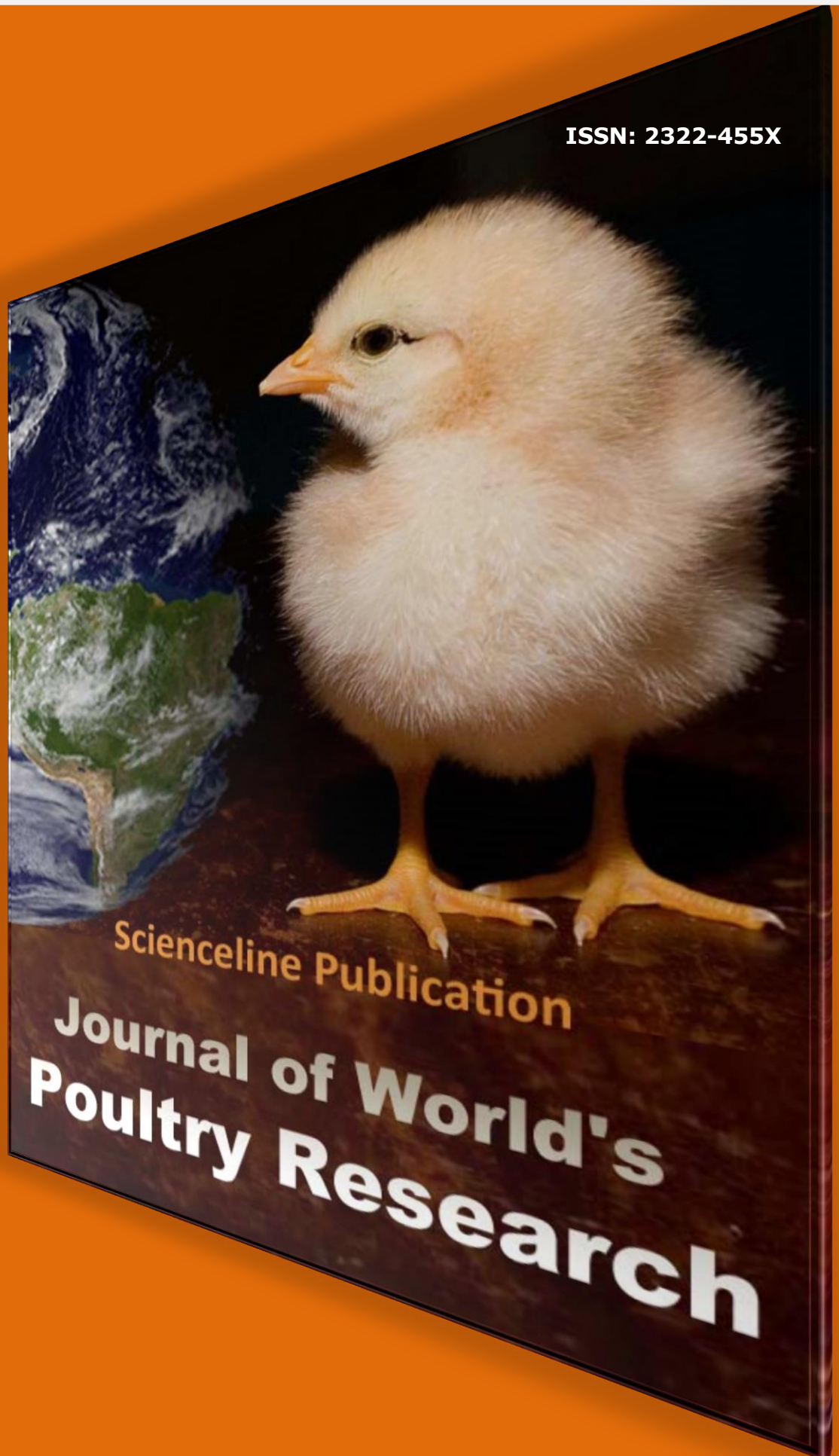




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Volume 15 (4); December 2025 [[Endnote XML for Agris](#)]

Research Paper

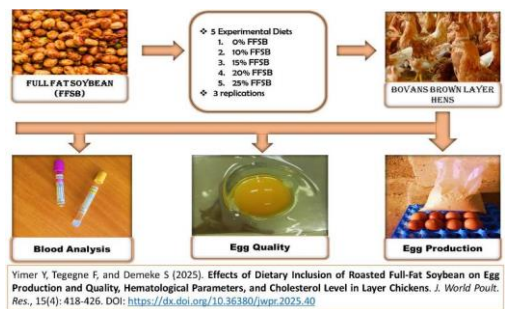
Effects of Dietary Inclusion of Roasted Full-Fat Soybean on Egg Production and Quality, Hematological Parameters, and Cholesterol Level in Layer Chickens

Yimer Y, Tegegne F, and Demeke S.

