints due to higher feed prices and a lack of quality ducklings for Pekin duck production. Therefore, improving management practices, biosecurity measures, and access to better inputs could enhance the profitability and sustainability of Pekin duck farming.

Keywords: Benefit-cost ratio, Carcass trait, Dressing percentage, Growth performance, Pekin duck

INTRODUCTION

White Pekin ducks originated from China and have become widely popular across the world (Elkin, 2007) due to their remarkable adaptability to diverse environments, including the varied climate of Bangladesh (Ahmed et al., 2021). White Pekin ducks are characterized by their pure white feathers, orange to yellowish bills, shanks, and webbed feet. Pekin ducks are highly prized for their premium-quality meat and are widely raised on commercial duck farms (Ghosh et al., 2022). Global duck meat production in 2023 was dominated by China, with 3.6557 million metric tons, which is significantly higher than in other countries. Myanmar and France followed at

0.22251 and 0.20553 million metric tons, respectively (ReportLinker Research, 2023). White Pekin duck meat has gained significant popularity among urban consumers across various regions of Bangladesh, driving the growth of value-added, ready-to-eat duck products and expanding the frozen meat sector in the metropolitan areas. In India, traditional duck farmers, particularly women's self-help groups in rural areas, are rapidly adopting White Pekin duck farming, driven by robust market demand, convenient forward marketing linkages, and promising economic returns (Ghosh et al., 2021). In many regions of Bangladesh, duck farming is often favored over chicken farming due to lower disease outbreaks, reduced mortality

rates, and simpler feeding management. Recently, the rise in duck rearing and production, particularly white Pekin duck farming, has been driven by a growing number of women farmers in villages and peri-urban areas of Bangladesh, who are increasingly drawn to its promising potential (Islam et al., 2016). Under the traditional backyard duck farming system in lowland Hoar, Flood-prone, and Coastal areas, ducks are primarily reared on rice bran-based diets with limited supplementary green feedings, which often fall short of meeting the proper nutritional needs of ducks. To minimize the high costs of commercial feeds, many village duck farmers rely on a single, cost-effective feeding approach, using a mixture of rice bran, broken rice, and other locally available feed ingredients throughout the entire life cycle of ducks (Mavromichalis, 2014). It is widely recognized that management practices and feeding conditions are essential factors influencing the growth and meat characteristics of food animals (Mir et al., 2017). Management practices and feeding conditions influence the growth patterns and meat quality by affecting various metabolic pathways (Lebret, 2008; Park et al., 2018) either alone or in combination. The meat quality of Pekin ducks is a crucial consideration, as their meat is typically sold in the frozen sector in packaged form. Prolonged storage can lead to lipid oxidation, which may affect the taste and texture of the meat (Biswas et al., 2019). Meat production mainly relies on commercial strains of Pekin duck that vary in growth performance, carcass conformation, and meat quality. Furthermore, large differences exist in their housing conditions that affect welfare, growth, and carcass characteristics. Meat-producing duck strains exhibit rapid growth as a result of genetic selection, efficient housing systems, and superior nutrition. Several strains of Pekin ducks are frequently utilized in commercial meat production because of their impressive growth rates, efficient feed conversion, desirable body conformation, and high 'dressing %' (Starcevic et al., 2021). Pekin ducks are typically slaughtered between 6 and 8 weeks of age, by which time they have completed their rapid growth phase and reach an average weight of approximately 3.5 kilograms (Kokoszynski et al., 2019a). However, the emphasis on selecting for rapid growth and high meat yield may negatively influence meat quality (Kwon et al., 2014). Since duck meat production has intensified in recent decades, there is a growing demand to develop production systems that not only support optimal animal welfare but also ensure the delivery of excellent-quality meat (Chen et al., 2015). Pekin ducks are highly susceptible to environmental stress, which is significantly

influenced by their housing conditions. Additionally, high stocking density can impair the growth performance, health, and welfare of Pekin ducks (Xie et al., 2014). Numerous studies have been conducted to examine the growth performance and meat quality of various commercial Pekin duck strains (Kwon et al., 2014; Kokoszynski et al., 2019b). Although substantial literature exists on numerous aspects of white Pekin duck production in the confined rearing system under the commercial feeding regime, there is limited research regarding the growth performance and meat quality of this duck breed raised in a backyard farming system (Steczny et al., 2017; Rabbani et al., 2019). The present study could explore the growth pattern, meat quality, and profitability of raising white Pekin ducks in existing backyard farming conditions with locally available ingredients-based feeding management. Therefore, this study aimed to investigate the growth performance, meat quality, socio-economic aspects, profitability, and constraints regarding White Pekin duck farming under existing backyard farming conditions in the Dhamrai area of Bangladesh.

MATERIALS and METHODS

Ethical approval

This study was conducted in strict accordance with established ethical guidelines for animal research and welfare. Ethical approval was obtained under the "Establishment of 'BLRI Technology village' at BLRI Regional station" project from the Ethics Committee of the Bangladesh Livestock Research Institute (BLRI), ensuring that all procedures involving animal care and handling complied with the standards set forth by the World Organization for Animal Health (OIE) for the ethical treatment of animals in research.

Study area and time

The present research was carried out at the "BLRI Technology Village" (Shraifbag) of the Dhamrai area in Dhaka district under the Dhaka division of Bangladesh. Dhamrai Sub-district is located about 40 kilometers northwest of the capital city of Dhaka. Dhamrai Sub-district has an area of 307.41 square kilometers, located between 23°49' and 24°03' north latitudes and between 90°01' and 90°15' east longitudes. Figure 1 represents the geographical location of the experimental site of Dhamrai Sub-district, Dhaka, Bangladesh. The experimental and data collection period was considered from the first of January to the 30th of April 2024.

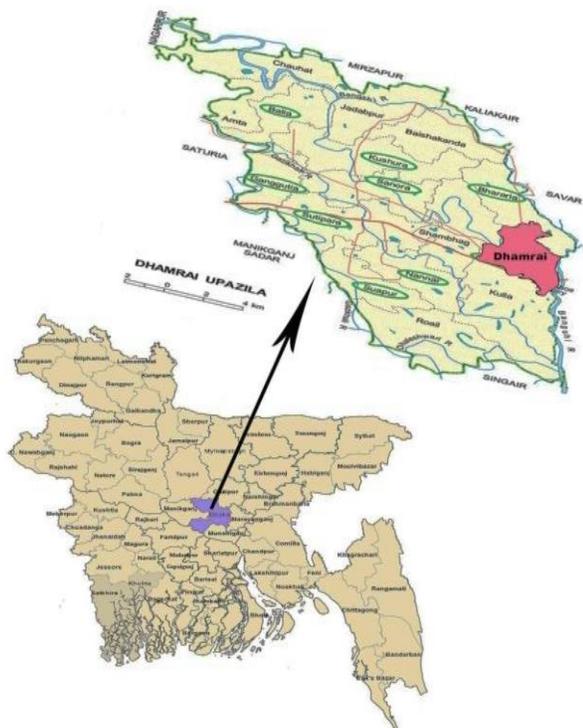


Figure 1. Geographical location of the study site, Dhamrai Sub-district, Dhaka, Bangladesh

Study population and design

A total of 25 farmers were selected based on their financial capacity, housing facility, adequate feed resources, and willingness to rear Pekin Duck, etc. A total of 250 one-day-old Pekin ducklings were collected from the Poultry Production Research Division by the coordination of the Farming System Research Division, BLRI, Savar, Dhaka. One farmer was selected from the total, based on his ability to provide sufficient brooding space and electricity supply. The brooding of White Pekin ducklings was conducted from day-old to 6 days of age under controlled environmental conditions to ensure optimal growth and health during the critical early stages. The ducklings were housed in well-ventilated, clean, and dry brooders equipped with adequate heating sources to maintain a consistent temperature range of 32-35°C during the first three days, gradually reducing to 28-30°C by the sixth day. The floor was covered with absorbent bedding material to prevent moisture accumulation and reduce the risk of infections. A 24-hour light cycle was maintained to encourage early feeding and activity. After completing the brooding up to 6 weeks of age, a total of 250 Pekin ducklings were distributed among the selected 25 farmers to assess the growth performance, carcass traits, and economic values of Pekin ducks at the community level. Farmers reared Pekin ducks on a semi-intensive system

with scavenging feeding management, where they supplied only a limited amount of locally available concentrate feed ingredients as a feed supplement from their household or purchased from the local market. Figure 2 shows the feeding and rearing management of Pekin ducks at Dhamrai Sub-district, Dhaka, Bangladesh. All farmers were interviewed with a structured questionnaire to assess the socio-economic conditions, rearing facilities, profitability, and constraints associated with Pekin duck rearing.

Data collection and recording

Data were collected and recorded on feed ingredients offered/day, body weight (gm), daily weight gain (gm) on a weekly basis from day old to nine weeks, and the market age of Pekin ducks was considered at 10-12 weeks. Four male and four female Pekin ducks were randomly selected from experimental farmers' households for this study and slaughtered to observe the physical quality as well as the carcass characteristics. Ducks were slaughtered manually at 10 weeks of age. In the beginning, the live weight (gm) was measured for each duck through a digital weight balance, and then slaughter was performed according to the standard procedure to ensure minimal pain and distress following the Halal method as customary in a Muslim country. The Halal approach ensured that the animals were slaughtered with the utmost care and respect, in compliance with ethical and religious standards. All procedures were carried out by trained personnel to maintain high ethical standards and ensure compliance with veterinary guidelines. Then the data were recorded on slaughtered weight (gm), Carcass weight (gm), Edible carcass weight (gm), muscle, bone, and Skin weight (gm) of Breast, Thigh (Thigh with drumstick), and lumbosacral region. The weight (gm) of edible parts and inedible parts, giblets, abdominal fat, skin, as well as other by-products of Pekin duck, was also recorded. The muscles from the Breast and Thigh region (50 gm) from each portion of both sexes (two males and two females) were considered as samples for laboratory analysis to determine the nutrient composition. Then the laboratory analysis was performed on nutrient composition, and the data were used for statistical analysis.

Economic assessment

The parameters on the nutrient composition (Dry matter, water content, crude protein, crude Fat, ether extract, and Ash) of the meat sample were calculated as per the standard methods (Proximate analysis). Proximate analysis was employed to assess the nutrient composition

of the samples, following the standard procedures outlined by the Association of Official Analytical Chemists (AOAC, 2005). Moreover, carcass traits were recorded for the whole carcass and individual cut-up parts. The ‘dressing %’ was calculated by the following formula.

$$\text{Dressing (\%)} = \text{Dressed weight} / \text{Live weight} \times 100$$

For calculating the Net return, the following formula was used.

$$\text{Net return} = \text{GR} - \text{GC}; \text{ Where GR: Gross return, GC: Gross cost}$$

Here, $\text{GC} = \text{TFC} + \text{TVC}$; Where TFC: Total fixed cost and TVC: Total variable cost

To calculate the Benefit-cost ratio, the following formula was used.

$$\text{Benefit-cost ratio} = \frac{\text{Gross Return (GR)}}{\text{Gross Cost (GC)}}$$

The gross return indicates the average return from the raising of Pekin Ducks, including the family consumed duck value and the sold value of ducks. Gross cost includes the total cost of Duck rearing, such as feed cost, veterinary cost, housing cost with 10% depreciation, cost of family labor involved, transportation cost, miscellaneous cost, etc. The benefit-cost ratio was a relative measure employed to compare the benefit to the cost. It assisted in analyzing the financial efficiency of the Pekin duck farms.

Statistical analysis

Initially, collected and recorded data were entered, sorted, compiled, and analyzed by using a Microsoft Excel worksheet. Descriptive statistics including frequency distribution, percentage, mean value and standard error of mean were considered to test the differences among the variables of feed supplying amount (gm/day), growth performance at different ages (gm/day), the cost involved in rearing and management practices (USD), fresh and slaughtered weight (gm), carcass weight (gm), nutrient compositions of meat under the basic economic performance indicators using SPSS (Statistical Package for the Social Science) Software, IBM Corporation, version-25.

RESULTS

Socio-economic demography of Pekin duck raising farmers

Table 1 represents the demographic and family-related characteristics of Pekin duck-raising farmers, where the mean age was 46.12 ± 2.58 years, indicating the

farmers were in their mid-life stage. The farmer’s family composed an average of 4.16 ± 0.19 members, with the number of earning members of 1.16 ± 0.07 , and most households had slightly more than one individual contributing to the family income. A moderate level of farming experience was observed at 9.08 ± 1.43 years within the sample population.

The distribution of respondents across different educational levels, occupations, training facilities, and purposes of Pekin duck rearing is presented in Table 2. The data reflected a diverse range of educational backgrounds, where the highest percentage, comprising 36% of the farmers, had completed Secondary School Certificate (SSC) education, followed by two groups, each representing 24% attained a Higher Secondary Certificate (HSC) level, and another had education below the SSC level. The lowest percentage (16%) had completed only primary education in the study area.

The majority of farmers (72%) were engaged in livestock and poultry rearing as their primary occupation. A smaller portion of farmers (16%) was involved in business activities. Agriculture was the primary occupation for only 8% of the respondents, while only 4% identified as day laborers. Regarding secondary occupation, a significant proportion (52%) was involved in agriculture, 12% were also engaged in livestock rearing and day labor. Business was an occupation for 16% of the farmers, and 8% practiced fish farming as their secondary occupation.

A combination of family need and extra income was the most common practice by 40% of the farmers, whereas 32% reared ducks for extra income, and 28% kept ducks primarily to meet family needs in the study area. Overall, 88% of the respondents had received training, whereas 64% of farmers attended training on livestock and poultry farming from the Bangladesh Livestock Research Institute (BLRI), and 24% got training organized by the Department of Livestock Services (DLS), Bangladesh. Moreover, 12% of the respondents did not receive any formal training related to duck rearing.

Table 1. Family status of Pekin duck raising farmers at Dhamrai Sub-district, Bangladesh, during 2024

Parameters	Mean ± SE (n = 25)
Age of the farmer	46.12 ± 2.58
Family size	4.16 ± 0.19
Earning member	1.16 ± 0.07
Farming experience	9.08 ± 1.43

SE: Standard error, n: Number of observations

Table 2. Education, occupation, rearing purpose, and training status of farmers at Dhamrai area, Bangladesh, in January to April 2024

Educational level	Percentage (n)	Primary occupation	Percentage (n)
Primary	16.00 (4)	Agriculture	8.00 (2)
Below SSC	24.00 (6)	Livestock and poultry rearing	72.00 (18)
SSC	36.00 (9)	Day labor	4.00 (1)
HSC	24.00 (6)	Business	16.00 (4)
Total	100.0 (25)	Total	100.00 (25)
Purpose of Pekin duck rearing		Secondary occupation	
Family need	28.00 (7)	Agriculture	52.00 (13)
Extra income	32.00 (8)	Fish farming	8.00 (2)
Family needs and extra income	40.00 (10)	Livestock rearing	12.00 (3)
Total	100.0 (25)	Day labor	12.00 (3)
Training facility		Business	16.00 (4)
DLS	24.00 (6)	Total	100.00 (25)
BLRI	64.00 (16)	-	-

*SSC: Secondary school certificate; HSC: Higher secondary school certificate; DLS: Department of Livestock Services; BLRI: Bangladesh Livestock Research Institute; n: Number of observations

Table 3. Housing facility and rearing system of Pekin duck at Dhamrai area, Bangladesh, from January to April 2024

Housing facility	Percentage (n)	Rearing system	Percentage (n)
Separate duck house	84.00 (21)	Scavenging	12.00 (22)
Same house with chicken	16.00 (4)	Semi-intensive	88.00 (3)
Housing material		House cleaning practice	
Tin and wood	60.00 (15)	Water	40.00 (10)
Bamboo and tin	24.00 (6)	Water and broom	24.00 (6)
Brick	16.00 (4)	Water and disinfectant	36.00 (9)
Floor type		Total	100.00 (25)
Wood	84.00 (21)	-	-
Brick	16.00 (4)	-	-
Total	100.00 (25)	-	-

Housing and rearing management

The housing facilities and rearing systems for Pekin ducks are shown in Table 3. A majority percentage (84%) of the duck farmers provided shelter to their Pekin ducks in a separate house, while 16% were kept in the same house with chickens. The materials used for constructing the duck houses varied, where 60% of the houses were made of tin and wood, 24% of farmers' duck houses were made of bamboo with tin, and 16% were made of brick. The floor types of the housing structures included 84% of wood and 16% of brick or concrete floors. In terms of the rearing system, most of the farmers adopted semi-intensive rearing management and allowed ducks to scavenge in the ponds near their household, while 12% of respondents raised Pekin ducks under the scavenging system. Water was the most common cleaning method for

house cleaning practices, used by 40% of the farmers. Other cleaning methods included the use of both water and a broom (24%) and water combined with disinfectant (36%).

Concentrate feed supplements are provided by the farmers

Most of the farmers did not follow any specific diet for duck production under traditional rearing and feeding management in Bangladesh. They mainly depended on natural feeding sources for feeding their ducks under the scavenging rearing system. However, they provide the minimum proportion of locally available concentrate feed ingredients 2-3 times a day. Table 4 shows the frequency and percentage of concentrate feed ingredients provided by farmers to their Pekin ducks at different growth stages. At the early stage, rice was the most commonly used feed

component, whereas 80% of farmers offered broken rice to their ducks, followed by rice polish 72%, whole rice 48%, wheat 36% and maize provided by 20% of the duck farmers. Boiled rice was supplied by a smaller proportion of farmers (16%), while 44% of farmers provided ready feed, and all farmers (100%) used common salt in the duck diet. At the growing stage, there was a noticeable shift in feed preferences. The use of rice increased to 84%, while rice polish (80%) remained a common choice; broken rice was used by 60% of farmers. The frequency of ready feed, wheat, and maize bran usage slightly decreased to 36%, 28%, and 20%, respectively, due to fluctuating market prices and the seasonal availability of these feed ingredients in the local market. Notably, a vitamin-mineral premix was incorporated by 20% of farmers at the growing stage. The distribution of feed ingredients highlights the farmers' adaptability in adjusting the duck feed formulation as the ducks progressed through different growth stages.

Growth performance

Figure 3 shows the growth performance and average daily gain of Pekin ducks in the study area. The average body weight of day-old ducklings was 53.17 ± 0.41 gm. Ducks gained an average of 17.37 gm per day during the first week, with their body weight reaching 121.63 ± 1.67 gm. Throughout the second to ninth week period, the Pekin ducks exhibited increasing average body weight (gm) of 340.77 ± 12.32 , 505.62 ± 12.45 , 648.36 ± 17.46 , 771.67 ± 23.97 , 957.66 ± 26.98 , 1223.94 ± 36.35 , 1517.58 ± 37.04 , and 1812.82 ± 49.91 gm, respectively. Moreover, the average daily gain (gm) from the first to the ninth weeks of age was observed at 17.37 gm, 48.68 gm, 35.23, 46.62, 52.23, 57.80, 65.84, 74.79, and 82.97 gm, respectively. Pekin ducks exhibited a remarkable growth performance, characterized by a steady weekly increase in both body weight and daily weight gain.

Table 4. Concentrate feed supplement provided by the farmers to their Pekin ducks at Dhamrai, Bangladesh, from February to April 2024

Feed ingredients	Frequency (n)	Percent at early stage (0-8 weeks)	Frequency (n)	Percent of growth stage (9-12 weeks)
Rice	12	48.00	21	84.00
Broken rice	20	80.00	15	60.00
Boiled rice	4	16.00	5	20.00
Rice polish	18	72.00	20	80.00
Wheat bran	9	36.00	7	28.00
Maize crushed	5	20.00	5	20.00
Vitamin-mineral premix	-	-	5	20.00
Ready feed	11	44.00	9	36.00
Salt	25	100.00	25	100.00

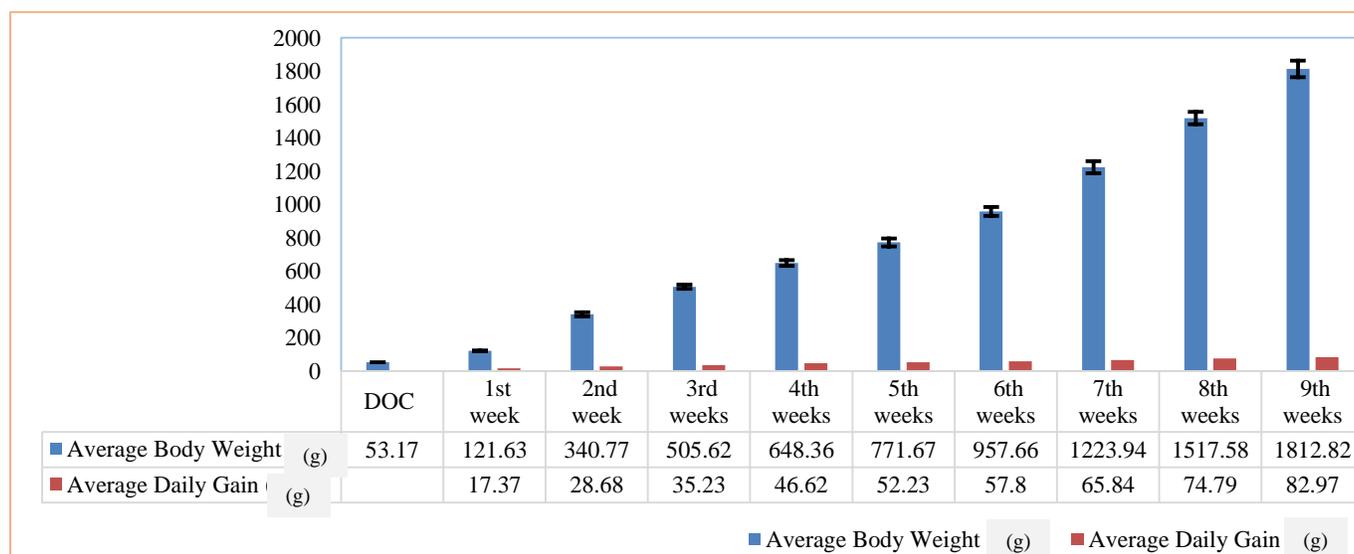


Figure 3. Growth performance (Mean \pm Standard Error) and average daily gain of Pekin Duck at Dhamrai area of Bangladesh in 2024.



Figure 2. Feeding and rearing management of Pekin ducks at Dhamrai Upazila, Dhaka, Bangladesh in 2024

Health and biosecurity management of Pekin ducks

Table 5 shows the health and biosecurity management of Pekin ducks and reveals that 84% of farmers followed regular vaccination protocols, with 48% vaccinating at 21 days of age and 36% at 28 days. About 56% of farmers reported their ducks were free from disease. Moreover, farmers experienced Duck Plague and Duck Cholera at 8%, because farmers reared Pekin ducks only up to reach their marketing age (12 weeks), while 16% Duck Brooder Pneumonia and 12% other diseases. Biosecurity management practices indicated that 88% of farmers' ducks came into contact with wild birds. Nearly 52% of farmers isolated sick ducks in a separate shed, while 20% kept them in the same shed, another 20% opted to slaughter the sick ducks, and 8% of farmers sold their sick

ducks to the local consumers. Excrement management was usually practiced by 100% of farmers, and they performed regular cleaning. About 32% of farmers disposed of excreta by dumping it with soil, 28% used it as fish feed, 20% threw it into the water, and another 20% used it as fertilizer in their household crop and vegetable garden. In terms of dead duck disposal, 44% of farmers practiced burning, 24% threw it into the water, 20% threw it into open fields, and 12% burned it. As the Sharifbag village has already been declared as the “BLRI Technology Village” which is located under Dhamrai Sub-district. So, the treatment of sick pek in ducks was primarily handled by veterinary experts from the Bangladesh Livestock Research Institute (68%), with a smaller proportion treated by veterinary hospitals (12%) and quacks (12%) or managed by the farm owners themselves (8%).

Table 5. Health and biosecurity management of Pekin duck at Dhamrai area, Bangladesh, from January to April 2024

Parameters	Frequency (n)	Percentage
Followed vaccination regularly	21	84.00
Age at vaccination		
21 days	12	48.00
28 days	9	36.00
Disease outbreaks		
Duck plague	2	8.00
Duck cholera	2	8.00
Duck brooder pneumonia	4	16.00
Others	3	12.00
Free from disease	14	56.00
Biosecurity management		
Contact with a wild bird	22	88.00
Sick duck management		
Kept in the same shed	5	20.00
Kept in a separate shed	13	52.00
Slaughtered	5	20.00
Sold a sick duck	2	8.00
Regular cleaning of excrement	25	100.00
Method of excreta management		
Throw it into the water	5	20.00
Used as a household fertilizer	5	20.00
Dumping with soil	8	32.00
Used as fish feed	7	28.00
Death duck management		
Throw in field	5	20.00
Buried	11	44.00
Burnt	3	12.00
Throw in water	6	24.00
Treatment facilities for ducks		
By a veterinary expert from BLRI	17	68.00
By the veterinary hospital	3	12.00
By quack	3	12.00
By own self	2	8.00

BLRI: Bangladesh Livestock Research Institute; n: Number of observations

Carcass characteristics of Pekin ducks

The carcass characteristics of Pekin ducks at their marketable age (10 weeks) are given in Table 6. The overall live weight of the Pekin duck was 2017.50 ± 44.63 gm, where the live weight of 2025.50 ± 61.48 gm was observed for males and 2009.50 ± 73.98 gm for females. Higher blood (7.50 ± 0.28 ml) but lower feather (152.50 ± 4.78 gm) weight was found for males; lower blood (7.00 ± 0.91 ml) and higher feather (157.50 ± 4.78 gm) weight were observed in female Pekin ducks. Sex had a minimum effect on dressing weight, and the overall dressing weight of the Pekin duck was 1494.12 gm. Results from the study revealed that male ducks possessed the more weighted head, wing, shank, heart, gizzard, liver, lungs, intestine and giblets was of 85.00 ± 1.91 gm, 146.75 ± 8.23 gm, 45.75 ± 1.43 gm, 21.00 ± 1.78 gm, 74.50 ± 3.47 gm, 55.75 ± 2.46 gm, 24.00 ± 2.27 gm, 118.00 ± 3.34 and 295.50 ± 9 gm, respectively. Although almost similar, the duck-bill 24.50 ± 0.88 gm and spleen (1.00 ± 0.00 gm) weight was measured in male and female ducks. However, a higher fat percentage on body weight (2.10%) was observed in female Pekin ducks than in males (1.97%). In this study, 74% of dressing weight was accounted from the overall live weight with 3.98% head, 1.21% bill, 6.84% wing, 2.22% shank, 2.04% fat, 0.96% heart, 3.61% gizzard, 2.71% liver, 0.05% spleen, 1.11% lungs, 5.76% intestine, and 14.50% giblet weight were also measured from the overall weight.