J. World Poult. Res. 15(4): 418-426, 2025; pii: S2322455X2500040-15

DOI: <https://dx.doi.org/10.36380/jwpr.2025.40>

ABSTRACT: The scarcity and high costs of poultry feed ingredients, such as soybean meal (SBM), present significant challenges for modern poultry production systems in Ethiopia. To explore potential alternatives, this study evaluated the effects of varying inclusion levels of roasted full-fat soybean (FFSB) in layer diets on productive performance, egg quality traits, and selected blood parameters. A 12-week feeding trial was conducted using 120 Bovans Brown hens aged 34-46 weeks, with an average initial body weight of 1.44 ± 0.01 kg. The hens were randomly assigned to five dietary treatment groups (0%, 10%, 15%, 20%, and 25% FFSB), each replicated three times with eight hens per replicate. The control diet contained 20% SBM for comparison. The results showed that hens in the control group exhibited significantly higher hen-day egg production (89.72%), egg mass (54.28 g/hen/day), and body weight gain (314 g) compared with those fed diets containing different inclusion levels of FFSB. However, no significant differences in egg production were observed among the groups fed diets containing different inclusion levels (10-25%) of FFSB. The group fed 10% FFSB exhibited a higher egg mass (38.44 g/hen/day) than those receiving 15% and 25% inclusion levels. The feed conversion ratio (FCR) increased with higher levels of FFSB inclusion, with the 25% FFSB group showing a significantly higher FCR (3.37) compared to the control group (2.06). Full-fat soybean inclusion had no significant influence on most egg quality traits, nor on hematological indices or serum cholesterol levels. The findings indicated that FFSB inclusion in layer feeds at levels up to 25% did not compromise egg quality or hen health. Although higher FFSB inclusion lowered feed costs, reduced egg production offset these savings, making full substitution of SBM economically unprofitable.



Keywords: Diet, Egg production, Egg quality, Full-fat soybean, Inclusion level, Layer

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Research Paper

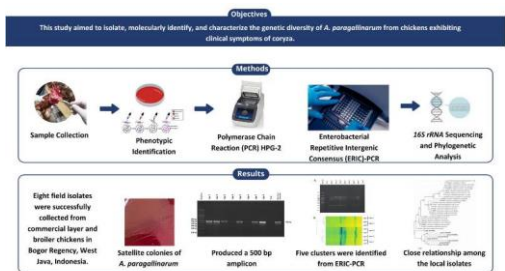
Molecular Identification and Genetic Diversity of *Avibacterium paragallinarum* Isolated from Chickens with Infectious Coryza in Bogor, Indonesia

Karunia N, Kurnia RS, Nugroho CMH, Putra MA, Indrawati A, and Safika.

J. World Poult. Res. 15(4): 427-437, 2025; pii: S2322455X2500041-15

DOI: <https://dx.doi.org/10.36380/jwpr.2025.41>

ABSTRACT: Infectious coryza, a respiratory disease caused by *Avibacterium paragallinarum* (*A. paragallinarum*), poses a major threat to poultry health and productivity, particularly in tropical countries such as Indonesia. This study aimed to isolate, molecularly identify, and characterize the genetic diversity of *A. paragallinarum* from chickens exhibiting clinical symptoms of coryza. A total of 60 infraorbital sinus swab samples were aseptically collected from commercial layer and broiler chickens in Bogor Regency, West Java, Indonesia. The method consists of phenotypic identification (culture on Nicotinamide Adenine Dinucleotide [NAD], Gram staining, and biochemical tests) and molecular confirmation using Polymerase Chain Reaction (PCR) HPG-2, Enterobacterial Repetitive Intergenic Consensus (ERIC)-PCR, and 16S rRNA sequencing. Eight field isolates were successfully cultured on NAD-supplemented blood agar, Gram-negative, catalase and oxidase-negative. Molecular confirmation was conducted using species-specific HPG-2, with all isolates amplifying the expected 500 bp product. The evaluation of genetic diversity was conducted through ERIC-PCR, which identified five unique clusters among the isolates, demonstrating considerable genomic variation. Furthermore, partial 16S rRNA gene sequences were amplified and analyzed through phylogenetic tree construction and BLAST comparison. Sequence analysis revealed 95.7–99.2% homology with reference *A. paragallinarum* strains in GenBank, and 96.5–99.2% homology among the study isolates themselves. The phylogenetic tree highlighted a close relationship among the local



isolates, yet also indicated a distinct genetic lineage from several international reference strains, suggesting possible regional specificity. This study provided the first detailed genetic characterization of *A. paragallinarum* field isolates in Indonesia using 16S rRNA sequencing. These findings highlighted the need for continuous molecular surveillance to guide accurate diagnosis.

Keywords: 16S rRNA sequencing, *Avibacterium paragallinarum*, Field isolate, Molecular identification

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Research Paper

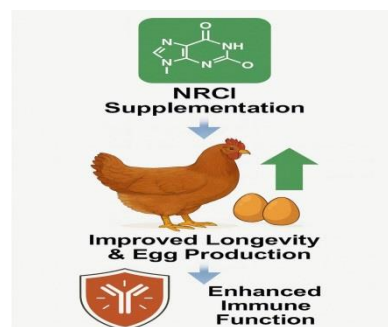
Impacts of Nicotinamide Adenine Dinucleotide Supplementation on Longevity, Egg Quality, and Immunity in Laying Hens

Yan BXZ, Zhang X, and Shahbaz R.

J. World Poult. Res. 15(4): 438-445, 2025; pii: S2322455X2500042-15

DOI: <https://dx.doi.org/10.36380/jwpr.2025.42>

ABSTRACT: The productivity and health of laying hens are major challenges for the global poultry industry, especially as flocks age and egg production declines. The present study aimed to evaluate the effects of nicotinamide riboside chloride (NRCI), a bioavailable precursor of nicotinamide adenine dinucleotide (NAD⁺), on longevity, egg quality, and immune performance in laying hens. A total of 180 Hy-Line Brown hens, 24-week-old, were randomly assigned to three dietary groups for 24 weeks, including a control group receiving standard layer feed, a low-dose NRCI group receiving 25 mg NR/kg feed, and a high-dose NRCI group receiving 50 mg NR/kg feed. The present study assessed survival rate, daily egg production, egg weight, shell strength, yolk nutrient composition, and immune parameters, including white blood cell (WBC) count, immunoglobulins (IgA and IgG), and cytokines (IL-6 and IFN-γ). The present findings indicated that supplementation with NRCI significantly increased the survival rate, resulting in 100% survival in the high-dose group compared to 93.3% in the control group. Egg production and feed conversion ratio were also enhanced in NRCI groups, indicating improved physiological resilience. The NRCI groups demonstrated significant improvements in egg quality, including stronger, thicker shells and enhanced nutritional content such as higher yolk protein, vitamin D3, and omega-3 fatty acid levels. Furthermore, immune markers were markedly elevated, reflecting enhanced immune modulation and potential disease resistance. The NRCI supplementation effectively supported metabolic efficiency, immune competence, and egg quality in laying hens. The current findings highlighted the potential of NAD⁺ precursor supplementation as a nutritional strategy to prolong the productive lifespan of commercial layers while promoting sustainable poultry production.



Yan BXZ, Zhang X, and Shahbaz R. (2025). Impacts of Nicotinamide Adenine Dinucleotide Supplementation on Longevity, Egg Quality, and Immunity in Laying Hens. *J. World Poult. Res.*, 15(4): 438-445. DOI: <https://dx.doi.org/10.36380/jwpr.2025.42>

Keywords: Egg quality, Immune response, Laying hens, Longevity, Nicotinamide adenine dinucleotide supplementation, Poultry feed

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Research Paper

Analysis of Poultry Product Market Integration at the Producer, Wholesaler, and Retail Levels in Jambi Province, Indonesia

Firmansyah, Hoesni F, Harahap A, and Gusri L.