Major tissue component of Pekin duck

Table 7 shows the different tissue components of Pekin ducks at the Breast, Leg quarter, and Lumbosacral parts of the body. The tissue components of the whole carcass of male and female Pekin ducks were divided into three categories: Breast part, Leg quarter, and Lumbosacral part. From these three different components, the muscle of males (430.75 ± 16.99 gm) and females (414.75 ± 21.48 gm) covered the maximum portion of the total carcass as Pekin was popular as a meat type duck whereas skin weight ranked second (353.25 ± 24.00 gm; 335.75 ± 13.22 gm) and bones covered the third one (257.5 ± 7.28 gm; 251.25 ± 18.69 gm). Muscle and skin from the breast part generally covered the maximum portion of the total carcass, which was found to be 194.75 ± 11.96 gm, and 167.75 ± 13.79 gm in males, respectively, and 188.25 ± 13.63 gm and 154.5 ± 3.41 gm in females Pekin duck, respectively. However, bones of the lumbosacral part covered the maximum portion, where 257.5 ± 7.28 gm in male and 251.25 ± 18.69 gm in female ducks.

Nutrient composition of Pekin ducks' breast and leg quarter muscle

Table 8 represents the measured percentage of nutrients from both male and female Pekin ducks' thigh and breast muscles. Drakes exhibited a higher dry matter of 29.13% compared to ducks' 24.14%, indicating lower water content in drake thigh muscle. Conversely, ducks generally showed higher water content in both thighs at 75.86% and breast muscles at 77.12% compared to drakes. Crude protein levels were similar between drakes and ducks across both muscle types, with slight variations observed. Crude fat content was notably higher in thigh muscles, exhibited at 12.59% in drakes and 14.88% in ducks, than in the breast muscles of drakes (5.12%) and ducks (5.10%). Additionally, ether extract values were higher in thigh muscles (2.74% in drakes and 2.13% in ducks) than in breast muscles of drakes (0.42%) and ducks (0.48%). Ash content was slightly elevated in thigh muscles, with values of 3.86% in drakes and 4.00% in ducks, compared to 3.60% in drakes and 3.33% in ducks' breast muscles, respectively.

Benefit-cost ratio of Pekin duck raising farmers

Table 9 represents the benefit-cost ratio (BCR) of Pekin duck farming in the Dhamrai area of Bangladesh. The various cost components for Pekin duck rearing up to their marketing age (12 weeks) were calculated, including 17.93 USD for feed cost, veterinary expenses of 2.80 USD, housing cost with 10% depreciation of 3.82 USD, the cost involved in family labor engaged 11.38 USD, transportation cost 2.060 USD, and miscellaneous expenses of 1.20 USD. The total cost incurred by farmers was observed at 38.99 USD. The income generated from the Pekin duck farming operation was also assessed, with the average value of family-consumed ducks at 10.97 USD and the value of sold ducks at 51.09 USD, resulting in a total income of 62.05 USD with a calculated Net income of 26.06 USD. The BCR was determined as 1.59, which reflected the efficiency of the duck farming operation and indicated that for every unit of currency spent on duck farming, a return of 1.59 units was generated.

Operational constraints in Pekin duck farming

Farmers faced operational constraints in Pekin duck rearing that were primarily related to the high price of feed, reported by 88% of farmers, followed by the lack of quality ducklings (72%) and the unavailability of ducklings at all times (60%). Attacks by predatory animals were a concern for 52% of duck farmers, while 40%

mentioned the high price of ducklings as a limiting factor. Disease outbreaks were reported as a constraint by 36% of farmers, and 24% reported the lack of vaccines and treatment facilities. About 20% of farmers noted that the profitability of Pekin duck farming was not always guaranteed due to fluctuations in feed prices,

unavailability of quality ducklings, market price volatility for duck meat and eggs, often reduced profit margins, the lack of efficient marketing and distribution channels, which significantly impacted the production costs. These factors were ranked by frequency and identified the challenges in Pekin duck farming presented in Table 10.

Table 6. Carcass characteristics of Pekin duck at 10 weeks of age at Dhamrai Sub-district, Bangladesh, in April 2024

Parameters (g)	Drake		Duck		Overall	
	Mean \pm SE	Percentage	Mean \pm SE	Percentage	Mean \pm SE	Percentage
Live wt.	2025.50 \pm 61.48	100.00	2009.50 \pm 73.98	100.00	2017.50 \pm 44.63	100.00
Feather	152.50 \pm 4.78	7.53	157.50 \pm 4.78	7.84	155.00 \pm 3.27	7.68
Blood	7.50 \pm 0.28	0.37	7.00 \pm 0.91	0.35	7.25 \pm 0.45	0.36
Dressing weight	1494.25	73.77	1494.00	74.35	1494.12	74.06
Head	85.00 \pm 1.91	4.20	75.50 \pm 1.32	3.76	80.25 \pm 2.09	3.98
Bill	24.50 \pm 1.65	1.21	24.50 \pm 0.95	1.22	24.50 \pm 0.88	1.21
Wing	146.75 \pm 8.23	7.25	129.25 \pm 3.19	6.43	138.00 \pm 5.25	6.84
Shank	45.75 \pm 1.43	2.26	43.75 \pm 2.05	2.18	44.75 \pm 1.22	2.22
Fat	40.00 \pm 3.39	1.97	42.25 \pm 6.35	2.10	41.13 \pm 3.36	2.04
Heart	21.00 \pm 1.78	1.04	17.75 \pm 0.85	0.88	19.38 \pm 1.10	0.96
Gizzard	74.50 \pm 3.47	3.68	71.25 \pm 3.25	3.55	72.88 \pm 2.28	3.61
Liver	55.75 \pm 2.46	2.75	53.50 \pm 1.55	2.66	54.63 \pm 1.41	2.71
Spleen	1.00 \pm 0.00	0.05	1.00 \pm 0.00	0.05	1.00 \pm 0.00	0.05
Lungs	24.00 \pm 2.27	1.18	20.75 \pm 1.54	1.03	22.38 \pm 1.41	1.11
Intestine	118.00 \pm 3.34	5.83	114.25 \pm 5.51	5.69	116.13 \pm 3.06	5.76
Giblet wt.	295.50 \pm 9.20	14.59	289.50 \pm 9.49	14.41	292.50 \pm 6.22	14.50

SE: Standard error, wt.: Weight, g: Gram

Table 7. Tissue component of breast, leg quarter, and lumbosacral parts of Pekin ducks at Dhamrai area, Bangladesh in April 2024

Parameters (g)	Pekin drake (Mean \pm SE)				Pekin duck (Mean \pm SE)			
	Breast part	Leg quarter	Lumbosacral part	Total	Breast part	Leg quarter	Lumbosacral part	Total
Muscle	194.75 \pm 11.96	90.0 \pm 2.55	146.00 \pm 2.48	430.75 \pm 16.99	188.25 \pm 13.63	83.50 \pm 5.60	142.75 \pm 2.28	414.75 \pm 21.48
Skin	167.75 \pm 13.79	137.5 \pm 5.80	48.00 \pm 4.41	353.25 \pm 24	154.5 \pm 3.41	128.75 \pm 7.22	52.50 \pm 2.59	335.75 \pm 13.22
Bones	92.50 \pm 2.75	52.75 \pm 2.28	112.25 \pm 2.25	257.5 \pm 7.28	90.50 \pm 5.90	51.25 \pm 1.49	109.50 \pm 3.30	251.25 \pm 18.69

SE: Standard error, g: Gram

Table 8. Nutrient composition of thigh and breast muscle of Pekin ducks reared at Dhamrai, Bangladesh, in April 2024

Parameters (%)	Drake		Duck	
	Thigh muscle	Breast muscle	Thigh muscle	Breast muscle
Dry matter	29.13	24.38	24.14	22.88
Water	70.87	75.62	75.86	77.12
Crude protein	17.57	16.90	17.36	16.70
Crude Fat	12.59	5.12	14.88	5.10
Ether extract	2.74	0.42	2.13	0.48
Ash	3.86	3.60	4.00	3.33

Table 9. Benefit-Cost ratio of Pekin duck farmers at Dhamrai Sub-district, Bangladesh, from January to April 2024

Parameters	Values (USD)
Feed cost	17.94
Veterinary cost	2.61
Housing cost with 10% depreciation	3.82
The cost of family labor involved	11.39
Transportation cost	2.60
Miscellaneous	1.20
Total cost	39.01
The family consumed duck value	10.97
Sold duck value	51.11
Total income	62.08
Net income	23.07
BCR	1.59

BCR: Benefit-cost ratio, USD: United States Dollar

Table 10. Major operational constraints of Pekin duck-rearing farmers at Dhamrai, Bangladesh, during January to April 2024

Parameters	Frequency (n)	Percent	Ranking
Higher feed price	22	88.00	I
Lack of quality duckling	18	72.00	II
Unavailability of ducking at all times	15	60.00	III
Attacked by a predatory animal	13	52.00	IV
The high price of ducking	10	40.00	V
Outbreak of disease	9	36.00	VI
Lack of vaccine and treatment facilities	6	24.00	VII
Profit not guaranteed	5	20.00	VIII

DISCUSSION

According to [Rahman et al. \(2020\)](#), the majority of Pekin ducks raising farmers were over 40 years old, accounting for 46% while 37% were in the middle-aged group (ranging from 25-49 years) in the Bhola district of Bangladesh. In the study of [Rahman et al. \(2009\)](#), a nearly similar result was observed, where 39% of farmers were from the middle-aged category. The mean age of duck-raising farmers of 33 and 36 years with the observation of [Afrin et al. \(2016\)](#), where [Jha et al. \(2015\)](#) also mentioned 52% of young-aged farmers, followed by 36% of middle-aged and 12% of old farmers.

[Rahman et al. \(2020\)](#) categorized the family size of Pekin duck-rearing farmers in the Bhola district, where most of the farmers (50%) belonged to medium family sizes, with 40% having small and Only 10% having large family sizes. [Rahman et al. \(2009\)](#) reported that about 50% of farmers had large families, with an average household size of 07 members per family. A relatively higher average family size (5.8) than the current study of duck-rearing

farmers in the Kishoreganj district was reported by [Afrin et al. \(2016\)](#).

In terms of education, [Rahman et al. \(2020b\)](#) reported that about 43% of the duck farmers in the Bhola district were illiterate, 33% completed primary education, and 24% of the respondents received a secondary level of education. Around 30% of duck farmers attained a primary level of education, 18% and 9% had received a secondary and higher education in the Noakhali and Lakshmipur districts of Bangladesh stated by [Rahman et al. \(2009\)](#). According to [Jha et al. \(2015\)](#), a literacy rate of 48% was recorded, with 28% of farmers receiving primary education. About 20% of farmers received primary education, which was higher, and only 5% had higher secondary or above educational level, which seemed to be lower than the current study reported by [Parvez et al. \(2020\)](#). [Afrin et al. \(2016\)](#) stated that 44% of duck farmers completed their secondary education, 28% completed the primary level, and only 18% completed their higher secondary education in the Kishoreganj district. The current study area may have offered better access to

secondary and higher education, reflecting a higher percentage of farmers with SSC or HSC qualifications. In contrast, other regions might have faced challenges in educational systems, socio-economic constraints, and regional disparities, leading to higher illiteracy rates or lower levels of formal education among farmers.

Rahman *et al.* (2020) mentioned that approximately 67% of the Pekin duck farmers in the Bhola district were housewives, 23% were occupied in business, and 10% provided service along with agriculture. Afrin *et al.* (2016) mentioned that 42% of farmers adopted duck farming as their primary occupation. About 25% of farmers were involved in duck farming with several supplementary occupations in the Kishoreganj district stated by Parvez *et al.* (2020), which was lower than the current study. Rahman *et al.* (2020) found that all of the respondents (100%) in the Bhola district did not have scientific knowledge of Pekin duck farming. Afrin *et al.* (2016) reported that 73% of duck-raising farmers did not receive any training in the Kishoreganj district. However, a higher percentage of trained farmers was observed in the current study area. In the findings of the current study, some differences may be attributed to several factors, including the geographical locations, years of study, variations in the sample populations, and farmers' resource availability compared to those in previous studies.

In the study of Rahman *et al.* (2020), a slightly higher percentage of Pekin duck-rearing farmers (100%) practiced a semi-intensive rearing system in the Bhola district of Bangladesh. About 90% farmers utilized tin and bamboo/betel nut shed houses, which was higher than the current study. About 56.57% of farmers cleaned duck houses 10-15 times in a month, 33.33% followed cleaning practices 5-10 times in a month, and only 10% of farmers cleaned duck houses regularly, which supported the present findings. Around 50-60% of farmers practiced regular cleaning of poultry houses, and 30% followed house cleaning once a week, reported by Alam *et al.* (2014). Rahman *et al.* (2009) stated that the majority of the farmers (67.5%) mainly utilized ponds as the scavenging place for ducks, which was well consistent with the present findings. They also mentioned that 93.5% of farmers provided a separate housing facility at a corner of their premises. Approximately 65.5% of duck houses were constructed of tin and wood. In the findings of Jha *et al.* (2015), it was reported that ducks were raised only on natural feed resources during the rainy season and reared under a scavenging management system. They also stated that 50% of houses were constructed with tin and wood, followed by 30% of straw-bamboo made, only 8.50% used

bamboo, and 11.50% provided houses made with soil and other materials. The results from the study of Jha *et al.* (2015) were closely in agreement with the observation of the present study and explained that the majority of the respondents utilized tin and wood-made houses, which were relatively permanent and long-lasting, as well as lower-cost involvement, which supported the present study. Farmers in the current study area mostly used cost-effective housing materials for constructing duck houses, because most of the farmers did not have enough economic solvency to provide bricks or concrete housing facilities for their Pekin ducks.

Rahima *et al.* (2023) reported that about 97.64% of farmers raised their poultry (Indigenous and Sonali chickens) in semi-scavenging conditions. The majority of respondents (88.82%) utilized tin and bamboo for constructing poultry houses, while a minimum proportion of farmers (11.2%) reared poultry without ensuring adequate housing facilities. These findings strongly aligned with the current outputs. Additionally, they reported that 73.53% of farmers usually cleaned poultry houses, which was supported by the results of the current study. These variations may have arisen due to differences in knowledge and farming practices, the availability of housing materials and resources in the findings across the above studies.

In the case of feeding management, Rahman *et al.* (2020) reported that 20% of the farmers provided natural feed, which was comparatively lower than the results of the current study. However, the study agreed with the present result, where they mentioned that about 80% depended on supplemental feed for Pekin duck-rearing. Farmers were provided supplemental feeds, particularly in the form of wheat bran (74%), commercial feed (13%), and rice polish (13%). Additionally, 87% of the farmers utilized feed ingredients from domestic sources. In a study by Jha *et al.* (2015), they mentioned that 46.50% of duck farmers did not offer any supplemental feed ingredients to their ducks. However, a different observation was seen in the current study, where 100% of farmers were provided additional feed during the entire study period. Parvez *et al.* (2020) explained that 50% of farmers provided supplemental feed ingredients to optimize the egg production of ducks. The major ingredients of additional feeds were paddy, a mixture of rice and broken rice and a combination of rice polish and wheat bran, and a mixture of different feed materials, which strongly supported the present findings. Zahan *et al.* (2016) mentioned that nearly 67% of respondents feed their ducks with rice polish and wheat bran. According to the study of Rahima *et al.*

(2023), approximately 84.71% of duck-raising farmers utilized whole rice as a supplement feed, followed by boiled rice, paddy, broken rice, wheat, and commercial ready-made feed. These findings were almost in agreement with those of the current study. However, a higher percentage of duck-rearing farmers selected locally available feed ingredients and used paddy, rice, and rice bran as a supplemental feed for Pekin duck rearing in the Dhamrai area of Bangladesh. Several variations in outputs were observed due to the study location, financial capability, and proper knowledge of farmers on daily feed requirements and supply were different in the present study compared to the above studies.

Rahman et al. (2020) conducted a study on Pekin ducks in the Bhola district of Bangladesh where they observed the average body weight (gm) of 60.43 ± 2.08 , 113 ± 2.65 , 282.87 ± 9.26 , 743.5 ± 26.48 and 1885 ± 34.56 , respectively at day-old-ducklings with 7 days, 15 days, 30 days and 60 days of age. According to Bhuiyan et al. (2005), the live weight of the Pekin duck was 1763 gm at 9 weeks, and they also suggested that the Pekin breed was superior to both Muscovy and Deshi white ducks in the Sylhet area of Bangladesh. The superior growth performance could be attributed to the fact that the Pekin ducks consumed varying amounts of fallen grains from the paddy fields along with earthworms and small insects during grazing, which helped to meet their daily protein and energy requirements in the body and gain desirable growth in the Pekin ducks. In the study conducted by Rabbani et al. (2019), the growth performance of meat-type Pekin ducks reared under a complete confinement system for 56 days was evaluated by feeding four different diets with varying nutrient concentrations and observed that the mean body weight (gm) of 1530.91, 1546.35, 1518.62 and 1595.13 which were in strong agreement with the findings of the present study. Ghosh et al. (2022) mentioned an impressive mean body weight of 2.003 kg attained by Pekin ducks at 56 days of age under backyard farming conditions. They also observed that the average daily weight gain (ADG) of White Pekin ducks was highest during the 43-56-day period, followed by the 29-42-day period. The mean ADG (gm) for male and female hybrid SM3 Heavy Pekin ducks was 70.9 and 68.9 from up to 49 days of age, reported by Steczny et al. (2017). Alsaffar et al. (2023) mentioned that the body weight (gm) of blue and yellow beak Pekin ducks at 6 weeks of age was 2720.7 ± 80.84 and 2631.14 ± 21.75 . Starcevic et al. (2020) reported the ADG (gm) of STAR 53 medium and SM3 heavy hybrids of Pekin duck up to 7 weeks of age of 177 and 184, under a semi-intensive management system.

The variations in data may have arisen from differences in the duck breeds, the duration of the growth periods, the specific feeding regimes, feed quality, housing, and genetic differences in duck populations across the above studies.

Approximately 80% of the Pekin duck-raising farmers were not conscious of duck diseases reported by Rahman et al. (2020). Alam et al. (2014) mentioned some common diseases, including Duck plague, Duck cholera, and Limber neck poisoning in duck farms in the Mymensingh district of Bangladesh. Jha et al. (2015) explained that the majority of the farmers had incomplete ideas and limited knowledge about duck diseases. A nearly similar finding was observed by Rahima et al. (2023) in the case of Pekin ducks mentioned that Duck plague and Duck cholera were the more frequent duck diseases. They also reported that a relatively lower proportion of respondents (30.50%) did not practice the scheduled vaccination under backyard poultry production, and only 8.82% of farmers vaccinated their poultry.

Regarding treatment sources, 56.67% of farmers had received treatment from a Local Service Provider (LSP), 33.33% from Non-Government Organization (NGO) workers, and 10% from Upazilla Veterinary Hospitals, which were consistent with the current results. A nearly similar output was observed in the present study, where they reported that approximately 90% of the respondents maintained a regular vaccination schedule in the Bhola district. According to Rahman et al. (2009), 85% of farmers in the Noakhali and Lakshmipur districts of Bangladesh did not practice scheduled vaccination against duck diseases. In the study of Zahan et al. (2016), it is mentioned that 60% of farmers regularly vaccinated their ducks. About 65% of the respondents in the Sylhet area of Bangladesh were not aware of the importance of vaccination, and they did not even vaccinate their ducks regularly, whereas only 14.50% followed the regular vaccination schedule stated by Jha et al. (2015). The variations in the results between the present study and the previous findings could have arisen from several factors, including differences in educational opportunity, lack of awareness, and access to resources among farmers in different regions.

Rabbani et al. (2019) reported nearly similar findings compared to the current study, where they mentioned the dressing percentage of 64.50, 64.60, 64.47, and 64.85% for meat-type Pekin ducks reared under concentrate-based four different diets in a complete confinement system up to 56 days of age. Ghosh et al. (2022) calculated the dressing percentage of White Pekin ducks and found the

average dressing percentage of 57.9% which was lower than the earlier studies of [Kokoszynski et al. \(2019\)](#). In the study of [Steczny et al. \(2017\)](#), they mentioned relatively higher average live weight (gm) and carcass weight (gm), wings (%) at 49 days old male (3518 ± 19.3 , 2465 ± 15.7 and 12.6 ± 0.2) with female (3433 ± 18.3 , 2418 ± 11.4 and 12.2 ± 0.3) in hybrid SM3 heavy Pekin ducks. However, a closely similar value for dressing percentage was observed at 70.1 ± 0.2 and 70.4 ± 0.2 . A nearly similar proportion of gizzard, liver, heart, and spleen in hybrid SM3 heavy male and female Pekin ducks was also reported by [Steczny et al. \(2017\)](#) compared to the present study. [Alsaffar et al. \(2023\)](#) stated nearly similar dressing percentages, and the percentage of liver, gizzard, heart, and head of Blue and Yellow beaked Pekin ducks at 6 weeks of age were 72.62, 71.66, 2.60, 2.76, 2.86, 2.96, 0.66, 0.67, 3.94, and 3.91, respectively. According to the findings of [Starcevic et al. \(2020\)](#), the weight of the wings of STAR53 (261 gm) medium and SM3 (269 gm) heavy hybrids of Pekin ducks under a semi-intensive management system was relatively higher compared to the present findings. The breed differences, environmental conditions, such as climate and farming practices, along with the availability of feed ingredients, could have influenced the variations in carcass characteristics and overall yield. Thus, these factors collectively led to differences in the findings between the present study and the above-mentioned findings.

[Starcevic et al. \(2020\)](#) mentioned comparatively higher values than the present findings for breast weight (gm) and drumstick with thigh (gm) of 606, 697, 427, and 419 in STAR 53 medium and SM3 heavy hybrids of Pekin ducks under a semi-intensive management system. According to the study results of [Lukaszewicz et al. \(2011\)](#), duck meat was found to have a higher nutritive value, with the chemical composition of the breast muscle revealed slightly higher protein (20.9-22.2%) and fat content (2.3-3.9%) where the percentage of water content (74.7-75.1) was also closely similar compared to the present results. Conversely, the water (72.5-75.1%) and protein content (18.0-18.9%) of leg muscle were slightly less; however, a higher percentage of fat content (4.6-7.2%) was observed compared to breast muscles. These results were in agreement with the observations of the current study. In the study of [Kokoszynski et al. \(2020\)](#), the nutrient content of Muscovy and Mule ducks was calculated and found that nearly similar water (70.9-72.1%) with higher protein (24.7-27.2%), and less fat content (1.0-1.4%) of their breast and leg muscles than in the present study. In another study by [Khaziev et al. \(2018\)](#), they showed a higher dry matter, protein, and fat

content in the breast muscles of Mule ducks compared to Muscovy ducks, both were mostly popular for meat production. Breed-specific differences in muscle development and fat deposition may have accounted for the higher fat content exhibited in the breast and thigh muscles of Pekin ducks in the current study compared to Muscovy and Mule, especially in female ducks. Pekin ducks are known for their rapid growth and higher fat deposition, particularly in meat production, which might result in a higher fat percentage in their muscles. In contrast, breeds such as Muscovy ducks may have a leaner body composition, as evidenced by their lower fat content in muscle tissues.

In a comparative study conducted by [Bhuiyan et al. \(2005\)](#) where they mentioned that the highest production costs were involved in raising Pekin ducks, while Deshi White ducks had the lowest. Nonetheless, Pekin ducks delivered a robust economic return, reflecting a Benefit-Cost Ratio (BCR) of 1.66. Slightly higher average net returns and BCR than the current study from duck rearing were estimated at 400.41 USD and 1.67 in the Haor areas, were reported by [Sheheli et al. \(2023\)](#). The differences in BCR and net income between the studies are likely due to a combination of regional factors, variations in farming practices, input costs, and market conditions in different study locations.

The outbreak of disease was the first-ranked problem, followed by the high price of feed second, Low prices of duck eggs and meat third, irregular supply of ducklings, and inadequate veterinary services for duck rearing in Haor areas mentioned by [Sheheli et al. \(2023\)](#). Infectious disease outbreaks were the most common constraint in duck rearing, of which Duck plague was the most prevalent one, as stated by [Khan et al. \(2018\)](#). The present findings were consistent with those of [Alam et al. \(2014\)](#), who identified conventional rearing methods, feed scarcity, poor housing facilities, disease outbreaks, inadequate access to vaccines and medicine, and attacks by predatory animals as the major challenges for backyard poultry (chicken and duck) farming in Mymensingh district of Bangladesh. Additionally, [Rahima et al. \(2023\)](#) reported that the most common constraints in backyard poultry farming systems were disease outbreaks, followed by the lack of adequate knowledge and predatory animal attacks. The variations in the constraints faced by farmers across different studies likely arise from differences in regional conditions, farm management practices, access to inputs, and local economic factors. These differences highlight the complexity of Pekin duck farming and the

need for tailored solutions based on specific regional challenges.

CONCLUSION

Pekin duck farming, particularly under existing rearing systems in the Dhamrai area of Bangladesh, offers a lucrative opportunity to become economically viable and get an excellent source of additional income for small to medium-scale rural farmers, especially women farmers. The steady growth performance, favorable carcass characteristics, and positive Benefit-cost ratio indicated a promising future for the sector. However, to enhance the sustainability and profitability of Pekin duck farming, addressing operational constraints such as high feed costs, the availability of quality ducklings, and disease control measures should be prioritized. Additionally, further training and improved access to veterinary care and biosecurity measures could help reduce disease risks and improve overall farm productivity. In-depth research into optimizing feeding strategies and improving disease management practices is required to find out both the sustainable and profitable pathway of Pekin duck farming for rural farmers in the study region, as well as a whole in Bangladesh.

DECLARATIONS

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Availability of data and materials

The data are available upon request from the corresponding author.

Ethical considerations

All authors have ruled and agreed on ethical issues, including fabrication of data, double publication and submission, redundancy, plagiarism, consent to publication, and misconduct for this article to be published with a high scientific quality in the present journal.

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Author's contributions

This research was completed in collaboration among all authors. Razia Khatun and Sharmin Sultana conceptualized and designed the study. Sharmin Sultana, Sydul Islam, and Md. Ashraful Islam implemented the experiment in the community of Pekin duck farmers. Sharmin Sultana wrote the research methodology, study protocol, and the manuscript. Sydul Islam and Md. Ashraful Islam assisted in the data collection and formal analysis. Shamin Ahmed reviewed and edited the manuscript for final submission. Razia Khatun provided guidelines for writing the manuscript and financial support for the research. All Authors read and agreed to the final version of the manuscript.

Competing interests

There is no conflict of Interest regarding this research and manuscript.

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Simulation of Multiple Mediation Variables for Finding the Ideal Model to Improve the Performance of the Chicken Farming Business in Indonesia

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ABSTRACT

The success of chicken farming can be assessed using performance indicators. The present study aimed to investigate internal and external environmental factors, entrepreneurial skills, innovation, financial management, and the business performance of chicken farms in the Penebel District, Indonesia. A total of 51 chicken farmers meeting the criteria were included as the study sample. Data collection methods included interviews, surveys, documentation, and literature review. The analysis employed quantitative descriptive methods, including simple tabulation and generalized structured component analysis software. The feasibility of the initial model was tested, and if any discrepancies were found, the model was re-specified and retested until it achieved overall goodness-of-fit criteria. The simulation model included 11 paths connecting variables. Five path coefficients demonstrated significant effects, while six did not. Significant effects were found between the internal environment and entrepreneurship, the external environment and entrepreneurship, the internal environment and innovation, the external environment and innovation, and the internal environment and financial management. The present findings indicated that entrepreneurship did not serve as a mediating variable. The internal and external environments significantly impacted farmers' entrepreneurial skills. However, entrepreneurial skills did not significantly enhance business performance. Furthermore, internal and external factors influenced innovation, but innovation did not affect business performance.

Keywords: Business competence, Business environment, Business performance, Chicken farming, Financial management, Innovation

INTRODUCTION

Failures in business stem from a failure to understand and accurately identify the conditions of the business environment. Al-Maskari et al. (2019) stated that the external and internal business environments are interconnected and each presents its own challenges for a company. According to Borodakfo et al. (2015) and Toppinen et al. (2019), achieving a deeper understanding of the external and internal environments is crucial for companies to operate effectively, as it enables them to

comprehend the market, consider strategic options, and compare optimal business strategies.

In a business environment that continues to evolve, entrepreneurs should ideally continue to enhance their entrepreneurial competence, innovation, and financial management skills. Entrepreneurial competence is essential for entrepreneurs because it enables them to advance their business, particularly in terms of business quality, coworker satisfaction, and forms of business cooperation with other parties (Kowal and Roztock, 2015). Entrepreneurial competence plays a crucial role in

implementing strategic business planning, including creating a vision and developing long-term priorities. Strategic business focuses on resource management, which can strengthen operations and adjust the company's direction according to environmental changes (Renfors, 2019). Furthermore, Nikitina and Lapiņa (2019) stated that entrepreneurial competence is a primary factor for effective business management in modern times, and this competence should be aligned with the interests of all stakeholders to have a positive impact on the business. In addition to entrepreneurial competence, innovation is a crucial factor in a business's progress. Innovation is closely related to the discovery of new combinations of resources that are generally more effective than existing ones (Tammekivi et al., 2024). Through business innovation, entrepreneurs can create more economic value by adding extra value to their innovations (Anokhin et al., 2016). The positive impact of innovation on the company is holding several dominant market positions, achieving long-term monopoly profits, generating substantial profits, and securing additional marginal market profits. The impact of innovation varies significantly by company, depending on the types of innovation they implement (Crowley and McCann, 2015). In addition, innovation plays a strategic role in business performance, as it can trigger increased business survival, facilitate significant business growth, and serve as a dynamic step in supporting business growth policies (Surya et al., 2021). In innovating, entrepreneurs should consider strategies that align with the aim of the business targets (Jo and Jang, 2022).

Financial management plays a crucial role in determining a business's stability. Effective financial management can significantly predict compulsive purchasing behavior in a business and lessen the impact of materialistic values on purchases (Alemis and Yap, 2013). An effective financial management system is essential for controlling costs in businesses, as it typically involves multiple parties (Xiao, 2016). Chen et al. (2023) stated that financial management offers practical benefits for policymakers, as it can be an effective way to enhance company performance and foster a sustainable business environment through proper implementation.

Poultry meat, particularly chicken, is an essential source of high-quality animal protein (Vlaicu et al., 2024). Chicken meat is superior to red meat because it contains less cholesterol and more vitamins, as well as balanced nutrients such as amino acids, energy, and micronutrients (Ali et al., 2019). Additionally, Household chicken farming helps to meet food security and nutrition goals (Ibrahim, 2020). On a broader scale, chicken meat production is more accessible, faster, and affordable than

mammalian meat production (Chunga et al., 2023; Connolly and Campbell, 2023). With the growing global population, there is a greater demand for high-quality protein sources; hence, maintaining food supplies, especially chicken meat, is crucial (Pius et al., 2021; Castro et al., 2023). Therefore, the availability of stable and affordable chicken meat is critical to preventing malnutrition and nutritional deficiencies in society. Consequently, chicken farming should be efficiently managed to ensure sustainability and to provide high-quality chicken meat (Gržinić et al., 2022). Sustainable chicken farming can be achieved by producing high-quality livestock that is consumer-friendly, financially rewarding for farmers, and has lower environmental effects (Castro et al., 2023). Internal and external factors, such as chicken genetics, breeding techniques, farmers' skills, financial management, processing and packaging, transportation and distribution, marketing, consumer preferences, and regulations, all affect the sustainability of chicken farming (Zielińska-Chmielewska et al., 2021; Yang et al., 2024). Farmers' entrepreneurial skills, experience, and farm management competence are critical factors influencing the sustainability of the poultry business (Ramukhithi, 2023). Furthermore, a positive relationship exists between entrepreneurial competence and both financial performance and operational efficiency in farming (Nieuwoudt et al., 2017). Poultry farming integrates all the critical aspects of business principles, environmental awareness, competence, innovation, and financial management while also serving as a sector with high economic, nutritional, and social importance.

The present study aimed to enhance the performance of chicken farming enterprises in Penebel District, Indonesia, by investigating the mediating roles of entrepreneurial skills, innovation, and financial management links to internal and external environmental dynamics and business outcomes, to analyze and simulate these mediating factors to develop a comprehensive model for enhancing business performance.

MATERIALS AND METHODS

Study area

Penebel district is located at coordinates 8°26'13.718" S 115°8'32.791" E, Indonesia. The Penebel district borders the Baturiti district and Marga district to the east, Buleleng Regency to the north, Pupuan district, Selamadeg district, and Selamadeg Timur district to the west, and Kerambitan district and Tabanan district to the south (Figure 1). The Penebel district is renowned for its successful agricultural and livestock sectors. Agricultural sector commodities, especially fertile rice farming, as well

as plants such as coffee, vegetables, and fruits, are also widely cultivated in this area. At the same time, the potential of the livestock sector is chicken, cattle, and pig farming. Overall, the Penebel district is an area rich in natural and cultural potential, which provides a calm and comfortable atmosphere. In February 2024, the population of Penebel village was 4,326 people with 1,528 heads of families, most of whom were farmers and ranchers (Statistical Agency of Tabanan Regency, 2024).

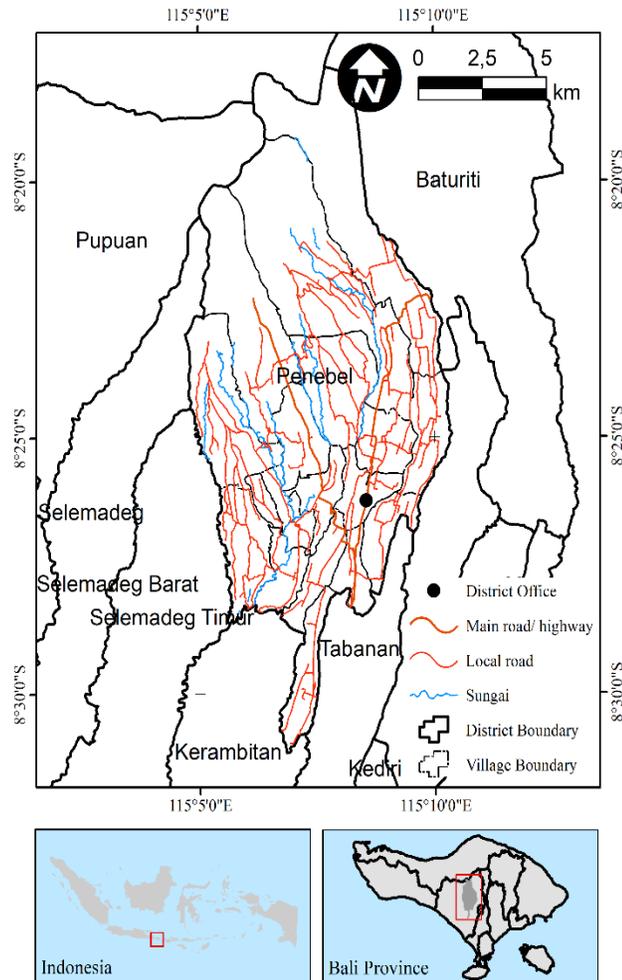


Figure 1. Study location in the Penebel district, Indonesia

Population and samples

The population in the present study comprised poultry farmers in the Penebel district who possessed more than 3,000 chickens. The population was primarily concentrated in three villages with the highest number of farmers, namely Jatiluwih, Senganan, and Babahan. According to the 2022 livestock business report (USPET) of Tabanan Regency, all 51 farmers were included as respondents for the present study; consequently, a census

sampling method was employed, whereby the entire population served as the sample.

Data collection

The present study employed interviews as the primary data collection method, involving a process of direct communication through verbal questions and answers with the chicken farm owners. Two interview methods were employed, including structured interviews using a prepared questionnaire and in-depth interviews. The purpose of these interviews was to collect information that would address the study's objectives through in-person interactions between the interviewer and the chicken farm owner.

Out of the 17 villages in the Penebel district, three villages with the highest number of farmers (Jatiluwih, Senganan, and Babahan) were selected as the study sites. The villages were chosen because of their high density of poultry farming activities, which provided a comprehensive overview of the actual conditions and primary challenges in the Penebel district, Indonesia. Additionally, a survey was conducted using a questionnaire to collect data on internal and external environmental conditions, entrepreneurial competence, innovation, financial management, and business performance of chicken farm businesses in the district. The documentation method and literature study were then employed to collect data and literature related to chicken farming businesses in the district. All participants involved in the survey provided informed consent before their participation. The data was collected anonymously and used solely for academic and study purposes.

Variables

The present study employed six study variables measured through 49 indicators (Table 1). Each variable was assessed using a Likert scale, a rating instrument designed to capture respondents' opinions, attitudes, and motivations. Respondents could choose from a range of answers, including strong agreement, strong disagreement, and a neutral option in between (Tanujaya *et al.*, 2022). The Likert scale consisted of statements or questions with response options of very good (VG), good (G), fairly good (FG), not good (NG), and not very good (NVG). Scores for each question ranged from one (not very good) to five (very good), based on the six study variables and 49 indicators. Respondents were asked to select the option that best suited their condition in relation to the statements or questions presented in the questionnaire.

Table 1. Variables and indicators for the simulation of multiple mediations in the chicken farming business performance in Indonesia

Variable (Code)	Indicator (Code)
Internal environment (LI)	Functional management (IS 1.1)
	Marketing (IS 1.2)
	Finance/accounting (IS 1.3)
	Production operations (IS 1.4)
	Research and development (LI1.5)
External environment (LE)	Bargaining power of buyers (LE 1.1)
	Product substitutes (LE 1.2)
	Economic power (LE 1.3)
	Social power (LE 1.4)
	Cultural power (LE 1.5)
	Demographic power (LE 1.6)
	Political power (LE 1.7)
	Governmental and legal power (LE 1.8)
	Technological power (LE 1.9)
Entrepreneurship competence (KW)	Making decisions under uncertainty (KW 1.1)
	Process adding value (KW 1.2)
	Ability to cope with failure (KW 1.3)
	Desire to grow (KW 1.4)
	Detecting and exploiting opportunities (KW 1.5)
	Self-concept (KW 1.6)
	People management skills (KW 1.7)
	Logical analytical skills (KW 1.8)
	Intellectual skills (KW 1.9)
	Interpersonal skills (KW 1.10)
	Adaptability skills (KW 1.11)
Innovation (I)	Product quality (I 1.1)
	Product development (I 1.2)
	Cost savings (I 1.3)
	New business (I 1.4)
	Marketing techniques (I 1.5)
	New marketing media (I 1.6)
	Developing new services (I 1.7)
	Creating new customer interactions (I 1.8)
Financial management (MK)	Planning (MK 1.1)
	Budgeting (MK 1.2)
	Management (MK 1.3)
	Searching (MK 1.4)
	Fund retention (MK 1.5)
	Controlling (MK 1.6)
	Auditing (MK 1.7)
	Financial reporting (MK 1.8)
Business Performance (KU)	Business scale level (KU 1.1)
	Profitability (KU 1.2)
	Market share (KU 1.3)
	Employment growth (KU 1.4)
	Sales growth (KU 1.5)
	Timeliness (KU 1.6)
	Cost-effectiveness (KU 1.7)
	Market growth (KU 1.8)

Data analysis

The respondents’ answers were analyzed using descriptive statistical methods. The percentage of

respondents who selected each indicator was calculated using Formula 1, where the proportion (P) is obtained by dividing the number of respondents in a given category (fi) by the total number of respondents (Σfi) and multiplying by 100.

$$P = \frac{f_i}{\sum f_i} \times 100\% \quad \text{Formula 1}$$

Furthermore, to measure the variability of responses, the standard deviation (δ) was computed using Formula 2 (Curran-Everett, 2008). This formula accounts for the distribution of individual values (X) from the mean (x) in relation to the total number of samples (n).

$$\delta = \sqrt{\frac{\sum(X-x)^2}{(n-1)}} \quad \text{Formula 2}$$

The criteria for interpreting the scores were calculated using the class interval method (de la Rubia, 2024). The lowest score was one, and the highest was five, yielding a range of $R = 5 - 1 = 4$. From this, the interval width was calculated as $w = 4/5 = 0.8$. The interpretation of questionnaire responses, categorized by interval and category, is presented in Table 2. Subsequently, respondents’ answer scores were measured using Formula 3.

$$R = \frac{R_s}{n} \times 100\% \quad \text{Formula 3}$$

R_s represents the average respondent’s answer score, and n represents the maximum respondent’s answer score.

The criteria for interpreting respondents’ answer scores were calculated using the class interval method (de la Rubia, 2024). The lowest value was 0% and the highest was 100%. The interpretation of questionnaire responses, presented by percentage scores and categories, is shown in Table 3.

The effectiveness of simulating entrepreneurial competency models, innovation, and financial management as mediating variables between the internal and external environments on the performance of chicken farming businesses in the Penebel district, Indonesia, was analyzed using generalized structured component analysis (GSCA). The first stage in the SEM model analysis was to test the feasibility of the initial model. If any discrepancies were identified, the model was adjusted and testing resumed until an adequate level of feasibility was reached, based on overall goodness-of-fit criteria. The next step involved examining the relationships among variables, including mediators, using the structural model evaluation. The GSCA analysis in the present study was conducted through several stages (Jung et al., 2012; Ramadhani et al., 2023). The process began with collecting interview results from chicken farm owners in the Penebel district, Indonesia, regarding internal and external environmental conditions, entrepreneurial competence, innovation, financial management, and business performance. The

interview data were then converted into ordinal data using a 5-point Likert scale, entered into Microsoft Excel, and grouped according to analytical requirements. Subsequently, a GSCA model was constructed using GSCA Pro Windows 1.2.1.0 software. The tabulated data from Excel were imported into the GSCA program, where a path diagram of the variables, including internal and external environmental conditions, entrepreneurial competence, innovation, financial management, and business performance, was compiled. Indicator estimates were generated for each variable, and the variables were connected through an Add Path process to establish the GSCA model framework.

Mediation testing was then performed by examining coefficient differences (Hwang et al., 2023). This procedure involved assessing the direct and indirect effects of independent variables on dependent variables, both with and without the mediation of intervening variables. The role of the mediation variables was classified into four categories, namely, complete mediation, partial mediation, non-mediation, or no mediation, depending on the significance and comparative strength of coefficients. If the significance test was not valid, the analysis returned to the path diagram stage for re-specification, after which the subsequent steps were repeated (Hermanu et al., 2024). Finally, the model was tested and its overall fit evaluated. The model framework representing the three objectives of the present study is presented in Figure 2.

Structural model evaluation was conducted using path coefficients and their significance levels. Path coefficients (Pij) indicated the direct effect of exogenous variables (j) on endogenous variables (i), ranging from -1 to +1, with values closer to the extremes reflecting stronger relationships (Chaitanya et al., 2024; Haji-Othman et al., 2024). The study framework is shown in Figure 3.

Table 2. Intervals and categories for the questionnaire in the present study

No	Interval	Category
1	1.0 – 1.8	Not very good
2	1.8 ≥ 2.6	Not good
3	2.6 ≥ 3.4	Fairly good
4	3.4 ≥ 4.2	Good
5	4.2 ≥ 5.0	Very good

Table 3. Scores, percentages, and categories for the questionnaire in the present study

No	Score (%)	Category
1	20 - 36	Not very good
2	36 - 52	Not good
3	52 - 68	Fairly good
4	68 - 84	Good
5	84 - 100	Very good

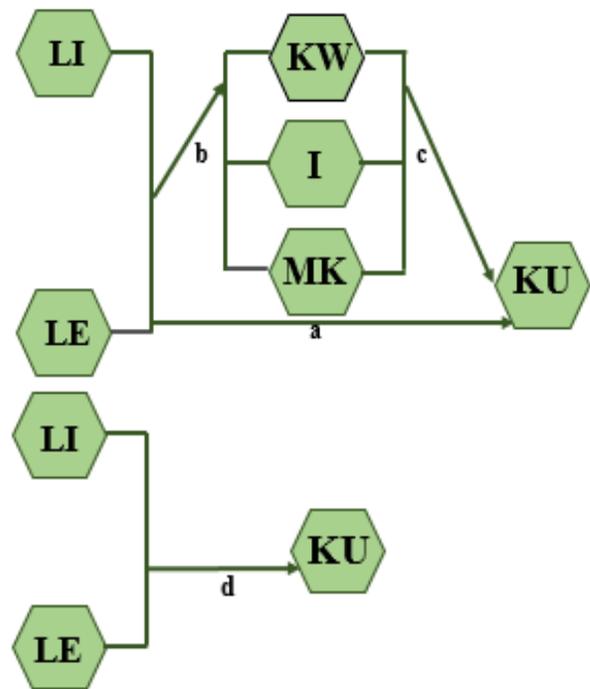


Figure 2. The generalized structured component analysis method was used in the present study. On the left: The GSCA model framework is successfully mediated by entrepreneurship, innovation, and financial management competencies. On the right: The GSCA model framework is not mediated by entrepreneurship, innovation, and financial management competencies. LI: Internal environment, LE: External environment, KW: Entrepreneurial competence, I: Innovation, MK: Financial management, KU: Business performance.

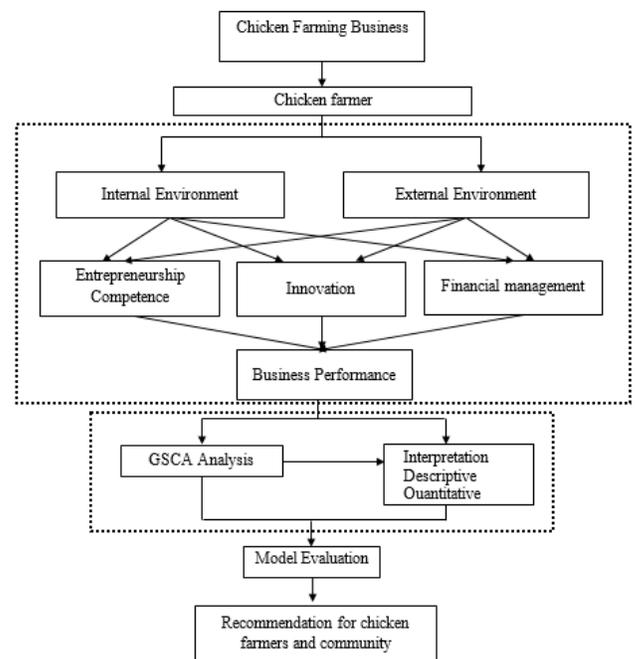


Figure 3. Study diagram

RESULTS AND DISCUSSION

Table 4 presents the descriptive statistics of the internal environment indicator in chicken farming in the Penebel district, Indonesia. The highest mean score among the internal environment indicators was for the marketing indicator (LI1.2), indicating that chicken farmers could effectively sell their eggs without concern for unsold stock. Marketing was often handled through intermediaries, with distribution reaching beyond Tabanan to places such as Denpasar. Conversely, the lowest mean score was in the development indicator (LI1.5), indicating limited efforts in this area. Farmers generally relied on traditional methods, and financial constraints, along with concerns about possible failure, discouraged investment in research and development. Consistent with the present findings, [Khan et al. \(2024\)](#) observed that financial barriers and risk considerations limited farmers' willingness to pursue innovation.

Table 5 indicates descriptive statistics of the external environment. An average standard deviation of 0.58 with a score of 55.47%, categorized as poor. External environmental management in chicken farming was still limited. The highest average score was recorded in the government and legal strength indicator (LE1.8), with a score of 4.31 or 86.27%, categorized as good. This result reflected the government's role in setting regulations related to animal health, safety, and environmental guidelines for livestock businesses, as well as its efforts to socialize these regulations. Most chicken farmers had successfully implemented the required guidelines in their operations. Conversely, the lowest average score was found in the social strength indicator (LE1.4), with a score of 1.47 or 29.41%, categorized as poor. Social strength referred to cooperation or partnerships with other local farmers. Field conditions indicated that such partnerships were still minimal, as many farmers preferred to operate independently, believing their businesses could continue effectively despite several challenges.

Table 6 demonstrates the standard deviation, mean score, and categories of Entrepreneurship in the Penebel district, Indonesia. An average standard deviation of 0.66 with an average score of 76.86%, categorized as sufficient. Chicken farmers demonstrated adequate entrepreneurial competence, with several aspects of entrepreneurship being applied in managing their businesses. The highest average score was found in the ability to make decisions under uncertainty (KW1.1), with a score of 4.75 or 94.90%, categorized as very good. Uncertainty in chicken farming included price fluctuations, pest and disease

outbreaks, weather variability, and other external factors. Farmers generally considered their decisions effective in addressing these challenges. For instance, the farmers routinely administered vaccines and medicines to manage disease risks. To mitigate the impact of price fluctuations, farmers prepared savings or took loans to avoid bankruptcy. In contrast, the lowest average score was observed in the value-added process indicator (KW1.2), with a score of 1.92 or 38.43%, categorized as poor. [Adun et al. \(2024\)](#) describe value-added as enhancing a product's worth through activities such as processing, relocation, or storage. However, chicken farmers in the Penebel district, Indonesia, did not participate in additional processing of primary or by-products.

Table 7 illustrates the descriptive statistics of financial management in the Penebel district, Indonesia. An average standard deviation of 0.66 with an average score of 68.48%, categorized as sufficient. The highest score was found in the financial control indicator (MK1.6), with a score of 4.73 or 94.51%, categorized as very good. Financial control was implemented by identifying and addressing financial deviations that occurred in chicken farming operations. Farmers considered financial control a crucial aspect, and the majority consistently applied it to anticipate potential problems in their businesses. Conversely, the lowest score was recorded in the fund storage indicator (MK1.5), with a score of 2.80 or 56.08%, categorized as poor. Farmers faced difficulties in saving funds from their chicken businesses due to frequent fluctuations in egg prices, which resulted in unstable income and limited their ability to save consistently. As noted by [Kalangi et al. \(2024\)](#), volatile egg prices made it difficult for farmers to maintain regular savings from their profits.

Table 8 presents the standard deviation, mean score, and categories of innovation in the Penebel district, Indonesia. The average standard deviation was 0.47, with an average score of 43.33%, categorized as poor. Chicken farming businesses in the Penebel district still lacked innovation, as most farmers managed their operations conventionally and followed established practices. The highest score was recorded in the product quality indicator (I1.1), which reached 100% in the very good category. Farmers considered product quality, particularly chicken eggs, the most critical aspect of their businesses, and they continued to make improvements in producing high-quality products. In contrast, the lowest score was in the new business indicator (I1.4), at 21.57%, categorized as poor. The indicator assesses the development of novel farming techniques, but farmers demonstrated minimal

innovation, largely adhering to traditional methods (Molina, 2021).

Table 9 demonstrates the standard deviation, mean score, and categories of business performance in the Penebel district, Indonesia. The average standard deviation was 0.71, with an average score of 54.85%, categorized as poor. Overall, chicken farmers faced significant challenges, particularly fluctuations in feed and egg prices, which led to instability in their business performance. The highest score was found in the timeliness indicator (KU1.6), which reached 4.53 or 90.59%, categorized as very good. The present results reflected the ability of farmers to maintain timely production processes, such as ensuring proper chicken care so that hens began laying eggs within the expected age range of 18 to 22 weeks. Conversely, the lowest score was recorded in the sales growth indicator (KU1.5), at 1.53 or 30.59%, categorized as poor. Limited capital and highly variable income made it difficult for farmers to expand their flocks, thereby restricting the growth of egg sales (Tenza *et al.*, 2024).

Although farmers may possess adequate entrepreneurial competencies, external factors such as fluctuations in feed and egg prices have more substantial and immediate influences on business performance. These external challenges directly affected production costs and revenue streams, thereby undermining the stabilizing role of internal mechanisms. In these contexts, leadership abilities, financial strategies, and innovation cannot fully protect farmers from market-driven risks. Smallholder chicken farms are structurally vulnerable, meaning that external market conditions, such as fluctuating prices, can easily outweigh the benefits of their internal skills and efficiencies.

Table 10 presents the path coefficients for each variable. A coefficient is considered statistically significant when the absolute critical ratio (CR) value exceeds 1.96, corresponding to the significance level ($p < 0.05$). This threshold indicates that there is less than a 5% probability that the observed relationship occurred by chance, thereby supporting the reliability of the estimated effect (Di Leo and Sardaneli, 2020). Conversely, CR values below this threshold suggest that the relationship is not statistically significant, implying that the corresponding path does not contribute meaningfully to the model. In the simulation model, there were 11 path relationships among variables, with five path coefficients showing significant effects and six showing insignificant effects.

The path coefficient from the internal environment to entrepreneurship was 2.792, indicating a positive effect.

The Internal environment significantly influenced entrepreneurship ($p < 0.05$). Marketing functions in livestock businesses, such as customer analysis, product or service sales, product and service planning, pricing, distribution, marketing research, and opportunity analysis, support the development of self-concept, people management skills, and intellectual abilities. Additionally, marketing and financial/accounting activities in chicken farming have shaped farmers' entrepreneurial traits, including decision-making, leadership, and knowledge.

The path coefficient from the external environment to entrepreneurship was 4.051, showing a positive and significant effect ($p < 0.05$). Factors such as product substitution, economic strength, and demographic strength significantly impacted farmers' self-concept, management skills, and intellectual abilities. These external factors impact the resilience of chicken farming, prompting farmers to enhance their entrepreneurial skills in order to sustain their operations. The dynamic economic conditions of the chicken farming sector, particularly price fluctuations, demand variations, and supply shifts, motivate farmers to enhance their intellectual and managerial capacities to adapt to market conditions. The path coefficient from the internal environment to innovation was 3.321, indicating a positive and significant effect ($p < 0.05$). The internal environment, shaped by marketing and financial/accounting indicators, significantly impacted farmers' ability to develop marketing techniques and create new services ($p < 0.05$). Market conditions drive innovation in techniques that meet industry needs. At the same time, financial factors influence decisions to offer new services such as forming partnerships, investing, joining groups, or developing alternative payment systems. For example, downturns in financial conditions often lead farmers to form partnerships to reduce risks. In contrast, the path coefficient from the external environment to innovation was -4.017, showing a significant negative influence ($p < 0.05$).

External factors, such as product substitution, economic, and demographic strength, tend to restrict rather than promote innovation in marketing and services. Finally, the path coefficient from the internal environment to financial management was 2.333, indicating a positive and significant effect ($p < 0.05$). Internal conditions, particularly marketing and financial factors, play a crucial role in shaping financial management practices, including planning, budgeting, sourcing, and saving.

The analysis indicated that entrepreneurship did not function as a mediating variable. Both internal and

external environments significantly influenced farmers' entrepreneurial competencies, but these competencies did not translate into improved business performance. Similarly, the internal and external environments significantly affected innovation, yet innovation had no impact on performance. Innovation in chicken farming in the Penebel district, Indonesia, has remained limited, particularly in terms of technology adoption, as most farmers continue to rely on conventional practices. This

finding aligns with the results of Wang et al. (2023) and Majeed et al. (2023), who suggested that innovation in renewable technology remains limited due to farmers' financial constraints in adopting technologies. The present study revealed that financial management did not affect business performance. Some farmers did not practice effective financial management in their operations, instead managing their farms informally.