J. World Poult. Res. 15(4): 446-459, 2025; pii: S2322455X2500043-15

DOI: <https://dx.doi.org/10.36380/jwpr.2025.43>

ABSTRACT: Price increases in poultry products remain inevitable, with a significant price disparity persisting between consumer and producer levels. This condition results from an unhealthy oligopolistic livestock market, leading to low purchase prices for farmers. The present study aimed to analyze market integration at the producer, wholesale, and retail levels for poultry products (chicken meat and eggs) and to develop a price-forecasting model for these products at the producer, wholesale, and retail levels for the future. A quantitative approach was used to analyze secondary data on average weekly prices for chicken meat and eggs at producer, wholesale, and retail levels in Jambi Province, Indonesia. The present data were obtained from the Center for Strategic Food Price Information from January 2020 to July 2023. The present results indicated a long-term cointegration relationship among chicken meat and egg prices at producer, wholesale, and retail levels in Jambi Province, Indonesia. The prices of chicken meat and eggs at the producer, wholesale, and retail levels influence each other not only in the current period but also concerning prices over the previous 2-6 weeks. Wholesale and retail chicken prices affect producer prices, and retail prices affect wholesale prices. For eggs, wholesale prices affect producer prices, and retail prices affect wholesale prices.



Firmansyah, Hoesni F, Harahap A, and Gusri L. (2025). Analysis of Poultry Product Market Integration at the Producer, Wholesaler, and Retail Levels in Jambi Province, Indonesia. *J. World Poult. Res.*, 15(4): 446-459. DOI: <https://dx.doi.org/10.36380/jwpr.2025.43>

and retail prices also affect producer and wholesale prices. The Vector Error Correction forecasting model for chicken and egg prices at producer, wholesale, and retail levels in Jambi Province demonstrated strong forecasting performance. Market integration occurs across different market levels, producer, wholesale, and retail, for chicken meat and eggs, both in the short and long term. This integration contributed to improving the efficiency of poultry marketing in Jambi Province, Indonesia.

Keywords: Market integration, Producer, Retail, Wholesaler

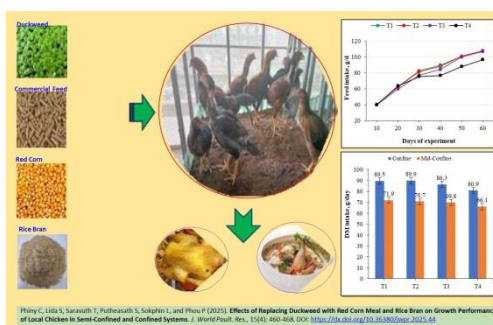
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Research Paper

Effects of Replacing Duckweed with Red Corn Meal and Rice Bran on Growth Performance of Local Chicken in Semi-Confined and Confined Systems

Phiny C, Lida S, Saravuth T, Putheasath S, Sokphin L, and Phou P.
J. World Poult. Res. 15(4): 460-468, 2025; pii: S2322455X2500044-15
 DOI: <https://dx.doi.org/10.36380/jwpr.2025.44>

ABSTRACT: Incorporating duckweed into poultry diets can improve feed efficiency and growth rate. However, its effectiveness as a replacement for traditional feeds such as red corn meal or rice bran remains less explored. The present study aimed to evaluate the feed intake, average daily gain (ADG), and feed conversion ratio (FCR) in local chickens reared under confined and semi-confined systems. The experiment was conducted at the campus of Svay Rieng University, Svay Rieng Province, Cambodia, from June to August 2024. The present study consisted of two factors. The first factor was the rearing system, including semi-confined and confined systems, and the second factor was the diet, with four treatments. A total of 120 local chickens, 35 days old, with an average initial body weight of 300 grams, were arranged in a 2 × 4 factorial layout using a completely randomized design within eight treatments and three replications. The first group included 5% duckweed, 22% commercial feed, 20% red corn meal, 52.5% rice bran, and 0.5% premix/salt (T1). The second group consisted of 10% duckweed, 19% commercial feed, 20% red corn meal, 50.5% rice bran, and 0.5% premix/salt (T2). The third group had 15% duckweed, 16% commercial feed, 20% red corn meal, 48.5% rice bran, and 0.5% premix/salt (T3), and the fourth group included 20% duckweed, 13% commercial feed, 20% red corn meal, 46.5% rice bran, and 0.5% premix/salt (T4). There were no significant interactions between four diets and two raising systems for feed intake, ADG, and FCR in the chickens, except for the crude fiber intake. However, significant differences were observed in T1 and T2 compared to T4 regarding feed intake and ADG, with a tendency toward improved FCR for chickens fed T3 compared to T4 and those raised under the confined system. The growth performance in chickens FCR raised under the confined system was higher than that of chickens raised under the semi-confined system. The present results indicated that using duckweed at 15% mixed with other ingredients led to higher feed intake, higher ADG, and improved FCR, even when chickens were raised in a confined or semi-confined system.