Table 4. Descriptive statistics of the internal environment indicator in chicken farming in the Penebel district, Indonesia (2024)

Indicator	Standard deviation	Average score*	Score (%)	Score category
Internal environmental				
Function management (li1.1)	0.84	3.24	64.71%	Moderate
Marketing (li1.2)	0.73	3.47	69.41%	Moderate
Finance/accounting (li1.3)	0.48	2.35	47.06%	Poor
Operation production (li1.4)	0.42	3.16	63.14%	Fair
Research and development (LI1.5)	0.61	1.71	34.12%	Poor
Average	0.62	2.78	55.69%	Fair

Table 5. Descriptive statistics of the external environment indicator in chicken farming in the Penebel district, Indonesia (2024)

Indicator	Standard deviation	Average score*	Score (%)	Score category
External environment				
Buyer bargaining power (LE1.1)	0.50	3.57	71.37%	Moderate
Product substitution (SP; LE1.2)	1.44	2.14	42.75%	Poor
Economic strength (KE; LE1.3)	0.60	3.20	63.92%	Fair
Social strength (KS; LE1.4)	0.88	1.47	29.41%	Poor
Cultural strength (KB; LE1.5)	0.50	3.47	69.41%	Moderate
Demographic strength (KD; LE1.6)	1.06	3.39	67.84%	Moderate
Political strength (KP; LE1.7)	1.25	2.04	40.78%	Poor
Government and legal strength (KPH; LE1.8)	0.47	4.31	86.27%	Good
Technological strength (KT; LE1.9)	0.99	1.88	37.65%	Poor
Average	0.86	2.83	56.60%	Fair

Table 6. Descriptive statistics of the entrepreneurship indicator in chicken farming in the Penebel district, Indonesia (2024)

Indicator	Standard deviation	Average score*	Score (%)	Score category
Entrepreneurship				
Decision-making under uncertainty (KW1.1)	0.52	4.75	94.90%	Very Good
Value-adding process (KW1.2)	1.68	1.92	38.43%	Poor
Failure management (KW1.3)	0.66	4.37	87.45%	Good
Growth orientation (KW1.4)	0.63	4.25	85.10%	Good
Opportunity detection and exploitation (KW1.5)	1.08	4.14	82.75%	Good
Self-concept (KW1.6)	0.87	4.08	81.57%	Good
People management skills (KW1.7)	0.84	4.25	85.10%	Good
Analytical logic skills (KW1.8)	0.42	3.78	75.69%	Moderate
Intellectual ability (KW1.9)	0.57	2.57	51.37%	Poor
Interpersonal skills (KW1.10)	0.52	4.23	85.10%	Good
Adaptability skills (KW1.11)	0.70	3.90	78.04%	Moderate
Average	0.77	3.84	76.86%	Moderate

Table 7. Descriptive statistics of the financial management indicator in chicken farming in the Penebel district, Indonesia, 2024

Indicator	Standard deviation	Average score*	Score (%)	Score category
Financial management				
Planning (MK1.1)	0.74	2.88	57.65%	Fair
Budgeting (MK1.2)	0.83	2.84	56.86%	Fair
Management (MK1.3)	0.51	3.76	75.29%	Moderate
Funding disbursement (MK1.4)	0.24	4.06	81.18%	Good
Fund storage (MK1.5)	0.69	2.80	56.08%	Fair
Control (MK1.6)	0.57	4.73	94.51%	Very good
Auditing (MK1.7)	0.66	3.14	62.75%	Fair
Financial report (MK1.8)	0.99	3.18	63.53%	Fair
Average	0.66	3.42	68.48%	Moderate

Table 8. Descriptive statistics of the innovation indicator in chicken farming in the Penebel district, Indonesia, 2024

Indicator	Standard deviation	Average score*	Score (%)	Score category
Innovation				
Product quality (I1.1)	0.00	5.00	100.00%	Very good
Product development (I1.2)	0.80	1.63	32.55%	Poor
Cost-saving measures (I1.3)	0.66	2.92	58.43%	Fair
New business (I1.4)	0.34	1.08	21.57%	Poor
Marketing technique (I1.5)	0.81	2.10	41.96%	Poor
New marketing media (I1.6)	0.40	1.20	23.92%	Poor
Developing new services (I1.7)	0.73	1.41	28.24%	Poor
Engaging with new customers (I1.8)	0.00	2.00	40.00%	Poor
Average	0.47	2.17	43.33%	Poor
Business performance				
Business scale level (KU1.1)	0.69	2.86	57.25%	Fair
Profitability (KU1.2)	0.56	2.35	47.06%	Poor
Market share (KU1.3)	0.81	2.47	49.41%	Poor
Workforce growth (KU1.4)	0.50	2.10	41.96%	Poor
Sales growth (KU1.5)	0.92	1.53	30.59%	Poor
Timeliness (T; KU1.6)	0.50	4.53	90.59%	Very good
Cost-effectiveness (C; KU1.7)	0.80	3.27	65.49%	Moderate
Market growth (PPR; KU1.8)	0.91	2.82	56.47%	Fair
Average	0.71	2.74	54.85%	Fair

Table 10. Path coefficients

Number	Path coefficients	Estimate	SE	CR
1	LI→KW	0.402	0.144	2.792*
2	LE→KW	0.474	0.117	4.051*
3	LI→I	0.744	0.224	3.321*
4	LE→I	-0.711	0.177	-4.017*
5	LI→MK	0.385	0.165	2.333*
6	LE→MK	-0.207	0.236	-0.877
7	LI→KU	-0.054	0.237	-0.228
8	LE→KU	0.111	0.283	0.392
9	KW→KU	-0.243	0.266	-0.914
10	I→KU	0.043	0.238	0.181
11	MK→KU	-0.191	0.154	-1.240

LI: Internal environment, LE: External environment, KW: Entrepreneurial competence, I: Innovation, MK: Financial management, KU: Business performance, SE: Standard error, CR: Critical ratio. Notes: *: Significant at level of 5% ($p < 0.05$).

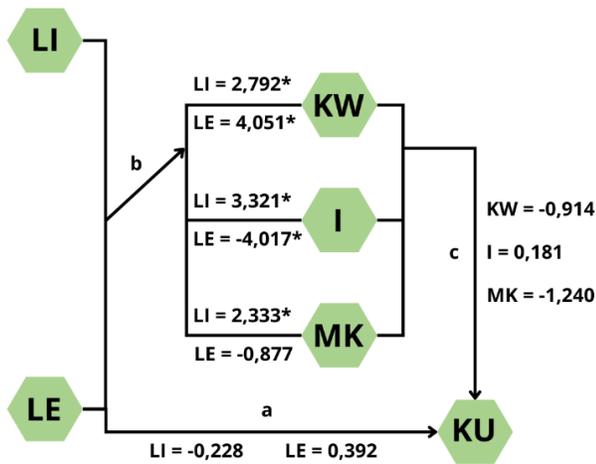


Figure 4. Mediating variables, internal and external environments of chicken farming business in the Penebel district, Indonesia, 2024. LI: Internal environment, LE: External environment, KW: Entrepreneurial competence, I: Innovation, MK: Financial management, KU: Business performance.

The simulation of the entrepreneurial competency model, with innovation and financial management as mediating variables between the internal and external environments and the performance of chicken farming businesses in the Penebel district, is illustrated in Figure 4. The effectiveness of the mediating variables in the model was assessed by examining the significance of each variable's path coefficient value, and they were then grouped into perfect mediation, partial mediation, or non-mediating variables.

The test results on the effectiveness of entrepreneurship as a mediating variable revealed that entrepreneurship was not a mediating variable for either the internal or external environments (Liu et al., 2024). The CR value of criterion b from the internal environment to entrepreneurship indicated a significant difference at 2.792 ($p < 0.05$), while criterion b from the external environment to entrepreneurship indicated a significant difference at 4.051 ($p < 0.05$). However, in criterion c, the CR value from entrepreneurship to business performance was insignificant at -0.914. Thus, although criterion b was significant, the insignificance of criterion c indicated that entrepreneurship was not a mediating variable for the relationship between the internal and external environments.

Entrepreneurship indicated no significant effect on the performance of chicken farming businesses. Field study revealed that the entrepreneurial competence of farmers in managing their businesses had not been sufficient to drive performance. While the internal and external environments greatly influenced entrepreneurial

competence, entrepreneurship still had a limited impact on improving performance. The performance of chicken farming businesses was significantly affected by price fluctuations, particularly in feed and egg prices. Although entrepreneurial competence had improved, it was insufficient to counteract these fluctuations. Although the internal and external environments significantly influenced entrepreneurial competence, the mediating effect of entrepreneurial competence on business performance was not established.

The current outcome was primarily due to the absence of a significant relationship between entrepreneurial competence and business performance. In the Penebel district of Indonesia, poultry farming offers opportunities to develop entrepreneurial skills and enhance decision-making. However, these improvements have not yet resulted in noticeable improvements in business performance. The external shocks, especially fluctuations in egg and feed prices, appeared to overshadow the potential contributions of entrepreneurial competence. These market fluctuations had a greater impact on performance outcomes, rendering the role of entrepreneurial competence a statistically insignificant mediator. This finding suggests that, while competence is essential, its impact on performance is highly contingent upon stable market conditions and complementary support, such as access to capital, innovation, and technology adoption.

The current results on the effectiveness of innovation as a mediating variable were conducted through significance testing of the CR value. The CR value of criterion b from the internal environment to innovation was significant at 3.321, while criterion b from the external environment to innovation was significant at -4.017 ($p < 0.05$). However, in criterion c, the CR value from innovation to business performance was insignificant at 0.181. Therefore, innovation was not a mediating variable between the internal and external environments. Innovation demonstrated no significant effect on the performance of chicken farming businesses. Innovation was minimal in the Penebel district, Indonesia, particularly in technology adoption. Field interviews and observations revealed that nearly all farmers continued to rely on conventional methods. Innovation in renewable technology was rare due to farmers' limited capital. Production processes generally adhered to traditional practices, as farmers avoided the risks linked to new methods. Consequently, innovation had less influence on performance. It was necessary to enhance innovation, knowledge sharing, and technology transfer in the chicken farming industry. The current results on the effectiveness of financial management as a mediating variable also

relied on significance testing using the CR value. The CR value of criterion b, from the internal environment to financial management, was significant at 2.333 ($p < 0.05$), whereas the CR value of criterion b, from the external environment to financial management, was insignificant at -0.877. The CR value of criterion c, from financial management to business performance, was also insignificant at -1.240. Therefore, financial management was not a mediating variable between the internal and external environments. Additionally, financial management indicated no significant effects on business performance. Some farmers did not engage in financial practices such as bookkeeping and instead performed their businesses informally. Other farmers applied financial management such as planning, recording, controlling, and saving, but only at a basic level. This basic form of financial management was not enough to enhance the financial performance of chicken farming businesses. Overall, the simulation results indicated that entrepreneurial competence, innovation, and financial management did not function as mediating variables between the internal and external environments and business performance in the Penebel district, Indonesia. Furthermore, the internal and external environments did not have a direct and significant impact on performance. Other external factors strongly influenced outcomes, especially fluctuations in egg and feed prices. Farmers in the Penebel district struggled to improve performance due to these price changes, as they acted as price takers with no influence over market rates. Rising feed costs increased production expenses, while unstable egg prices resulted in significant fluctuations in income. The entrepreneurial skills, innovation, and financial management of farmers were not enough to overcome these fluctuations and therefore did not provide a solution for improving the performance of chicken farming businesses.

CONCLUSION

The present study indicated that the performance of chicken farming businesses in the Penebel district, Indonesia, remained weak, particularly due to minimal innovation and limited financial capacity. Entrepreneurial competence, innovation, and financial management did not function as mediating variables among internal and external environments and business performance. Furthermore, performance was not significantly affected by either internal or external environments. Instead, the primary factors influencing performance were external market conditions, particularly changes in feed and egg prices. These findings suggested that future efforts should

prioritize strengthening innovation capacity, improving financial resilience, and developing strategies to mitigate market volatility, thereby enhancing the sustainability of chicken farming businesses. Future studies should explore adaptive strategies that strengthen farmers' innovation capacity, enhance financial resilience, and mitigate market volatility. Comparative studies across different regions and production systems could also provide broader insights into effective models for sustainable chicken farming.

DECLARATIONS

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Competing interests

The authors declare no conflict of interest.

Authors' contributions

Dwi Putra Darmawan was responsible for conceptualization, methodology, supervision, funding acquisition, and manuscript review and editing. Gede Mekse Korri Arisena contributed to methodology, and original draft preparation. Putu Perdana Kusuma Wiguna handled software development, data interpretation and visualization. Ni Luh Made Indah Murdyani Dewi carried out formal analysis, project administration, data curation, and drafting. Anak Agung Istri Agung Peradnya Dewi, Gede Wisnu Sahatmana, and Ni Nyoman Ayu Prapti Rahayu contributed to validation and data curation. Anak Agung Keswari Krisnandika supported analysis and manuscript revision. All authors read and approved the final edition of the manuscript before submission.

Ethical considerations

All authors have contributed to the preparation of this original paper. The authors observed the final edition of the finished paper and evaluated any corrections and

updates. The authors checked the similarity index and plagiarism of the article.

Availability of data and materials

All data generated or analyzed during this study are included in the manuscript. Additional datasets are available from the corresponding author upon reasonable request.

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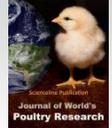
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Integrating Halal Management Systems and Control Points in Poultry Processing: A Transnational Compliance Case from Thailand to Malaysia

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ABSTRACT

Halal poultry production in Thailand is vital for strengthening its position in the global halal poultry market. The present study aimed to explore the strategic significance of halal management systems (HMS) and halal control points (HCPs) for the global halal poultry industry by investigating their application in Thailand and assessing compliance with Malaysian standards. The present study focused on certified Thai poultry slaughter and processing facilities located in Thailand and approved for export to Malaysia. Additionally, the present study explored the religious, technical, and institutional requirements compatible with poultry processing to fulfill the requirements of the Department of Veterinary Services and the Department of Islamic Development Malaysia. Employing a qualitative case study methodology that encompassed document analysis, in-depth interviews, and field observations, the present study identified eight critical HCPs immersed within a large 59-step operational process, focusing on key issues concerning animal welfare, ritual slaughter, hygiene, and prevention measures contamination. The integration of HMS and HCPs into the operational flow of poultry processing facilities required a structured approach, incorporating halal food-general requirements, the Malaysian protocol for halal meat and poultry production, and the Malaysian halal management. The present findings provided significant contributions to cross-border halal governance, supporting the development of a framework that enhances halal assurance in the international poultry processing industry and comprehensively addresses the halal poultry standards market.

Keywords: Halal control point, Halal management system, Poultry processing, Slaughterhouse

INTRODUCTION

The global halal food industry that adheres to Islamic dietary laws has experienced substantial growth, driven by increasing Muslim populations and heightened demand for halal products. It was estimated that the global halal food market would exceed 2 trillion USD by the year 2024, with meat and poultry products constituting a significant segment (DinarStandard, 2023). Therefore, ensuring religious compliance and hygienic integrity in poultry production has become essential for both Muslim-majority and non-Muslim countries involved in halal certification and production trade.

Thailand is one of the world's leading halal poultry exporters, which is widely accepted in the global markets such as the Middle East, the European Union, and Southeast Asia (Wongtangintharn et al., 2025). Among

these countries, Malaysia stands out as both a significant trading partner and a pioneer in halal certification and enforcement. The Department of Islamic Development Malaysia (JAKIM), in collaboration with the Department of Veterinary Services (DVS), has developed stringent guidelines for halal food imports, such as the MS 1500:2019 and the Malaysian protocol for halal meat and poultry production. These guidelines encompass animal welfare, slaughter procedures, hygiene, traceability, and halal governance systems (Department of Standards Malaysia, 2019).

For Thai poultry processors aiming to access the Malaysian market, adherence to these regulations is obligatory. Facilities should be certified by an authorized halal certification body (HCB), such as the Central Islamic Council of Thailand (CICOT), and should undergo on-site

audits conducted by Malaysian authorities. Additionally, poultry processing facilities should implement internal halal assurance systems that include trained Muslim slaughtermen, halal executives, and documentation of halal control points (HCPs) throughout the production process (JAKIM, 2020).

Halal poultry production involves applying Islamic legal principles (Shariah) to the breeding and feeding of chickens until slaughter and beyond distribution. According to Rahman *et al.* (2024), halal meat is authorized exclusively when it adheres to a specific procedure involving an animal deemed permissible for consumption, slaughtered by a mentally competent adult Muslim, utilizing a sharp instrument, and invoking the name of Allah. The slaughtering process should include cutting the trachea, esophagus, and carotid arteries to ensure it does not suffer before it dies, and allow complete blood drainage. The blood removal from the edible muscle is essential for religious adherence and hygienic considerations. Furthermore, halal assurance schemes have integrated operational methods, including good manufacturing practices (GMP), hazard analysis and critical control point (HACCP), and HCP within the halal poultry production process (Rahman *et al.*, 2021). These operational methods are crucial to ensure religious compliance, addressing not only the welfare of the slaughtered animal but also the implications for food safety in industrial processes (Bonne and Verbeke, 2008; Alqudsi, 2014).

The HCPs are critical operational stages, such as lairage, stunning, slaughtering, and carcass inspection, where risks of religious or procedural non-compliance should be mitigated. These control points require trained oversight, preventive measures, and detailed documentation in accordance with MHMS 2020 (JAKIM, 2020). The HCPs strategically serve as religious risk mitigation tools within a broader enterprise risk management framework, emphasizing continuous monitoring, control, and validation (Tieman, 2011). Failures in HCP governance can result in certification invalidation and loss of consumer trust (Tieman *et al.*, 2012).

Malaysia is recognized worldwide for its comprehensive and institutionalized halal management system (HMS). The JAKIM established several national halal standards, including MS 1500:2019, which includes general requirements on cleanliness, traceability, handling, and storage for halal food production (JAKIM, 2019). To address the technical and organizational aspects of halal regulation, including stunning techniques, personnel

certification, and internal auditing, the Malaysian protocol for halal meat and poultry production and the Malaysian halal management system are implemented (Department of Standards Malaysia, 2019; MHMS, 2020). The use of HMS by DVS and other authorities ensured that the religious and safety standards were firmly applied (Hashim *et al.*, 2019).

Thailand has emerged as a significant supplier of halal poultry, particularly to Muslim-majority countries such as Malaysia (Paramayudha and Budhisatrio, 2024). The CICOT is the only recognized halal certifying body by JAKIM. Thai exporters are obligated to adhere to the HMS established by the exporting country. Consequently, Thai poultry exporters willing to export to Malaysia should obtain halal certification from the appropriate Malaysian authorities and establish their operational and quality control systems in accordance with the certification requirements (Wongtangintharn *et al.*, 2025).

There is a limited understanding of how HCPs are employed and their compliance with regulatory standards such as MS 1500:2019 (MHMS, 2020). Furthermore, the HMS in Malaysia plays a crucial role in guiding halal certification processes; however, there is a limited number of empirical studies examining how poultry processing facilities in Thailand align their practices to facilitate export. The present study aimed to assess the implementation of Malaysia-aligned HMS in a Thai poultry processing facility, exploring halal compliance and the application of HCPs in the slaughtering and processing stages according to the MS 1500:2019, the Malaysian protocol for halal meat and poultry production.

MATERIALS AND METHODS

Ethical approval

For the present study, ethical approval was obtained from the research ethics committee for humanities, social sciences, and education, Prince of Songkla University, Pattani Campus, Thailand (Rec No. psu.pn.2-052/67), and all participants provided informed consent before data collection. Given the religious sensitivity of halal poultry operations, particularly the controversial aspect of pre-slaughter stunning, interviews explicitly addressed how stunning was conducted in accordance with the Malaysian protocol for halal meat and poultry production to ensure halal compliance. All responses were maintained with strict confidentiality and anonymity and were utilized solely for the current study.

Study design

A single case study design was utilized, focusing on a halal-certified poultry processing plant in Thailand. This

facility was officially registered with the DVS and JAKIM for exporting poultry products to Malaysia. The selected plants met the criteria for inclusion, as they fully adhere to

halal guidelines, traceability protocols, and veterinary and religious compliance procedures.

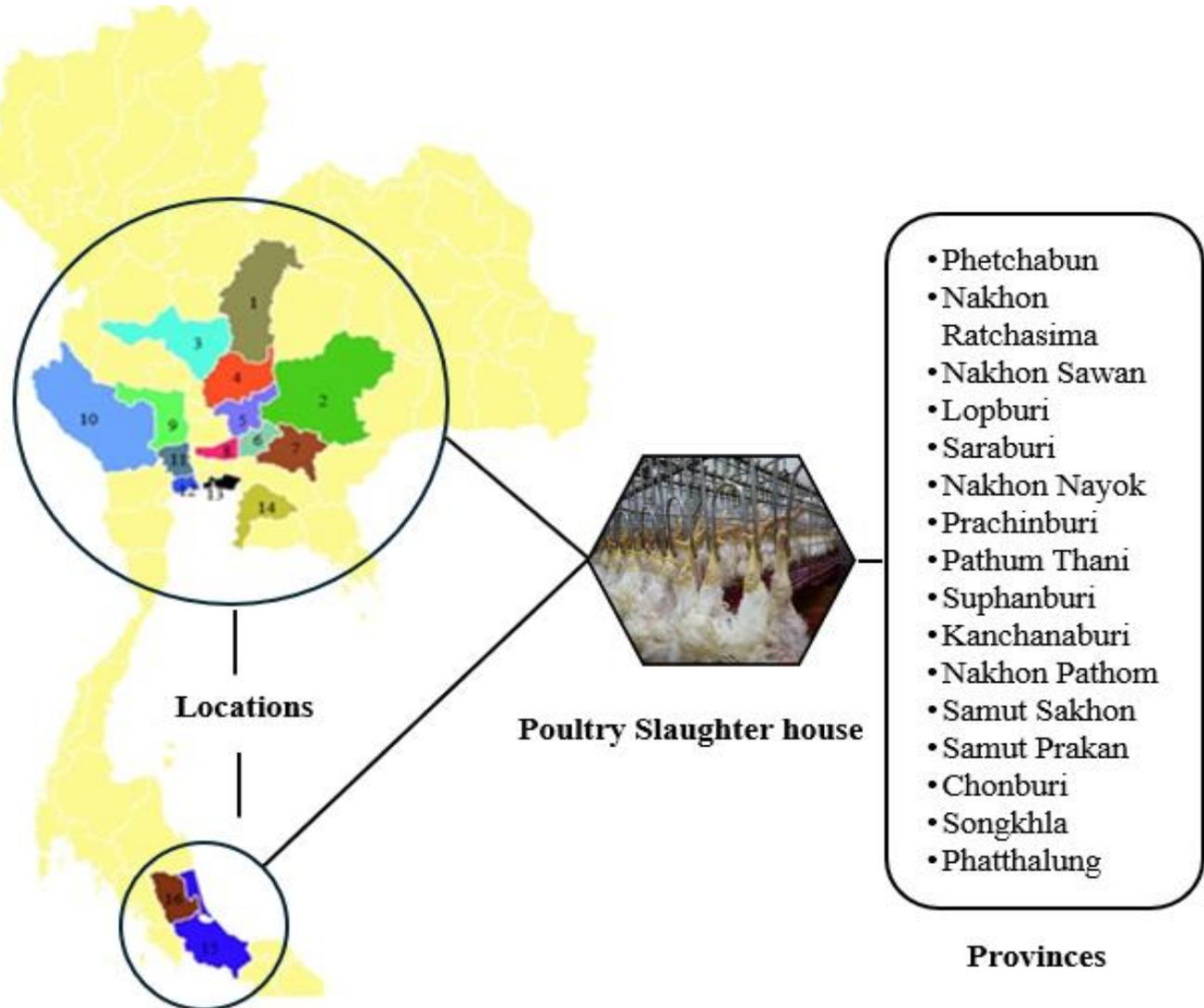


Figure 1. Location of the export-oriented slaughterhouse across 16 provinces in Thailand

Data collection

The present study utilized a qualitative case study approach, following the principles of interpretivist inquiry, to explore the differences of halal beliefs, organizational practices, and compliance with Malaysia's halal regulations, which are constantly evolving (Creswell and Poth, 2018). The present study analyzed documentary sources, including official Malaysian halal certification standards such as MS 1500:2019 (MHMS, 2020), HACCP, GMP, and internal halal audit documents. These were supplemented by semi-structured interviews, which were designed and pilot-tested to ensure clarity, relevance, and alignment with the objective of the study. In-depth

interviews were conducted with key personnel utilizing semi-structured guides. Data collection occurred at a 24 Halal poultry slaughterhouse with export orientation across 16 provinces in Thailand (Figure 1). The study was conducted from September 2024 to February 2025.

The study encompassed a total of 25 participants, with five individuals representing each key role category, including halal executive, halal supervisor, halal checker, slaughtermen, and quality control (QC) officer. Participants were required to have at least three years of experience in halal poultry processing and be involved in ensuring compliance with Malaysian halal guidelines. Interviews lasting 60-90 minutes were conducted to

understand their operational practices and religious compliance.

Data analysis

The data were analyzed employing thematic analysis as described by [Braun and Clarke \(2006\)](#). The analysis started with inductive immersion in the data, including transcripts and documents, to establish a comprehensive understanding of the context. Then followed the initial coding and the development of preliminary conceptual themes, such as slaughter, stunning, documentation, and training. Essential themes, according to the Malaysian halal guidelines, were defined, focusing on the implementation of HCPs within designated processing zones, internal halal auditing and governance procedures, and internal traceability systems. Finally, a framework was established to assess the usability and consistency of these themes through reviewing the documents and the observations of the investigators. Thematic categories were subsequently refined using manual coding and memos. The data was coded and then verified by an independent Halal Systems team expert. The data were coded and checked using thematic categories derived from reflexive coding, memoing, and participant input verification. Any discrepancies or contradictions across interviews, observations, or documentation were resolved with clarification, discussion with participants to garner clarity and consensus, and ensured trustworthiness and credibility ([Denzin, 2012](#)). To ensure accuracy, participants were asked to review and validate the present findings.

RESULTS AND DISCUSSION

Convergence of religious and technical standards

A key theme emerging from the present findings was the alignment of Islamic jurisprudential principles (fiqh) with technical food safety systems. The Muslim-trained slaughtermen, the invocation of Allah's name, and the complete cutting of the trachea, esophagus, and major blood vessels reflected the traditional Islamic slaughtering rules. These were integrated into the statutory operating procedures supported by structured audits and controlled documentation. Shariah principles were integrated into the audit process by including criteria for ritual compliance. Each auditor confirmed that each slaughterman was a Muslim, had received training, and they recited the tasmiyyah before slaughtering. The integration of Shariah with systems such as HACCP and GMP signified that religious and scientific epistemologies can coexist within contemporary poultry processing ([Bonne and Verbeke, 2008](#)). In contrast, facilities that do not follow these

guidelines may have changes at critical stages, such as stunning, slaughtering, and bleeding, leading to violations of Shariah compliance and food safety, and increasing the risk of losing halal certification penalties. Therefore, facilities maintain active internal audit processes to prevent non-compliance and protect their halal status certification.

Poultry processing and halal compliance

The present study revealed that, to meet the halal poultry export demands, particularly to Malaysia, the poultry processing facility has adopted a comprehensive production system that includes halal compliance, different operational zones, HCPs, food safety protocols, industry hygiene, and MHMS. It covered the entire process to guarantee that both religious and health standards were maintained. The poultry slaughtering and processing facility functioned through a systematic workflow comprising 59 sequential stages, which were categorized into three operational phases. The unclean zone covered initial handling stages, including transportation, reception, and lairage, during which risks of contamination and animal stress were observed at their highest. The clean zone included slaughtering, bleeding, washing, and inspection. The chilled/export zone included chilling, packaging, and storage to prepare traceable and compliant products for export.

Figure 2 highlights key features of a halal-compliant poultry production, showcasing HCPs that monitor Shariah compliance in real-time, along with relevant halal practices aligned with the Malaysian HMS ([MHMS, 2020](#)), operational zones for processing, as well as food safety systems such as HACCP and GMP. It demonstrated how all these contribute to constructing the comprehensive framework of a poultry processing facility that adheres to halal-compliant production guidelines. This systematic approach highlighted the establishment's efforts to integrate Shariah compliance into modern food safety systems, facilitating the follow of food safety regulations and plans. Prior studies have established that halal assurances rely on religious compliance and technical compliance. The combination of operational zoning, HCP monitoring, and MHS strategies provided another facility that exports to Malaysia, ensuring its models meet the strict requirements needed for Malaysia's halal standards imports. However, compliance requires ongoing training, completing documentation, and conducting internal audits to ensure that no violations occur, which could invalidate the process ([Umar and Parakkasi, 2025](#)).

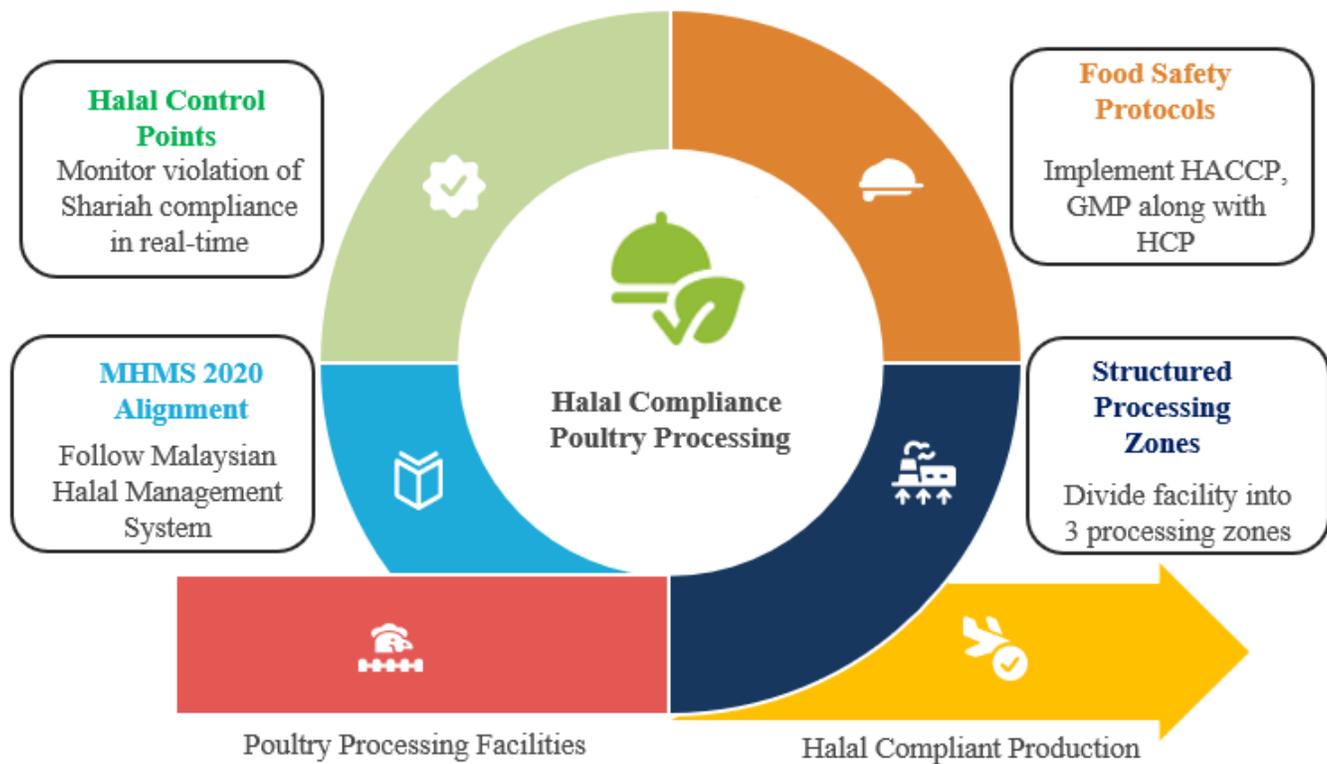


Figure 2. Poultry production with Halal compliance slaughtering and processing facilities.

Halal control point and risk management

To maintain halal authenticity, the facility introduced HCPs at key stages such as slaughtering and evisceration, focusing on potential Shariah violations (e.g., najis-ritually impure substances prohibited in Islamic law, improper slaughter, non-Muslim slaughterer). Unlike traditional Critical Control Points (CCPs), HCPs are designed to address religious non-compliance in real time, overseen by trained Halal Checkers. These controls are integrated with food safety protocols under HACCP, GMP, and Good Hygiene Practices (GHP), covering temperature regulation, sanitation routines, staff hygiene, and physical separation across zones. Consistent with the Malaysian Halal Management System (MHMS, 2020), the facility applies a structured compliance regime that includes Halal Committees, Muslim-trained personnel, Standard Operating Procedures (SOPs), and continuous internal audits to a unique Shariah-based production ideology (Desa et al., 2022). This study identified eight HCPs within a 59-step operational workflow, each marking a point of potential religious or hygienic non-compliance. Table 1 presents the corresponding risks and mitigation strategies. The integration of trained personnel, institutional Standard Operating Procedures (SOPs), and real-time monitoring provides an appropriate halal assurance framework (Alqudsi, 2014; Asa, 2017). It exemplifies effective halal risk management, aligning Islamic jurisprudence with industrial standards, and offers

a replicable model for producers and certification bodies in halal-sensitive export markets.

Halal management system implementation

The current study indicated that the Malaysian government plays a significant role in the halal industry through its establishment of halal standards, control mechanisms, and incentives, which speed up industry development and growth (Rashid and Bojei, 2020). While Malaysia's Halal Management System (HMS) focuses primarily on domestic certification and regulating imports, Thailand's HMS is designed to facilitate international trade compliance (Shafii and Zubir, 2018). Consequently, Thai poultry exporters should operationalize their halal standards in alignment with both Thailand's CICOT procedures and the specific requirements of the Malaysian market. In response to Malaysia's stringent halal import regulations, Thailand's poultry facilities have adopted an integrated HMS, synthesizing religious, technical, and operational standards across all phases of production.

The present study indicated that the Thai poultry industry employed a multi-variable HMS approach, tailored explicitly to Malaysian halal practices encompassing regulatory, religious, and operational compliance. These guidelines ensured that all critical control points, from live poultry handling and Shariah-compliant slaughter to final packaging hygiene, were rigorously maintained. Therefore, the implementation of a

Malaysian-standard HMS required several key components, including an internal governance structure with a halal committee, executive, and auditors, enforced SOPs for segregated unclean, clean, and chilled/export zones, and the management of eight HCPs throughout the slaughter process. Furthermore, facilities underwent regular audits by both Thailand's CICOT and Malaysia's JAKIM to maintain certification and ensure ongoing compliance. The integrated halal management system framework for the export-oriented poultry slaughtering

and processing facilities in Thailand was illustrated in Figure 3. The framework highlighted the important connections between regulatory, institutional, and operational aspects, which included identifying HCPs, utilizing digital traceability tools, maintaining proper documentation, and involving trained Muslim personnel. By integrating these elements with Malaysia's HMS, the facility demonstrated its appropriate commitment to blending religious obligations with international food safety standards, particularly for export markets.

Table 1. Halal control point and risk mitigation strategies

HCP	Process step	Location	Risk addressed	Control measures
HCP1	Lairage	Holding area	Stress, respiratory distress	Rest poultry for ≥ 30 mins; control temperature and humidity
HCP2	Hanging	Shackling section	Inclusion of dead/ infected poultry	Train staff; separate dead or infected poultry
HCP3	Stunning	Stunning station	Over-stunning or pre-slaughter death	Calibrate voltage; confirm recovery within 5 minutes
HCP4	Slaughtering	Slaughter hall	Improper slaughter; non-Muslim personnel	Use a sharp knife; trained Muslim slaughterman; recite <i>Bismillah</i>
HCP5	Bleeding	Bleeding tunnel	Residual blood; insufficient bleeding time	Monitor bleeding for ≥ 180 seconds; visual inspection by Halal Checker
HCP6	Red carcass inspection	Inspection point	Incomplete bleeding; visual impurities (najis)	Inspect and remove red/incomplete carcasses
HCP7	Carcass washing	Washing section	Contamination from blood or impure water	Use clean, cold water; visual cleanliness check
HCP8	Carcass destruction	Condemnation area	Improperly slaughtered carcass inclusion	Heat $\geq 85^{\circ}\text{C}$ (internal temp $\geq 75^{\circ}\text{C}$); grind condemned carcasses

HCP: Halal control point

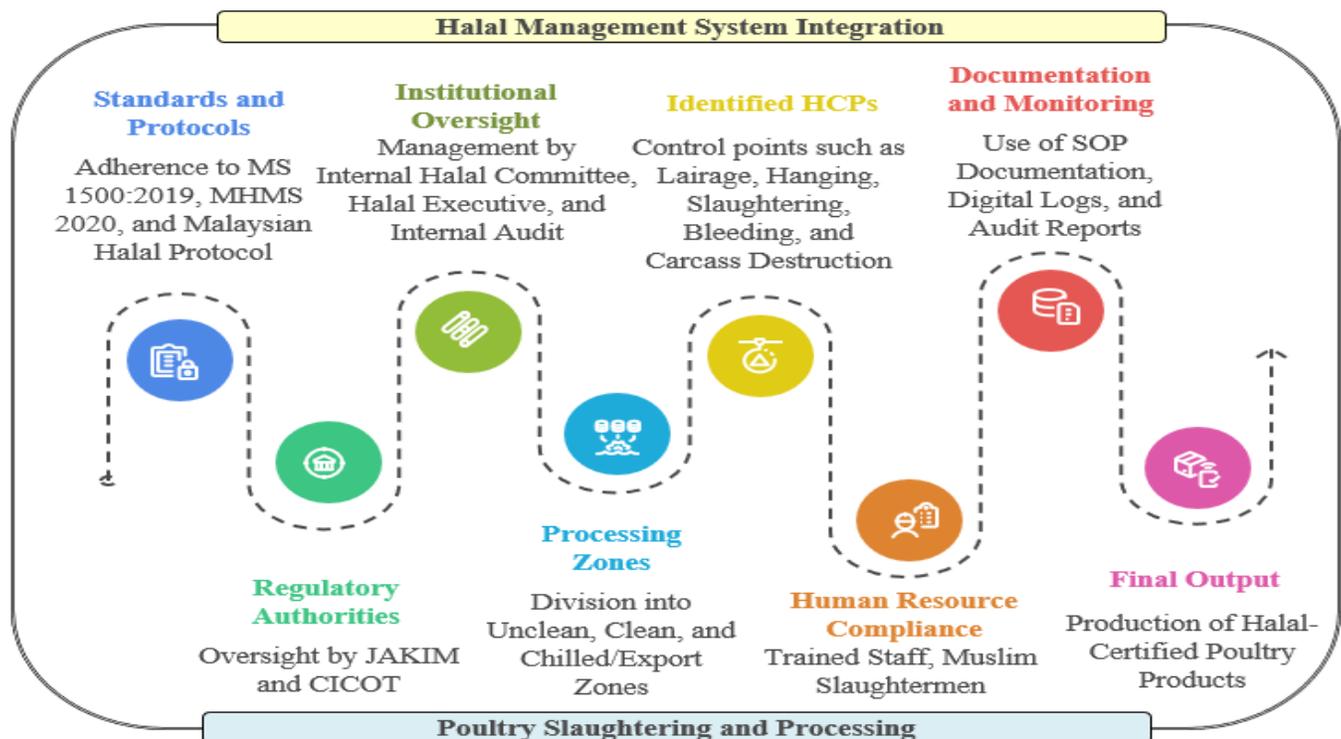


Figure 3. Integrated halal management system framework for halal poultry production in Thailand aligned with Malaysian standards.

Stakeholder perspectives on halal compliance

The thematic analysis of stakeholder interviews revealed a high organizational commitment to balancing religious integrity with regulatory compliance. A key finding emphasized a dual responsibility, including meeting technical requirements while upholding core Shari'ah principles. Participants described a system of integrated governance that includes regular internal audits and close collaboration with authoritative organizations such as JAKIM and CICOT to ensure compliance. The slaughtering process was consistently identified as the most critical and sensitive HCP. Participants demonstrated a clear understanding of the precise ritual requirements, strictly following the MS 1500:2019 standard. This included employing trained Muslim slaughtermen who ritually recite the Tasmiyah (Bismillah) before each cut, underscoring the religious significance beyond mere procedural duty. Strict documentation protocols at each control point further demonstrated operational clarity. Any case of improper bleeding was carefully documented, resulting in the carcass being culled. This reflected a systematic approach to risk management, especially regarding blood retention and cleanliness, acting as a continuous preventative and corrective measure to ensure Shariah compliance throughout the production line. According to the QC officer, the collaboration between the halal and hygiene teams highlighted the operational synergy needed to meet religious and food safety

requirements, further confirming the integrated approach of the HMS in Thai poultry slaughter facilities.

Halal compliance gaps

Poultry supply chain

The current study identified significant gaps in halal compliance across upstream and downstream activities of the poultry supply chain, although midstream processing generally followed halal requirements. Although inputs such as animal feed and vaccines underwent a degree of regulatory supervision, they frequently did not possess formal certification from an accredited halal body. Moreover, downstream products, particularly ready-to-eat items, lacked halal certification for export to Malaysia, reflecting an absence of a comprehensive halal assurance system from farm to fork. To meet Malaysia's halal processing standards, several Thai poultry producers encountered challenges involving regulatory interpretation, operational alignment, and procedural problems in the certification process (Tieman, 2011; Talib et al., 2014; Ngah et al., 2015).

In addition, recruiting and training qualified Muslim slaughtermen is still difficult in Thailand, where the majority of the population is non-Muslim (Talib et al., 2014). Discrepancies between local documentation and Malaysia's halal certification requirements caused procedural delays and increased costs. These findings aligned with the findings of Ngah et al. (2015), highlighting the importance of legal harmonization and bilateral technical cooperation in solving halal compliance and logistical issues.

Table 2. Strategic recommendations for enhancing halal system management for export-oriented Thailand's poultry industry

Focus area	Objective	Key actions	Expected outcomes
Capacity building	Enhance skills in halal operations	Train slaughtermen, auditors; develop SOPs	Improved compliance; reduced non-conformance
Traceability systems	Boost transparency and verification	Adopt blockchain, QR codes, sync with audits	Higher traceability; greater stakeholder trust
Regulatory alignment	Harmonize Thai–Malaysia standards	Conduct CICOT–JAKIM–DVS dialogues; align audits	Fewer delays; smoother certification process
Halal R&D	Foster innovation in halal compliance	Study stunning, feed, audit models; form study consortia	Evidence-based practices; academic–industry links
Policy integration	Mainstream halal in national agendas	Draft halal masterplan; offer incentives to exporters	Institutional support; increased market access
Bilateral collaboration	Deepen Thai–Malaysia cooperation	Run joint training, forums, and share inspection expertise	Regional capacity-building; mutual recognition

SOPs: Standard operating procedures, QR codes: Quick response codes, CICOT: The Central Islamic Council of Thailand; JAKIM: Department of Islamic Development Malaysia, DVS: Department of Veterinary Services (Malaysia), R&D: Research and development.

Strategic implications

The current study offered a scalable halal governance model for the international poultry trade. The model

included staff training, internal halal committees, and maintained controlled records related to export compliance. The sustainability of the industry requires

commitment to capacity building, the integration of an online traceability system, alignment with regulatory frameworks, and the establishment of robust legal structures to facilitate ethical and sustainable halal practices (Ramli *et al.*, 2023; Rahman *et al.*, 2024).

To improve Thailand's halal system to make it suitable for export, the current study has identified several strategic measures as priorities. These included training Muslim slaughterers, halal supervisors, and auditors to increase technical capacity; introducing traceability technologies, such as blockchain and QR codes; and promoting greater partnerships between CICOT, JAKIM, and DVS for regulatory harmonization (Nashirudin and Ulfah, 2024). Additionally, the halal industry should invest greater engagement in halal-related studies and development through university-industry collaboration partnerships, and promote halal considerations in national trade policies, offering financial incentives to foster halal trade relationships (Rahman and Ahmad, 2024). Training Muslim slaughterers, supervisors, and auditors is one of these priorities and a short-term, urgent move that offers immediate compliance benefits. On the other hand, implementing blockchain-based traceability and advanced R&D programs are long-term strategic priorities that require significant investment and infrastructure. As a result, the interview and document analysis identified six strategic priority areas for improving Thailand's halal system to support export-oriented poultry production. These priorities emerged directly from observed gaps in operational capacity, traceability, regulatory alignment, policy integration, and bilateral collaboration. Table 2 presents the strategic recommendations for enhancing halal system management in Thailand's export-oriented poultry industry.

CONCLUSION

The current study indicated that Thai poultry slaughtering facilities have effectively implemented halal HMS and HCPs that adhere to Malaysia's rigorous halal standards in applying religious requirements alongside food safety measures across 59 operational bodies. The Thai poultry industry, which adhered to halal standards, was compromised by upstream and downstream uncertainties, such as uncategorized feed sources and ready-to-eat products. Despite high midstream compliance, these gaps indicated the critical lack of an integral farm-to-fork halal assurance system. The issues outlined in the present study highlighted the need to certify additional components, improve digital traceability, and strengthen regulator

communication through bilateral engagement. These improvements aim to preserve religious and hygienic guidelines while boosting Thailand's global competitiveness in halal market sectors. Furthermore, the study offered other producers and policymakers a replicable model that aligns operational goals with Shariah-compliant methods for the international halal industry. To improve halal assurance across the supply chain, Thai certification bodies should expand the scope of certification to cover feed suppliers, veterinary drug manufacturers, and poultry farms. Policymakers should implement mandatory halal standards for upstream inputs, following Malaysia's integrated halal framework. Future studies should examine the long-term effectiveness of these strategic measures, especially in enhancing supply chain resilience and ensuring cross-border compliance. Additionally, further studies could explore consumer perspectives on Thai halal poultry in Malaysia to demonstrate how governance improvements influence purchasing decision behavior.

DECLARATIONS

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Authors' contributions

Hafit Khamnurak conducted the study as part of his doctoral studies, overseeing data collection, analysis, and the development of the halal control point model. Asman Taeali served as the academic supervisor, providing essential guidance on the application of Islamic jurisprudence and Shariah principles throughout the study process. Md. Mahfujur Rahman offered supervisory support with an emphasis on halal compliance and the integration of Halal Control Points within the management system of poultry production. All authors participated in refining the manuscript and approved the final edition.

Ethical considerations

Ethical approval was obtained from the institutional research board at Prince of Songkla University. Informed consent was secured from all participants before data collection, and confidentiality was maintained throughout the study. The authors affirmed that all ethical issues have been addressed, including plagiarism, consent to publish, misconduct, double publication and/or submission, and redundancy.

Availability of data and materials

The data obtained in this study can be obtained upon reasonable request.

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Competing interests

The authors declared no conflicts of interest in publishing this article.

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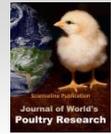
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Evaluation of Broiler Chicken Farmers' Knowledge and Antibiotic Usage Practices and the Resistance of *Staphylococcus* spp. in Bali, Indonesia

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ABSTRACT

Antimicrobial resistance (AMR) is a global threat that seriously affects public health, animal welfare, and the sustainability of food production systems. In the poultry sector, irrational use of antibiotics is a key factor contributing to the emergence of resistance. This study investigated the association between broiler farmers' knowledge and their antibiotic usage practices with the resistance of *Staphylococcus* spp. in broiler chickens. The study employed a cross-sectional design and included 20 broiler farms located in Tabanan Regency, Indonesia. Knowledge and practices were assessed using structured questionnaires, while five tracheal swab samples were collected from randomly selected chickens on each farm. These samples were pooled and tested in the laboratory to identify *Staphylococcus* spp. and determine their susceptibility to amoxicillin, ciprofloxacin, erythromycin, and doxycycline. The antibiotic susceptibility test followed the guidelines of the Clinical and Laboratory Standards Institute (CLSI), and the associations between variables were examined using cubic regression analysis. Based on the scores obtained from the structured questionnaires, the majority of farmers demonstrated a moderate level of knowledge (65%) and a moderate level of antibiotic usage practices (60%). Laboratory testing revealed that *Staphylococcus* spp. isolates showed the highest resistance to amoxicillin (75%), followed by erythromycin (60%), doxycycline (55%), and ciprofloxacin (30%). The results showed that most farmers had moderate knowledge (65%) and practices (60%), with the highest resistance observed against amoxicillin (75%), followed by erythromycin (60%), doxycycline (55%), and ciprofloxacin (30%). A significant positive correlation was found between farmers' knowledge scores and their antibiotic usage practice scores ($r = 0.683$, $R^2 = 0.467$), indicating that higher knowledge was associated with better practices; however, practices were not significantly associated with inhibition zone diameters. These findings suggested that low antibiotic literacy may lead to improper use, while bacterial resistance is also influenced by external factors such as environmental contamination, horizontal gene transfer, and centralized medication protocols in contract farming.

Keywords: Antimicrobial resistance, Broiler, Knowledge, Practice, *Staphylococcus* spp.

INTRODUCTION

Antimicrobial resistance (AMR) represents an urgent global issue, threatening the health of humans, animals, and the environment, as well as undermining the stability of food supplies worldwide. Within the One Health perspective, the World Health Organization (WHO) places AMR among its highest priorities, emphasizing the concern over the rising number of bacterial infections that no longer respond to common medical treatments (WHO, 2023). In the poultry industry, the widespread use of

antibiotics without adequate understanding or regulatory oversight is considered a major driver of AMR emergence (Van Boeckel et al., 2015).

In broiler production systems, antibiotics are often administered not only for therapeutic purposes but also for disease prevention and growth promotion (Chowdhury et al., 2021; El-Fateh et al., 2024). However, irrational use, including administration without clinical indications, inappropriate dosage and duration, and poor compliance with withdrawal periods, can exert selective pressure that

promotes the development of resistant bacterial strains (McEwen and Collignon, 2018). Among the pathogens commonly exhibiting resistance in poultry is *Staphylococcus* spp., particularly *S. aureus*, which is known to carry various resistance genes such as *blaZ*, *erm*, and *tet* (Mak *et al.*, 2022).

Staphylococcus species are normally present within the poultry microbiota but can become opportunistic pathogens responsible for diseases such as dermatitis, arthritis, and omphalitis (Szafraniec *et al.*, 2022). Continuous and repeated use of antimicrobial agents in poultry production contributes to the development of resistant strains, including those resistant to multiple drugs. Thus, surveillance of resistance trends in *Staphylococcus* spp. is vital to understand how antibiotic use by farmers affects animal health and poses potential risks to public health. Previous studies conducted in Indonesian broiler farms have highlighted considerable resistance of *Staphylococcus* spp. to frequently administered antibiotics (Khusnan *et al.*, 2016; Hermana *et al.*, 2021). However, most of these studies have focused solely on resistance patterns, without examining human behavioral factors such as farmers' knowledge and antibiotic use practices. In fact, farmer literacy is a critical determinant of on-farm antimicrobial usage (Rware *et al.*, 2024). Despite this, specific data linking farmer behavior to bacterial resistance are still lacking, especially in contract farming regions such as Tabanan Regency in Bali, which is a major broiler production center with a population exceeding 2 million broilers (BPS Bali, 2023). Therefore, this study aimed to evaluate the relationship between broiler chicken farmers' knowledge and practices regarding antibiotic use and the resistance patterns of *Staphylococcus* spp. isolated from broiler chickens in Tabanan, Bali, Indonesia.

MATERIALS AND METHODS

Ethical approval

This study was approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, Udayana University, Denpasar, Indonesia, under approval number: B/81/UN14.2.9/PT.01.04/2025.

Study area and sample size

A cross-sectional study was conducted from April to May 2025 on 20 broiler chicken farms located in Tabanan Regency, Bali Province, Indonesia (coordinates: 8.4596°S, 115.0466°E). Farms were randomly selected based on inclusion criteria: having a minimum population of 5,000 broiler chickens and having been operational for at least

one year. According to the available population data, 21 farms met the inclusion criteria. The required number of farms was determined using the Slovin formula (Riyanto and Hatmawan, 2020), resulting in a sample size of 20 farms. The selected farms varied in size and management systems, ranging from small-scale traditional operations to larger commercial enterprises. In terms of ownership, the farms included both privately owned operations and contract farms collaborating as plasma farms with major companies, reflecting the diversity of broiler production in the region. Tracheal swab samples were collected from five randomly selected live broiler chickens at each farm. The chickens were carefully handled without sedation to minimize stress during sampling. The five swab samples were then pooled and analyzed as a single representative sample per farm, considering the observed homogeneity of results among individuals within each location.

Farmer knowledge and practice survey

Data on farmers' knowledge and practices regarding antibiotic usage were collected using a structured questionnaire employing a four-point Likert scale, which was administered directly to the respondents. Educational levels were categorized as primary school, junior high school, senior high school, and university from primary school to university. Total knowledge and practice scores were classified into five categories: very poor, poor, moderate, good, and excellent. The influence of education level on knowledge and practices was analyzed using the Kruskal-Wallis test.

Bacterial sampling and isolation

Samples were collected only from broilers exhibiting clinical symptoms such as swollen head syndrome or bumblefoot to increase the likelihood of isolating *Staphylococcus* spp. This targeted sampling approach was chosen because symptomatic broilers are more likely to carry pathogenic strains, facilitating the study of antibiotic resistance profiles. Sampling asymptomatic broilers was not conducted due to the lower probability of isolating clinically relevant *Staphylococcus* strains from healthy broilers. Samples were aseptically collected and transported to the Veterinary Microbiology Laboratory, Faculty of Veterinary Medicine, Udayana University, Denpasar, Indonesia. Swabs were inoculated onto Mannitol Salt Agar (MSA; Merck KGaA, Darmstadt, Germany; Cat. No. 1.05404.0500) and incubated at 37°C for 24 hours. Grown colonies were identified as *Staphylococcus* spp. based on Gram staining and

biochemical tests (Catalase and oxidase; Musliu et al., 2021).