Keywords: Commercial feed, Confined system, Duckweed, Performance, Red corn

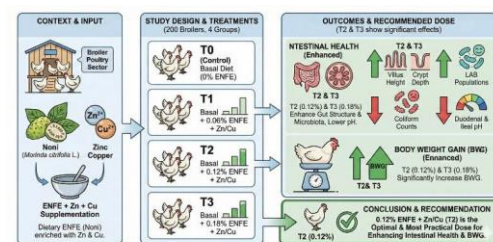
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Research Paper

Evaluation of Body Weight and Intestinal Health in Broiler Chickens Supplemented with Encapsulated *Morinda citrifolia* L. Extract, Zinc, and Copper

Baetavianti LN, Yuniyanto VD, Mulyono, and Krismiyanto L.
J. World Poult. Res. 15(4): 469-477, 2025; pii: S2322455X2500045-15
 DOI: <https://dx.doi.org/10.36380/jwpr.2025.45>

ABSTRACT: The broiler poultry sector is strategically important, serving as a major contributor to animal protein supply and an essential component of national food security. The present study aimed to examine the effects of dietary supplementation of encapsulated noni fruit (*Morinda citrifolia* L.) extract (ENFE) enriched with zinc (Zn) and copper (Cu) on intestinal health and body weight in broiler chickens. A total of 200 eight-day-old broiler chickens, with an initial weight of 233.69 ± 7.28 grams, were assigned to four treatments using a completely randomized design, with five replications per treatment. The treatment groups included a control group with a basal diet (T0), a basal diet with 0.06% ENFE, Zn at 40 ppm and Cu at 5 ppm (T1), a basal diet with 0.12% ENFE, Zn at 40 ppm and Cu at 5 ppm (T2), and a basal diet with 0.18% (T3) ENFE, Zn at 40 ppm and Cu at 5 ppm (T3). The body weight gain (BWG), counts of total lactic acid bacteria (LAB), coliform bacteria, pH value, villus height, crypt depth, and weight of each segment of the small



Baetavianti LN, Yuniyanto VD, Mulyono, and Krismiyanto L. (2025). Evaluation of Body Weight and Intestinal Health in Broiler Chickens Supplemented with Encapsulated *Morinda citrifolia* L. Extract, Zinc, and Copper. *J. World Poult. Res.* 15(4): 469-477. DOI: <https://dx.doi.org/10.36380/jwpr.2025.45>

intestine were assessed. The current results indicated that chickens in 0.12% (T2) and 0.18% (T3) significantly enhanced BWG, LAB populations, villus height, crypt depth, and length, and relative weight of the duodenum, jejunum, and ileum. Additionally, reduced duodenal and ileal pH, as well as coliform counts across all intestinal segments, were observed in 0.12% (T2) and 0.18% (T3). However, ENFE supplemented with Zn and Cu did not significantly affect jejunal pH. Administering ENFE at 0.12% supplemented with Zn and Cu represented the most optimal dose. Although the higher dose of 0.18% (T3) yielded statistically comparable results and was superior in some parameters, the 0.12% (T2) inclusion level is recommended as the most practical dose for enhancing BWG and intestinal health in broiler chickens.

Keywords: Body weight gain, Broiler chicken, Encapsulation, Intestinal morphology, *Morinda citrifolia*

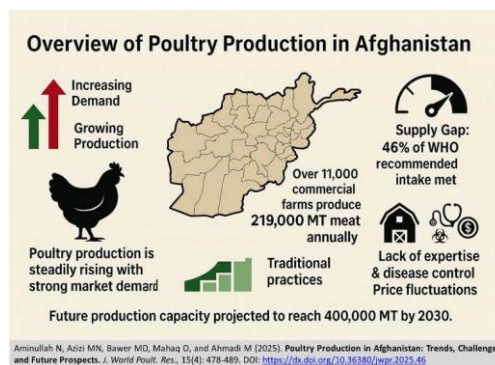
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Review