Antibiotic susceptibility testing

Antibiotic susceptibility testing was conducted using the Kirby-Bauer disk diffusion method. Bacterial suspensions were prepared by diluting cultures in 0.9% sodium chloride solution and standardized to a turbidity equivalent to 0.5 McFarland standard (Approximately 1.5×10^8 CFU/ml; Rubin and Damborg, 2024). Then, 0.2 ml of each suspension was uniformly spread onto Mueller-Hinton Agar plates (MHA; Merck KgaA, Darmstadt, Germany; Cat. No. 1.05437.0500). Disks impregnated with antibiotics are frequently applied in broiler production, including amoxicillin (AML, 25 µg; Oxoid Ltd., Hampshire, UK; Cat. No. CT0061B), ciprofloxacin (CIP, 5 µg; Oxoid Ltd.; Cat. No. CT0425B), erythromycin (E, 15 µg; Oxoid Ltd.; Cat. No. CT0020B), and doxycycline (DO, 30 µg; Oxoid Ltd.; Cat. No. CT0018B). Plates were incubated at 37°C for 24 hours. The diameter of the inhibition zones was measured and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2021).

Data analysis

Data on farmers' knowledge and practices were analyzed descriptively and statistically using the Kruskal-

Wallis test to compare differences across groups, with p-values reported accordingly. The resistance profile of *Staphylococcus* spp. isolates were also analyzed descriptively based on the antibiotic susceptibility test results (Besung et al., 2024). Furthermore, linear regression analysis was performed to assess the associations between farmers' knowledge and practices and the correlation between antibiotic use practices and the inhibition zone diameters of bacterial isolates. All statistical analyses were conducted using IBM SPSS Statistics version 25, with significance set at $p < 0.05$.

RESULTS

Out of the 20 broiler farmers surveyed in Tabanan Regency, 65% demonstrated a moderate level of knowledge regarding antibiotics, 30% exhibited low knowledge, and only 5% showed good knowledge. A similar trend was observed in antibiotic use practices, where 60% of farmers fell into the moderate category, 30% were classified as low, and only 10% reported good practices. The results showed a significant difference ($p < 0.05$) in knowledge and practices associated with the farmers' educational background. The detailed results are presented in Table 1.

Table 1. Differences in knowledge and practices related to antibiotic use based on the educational background in broiler chicken farmers in Tabanan Regency, Bali Province, Indonesia

Education Level	Knowledge score (Mean ± SD)	Practice score (Mean ± SD)
Primary School	51.500 ± 8.0234 ^a	51.500 ± 5.1841 ^a
Junior High School	61.250 ± 1.7678 ^{ab}	60.000 ± 7.0711 ^{ab}
Senior High School	61.250 ± 10.000 ^{ab}	58.438 ± 4.9888 ^{ab}
University	71.500 ± 2.8504 ^b	68.000 ± 8.9093 ^c

^{abc}Different superscript letters in the same column indicate significant differences ($p < 0.05$). SD: Standard deviation

The lowest levels of knowledge and practice were recorded among farmers with only primary school education, which significantly differed from those with secondary school, high school, and university-level education. Farmers with university-level education had the highest average scores in both knowledge and practice compared to the farmers with a primary level of education ($p < 0.05$). Amoxicillin was the most frequently used antibiotic among respondents (40%), followed by ciprofloxacin, erythromycin, doxycycline, and enrofloxacin (each used by 25% of farmers). Other antibiotics reported included norfloxacin, oxytetracycline,

and a combination of sulfadiazine-trimethoprim (20% each), while Tylosin tartrate and neomycin sulfate were used by only 10% and 5% of farmers, respectively.

Antibiotic susceptibility testing of 20 *Staphylococcus* spp. isolates revealed that resistance was highest to amoxicillin (75%), followed by erythromycin (60%), doxycycline (55%), and ciprofloxacin (30%). Ciprofloxacin showed 40% sensitivity, while 30% of isolates exhibited intermediate response and 30% were resistant. Sensitivity to erythromycin and doxycycline was observed in 35% and 45% of isolates, respectively (Figure 1).

The pattern of multiple resistance showed that 40% of isolates were resistant to a single antibiotic (amoxicillin), 15% to two antibiotics, 20% to three, and 20% were resistant to all four antibiotics tested. None of the isolates were fully sensitive to all antibiotics. Regression analysis results regarding knowledge, practices, and antibiotic resistance are summarized in Table 2. A significant

correlation was found between farmers' knowledge and their practices in antibiotic use ($r = 0.683$; $R^2 = 0.467$; $p < 0.05$). However, no significant difference was found between antibiotic use practices and the inhibition zone diameters of *Staphylococcus* spp. against the four antibiotics tested ($p > 0.05$).

Table 2. Regression analysis between knowledge, practices, and antibiotic resistance variables in Tabanan Regency, Bali Province, Indonesia

Relationship tested	r	R ²	p-value
Knowledge versus antibiotic use practices	0.683	0.467*	0.001
Practices versus inhibition zone diameter (Amoxicillin)	0.156	0.024 ^{ns}	0.858
Practices versus inhibition zone diameter (Ciprofloxacin)	0.257	0.066 ^{ns}	0.366
Practices versus inhibition zone diameter (Erythromycin)	0.267	0.071 ^{ns}	0.249
Practices versus inhibition zone diameter (Doxycycline)	0.365	0.133 ^{ns}	0.086

r: Correlation coefficient; R²: Coefficient of determination. Vs: Versus, *: Significant, ns: Not significant

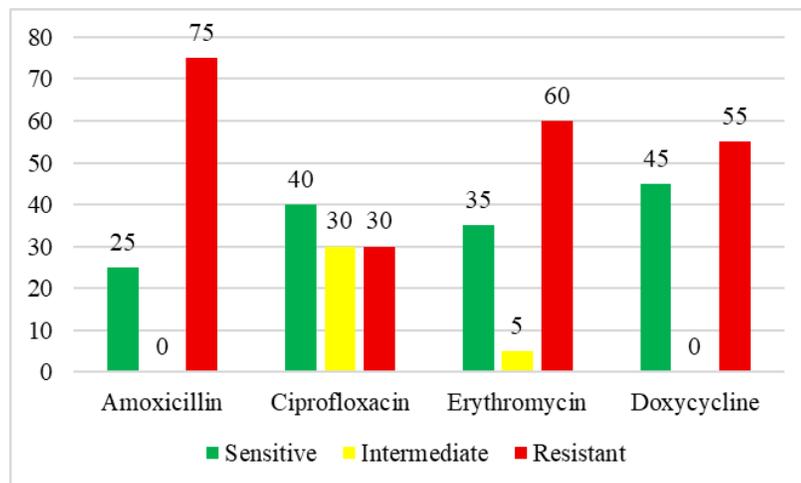


Figure 1. Percentage of antibiotic susceptibility test results for *Staphylococcus* spp. isolates collected from broiler farms in Tabanan Regency, Bali Province, Indonesia, between April and May 2025.

DISCUSSION

The findings of this study revealed that the majority of broiler farmers in Tabanan Regency possess only moderate knowledge and practices regarding antibiotic use. This finding indicated a limited level of veterinary health literacy, which may contribute to the irrational use of antibiotics in poultry production. Inadequate understanding of appropriate antibiotic use has been previously linked to poor on-farm practices in various studies (McEwen and Collignon, 2018; Rware et al., 2024). Low awareness of withdrawal periods, differentiation between bacterial and viral infections, and the broader implications of antimicrobial resistance

(AMR) for human and environmental health are key issues that require urgent attention.

Ongoing education and the active involvement of veterinarians in treatment decisions are essential to improving antibiotic stewardship on farms (FAO, 2021; WHO, 2022). This is supported by the regression analysis in the present study, which demonstrated a significant relationship between farmers' knowledge and their antibiotic use practices with a correlation coefficient of 0.683 and a determination coefficient (R^2) of 0.467. These results suggest that improving farmers' understanding may positively influence their practices, including the selection, dosage, and duration of antibiotic administration (Sadiq et al., 2018).

Questionnaire results showed that amoxicillin was the most frequently used antibiotic among respondents. Consistently, amoxicillin exhibited the highest resistance rate (75%) among the isolates of *Staphylococcus* spp. in this study. This correlation between frequent use and resistance development aligned with previous reports (Van Boeckel et al., 2019). Similarly, high levels of resistance to erythromycin and doxycycline may reflect repeated use without accurate diagnosis or laboratory confirmation. Furthermore, knowledge of antibiotic use among poultry farmers was also reported in Kwara State, Nigeria, where farmers' knowledge levels were significantly associated with their perceptions but showed no significant correlation with actual antibiotic usage practices (Al-Mustapha et al., 2020).

The observed multiple resistance patterns, where isolates were resistant to up to four antibiotics, suggest a high selective pressure resulting from prolonged or inappropriate antibiotic exposure (Sunartatie et al., 2024). This result further emphasizes the possibility of resistance genes being horizontally transferred through plasmids, transposons, and various other mobile genetic elements (Michael and Schwarz, 2016). Although this study did not include molecular detection of resistance genes, genes such as *bla_Z*, *erm*, *mecA*, *msr(A)*, and *tet(K/L)* have been widely reported to mediate resistance to the tested antibiotics (Hooper and Jacoby, 2015; Mak et al., 2022).

Interestingly, no significant correlation was found between knowledge of antibiotic use and the inhibition zone diameters of *Staphylococcus* spp. against the tested antibiotics. This finding suggests that resistance is not solely influenced by individual behaviors but also by external factors such as centralized treatment protocols in contract farming systems, environmental antibiotic exposure, and the broader circulation of resistance genes (Carrique-Mas et al., 2015; Nhung et al., 2016). In many nucleus-plasma systems, treatment regimens are determined by the integrator company, which often administers antibiotic packages en masse without considering specific on-farm conditions, consequently limiting farmers' control over actual antibiotic exposure and overlooking the role of their knowledge (Adam et al., 2020).

Additional contributing factors such as subtherapeutic dosing, cross-contamination from the environment, and lack of antibiotic rotation further intensify selection pressure and promote resistance (Tang et al., 2017). Therefore, the control of AMR in the poultry sector must be systemic and cross-sectoral, rather than relying solely on changes in individual farmer behavior.

Overall, the results of this study underscore the urgent need to adopt the One Health approach in addressing AMR in poultry production. The significant association between antibiotic usage practices at the farm level and the potential risks to public health underscores the need for comprehensive, multidisciplinary strategies incorporating educational initiatives, improvements in farm management, and enhanced regulatory oversight of antibiotic application. An effective approach to AMR mitigation, therefore, requires a comprehensive strategy that combines farmer education, strict policy implementation, and enhanced farm management practices within the One Health perspective. Building farmers' capacity refers to enhancing their knowledge, skills, and practices related to responsible antibiotic use and biosecurity measures. Fostering cross-sector collaboration involves coordinated efforts among stakeholders from agriculture, veterinary, public health, and regulatory sectors to effectively manage and reduce antimicrobial resistance risks along the entire food production chain (FAO, 2021; WHO, 2022).

CONCLUSION

Most broiler chicken farmers in Tabanan exhibited limited knowledge and suboptimal practices regarding antibiotic use, contributing to the high resistance levels of *Staphylococcus* spp. While knowledge significantly influenced practice, practices did not show a direct effect on bacterial resistance, indicating that AMR is a multifactorial issue. A comprehensive control strategy based on the One Health approach is essential to effectively address antimicrobial resistance in the poultry farming sector.

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Authors' contributions

I Gusti Bagus Aryanta Kusuma Putra designed the study, collected the samples, and analyzed the data. Kadek Karang Agustina, I Wayan Suardana, and Ni Ketut Suwiti collected the samples and provided media for the study. I Wayan Masa Tenaya and Putu Henrywaesa Sudipa conducted the study process in the laboratory. Yeocelin Meida Utami and I Nengah Kerta Besung analyzed the data and wrote the manuscript. All authors reviewed the analyzed data and gave their approval to the final draft of the manuscript.

Competing interests

The authors declare that there are no conflicts of interest related to this publication.

Ethical considerations

All ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been carefully reviewed and addressed by all authors.

Availability of data and materials

The original data presented in this study are included in the article. For inquiries, please contact the corresponding author.

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Effects of Fermented *Sargassum binderi* Meal on Productivity and Egg Quality of Laying Hens

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ABSTRACT

Brown algae, such as *Sargassum binderi* (*S. binderi*), are abundantly present in marine ecosystems and constitute a valuable natural resource. The present study aimed to investigate the effect of fermented *S. binderi* meal on the performance of laying hens and egg quality, as well as to establish the safe limits of its usage in the diet of laying hens. The 200 Isa Brown laying hens were randomly assigned to five different treatment groups based on fermented *S. binderi* levels, including the control group, which received 0% fermented *S. binderi*, while the other treatment groups received 4%, 8%, 12%, and 16% fermented *S. binderi*, respectively, over six weeks. At the end of the experiment, 80 eggs were assessed for quality. The variables included feed intake, hen day egg production, egg weight, egg mass, and feed conversion ratio, as well as external egg quality measures such as eggshell weight, percentage of eggshell, eggshell thickness, eggshell strength, egg length, egg width, and egg shape index and internal egg quality parameters including yolk weight, percentage of yolk, albumin weight, percentage of albumin, yolk color index, and haugh unit (HU). The current results indicated that the inclusion of fermented *S. binderi* at levels of 0, 4, 8, 12, and 16% in laying hens' diets had no significant effect on performance or external and internal egg quality. The utilization of fermented *S. binderi* is considered safe when incorporated at levels up to 16% within diets for laying hens.

Keywords: Egg quality, Fermented *Sargassum binderi*, Laying hen, Performance, *Sargassum binderi*

INTRODUCTION

Algae, encompassing brown, red, and green varieties, serve as biomass sources that grow and develop naturally and are readily cultivated. Consequently, the availability of this biomass is substantial (Cabrita et al., 2016). The Food and Agriculture Organization of the United Nations reported that global algae production amounted to 35.8 million tons, contributed by 54 countries, with 97% of the production derived from cultivation (FAO, 2021). The leading nations in algae production are Norway, China, and Chile for wild brown and red algae; China, Indonesia, the Philippines, and South Korea engage in the cultivation of *Undaria pinnatifida*, *Eucheama*, *Gracilaria*, and Japanese kelp (FAO, 2018).

The rising cost of conventional poultry feed ingredients has prompted increasing interest in exploring

alternative, sustainable feed resources. Marine macroalgae, such as brown algae, have emerged as promising candidates due to their rich nutritional profiles and bioactive compounds (Michalak and Chojnacka, 2015). Brown algae consisting of *Ascophyllum* spp., *Fucus* sp., *Hizikia* spp., *Laminaria* spp., *Macrocystis* spp., *Padina* sp., *Sargassum* spp., *Turbinaria* spp., and *Undaria* spp. (Michalak and Mahrose, 2020). *Sargassum binderi* (*S. binderi*) is a species of brown algae that has been investigated for its potential application as poultry feed (Dewi et al., 2024).

The recent studies demonstrated that the nutritional content and polyphenolic compounds of algae have beneficial effects on health, which has increased scientific interest in exploring the potential applications of algae (Li et al., 2025). Algae contain different components, including polysaccharides such as alginate, fucoidan,

laminarin, and cellulose in brown algae, carrageenan, agar, xylan, cellulose, and porphyran in red algae, and xylan, ulvan, cellulose, inulin, and pectin in green algae. Algae also have proteins, essential amino acids, minerals, vitamins, lipids, polyunsaturated fatty acids, pigments such as carotenoids, chlorophyll, phycobilins, and fucoxanthin, as well as antioxidant compounds, particularly polyphenols (Michalak and Chojnacka, 2015; Øverland et al., 2018). Polyphenols, which are found in algae, have antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, and immunomodulatory properties (Michalak and Chojnacka, 2015; Corino et al., 2019). These compounds have hypocholesterolemic, antithrombotic, anticoagulant, antilipidemic, hypocholesterolemic, and antitoxic activities (Kumar et al., 2021). In addition to algae's abundant availability, the unique and diverse nutritional compositions of different algae species make them attractive and potentially advantageous for inclusion in poultry feed formulations.

Supplementation of algae in poultry diets has been widely investigated and developed. Including algae in poultry feeding has several positive impacts on different performances, including increased egg-laying rate, improved egg composition and quality parameters, enhanced growth performance, and acting as a prebiotic. Additionally, supplementation of algae enhances immune activities, improves intestinal villi, and boosts useful bacteria (Michalak and Mahrose, 2020). Alga is a new, natural, environmentally friendly, and healthy feed ingredient that can produce eggs enriched with active compounds such as fatty acids, amino acids, vitamins, and pigments, along with functional properties such as antioxidant, anti-inflammatory, and antimicrobial effects (Michalak and Chojnacka, 2015; Qadri et al., 2019).

However, despite these nutritional and functional benefits, the direct application of algae in poultry diets encounters several limitations. Algae contain anti-nutritional factors such as high sodium chloride (NaCl) (Dewi et al., 2018), as well as heavy metals, tannins, and mannitols (Stengel et al., 2011), which may interfere with nutrient absorption. In addition, algae have low energy content (Dewi et al., 2024) and indigestible nutrients that reduce feed efficiency (Holdt and Kraan, 2011; Dewi et al., 2019). High NaCl and alginate levels limit the use of *S. binderi* as poultry feed (Dewi et al., 2018; 2019). According to Dewi et al. (2018), it has been found that the alginate and NaCl contents of *S. binderi* were 20.68% and 17.20%, respectively. Laying rations contain NaCl at a concentration of 0.33% (NRC, 1994). Previous studies have demonstrated that elevated levels of NaCl in poultry

feed can induce diarrhea, mortality, and ascites (Zhang et al., 2013; Dewi et al., 2018). Adding algae-derived polysaccharides to poultry feed may cause a decrease in performance due to their ability to bind and reduce nutrient absorption in the digestive tract (Jacob, 2025).

To address the limiting factors associated with the utilization of *S. binderi*, processing procedures are necessary. The NaCl content can be reduced by immersing the seaweed in flowing water for a duration of 15 hours, resulting in a reduction of the salt content from 16.86% to 0.94% (Dewi et al., 2018). Meanwhile, the alginate content can be reduced through fermentation. Fermentation can enhance the nutritional quality of seaweed and extend its shelf life (Choi et al., 2018; Dewi et al., 2019). In brown seaweed, alginate can be depolymerized using the alginate lyase enzyme (Guarino et al., 2015). *Bacillus megaterium* (*B. megaterium*) S245 is one of the bacterial strains capable of producing alginate lyase enzyme (Subaryono et al., 2016). Fermentation with *B. megaterium* S245 can reduce the alginate content of *S. binderi* from 37.10% to 31.77%, representing a 14.37% decrease (Dewi et al., 2019). The present study aimed to determine the safe usage limits of fermented *S. binderi* meal within the diet of laying hens and to assess its effects on egg quality as well as the performance of the laying hens.

MATERIALS AND METHODS

Ethical approval

The present study was conducted in compliance with the regulations established by the Official Ethics Committee for Animal Care and Welfare of Andalas University, Padang, Indonesia (577/KEP/FK/2019).

Collecting and processing of *Sargassum binderi*

The collection of *S. binderi* was carried out using a random sampling method at Sungai Nipah Beach (coordinates: -1.371664, 1.371664, 100.579617/1022°18.0" S 100°34'46.6" E), West Sumatra, Indonesia. The present study used *S. binderi* individual parts, such as the talus, bladder, and holdfast. The alga was then submerged in the flowing water of the River Gunung Nago, located in the Pauh District of Padang, West Sumatra, Indonesia, with a flow rate of 0.6745 m³ per second and a depth of 1.3 meters for 15 hours (Dewi et al., 2018). Following immersion, the alga was harvested and dehydrated in an oven at 60°C until the moisture content reached around 14%, then milled into a powder with a particle size of 0.5 mm. Furthermore, the alga flour was fermented with

Bacillus megaterium S245. The fermentation process involved liquid fermentation. *S. binderi* flour, palm sugar, and water were used as the substrate. The ratio of alga and water was 1:5, with palm sugar added at 3% of the total volume of substrate. Before the fermentation process began, the substrate was sterilized using an autoclave at 121°C for 15 minutes. Then, inoculated with *B. megaterium* S245 at a concentration of 27×10^7 CFU/mL. The fermentation process was conducted under anaerobic conditions for nine days at ambient temperature (25-35°C). After the fermentation process finished, the fermented alga was removed from the plastic and then aerated. Additionally, the fermented alga was dried, placed into a plastic bag, and stored at room temperature. The following process was carried out on proximate, metabolic energy, alginate, and fucoxanthin (Table 1), based on Sibbald (1986), AOAC (1990), Hegazi et al. (1998), and Dewi et al. (2019), respectively. Amino acid content determination was carried out based on the method of IK.LP-04.7-LT1.0 (HPLC; Table 2).

Animals and diets

Two hundred Isa Brown laying hens (body weight: 1571-1586 g; age: 62 weeks old) were randomly assigned to five treatments with four replications. Each replication consisted of ten layers for each group. The treatment groups were based on fermented *S. binderi* levels, including the control group, which received 0% fermented *S. binderi*, while the other treatment groups received 4%, 8%, 12%, and 16% fermented *S. binderi*, respectively, over six weeks as-fed in daily rations. The diet calculation was conducted based on NRC (1994), and water was prepared *ad libitum* during the study period (Table 3).

Performance and egg qualities

Each day, eggs were collected, and their production was recorded. Weekly summaries were provided for feed intake, conversion ratio, production, and egg weight. Eighty eggs were randomly selected (Four eggs from each replicate of treatment) on the last two days of the study and used to evaluate egg quality. Internal egg quality was measured by yolk weight, albumin weight, and albumin percentage, while external egg quality was assessed by the weight, thickness, haugh unit (HU), strength, length, and width of the eggshell. Egg length and width were measured using a caliper. The percentage of egg yolk, shell, and albumin was obtained by dividing the weight of the yolk, shell, and albumin by the weight of the egg and multiplying by 100%. Yolk color was evaluated using a Roche yolk color fan. Eggshell strength was assessed

using an EGG Shell Force Gauge EFG-0502, while eggshell thickness was determined with a micrometer accurate to 0.01 mm. The shape index (SI) was calculated using the following formula. $\text{Width/length} \times 100$ (Anderson et al., 2004). The HU was calculated using the following formula.

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

H means the height of the thick albumen in millimetres, and W is the egg weight in grams (Silversides and Villeneuve, 1993).

Statistical analysis

The data was evaluated by variance analysis using the WPS Excel-Statistics 2022 version 11.2.0.11254 software in a properly randomized design. To normalize data distribution, the hen-day egg production (HDEP) and eggshell percentages were logarithmically adjusted using $\log_{10}(x + 1)$. The Duncan Multiple Range Test (DMRT) was performed to compare mean values for each treatment, and $p < 0.05$ indicated a significant difference (Steel et al., 1997).

Table 1. The nutrient content of fermented *Sargassum binderi* meal

Nutrient	In dry wet
Dry matter (%)	92.57
Ash (%)	16.18
Organic matter (%)	76.34
Crude protein (%)	11.68
Crude lipid (%)	0.80
Crude fiber (%)	15.17
Calcium (Ca) (%)	1.19
Phosphor (P) (%)	0.26
Natrium (Na) (%)	0.37
Metabolisable energy (kcal/ kg)	477.87
Alginate (%)	29.13
Fucoxanthin (µg/g)	2.3458

Table 2. Amino acid composition of fermented *Sargassum binderi* meal

Amino acid	Percentage
Aspartic acid	0.56
Glutamic acid	0.32
Serine	0.28
Histidine	0.74
Glycine	0.31
Threonine	0.37
Arginine	0.27
Alanine	0.15
Tyrosine	0.29
Methionine	0.48
Valine	0.15
Phenylalanine	0.28
Isoleucine	0.36
Leucine	0.30
Lysine	2.45
Total amino acid	7.32

Table 3. Different fermented *Sargassum binderi* meal (%) levels in the experimental diet compositions of laying hens

Ingredients (%)	Control	4 (%)	8 (%)	12 (%)	16 (%)
Concentrate K 38 Royal Maize	30.10	30.10	30.10	30.10	30.10
Maize	45.35	46.36	46.36	46.36	46.36
Rice bran	20	13.94	9.14	4.34	0
Palm oil	0	0.60	1.40	2.20	2.54
Limestone	4.55	5	5	5	5
Fermented <i>S. binderi</i> meal	0	4	8	12	16
Chemical analysis	100	100	100	100	100
Crude protein (%)	16.08	16.16	16.25	16.34	16.47
Crude lipid (%)	2.67	3.16	3.86	4.51	4.82
Crude fiber (%)	6.32	5.94	5.74	5.54	5.42
Ca (%)	3.33	3.44	3.45	3.47	3.48
P (%)	0.36	0.35	0.34	0.34	0.33
Metabolisable Energy (kcal/kg)	2603.69	2614.92	2628.91	2642.91	2624.43
Methionine (%)	1.03	1.03	1.04	1.04	1.05
Lysine (%)	1.42	1.48	1.55	1.45	1.70
Alginate (%)	0	1.17	2.33	3.50	4.66

Note: Concentrate K 38 Royal is a commercial concentrate feed for laying hens from PT Leong Hup Jayapindo; Ca: Calcium; P: Phosphorus

Table 4. Laying hen productive performance of laying hens

Level of fermented <i>Sargassum binderi</i> (%)	Feed intake (g/hen/d)	HDEP (%)	Egg weight (g/egg)	Egg mass (g/hen/d)	Feed conversion ratio
Control	116.59	84.11	60.29	50.76	2.30
4	114.26	85.48	60.88	52.12	2.20
8	116.65	85.71	60.01	51.30	2.28
12	115.16	81.55	60.73	49.50	2.33
16	114.39	81.41	60.74	49.41	2.32
SEM	1.28	1.77	0.93	0.89	0.04
P-value	0.69	1.19	0.11	1.64	1.95

Note: HDEP: Hen day eggs production, SEM: Standard error of the mean

Table 5. Egg external quality criteria of laying hens

Egg external quality	Level of fermented <i>Sargassum binderi</i> in Diet (%)					SEM	P-value
	Control	4	8	12	16		
Eggshell weight (g)	6.60	6.96	6.66	6.84	6.84	0.12	1.54
Eggshell (%)	10.95	11.49	10.93	11.25	10.88	0.17	2.45
Eggshell thickness (mm)	0.34	0.33	0.35	0.37	0.35	0.01	1.54
Eggshell strength (kg/cm ²)	3.00	3.68	3.51	3.55	3.37	0.25	1.08
Egg length (cm)	5.65	5.60	5.68	5.63	5.75	0.05	1.21
Egg width (cm)	4.27	4.29	4.28	4.31	4.30	0.03	0.27
Egg shape index/SI	75.66	76.56	75.37	76.67	74.93	0.71	1.14

Note: SEM: Standard error of the mean

Table 6. Egg internal quality criteria of laying hens

Egg internal quality	Level of fermented <i>Sargassum binderi</i> in diet (%)					SEM	P-value
	Control	4	8	12	16		
Yolk weight (g)	14.63	14.84	14.67	14.51	14.81	0.34	0.15
Yolk (%)	24.24	24.51	24.06	23.87	23.53	0.37	1.00
Albumin weight (g)	39.13	38.76	39.65	39.45	41.21	0.78	1.46
Albumin (%)	64.81	64.00	65.01	64.89	65.58	0.45	1.63
Yolk color index	7.95	8.41	8.13	8.48	8.37	0.17	1.61
Haugh unit (HU)	96.56	94.87	98.04	94.55	94.41	0.66	1.46

Note: SEM: Standard error of the mean

RESULT AND DISCUSSION

Laying hen performance

Table 4 shows the effect of fermented *S. binderi* meal in laying hens' diets on production performance. The current results revealed that including fermented *S. binderi* meal in the laying hen feed had no significant effect on production performance ($p > 0.05$).

Feed intake

The inclusion of fermented *S. binderi* in the diet of laying hens up to a level of 16% did not influence feed intake. This outcome can be attributed to the fermented *S. binderi* utilized in the present study, which contained a low sodium chloride (NaCl) content, as a processing method was employed to decrease the NaCl concentration in flowing water. Consequently, the NaCl level was reduced to 0.94%, with 0.37% sodium (Na). The inclusion of fermented *S. binderi* up to 16% in the diet of laying hens, with a Na concentration of 0.13%, was considered safe and did not negatively impact their feed consumption. The recommended Na levels in poultry diets generally range from 0.17% to 0.19% (Baloš et al., 2016). Furthermore, the fermentation process utilizing *B. megaterium* S245 facilitated the depolymerization of alginate in the seaweed, thereby enhancing its potential as a feed ingredient (Dewi et al., 2019).

The alginate content in diets incorporating 0%, 4%, 8%, 12%, and 16% fermented *S. binderi* was recorded as 0, 1.17, 2.33, 3.50, and 4.66%, respectively. It has been documented that the inclusion levels of alginate and its derivatives, such as polymannuronates, in poultry diets are 0.04% and 0.2%, whereas Na alginate oligosaccharides are utilized at levels ranging from 0.1% to 0.4%. (Yan et al., 2011; Zhu et al., 2015). While the alginate content in the present study exceeded the levels previously reported, the alginate in the diets was broken down into its simpler form, alginate oligosaccharides, which facilitated digestive enzymes to penetrate the laying hens' gastrointestinal tract, breaking down nutrients more effectively. As a result, nutrients can be digested and absorbed without being hindered by the alginate present in the diet. Ertesvåg (2015) reported that the alginate lyase depolymerizes alginate to form unsaturated uronic acid. In addition, low molecular weight polymannuronates, a derivative of alginate, in broiler diets are more efficient than natural seaweed fibers (alginate) with higher molecular weights (Zhu et al., 2015).

The previous study has shown that depolymerized alginate or alginate oligosaccharides are useful as a prebiotic (Wang et al., 2006). According to Kulshreshtha et al. (2014), prebiotics can increase the proliferation of beneficial bacteria, such as *Streptococcus salivarius* (4- to 15-fold) and *Bifidobacterium longum* (4- to 14-fold), and reduce the unfavourable bacterial population of *Clostridium perfringens* in the laying hen digestive tract. These bacteria facilitate the production of secondary metabolic enzymes to degrade undigested feed ingredients in the digestive tract (Flint et al., 2012). Wan et al. (2017) reported that utilizing alginate oligosaccharides in pork rations can improve the nutrient digestibility, including crude protein, fat, and ash.

In addition to the alginate content that did not affect the laying hen feed intake in the present study, the darker color of the ration containing fermented *S. binderi* meal, compared to the control ration (0% fermented *S. binderi* meal), did not impact the palatability of the feed for the hens. The current results supported the findings of Farghly and Abou-Kassem (2014) and Farghly and Mahrose (2017), indicating that providing colorless or colored feed (red, orange, and green) does not affect turkey feed consumption at 0-16 weeks of age. Adding fermented *S. binderi* to the diet made the color of laying hen feed darker, but it did not affect its taste and was believed to meet the poultry's nutritional requirements. Feed energy substances in the laying hen diet in the present study were in accordance with the laying hen's requirements and were given every day. According to Classen (2017), a characteristic specific to poultry is their feed intake for energy acquisition, and consequently, daily feed intake is likely correlated with feed energy content.

The feed intake of laying hens in the present study aligns with the findings of Fan et al. (2021), indicating that the inclusion of up to 5% *Sargassum* meal did not influence feed intake. However, supplementing the diet of laying hens with *S. dentifebium* 3% reduced feed intake (Al-Harathi and El-Deek, 2011), and supplementing with *Sargassum* sp. 2% decreased feed intake in laying hens (Carrillo et al., 2012).

Unaffected feed intake helped stabilize HDEP, which was possible because the digestive tract of laying hens continued to digest and absorb nutrients normally, meeting their energy and nutrient requirements for egg production. According to Jacob et al. (2014), laying hens require a balanced diet to maintain egg production. A lack of energy, essential nutrients such as protein, especially lysine and methionine, or calcium can disrupt egg

production, leading to a halt in egg-laying (Alagawany et al., 2021).

Hen day egg production

The HDEP observed during the current study ranged from 81.41% to 85.71%, aligning within the HDEP interval reported by previous studies, specifically between 58.3% and 91.28%, through the administration of different types of algae at levels of 0.25% to 3% in the diet of laying hens (Halle et al., 2009; Al-Harathi and El-Deek, 2011; Carrillo et al., 2012; Kulshreshtha et al., 2014; Park et al., 2015). However, previous studies indicated a decrease of more than 3% in HDEP caused by *Sargassum* (Al-Harathi and El-Deek, 2011; Carrillo et al., 2012) and 5% in laying hens' diet (Fan et al., 2021), which could be due to the high Na content. A decrease in HDEP can also be caused by high algal tannins, which interfere with the digestion process and protein absorption. The difference in HDEP caused by adding alga to laying hen diets can be affected by chicken strain, age, algae type, algae concentration, and several environmental factors (Zhang et al., 2012).

The inclusion of fermented *S. binderi* in the diet at levels up to 16% did not influence the HDEP of laying hens, which was attributed to the observation that the feed intake of the laying hens was not inhibited by the incorporation of fermented *S. binderi* into their diet. The HDEP of laying hens is influenced by multiple factors, including the quality of the diet, the quantity of feed consumed, and its nutritional and energetic content (Jacob et al., 2014). Furthermore, the HDEP among different treatments remained consistent, given that *S. binderi* underwent processing through soaking to reduce salt content and fermentation to depolymerize alginate into simpler compounds. Consequently, the increased incorporation of fermented *S. binderi* meal into the diet of laying hens in the present study did not hinder nutrient digestion and absorption necessary for egg production.

Egg weight

In the present study, the inclusion of 4%, 8%, 12%, and 16% fermented *S. binderi* in the diet exhibited a comparable effect on egg weight. The lack of significant differences in egg weight among treatments may be attributed to the alginate presented in fermented *S. binderi*, which has depolymerized into its simpler form, namely alginate oligosaccharide. Consequently, alginate in the form of alginate oligosaccharide does not hinder the absorption of protein, fat, and minerals, including calcium and phosphorus (Wan et al., 2017), necessary for egg

formation, thereby influencing egg weight. The egg weight obtained in the present study (60.01-60.88 g/egg) remained within the egg weight range reported by several previous studies, including those by Halle et al. (2009), Al-Harathi and El-Deek (2011), Carrillo et al. (2012), Kulshreshtha et al. (2014), and Park et al. (2015). These studies indicated that the addition of algae at different types and concentrations, ranging from 0.25% to 8%, did not significantly affect the egg weight of laying hens. The reported egg weight range in these studies was between 52.30 g and 66.82 g per egg. Nonetheless, Al-Harathi and El-Deek (2011) observed that dietary inclusion of 6% *Sargassum dentifebium* resulted in a significant reduction in egg weight.

Egg mass

The egg mass is influenced by HDEP and egg weight. The HDEP and egg weight measured during the present study were unaffected by adding all levels of fermented *S. binderi* (4, 8, 12, and 16%) to the laying hen diet. According to Figueiredo et al. (2012) and Cesari et al. (2014), egg mass is calculated by multiplying egg production by the average egg weight during each period. The lack of effect on HDEP and egg weight may be due to stable energy intake and nutrient availability, as the inclusion of fermented *S. binderi* in the diet did not interfere with digestive function or nutrient absorption in the hens.

The egg mass obtained in the present study (44.73-52.12 g/hen/day) was within the range reported by previous studies. According to Halle et al. (2009), Al-Harathi and El-Deek (2011), and Choi et al. (2018), adding algae at different concentrations from 0.25% to 3% did not affect the egg mass of laying hens, with reported values of 40.04-60.2 g/hen/day. Studies by Al-Harathi and El-Deek (2011) exhibited a decline in egg mass when *Sargassum* was used at 3% in the diet, and Fan et al. (2021) found a similar decrease at 5%. Egg mass levels stayed below the established level for brown laying hens during the current study.

Feed conversion ratio

There was no significant effect ($p > 0.05$) on the feed conversion ratio due to feed consumption, HDEP, and egg weight, which were unaffected by providing fermented *S. binderi* at 4, 8, 12, and 16%. Feed conversion ratio compares feed intake to egg production and the average weight of eggs laid by hens in one day (Kulshreshtha et al., 2014). The addition of fermented *S. binderi* at levels of 4%, 8%, 12%, and 16% did not influence the digestion or

absorption of nutrients. Energy and essential nutrients such as protein, including amino acids (lysine and methionine), fatty acids (linoleic acid), calcium, and vitamins, are vital not only for basic physiological functions but also for optimal egg production (Leeson and Summer, 2005).

The current results on feed conversion ratio ranged from 2.20 to 2.49, which were close to the feed conversion reported by several studies. Halle et al. (2009), Carrillo et al. (2012), and Kulshreshtha et al. (2014) reported that adding algae of different types and at different concentrations from 0.25% to 8% did not affect the feed conversion ratio of laying hens' diets, with the feed conversion ratio in these studies ranging from 1.69 to 2.19. However, there was an increase in feed conversion ratio with the addition of alga at 3% and 6% (Al-Harathi and El-Deek, 2011) and *Codrus crispus* by 1% in laying hen feed (Fan et al., 2021). Changes in feed conversion ratio values can be influenced by the age and breed of chickens, species, and algae concentration, as well as differences in experimental environments (Zhang et al., 2012).

Based on previous studies, it can be observed that the inclusion of alga in the diet is $\leq 8\%$ (Carrillo et al., 2012). However, in the present study, the utilization of fermented *S. binderi* at levels up to 16% did not adversely affect feed intake, HDEP, egg weight, egg mass, or feed conversion ratio. The processing of *S. binderi* may be impacted by fermentation with *B. megaterium* S245.

External egg quality

Table 5 illustrates the effect of fermented *S. binderi* meal supplementation on external egg quality in laying hens. The current results demonstrated that its inclusion did not have a statistically significant impact on external egg quality ($p > 0.05$).

The addition of fermented *S. binderi* at 4%, 8%, 12%, and 16% in laying hen diets indicated no difference in external egg qualities compared to a control diet (Fermented *S. binderi* 0% meal). Adding fermented *S. binderi* at these levels did not interfere with the absorption of calcium, phosphorus, vitamin D, and fat in the laying hen's digestive system tract. This was influenced by the low Na content of the diets, specifically the fermented *S. binderi* at different inclusion levels, including 0% (0.08%), 4% (0.09%), 8% (0.11%), 12% (0.12%), and 16% (0.13%). Therefore, the external egg qualities, including eggshell weight, eggshell percentage, eggshell strength, and thickness, were not disturbed. These findings align with Persson (2009), who stated that high Na content

negatively impacts eggshell calcium deposition. Persson (2009) further elucidated that elevated NaCl levels significantly diminish eggshell quality due to a reduction in carbonic anhydrase activity within the glands responsible for eggshell formation. The strength of the eggshell is dependent on the mineral and vitamin composition of the feed, notably calcium, phosphorus, manganese, and vitamin D. Laying hens produce eggs with fragile or compromised shells when their feed lacks sufficient calcium (de Abreu Fernandes and Litz, 2017). Furthermore, *S. binderi* underwent fermentation to depolymerize alginate into simpler compounds. The increased inclusion of fermented *S. binderi* meal in the diet did not disrupt the digestion and absorption of nutrients within the gastrointestinal system. Consequently, it did not impact the external quality of the eggs.

The length, width, and shape index of eggs treated with fermented *S. binderi* at levels of 4%, 8%, 12%, and 16% displayed results comparable to the control diet (Fermented *S. binderi* 0%). Furthermore, the measurements of egg length and width are associated with the egg shape index (Duman et al., 2016). According to Duman et al. (2016), the egg shape index represents the ratio of the width of the egg to its length. The egg shape index of the current results indicated the criteria for regular and round eggs. The SI criteria are sharp eggs with an SI value of $SI < 72$, as regular, ranging from 72 to 76, and round eggs with an $SI = 76$ (Altuntaş and Şekeroğlu, 2008). Factors influencing the egg shape index include egg albumin quality, egg length, width, eggshell strength (Altuntaş and Şekeroğlu, 2008), albumin index, and egg HU (Duman et al., 2016).

Eggshell strength and thickness of the current results were similar to those reported by Park et al. (2015) for the addition of 0.5 and 1% microalga Schizochytrium, or for diets without microalga, which did not affect eggshell strength (4.20-4.23 kg/cm²) and eggshell thickness (0.37-0.38 mm). The current results indicated that eggshell percentage and eggshell thickness, regarding the supplementation of *S. dentifebium* 0% and 3% in the laying hen diet, did not affect eggshell percentage (13.6-13.8%) and eggshell thickness (0.43-0.44 mm), which was consistent with the result of Al-Harathi and El-Deek (2011).

Internal egg quality

Table 6 presents the impact of fermented *S. binderi* meal supplementation on internal egg quality in laying hens. The current findings indicated that its inclusion had no significant effect on internal egg quality ($p > 0.05$).

Adding fermented *S. binderi* at 4%, 8%, 12%, and 16% in the laying hen diet exhibited no difference compared to the control diet in egg yolk weight and egg yolk percentage, which was influenced by fulfilling nutritional requirements, such as protein and fat. The yolk of the egg is primarily composed of fat and protein constituents. According to [Puertas and Vázquez \(2018\)](#), the egg yolk contains approximately 50% solids. The majority of these solids consist of fat (65-67%) and protein (30%; [Laca et al., 2010](#)).

The inclusion of fermented *S. binderi* at levels of 4%, 8%, 12%, and 16% in the diet did not significantly affect albumin weight or albumin percentage compared to the control group, which was influenced by the nutritional requirements of laying hens, which necessitate protein intake for albumin synthesis. According to [Bouvarel et al. \(2011\)](#), a reduction in protein content in feed may result in decreased levels of albumin.

Yolk weight and albumin weight of the present study are consistent with [Ozaki et al. \(2013\)](#), who indicated that *Gracilaria vermiculophylla* 2% supplementation did not affect yolk and albumin weight. The current results align with [Al-Harathi and El-Deek \(2011\)](#), who found that supplementing laying hen diets with 0%, 3%, and 6% *S. dentifebium* did not significantly influence yolk and albumin percentages.

Adding fermented *S. binderi* at concentrations of 4%, 8%, 12%, and 16% to the laying hen diet did not affect the yolk color index compared to the control diet, which was caused by the damage to fucoxanthin in fermented *S. binderi* due to processing methods such as immersion in flowing water to reduce NaCl content, sterilisation using heating during fermentation, and drying. Fucoxanthin is highly sensitive to UV light and high temperatures ([Baek et al., 2021](#)).

The yolk color index in the present study aligns with the findings of [Al-Harathi and El-Deek \(2011\)](#), showing that supplementation with *S. dentifebium* at levels of 0%, 3%, and 6% does not affect the yolk yellow index. Furthermore, [Park et al. \(2015\)](#) reported that using 0.5% and 1% microalga *Schizochytrium* or not including microalga in the laying hen diet did not increase yolk color. A significant increase in yolk color was observed with the inclusion of *Sargassum* spp., at levels of 4%, 6%, and 8% which is inconsistent with the findings of [Carrillo et al. \(2012\)](#). [Halle et al. \(2009\)](#) reported that microalga *Chlorella vulgaris* supplementation at 2.5, 5, and 7.5 g/kg, treated with spray drying, had a significant effect on increasing the yolk color index. Differences in yolk color index among studies may result from several variables,

including whether algae were processed or not, the concentration and type of algae used, the carotenoid content in the basal diet, and the addition of extra carotenoids in the experimental feed.

The unaffected HU of eggs resulting from the addition of fermented *S. binderi* to the laying hen diet suggested that the nutritional requirements of the hens were satisfied. Several studies have reported a decrease in HU with increasing dietary energy ([Pérez-Bonilla et al., 2012](#)). Additionally, [Valkonen et al. \(2006\)](#) documented elevated HU levels in laying hens subjected to a low-protein diet (14%) in comparison to a high-protein diet (19%). Conversely, HU appears unaffected by the nutrient content of the ration ([Wall et al., 2010](#); [Ribeiro et al., 2014](#); [Khatibi et al., 2021](#); [Scappaticcio et al., 2021](#)).

CONCLUSION

The inclusion of fermented *Sargassum binderi* meal at a level of 16% in the diet of laying hens did not adversely impact their performance, external or internal qualities of eggs. Algae have nutrients and bioactive compounds that can meet poultry's nutritional needs, boost their health, and have a positive impact on the environment. Further studies on algae need to be conducted to investigate their potential as a functional feed in poultry diet.

DECLARATIONS

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Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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Authors' contributions

Mahata contributed to the conceptualization and project administration. Dewi conducted data collection and drafted the manuscript. Sofyan reviewed and approved the final edition of the manuscript. All authors provided their

informed consent to participate in the study, read and approved the final edition of the manuscript.

Competing interests

The authors declared that there are no conflicts of interest related to this research.

Ethical considerations

No ethical issues such as plagiarism, informed consent violations, misconduct, data falsification, duplicate submission or publication, or redundancy were found in this article before submission to the journal.

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Newcastle Disease Virus Infection in Domestic Pigeons: Epidemiology, Pathogenesis, Diagnosis, and Vaccination Strategies with Emphasis on Chitosan Nanoparticles

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ABSTRACT

Newcastle disease virus (NDV), also known as avian paramyxovirus-1 (APMV-1), is a highly contagious pathogen that affects most avian species, including domestic pigeons (*Columba livia*), leading to Newcastle disease (ND). The ND in pigeons is attributed to pigeon-specific strains of NDV, predominantly characterized by the emergence of pigeon paramyxovirus-1 (PPMV-1). This viral strain is specifically adapted to affect avian species, particularly within the pigeon population, resulting in distinct pathological features associated with the disease. The ND was correlated with severe respiratory, neurological, and gastrointestinal manifestations, resulting in elevated morbidity and mortality rates, which may reach up to 80%. The present study provided an updated overview of the pathogenesis, clinical and pathological features, and diagnostic approaches related to NDV infection in domestic pigeons worldwide. Conventional and modern vaccination strategies were discussed in the present study, with a focus on mucosal immunization. Chitosan-based nanoparticles (CS-NPs) have emerged as a promising vaccine delivery platform due to their compatibility with biological systems, strong adhesion to mucosal surfaces, and ability to enhance antigen stability and stimulate the immune response. The CS-NPs improved antigen uptake at mucosal surfaces in poultry and stimulated both humoral and cellular immune responses, which included activating cytotoxic T cells, producing cytokines, and secreting immunoglobulins at mucosal sites. The present review may contribute to the advancement of more effective and targeted vaccine strategies against NDV in pigeons and other avian species.

Keywords: Chitosan, Live vaccine, Nanoparticle, Newcastle disease, Paramyxovirus, Pigeon

INTRODUCTION

Newcastle disease virus (NDV) is the causative agent of Newcastle disease (ND), a highly contagious infection affecting poultry. The economic impact of ND is significant, posing considerable challenges to the poultry industry worldwide (Mao et al., 2022). The ND was first reported in 1926 on Java Island, Indonesia, and in Newcastle-upon-Tyne, England; the disease later spread worldwide, causing substantial economic losses to the poultry industry (Mao et al., 2022; Dharmayanti et al., 2023). The ND is endemic in parts of Asia (Ansori and Kharisma, 2020), Africa (Ansori and Kharisma, 2020), the Middle East (Dzoghema et al., 2021), Central and South America, and Indonesia (Dharmayanti et al., 2023).

The NDV, recognized as avian paramyxovirus-1 (APMV-1), belongs to the family Paramyxoviridae, subfamily Avulavirinae, genus Orthoavulavirus, and species avian paramyxovirus 1 (Zerbini et al., 2024). The NDV possesses an envelope and carries a single-stranded, negative-sense RNA genome (Biswas et al., 2024). The RNA genome encodes six structural proteins, including nucleoproteins (NP), phosphoproteins (P), matrix protein (M), fusion protein (F), hemagglutinin-neuraminidase (HN), and large polymerase protein (L) (Moustapha et al., 2023). The complete nucleotide sequence of the *F* gene indicates that the virus is categorized into class I, which contains a single genotype, and class II, which comprises 20 genotypes (I-XXI) (da Silva et al., 2020). The classification of NDV pathogenicity encompasses

velogenic, mesogenic, lentogenic, and asymptomatic categories, based on intracerebral pathogenicity index (ICPI) values of over 1.5, between 1.5 and 0.7, less than 0.7, and 0, respectively (Dzobema *et al.*, 2021). The velogenic NDV pathotype (Viscerotropic and neurotropic) shows very high mortality, the mesogenic pathotype reveals moderate respiratory signs and low mortality, the lentogenic strain induces a mild respiratory infection with no mortality, and the asymptomatic pathotype may exhibit no clinical signs or subclinical enteric infection in poultry (Nurzijah *et al.*, 2022).

The domestication of the pigeon (*Columba livia*) dates back approximately 6,000 years, originating during the Neolithic period in the Mesopotamian Valley (Giunchi *et al.*, 2020). The selection of different domestic pigeon breeds marks the early stages of their development. The pigeon industry has grown into a diverse sector, including birds used for racing, ornamental purposes, and meat production (Gao *et al.*, 2016; Jin *et al.*, 2023). Pigeon meat and eggs are highly nutritious and have become a popular food source worldwide. Recently, the domestic pigeon farming industry has evolved as a significant component of the poultry sector (Wang *et al.*, 2025). Pigeons are susceptible to many important viral, bacterial, fungal, and parasitic diseases; one of the significant infections of pigeons is APMV-1 (Abd El-Ghany, 2023). Pigeons are effective carriers of APMV genotype VIId (Velogenic viscerotropic strain) due to the cross-transmission of the virus among avian species (He *et al.*, 2018). Infected pigeons exhibit high mortality rates exceeding 75%, accompanied by severe digestive (Greenish diarrhoea), respiratory (Dyspnoea, rales), and neurological signs (Torticollis, paralysis), as well as high viral shedding in the trachea and cloaca (Thomazelli *et al.*, 2021).

Infectious viral diseases in poultry, particularly those affecting the respiratory system, pose a significant threat, resulting in severe economic losses, as well as their zoonotic importance (Biswas *et al.*, 2024; Hassman *et al.*, 2025; Kilany *et al.*, 2025). Due to the difficulties in controlling viral diseases, vaccination is the only recommended method to prevent and overcome viral diseases (Moustapha *et al.*, 2023). Vaccines against viral infections include live (Naturally weak or attenuated), killed or inactivated, subunit, or recombinant types (Abdelaziz *et al.*, 2024). The development of innovative vaccines aims to induce specific adaptive immune responses that protect chickens against different viral diseases, which can be achieved by optimizing factors such as the route of administration, the use of effective adjuvants, and the implementation of a well-structured

vaccination program (Yu *et al.*, 2020). However, delivering the vaccine antigen can be hindered by several barriers, including low immunogenicity, degradation within the body, and the risk of inducing immune tolerance (Moghaddam, 2021). To address the challenges associated with conventional vaccine delivery mechanisms, there has been a growing interest in carbohydrate-based polysaccharides as effective agents for improving vaccine efficacy. Notably, chitin, a naturally occurring biopolymer sourced from insects and crustaceans, emerges as a particularly significant candidate due to its abundant availability and potential application in vaccine formulation (Renu and Renukaradhya, 2020). The deacetylation of the chitin polysaccharide chain produces chitosan. Chitosan is extensively utilised to target mucosal areas via oral, ophthalmic, nasal, implant, parenteral, and transdermal routes owing to its distinctive characteristics, including modifiable flexibility, positive surface charge, and the ability to conjugate with other polymers (Renu and Renukaradhya, 2020; Guo *et al.*, 2024). Additionally, chitosan is known for its bioavailability, biodegradability, safety, and immunostimulatory properties, making it a suitable carrier for all types of vaccines (Wijesekara and Xu, 2024).

The mucosal immunity triggered by viral vaccines is achieved through precise and optimal delivery methods (Nasal and oral) in most poultry species (Akter *et al.*, 2024). The mucosal immune response begins with microfold cells, specialized epithelial cells located in the mucosa-associated lymphoid tissue, mainly in the Peyer patches of the small intestine. These cells take up the CS-NP-based vaccines and then transport the nanoparticles to areas rich in antigen-presenting cells (Jazayeri *et al.*, 2021). This process activates antigen-specific CD4+ T helper (Th) cells, which then interact with B lymphocytes, encouraging their differentiation into IgA-committed B cells (IgA+ B cells). These IgA+ B cells migrate to mucosal effector sites, where they further mature into plasma cells that produce immunoglobulin A (IgA) (Jin *et al.*, 2019; Thakur and Foged, 2020). Meanwhile, the cellular immune response is initiated by antigen-presenting cells (APCs), such as dendritic cells, macrophages, and B lymphocytes. These APCs stimulate the development of cytotoxic T lymphocytes (CTL) by activating CD8+ T cells and CD4+ T helper type 1 (Th1) cells (Renu and Renukaradhya, 2020; Guo *et al.*, 2024).

The present study aimed to provide an updated overview of NDV infection in domestic pigeons, including pathogenesis, clinical features, and diagnostic methods, highlighting current vaccination challenges and exploring the potential of chitosan-based nano vaccines as an innovative approach for mucosal immunization against NDV in pigeons.

PARAMYXOVIRUS INFECTION IN PIGEONS

Pigeons are highly susceptible to NDV infection, resulting in significant economic losses due to immunosuppression, mortality, and vaccination costs. Additionally, outbreaks of NDV in pigeons can result in trade limitations regarding the movement of live birds and involvement in exhibitions or races, especially in accordance with international animal health regulations (Abd El-Ghany, 2023). The NDV that affects pigeons of all ages is an antigenic variant of APMV-1, known as pigeon paramyxovirus type 1 (PPMV-1), which belongs to the genus *Orthoavulavirus*, subfamily Avulavirinae, within the family Paramyxoviridae (Zerbini et al., 2024).

Over the past three to five years, there has been a significant increase in APMV-1 infections among pigeons, causing substantial losses in the global poultry and pigeon breeding industries (Tong et al., 2024). The PPMV-1 is a host-adapted variant of the traditional NDV genotype VI in chickens and pigeons (Abd El-Ghany, 2023; Tong et al., 2024). Some recent PPMV-1 demonstrated the virulence of mesogenic strains, as evidenced by the mean death time of chicken embryos, which was recorded at 76.8 hours, and an ICPI of 1.25. The morbidity and mortality rates in pigeons were recorded at 100% and 80%, respectively, while chickens exhibited both rates at 80%. Consequently, these recent PPMV-1 isolates were determined to be velogenic for both species (Tong et al., 2024). The ND in pigeons is known as paramyxovirus, which leads to neurological symptoms and high mortality rates, especially in those infected with viscerotropic strains (Pestka et al., 2014; Abdulrasool and Seger, 2023). Currently, PPMV-1 has been documented as an enzootic infection affecting racing, feral, and fancy pigeons (Alexander, 2011; Abd El-Ghany, 2023). Young pigeons (4-6 months old) are the most susceptible to PPMV-1 infection (Badr et al., 2022).