Poultry Production in Afghanistan: Trends, Challenges, and Future Prospects

Aminullah N, Azizi MN, Bawer MD, Mahaq O, and Ahmadi M.
J. World Poult. Res. 15(4): 478-489, 2025; pii: S2322455X2500046-15
DOI: <https://dx.doi.org/10.36380/jwpr.2025.46>

ABSTRACT: The demand for poultry products is steadily increasing globally as well as in Afghanistan, driven primarily by human population growth, economic development, and urbanisation. A clear understanding of Afghanistan's poultry sector is crucial for policy direction towards food security and rural development. The present study aimed to evaluate the current status of poultry farming and highlight the key challenges and future view of poultry production in Afghanistan. The study was synthesized from the available literature, government reports, and international datasets to provide a coherent overview of Afghanistan's poultry sector. Particular attention was given to identifying data inconsistencies and research gaps to guide evidence-based policies. The trend of investment in Afghanistan's poultry sector increased from 0.5 billion AFN (71.42 million USD) in 1986 to 200 billion AFN (2.85 billion USD) in 2022. Currently, a total of approximately 11,000 commercial poultry farms are operating across the country, which can produce about 219,000 metric tons (MT) of meat, while the total market demand is estimated as 295,000 MT in 2024. The available poultry meat in Afghanistan covers only approximately 46% of the World Health Organization's recommended rate per capita. However, the sector development is hindered by different factors such as traditional practices, poor infrastructure, lack of technical expertise, disease outbreaks, uncontrolled use of antimicrobial agents, poor input quality, and end-product price variation. The poultry sector's average annual growth rate in Afghanistan is estimated as 13.8%, and the future production capacity is projected to reach 400,332 MT by 2030. Poultry production in Afghanistan is gradually increasing, driven by growing market demand. However, the current production level still cannot meet the market demands in the country. The present findings contribute to a clearer understanding of poultry development priorities for national food security and sustainable growth in Afghanistan.



Keywords: Chicken, Market, Meat, Poultry production

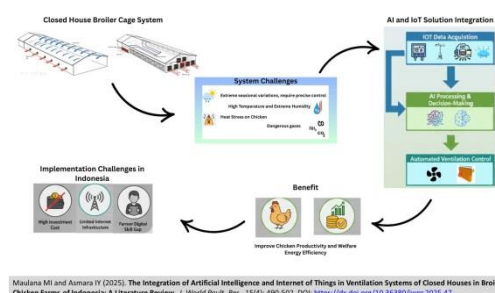
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Systematic Review

The Integration of Artificial Intelligence and Internet of Things in Ventilation Systems of Closed Houses in Broiler Chicken Farms of Indonesia: A Literature Review

Maulana MI and Asmara IY.
J. World Poult. Res. 15(4): 490-502, 2025; pii: S2322455X2500047-15
DOI: <https://dx.doi.org/10.36380/jwpr.2025.47>

ABSTRACT: The closed house system has been widely adopted in Indonesia due to its ability to control the microclimate; however, its implementation still faces several challenges, including high investment costs, limited monitoring, and maintenance management that is not yet adaptive. This literature review aimed to analyse the design of Artificial Intelligence (AI) and Internet of Things (IoT) integration in the automatic ventilation control system of broiler chicken closed houses in Indonesia. The method employed is a systematic review of relevant international and national articles. The literature review followed PRISMA guidelines, identifying 28,827 publications on AI and IoT, filtered to 636 studies on poultry and 335 studies on AI-IoT-based monitoring systems. Ultimately, 98 articles met the inclusion criteria, including 20 studies



specifically focused on studies in Indonesia. The findings indicated that AI-IoT integration has the potential to improve energy efficiency, optimize the microclimate, such as temperature, humidity, velocity, and support broiler chicken welfare through data-driven monitoring and automated decision-making systems. Nevertheless, the adoption of this technology continues to face challenges such as high initial costs, limited energy and internet infrastructure, and the digital skills gap among farmers.

Keywords: Adaptive ventilation, Energy-efficient, Microclimate, Poultry production, Smart farming, Tropical climate

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Short Communication

Impacts of Restricting Ribonucleotide Reductase on Performance, Meat Quality, and Intestinal Health in Broiler Chickens Evaluated by Evans Blue Dye

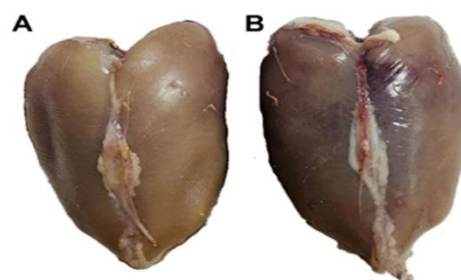
Shakeri M, Rafieian-Naeini HR, and Khanaki H.

J. World Poult. Res. 15(4): 503-507, 2025; pii: S2322455X2500048-15

DOI: <https://dx.doi.org/10.36380/jwpr.2025.48>

ABSTRACT: Ribonucleotide reductase (RNR) enzyme is essential for DNA synthesis and overall cellular health. Its inhibition interferes with metabolic pathways and mitochondrial function, leading to increased oxidative damage. Evans blue dye evaluates oxidative damage in tissues by penetrating through ruptured or destabilized membranes, thereby serving as an indicator of cell viability. The present study aimed to investigate the effects of restricting RNR on growth performance, meat quality, and intestinal health in broiler chickens after intestinal development was mainly completed. Twenty male broiler chickens, aged 14 days and weighing 445 ± 5 grams, were randomly divided into two equal groups: a control group and an experimental group, which received an RNR inhibitor (RR) at 20 mg/kg body weight/day for two weeks. Half of the chickens in both groups (5 broiler/group) were injected with Evans blue dye (EB) on the last day of the study. The oxidative damage was measured at the end of the study. The present results indicated that growth performance and feed intake were unchanged during the study. The RNR enzyme increased meat drip loss and lightness while reducing ultimate pH (pHu) compared to the control group. Evans blue concentration increased in the muscle, duodenum, jejunum, and ileum of the experimental group compared to the control group. Although RNR inhibition did not affect growth performance, it led to reduced meat quality and increased oxidative damage. These findings were evidenced by elevated drip loss and increased EB concentration in muscle and in all segments of the small intestine. The present results highlighted the essential role of RNR in maintaining meat quality and tissue integrity of broiler chickens.

Breast muscles from untreated (A) or treated (B) with Hydroxyurea (RNR inhibitor) for 14 days. Evans Blue Dye was administered to evaluate tissue damage in the muscle.



Shakeri M, Rafieian-Naeini HR, and Khanaki H (2025); Impacts of Restricting Ribonucleotide Reductase on Performance, Meat Quality, and Intestinal Health in Broiler Chickens Evaluated by Evans Blue Dye. *J. World Poult. Res.*, 15(4): 503-507, DOI: <https://dx.doi.org/10.36380/jwpr.2025.48>

Keywords: Evans blue dye, Intestine, Meat quality, Ribonucleotide reductase

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Journal of World's Poultry Research



ISSN: 2322-455X

Frequency: Quarterly

Current Issue: 2025, Vol: 15, No: 4 (December)

Publisher: [SCIENCELINE](http://www.science-line.com)

The Journal of World's Poultry Research (ISSN: 2322-455X) is an international, peer reviewed open access journal aims to publish the high quality material from poultry scientists' studies to improve domesticated birds production, food quality and safety ... [view full aims and scope](#)

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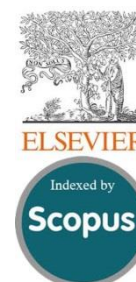
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Homepage: www.science-line.com

Email: administrator@science-line.com

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Effects of Dietary Inclusion of Roasted Full-Fat Soybean on Egg Production and Quality, Hematological Parameters, and Cholesterol Level in Layer Chickens

Yemsrach Yimer^{1,2*} , Firew Tegegne^{1,3} , and Solomon Demeke⁴ 

¹Collage of Agriculture and Environmental Science, Bahir Dar University, Bahir Dar, Ethiopia

²Collage of Agriculture and Environmental Science, Debark University, Debark, Ethiopia

³Ethiopian Agricultural Transformation Institute, Addis Ababa, Ethiopia

⁴Collage of Agriculture and Veterinary Medicine, Jimma University, Jimma, Ethiopia

*Corresponding author's E-mail: yemsrachyimer@gmail.com

Received: September 26, 2025, Revised: October 23, 2025, Accepted: November 19, 2025, Published: December 25, 2025