The first identification of APMV-1 in pigeons occurred in Egypt in 1967 (Mansour et al., 2021). More cases of pigeons showing neurological symptoms have been recorded (Elbhnsawy et al., 2017; Mansour et al., 2021; Abd El-Ghany, 2023). Furthermore, PPMV-1 was initially isolated in the Middle East, specifically in Iraq, in 1978. Kaleta et al. (1985), Pestka et al. (2014), and Abdulrasool and Seger (2023) provided a comprehensive characterization of the PPMV-1. During the early 1980s, outbreaks were reported in Europe and North America among pigeons, which occasionally spread to domestic poultry (Kaleta et al., 1992; Rogers et al., 2021). The global spread and evolution of PPMV-1, from early detections to modern outbreaks, are summarized in Table

1 (Rogers et al., 2021; Abd El-Ghany, 2023). Pigeons of all ages have been infected with PPMV-1, showing high morbidity and mortality (Abd El-Ghany, 2023; Abdulrasool and Seger, 2023). The PPMV-1 constantly circulates in healthy feral pigeons, which serve as asymptomatic carriers transmitting the virus to free-range chickens (Annaheim et al., 2022). Sub-genotype XXI.2 of PPMV-1 has been reported in collared doves in Italy and Iran and wild doves in North America (Esmaealzadeh-Dizaji et al., 2022). The PPMV-1 virus is highly virulent in pigeons, chickens, and turkeys, while quails and geese tend to resist infection (Alexander, 2011; Abd El-Ghany, 2023). Passaging in chickens increases its virulence (Śmietanka et al., 2014; Tong et al., 2024). Although some migratory pigeons might transmit the virus, documented cases of infection are restricted to members of the Columbiformes order, such as pigeons and doves, and have not been seen in species outside this group (Dortmans et al., 2011; Abd El-Ghany, 2023). The PPMV-1 virus spreads through nasal, buccal, and ocular secretions, as well as droppings from infected pigeons. Horizontal transmission happens through inhalation or ingestion; additionally, direct contact between healthy and infected pigeons accelerates the spread of the virus (Annaheim et al., 2022; Al-Hially et al., 2024).

CLINICAL SIGNS AND PATHOLOGY OF PIGEON PARAMYXOVIRUS TYPE 1 IN INFECTED PIGEONS

The incubation period of Paramyxovirus type 1 (PMV-1) infection in pigeons ranges from 7 to 14 days, with the severity of clinical manifestations influenced by the virulence of the infective strain, the host's immune status, and any concurrent infections (Alexander and Senne, 2008; Abd El-Ghany, 2023). Pigeons infected with PMV-1 often show clinical signs similar to those caused by neurotropic velogenic strains (Badr et al., 2022; Tong et al., 2024). Pigeons often exhibit polyuria and neurological signs, such as torticollis, shaking, head nodding, muscular tremors, and paralysis of the wings and legs (Figure 1), along with the potential for greenish diarrhea (Badr et al., 2022; Abd El-Ghany, 2023; Al-Hially et al., 2024).

Respiratory signs and swollen eyelids with a serous discharge may occasionally be observed in infected pigeons. As reported by Kraidi et al. (2024), mortality usually starts within five days after infection, and the pigeon dies throughout the observation period of 15 days. Post-mortem findings in pigeons infected with PPMV-1 are often variable and may be nonspecific. However,

several key lesions have consistently been identified, including haemorrhages in the brain (Figure 2), petechial haemorrhages in the gizzard, hemorrhagic enteritis, liver congestion, and soft or friable brain tissue (Badr *et al.*, 2022; Abd El-Ghany, 2023; Al-Hially *et al.*, 2024).

The histopathological changes include alterations in meningeal capillaries, hemorrhages in brain tissue, vacuolar degeneration, vasogenic oedema, and gliosis (Pereira *et al.*, 2022; Al-Hially *et al.*, 2024). Other affected organs exhibited interstitial haemorrhages, accompanied by significant epithelial sloughing of the renal tubular epithelium. Additionally, there was marked white pulp hyperplasia and peri-arteriolar fibrosis, giving a characteristic onion skin appearance in the spleen (Yuzbasioglu-Ozturk and Gurel, 2022). The infiltration of mononuclear cells was observed in the pigeon intestine, characterised by necrosis, severe destruction of the superficial intestinal mucosa, and intense inflammation at the site of tissue destruction (Thomazelli *et al.*, 2021; Yuzbasioglu-Ozturk and Gurel, 2022).

CROSS-TRANSMISSION OF PARAMYXOVIRUSES BETWEEN PIGEONS AND CHICKENS

Pigeons are considered a significant threat for transmitting NDV to domestic chickens because of their migratory behavior, free-living activities, and presence in live bird markets and backyard settings. As a natural host of APMV-1, they play a vital role in the virus's ecology (Pestka *et al.*, 2014; Abd El-Ghany, 2023).

Further evidence of chicken susceptibility to PPMV-1 came from experimental infection of 5-week-old chickens with pigeon-derived NDV of genotype XXI (Nooruzzaman *et al.*, 2021; Tong *et al.*, 2024). Infected chickens exhibited notable pathogenic changes in the lungs, thymus, spleen, and bursa of Fabricius, along with a high mortality rate of approximately 85% and typical signs of NDV. Additionally, histological examination of the brains of infected chickens revealed glial hyperplasia and neuronal degeneration (Zhang *et al.*, 2023). Remarkably, multiple passages of PPMV-1 in chickens may increase its virulence, leading to more severe disease manifestations and heightened neuro-invasiveness (Dortmans *et al.*, 2011; Tong *et al.*, 2024). Several NDV outbreaks in chickens have been associated with spillover events from PPMV-1, and genetic studies have identified specific point mutations that increase the pathogenicity of PPMV-1 in chickens (Werner *et al.*, 1999; Dortmans *et al.*, 2009; Abd El-Ghany, 2023).

DIAGNOSIS STRATEGIES

The signs of PPMV-1 infection closely resemble those of pigeon herpesvirus infection, vitamin B1 deficiency, sodium chloride toxicity, and ronidazole overdose (Pestka *et al.*, 2014; Hamouda *et al.*, 2017; Abd El-Ghany, 2023). This shared feature can complicate clinical diagnosis; therefore, laboratory diagnosis is essential.

The clinical signs and pathological lesions related to the disease are not considered definitive diagnostic tools; however, they may provide preliminary indications that support a tentative diagnosis (Moustapha *et al.*, 2023). Therefore, direct viral antigen detection through viral isolation and identification from swabs of live pigeons or organs taken from deceased ones is the most effective method for a definitive diagnosis of NDV (Prasad *et al.*, 2024). Virus isolation can be effectively performed using embryonated chicken eggs aged 9 to 11 days or different cell types, such as chicken embryo hepatocytes, fibroblasts, reticulum cells, and African monkey kidney cells (WOAH, 2024). Following incubation at 37°C for 4 to 7 days, the propagated eggs were stored at +4°C; after harvesting the allantoic fluid, the NDV was detected by analyzing the allantoic fluid through a hemagglutination assay and confirming NDV infection by a hemagglutination inhibition (HI) test or molecular techniques, such as reverse transcription polymerase chain reaction (RT-PCR) or real-time PCR (Moustapha *et al.*, 2023; WOA, 2024). Additionally, NDV is identified by other serological methods, including fluorescent antibody tests, agar gel immunodiffusion techniques, hemolysis tests, or electron microscopy through identification of viral particle morphology (Moustapha *et al.*, 2023).

Serological diagnosis using the HI test was typically employed as a confirmatory method, relying on specific antibodies to inhibit the hemagglutination activity of NDV (WOAH, 2024). Another serological test often used is the indirect enzyme-linked immunosorbent assay (ELISA), in which the viral antigens are first coated onto a microtiter plate. Serum from the test subject, such as pigeons, is then added, allowing specific antibodies to bind to the target antigen. Secondary antibodies, typically derived from another species and conjugated to an enzyme, including horseradish peroxidase, are introduced to produce a colorimetric reaction when the substrate (TMB) is added. The resulting color intensity is measured by a spectrophotometer at 450 nm, indicating antibody levels (Dzoghema *et al.*, 2021; Moustapha *et al.*, 2023). This method has been successfully applied in the diagnosis of PPMV-1 infection, as demonstrated by Yuzbasioglu-

Ozturk and Gurel (2022), who used ELISA to test sera from pigeons in Istanbul. The ELISA identified 89.18% of cases as positive, confirming its reliability as a diagnostic tool in field investigations. Additional serologic techniques, such as virus neutralization tests, immunofluorescence assays, and colloidal gold-based immunoassays, are employed to detect antibodies against NDV (Mao et al., 2022).

Additional significant methods, including nested PCR, fluorogenic probe-based RT-PCR, ligase chain reaction, SYBR Green intercalation, and light-extended fluorogenic primer assays, have been developed (Dzobgema et al., 2021; Mao et al., 2022). Despite their promising potential, these assays encounter several limitations, particularly in the detection of different viral strains, such as the distinct genotypes of NDV, including genotypes I, II, VII.1.1, and VII.2, as well as strains exhibiting mutations in the *F* gene cleavage site. These challenges underscore the necessity for further validation to establish the reliability and efficacy of these assays in a broader diagnostic context (Moustapha et al., 2023). Since the NDV *F* gene is essential in affecting viral pathogenicity, RT-PCR assays for NDV pathotyping mainly focus on this gene (Abd Elfatah et al., 2021; Moustapha et al., 2023). A novel real-time reverse transcription isothermal loop-mediated amplification method has recently been introduced, providing quicker and more precise detection compared to real-time RT-PCR (Song et al., 2023).

CONTROL AND VACCINATION STRATEGIES

As both immune complex vaccines and *in ovo* injection are widely used advanced strategies in poultry immunization programs, they play a crucial role in early and effective disease control (Abd El-Ghany, 2025). Immune complex vaccines are crucial for early-life immunization, in which the live virus is bound to specific antibodies, creating a complex. The formed antibody-virus complex slows down the release of the PPMV-1 in the pigeons' bodies, helping it to evade maternal antibodies and activate immunity at the optimal time. The immune complex vaccines are frequently used to combat infectious bursal disease and, in some instances, ND (Marchenko and Kolechko, 2024). On the other hand, *in ovo* vaccination, typically performed at 18 days of embryonic development, involves injecting attenuated ND vaccines directly into the egg. This method provides consistent vaccine delivery, quick protection after hatch, and less labor, making it a

safe and effective way for large-scale immunization programs (Hu et al., 2022; Abd El-Ghany, 2025).

Controlling PMV-1 infection in pigeons depends on strict biosecurity measures to prevent viral contact and effective vaccination strategies. For NDV, traditional live and inactivated vaccines, along with advanced recombinant and antigen-matched vaccines, have been widely used, with strains such as Ulster, LaSota, and Mukteshwar being particularly effective (Nurzijah et al., 2022; Moustapha et al., 2023). Recent advances in genetic engineering have enabled the development of advanced multipurpose vaccines, such as virus-like particle vaccines for ND. These vaccines mimic the virus's outer structure without containing its genetic material, making them both safe and effective. Moreover, they are designed to differentiate between infected and vaccinated animals (DIVA) (Raji et al., 2024). Emerging plant-based vaccines demonstrated potential due to their DIVA capability and reduced shedding (Smith et al., 2023). For PPMV-1, vaccination remains crucial, with inactivated NDV vaccines, particularly those derived from lentogenic strains such as LaSota, providing significant protection (Viaene et al., 1984; Soliman et al., 2019; Abd El-Ghany, 2023). Homologous PPMV-1 vaccines are preferred due to their enhanced protective capabilities compared to heterologous NDV vaccines, which may not adequately replicate within pigeon tissues (Soliman et al., 2019; Abd El-Ghany, 2023). Since slow release of vaccinal antigen gives long immunity, homologous live and inactivated vaccines, especially those formulated with oil adjuvants, have been shown to elicit robust immune responses and provide long-lasting protection, with some studies reporting rates as high as 100% against homologous challenges (Amer et al., 2013; Hamouda et al., 2024). Regular updates to vaccine strains, including local field isolates, are essential for preserving effectiveness against evolving PPMV-1 sub-strains (Zhang et al., 2024).

Recent advances in NDV vaccine techniques have increasingly focused on utilizing nanotechnology to enhance protective efficacy, particularly through the use of chitosan nanoparticles, as seen in the development of live attenuated NDV vaccines encapsulated in chitosan for mucosal delivery (Renu and Renukaradhya, 2020). Traditional strategies focused on matching vaccination strains to circulating viruses, while modern methods use innovative delivery systems that enhance mucosal immunity and minimize viral shedding (Zhang et al., 2024).

Chitosan, a natural polysaccharide known for its bioavailability, biodegradability, safety, and immunostimulatory potential, functions effectively as a vaccine carrier (Wijesekara and Xu, 2024). However, one of the critical challenges in its application is its poor solubility at neutral pH (6 to 6.5), which restricts the delivery of soluble and stable antigens in natural pH conditions (Renu and Renukaradhya, 2020). Therefore, numerous structural modifications have been made to the

leading amino group of chitosan, including quaternization with N-2-hydroxypropyl trimethyl and N, O-carboxymethyl, to enhance water solubility without compromising its biological properties (Pathak *et al.*, 2021). A common technique for forming CS-NPs-based vaccines involves mixing antigens with CS-NPs and inducing encapsulation using ionic gelation with sodium tripolyphosphate (Renu *et al.*, 2020; Bugybayeva *et al.*, 2024). This technique protects the vaccine virus from degradation, especially in the gastrointestinal tract of vaccinated animals such as pigs, which is a common reason for oral vaccine failure. As a result, oral and intranasal delivery methods for CS-NPs-encapsulated NDV vaccines have shown significant promise (Bernocchi *et al.*, 2017; Masimov and Wasan, 2024).

Experimental studies have shown that the intranasal delivery of CS-NP-based NDV vaccines significantly

boosts both mucosal and systemic immune responses. This results in the production of antigen-specific IgG and mucosal IgA, stimulates lymphocyte growth, and increases cytokine levels, including interleukins (IL-2) and IL-4, along with interferon- γ (IFN- γ). Ultimately, these immunological enhancements help to provide comprehensive protection against infection (Renu and Renukaradhya, 2020). These findings supported the increasing consensus on the improved immunogenicity and effectiveness of vaccines formulated with CS-NPs in poultry (Renu and Renukaradhya, 2020; Moghaddam, 2021), as further illustrated in Table 2. Despite these promising developments in the use of CS-NP-based NDV vaccines in poultry, no published studies have applied this delivery system for PPMV-1 vaccination in domestic pigeons (*Columba livia*).

Table 1. Global spread and incidence survey of pigeon paramyxovirus-1 infection

Region	Country/region	Year(s)	Isolation details
Middle East	Middle East	1978	First reported isolation of PPMV-1 in the Middle East in captive pigeons raised for meat.
	United Kingdom (Liverpool docks)	1984	Feral pigeons contaminated feed at the Liverpool docks, leading to 19 NDV outbreaks in chickens.
	Ireland	1994	Isolation of PPMV-1 reported in Ireland.
Europe	Slovenia	2000, 2008	PPMV-1 was isolated from pigeons between 2000 and 2008 in Slovenia.
	Macedonia	2007, 2008, 2010, 2011	Phylogenetically similar (NDV/chicken/Macedonia/231/2010)
	Italy (Eurasian collared doves)	1960s-2000 and 2001	Pigeon isolates of PPMV-1 have been reported in Italy. Mass mortality events in collared doves reach 90%.
Asia	Japan	1984, 1990s, 2000s, 2013	The PPMV-1 has been circulating in pigeon populations for a long period.
	China	1996-2018	Multiple isolations were reported in 2006, 2015, 2017, 2018, 2020, and 2022, indicating over 30 to 40 years of virus circulation in Chinese pigeon populations.
	Iran	2012-2018	Recurring isolation of PPMV-1 from pigeons in Iran was reported between 2012 and 2018, indicating sustained viral circulation during that period.
Africa	South Africa	1987, 2004, 2008	1987: Early outbreak of PPM-1 detection in pigeons.
	Egypt	1980, 1989, 1993, 2005, 2016, 2017	A series of isolations associated with PPMV-1 were reported.
Americas	Brazil	2001, 2018, 2021, 2022	A sequence of isolations from commercial and backyard pigeon outbreaks
	United States	1984, 1990	The PPMV-1 was first detected in captive and free-ranging feral pigeons in New York, then in California in 1990.
	United States (Florida, collared doves)	2001	A significant mortality event in collared doves, with ~5,000 deaths.
	United States (Western regions including AZ, TX, NV, and UT)	2009-2014	Recurring outbreaks in collared doves, with some native dove species, such as mourning, white-winged, and common ground doves, occasionally found dead, though confirmation in native species has been challenging.

PPMV-1: Pigeon paramyxovirus-1, NDV: Newcastle disease virus, AZ: Arizona, TX: Texas, NV: Nevada, UT: Utah.

Table 2. Summary of chitosan-based nanoparticle vaccines for Newcastle disease in poultry

Antigen encapsulated	Nanoparticle composition	Route of delivery	Immune responses	Country	Reference
Live NDV (LaSota strain)	Chitosan nanoparticles	Oral and intranasal	Enhanced mucosal and systemic immunity	China	Zhao et al. (2012)
NDV <i>F</i> gene plasmid DNA	Chitosan nanoparticles	Intranasal	Elevated serum IgG and mucosal IgA levels	China	Sun et al. (2014)
NDV <i>F</i> gene plasmid DNA	Ag@SiO ₂ hollow nanoparticles	Intranasal	Significant mucosal IgA and systemic IgG responses	China	Zhao et al. (2016)
Different bacterial and viral antigens	Chitosan nanoparticles	Oral and Intranasal	Robust mucosal and systemic immunity	United States	Renu and Renukaradhya (2020)
NDV vaccine	O-2'-Hydroxypropyl trimethyl ammonium chloride chitosan nanoparticles	Intranasal	Enhanced mucosal and systemic immune responses	China	Zhao et al. (2021)
Genotype VII NDV	Chitosan nanoparticles	<i>In vitro</i> study	Demonstrated antiviral activity against NDV	Egypt	Alkhalefa et al. (2022)
Inactivated NDV and H9N2 avian influenza virus	N-2-Hydroxypropyl trimethyl ammonium chloride chitosan-aluminum sulfate composite nanoparticles (N-2-HACC-Al NPs)	Intra-muscular	Elevated serum IgG, IL-4, and IFN- γ levels	China	Liu et al. (2023)

NDV: Newcastle disease virus, NPs: Nanoparticles, IgG/IgA: Immunoglobulin G / A, IL-4: Interleukin-4, IFN- γ : Interferon gamma, H9N2: Subtype of avian influenza virus, *F* gene: Fusion gene of NDV, Ag@SiO₂: Silver-doped silica nanoparticle, N-2-HACC-Al NPs: N-2-hydroxypropyl trimethyl ammonium chloride chitosan-aluminium sulfate composite nanoparticles, O-2'-Hydroxypropyl trimethyl ammonium chloride chitosan: A water-soluble quaternised chitosan derivative.



Figure 1. Clinical signs in an 8-month-old domestic pigeon infected with pigeon paramyxovirus-1. The pigeon exhibits neurological manifestations, including torticollis and loss of equilibrium, which are characteristic signs of pigeon paramyxovirus-1 infection.



Figure 2. Gross lesions in the brain of an 8-month-old domestic pigeon infected with pigeon paramyxovirus-1. The brain shows multiple haemorrhages indicative of virus-induced vascular damage associated with pigeon paramyxovirus-1 infection.

CONCLUSION

Newcastle disease virus (NDV) infection remains a significant concern for avian health and poultry productivity worldwide, particularly in pigeons. While conventional control measures, such as vaccination with homologous PPMV-1 strains, have achieved variable success, the modern nanotechnology-based approaches have exhibited exceptional promise in vaccination strategies. Among these, chitosan nanoparticles (CS-NPs) have demonstrated notable potential in enhancing mucosal and systemic immunity against NDV in poultry. However, this novel delivery strategy has yet to be applied or validated in pigeons, presenting a significant opportunity for further studies and innovation. It is strongly recommended that future vaccination strategies for pigeons include CS-NPs-based mucosal vaccines, as this approach could improve immune responses and effectively decrease viral shedding.

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Authors' contributions

Muhammadtaher Abdulrazaq Abdulrasol conceptualized the topic, conducted the literature review, and prepared the initial manuscript draft. Wafaa A. Abd El-Ghany supervised the scientific content, reviewed the immunological and virological sections, and edited the manuscript. Harith Abdulla Najem contributed to organizing the vaccination strategies section and revised the manuscript linguistically. All authors have read and approved the final edition of the manuscript.

Availability of data and materials

The present article is a review paper and does not include original experimental data. However, any supporting materials or information can be provided upon request from the corresponding author.

Ethical considerations

The present article is a literature-based review that does not involve any animal or human experiments. The

authors carefully observed and addressed ethical concerns regarding plagiarism, misconduct, data integrity, and duplicate publication.

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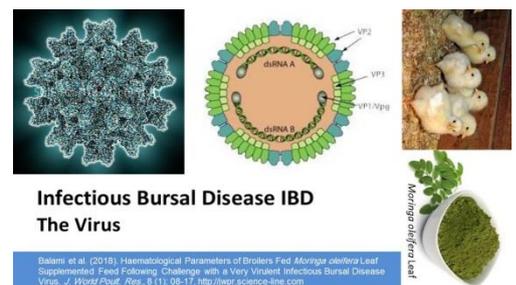
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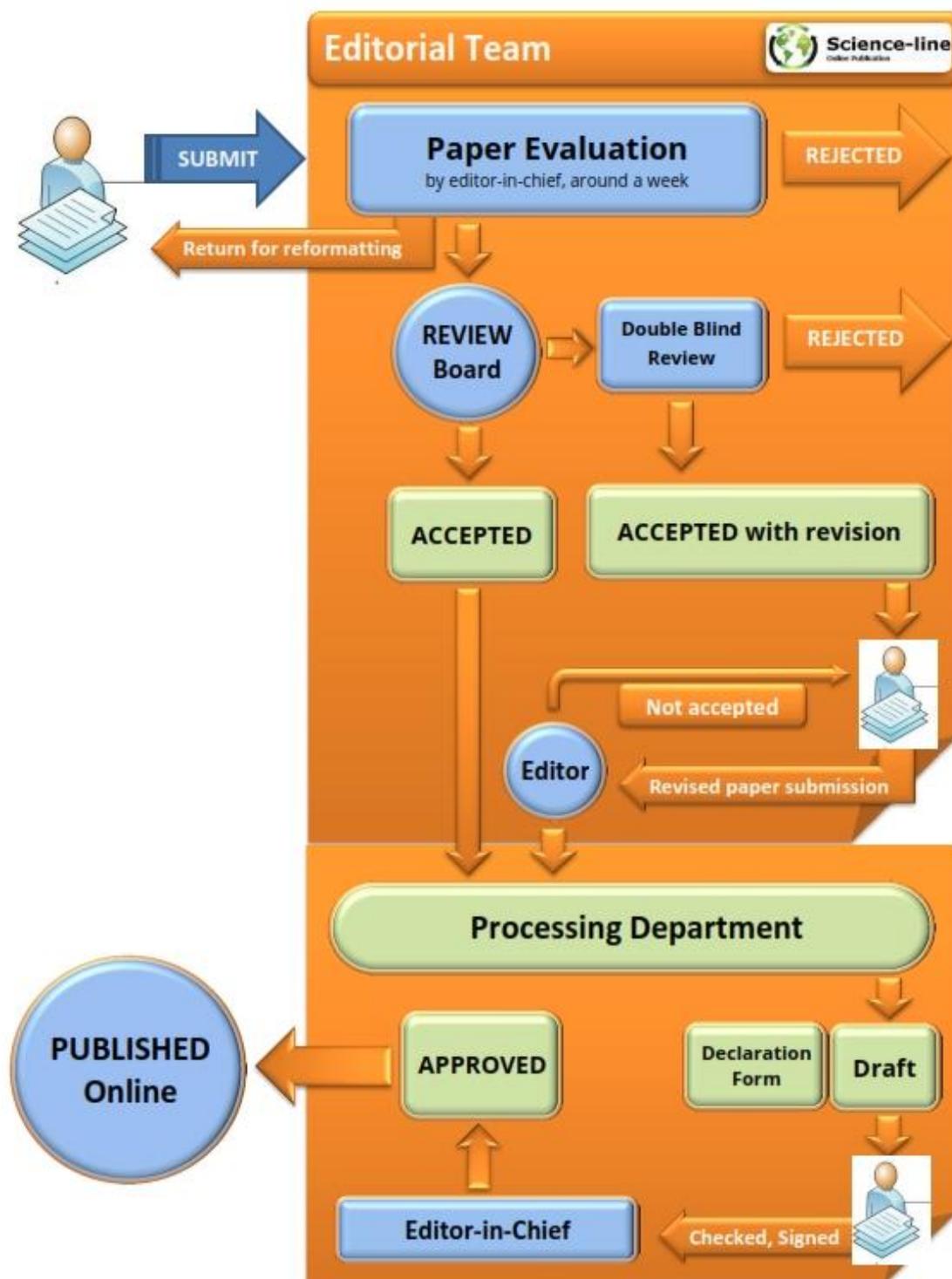
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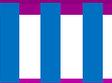
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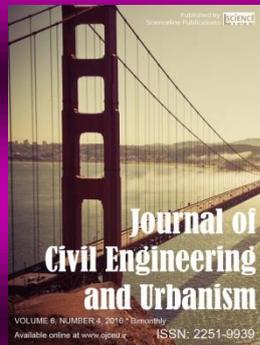
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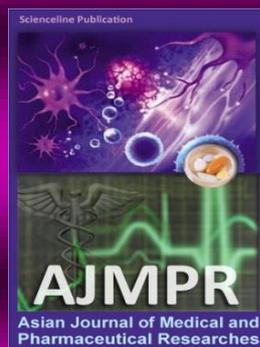
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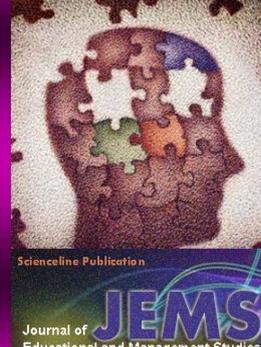
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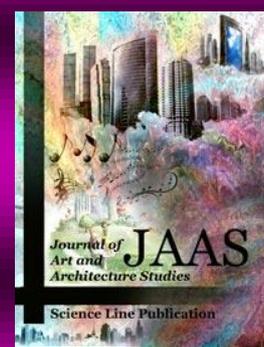
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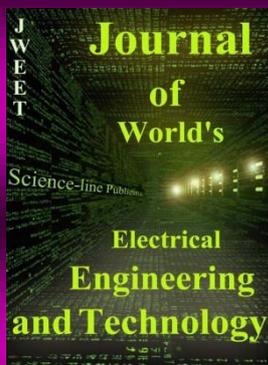
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