ABSTRACT

The scarcity and high costs of poultry feed ingredients, such as soybean meal (SBM), present significant challenges for modern poultry production systems in Ethiopia. To explore potential alternatives, this study evaluated the effects of varying inclusion levels of roasted full-fat soybean (FFSB) in layer diets on productive performance, egg quality traits, and selected blood parameters. A 12-week feeding trial was conducted using 120 Bovans Brown hens aged 34-46 weeks, with an average initial body weight of 1.44 ± 0.01 kg. The hens were randomly assigned to five dietary treatment groups (0%, 10%, 15%, 20%, and 25% FFSB), each replicated three times with eight hens per replicate. The control diet contained 20% SBM for comparison. The results showed that hens in the control group exhibited significantly higher hen-day egg production (89.72%), egg mass (54.28 g/hen/day), and body weight gain (314 g) compared with those fed diets containing different inclusion levels of FFSB. However, no significant differences in egg production were observed among the groups fed diets containing different inclusion levels (10-25%) of FFSB. The group fed 10% FFSB exhibited a higher egg mass (38.44 g/hen/day) than those receiving 15% and 25% inclusion levels. The feed conversion ratio (FCR) increased with higher levels of FFSB inclusion, with the 25% FFSB group showing a significantly higher FCR (3.37) compared to the control group (2.06). Full-fat soybean inclusion had no significant influence on most egg quality traits, nor on hematological indices or serum cholesterol levels. The findings indicated that FFSB inclusion in layer feeds at levels up to 25% did not compromise egg quality or hen health. Although higher FFSB inclusion lowered feed costs, reduced egg production offset these savings, making full substitution of SBM economically unprofitable.

Keywords: Diet, Egg production, Egg quality, Full-fat soybean, Inclusion level, Layer

INTRODUCTION

Poultry production plays a critical role in enhancing food security and economic stability in Ethiopia. Meeting consumer demand for meat and eggs heavily depends on the consistent availability of safe and cost-effective poultry feeds. However, the scarcity and rising prices of key feed ingredients, particularly soybean meal (SBM), pose significant challenges to the development of modern poultry production systems (Negash, 2022; Zegeye et al., 2023). This challenge highlights the need to identify sustainable, locally available, and cost-effective feed alternatives. Full-fat soybeans (FFSB) present a promising

option, offering protein (37-42%) and energy (18-22% fat) comparable to SBM, while eliminating the additional cost associated with oil extraction (Mihandoost et al., 2021; Karimi et al., 2022; Toomer et al., 2024a). The potential for using FFSB is particularly strong in the Amhara region, which accounts for approximately 39% of Ethiopia's soybean production (CASA, 2023). This substantial output presents a direct opportunity to utilize locally sourced FFSB in poultry feed formulation. However, raw FFSB contains several anti-nutritional factors (ANFs), such as trypsin inhibitors, lectins, and saponins, which can compromise poultry growth performance, feed efficiency, and egg quality by

interfering with protein digestion (Mishra et al., 2024). Heat treatment methods such as roasting, extrusion, and autoclaving have been used to deactivate ANFs, particularly the trypsin inhibitors that are sensitive to thermal processing (Macisaac et al., 2005; Senkoylu et al., 2005; Karimi et al., 2022).

Dry heating (roasting) provides a practical solution for Ethiopia, which often faces resource limitations and relies on smallholder production systems. This method entails low operational costs and requires minimal infrastructure, making it particularly suitable for these environments (Papanikou, 2020). Previous studies have indicated that heat-treated FFSB can be incorporated into layer diets at inclusion levels of 20-25%, resulting in optimal performance without adverse effects on laying hens (Senkoylu et al., 2005; Lázaro et al., 2006; Toomer et al., 2024b). However, its applicability in Ethiopia has not been thoroughly examined, given the variability in climatic conditions, feed management practices, and breed-specific responses. Additionally, the economic feasibility of incorporating roasted FFSB into layer feed formulations remains unassessed, hindering evidence-based decision-making for smallholder farmers and regional feed mills. Therefore, this study investigated the impact of varying dietary inclusion levels of roasted FFSB on egg production performance, egg quality characteristics, hematological indices, and serum cholesterol levels in Bovans Brown laying hens.

MATERIALS AND METHODS

Ethical approval

The animals were handled and cared for following welfare standards and experimental protocols approved by the College of Agriculture and Environmental Sciences Animal and Research Ethics Committee of Bahir Dar University, Ethiopia (Ref. No. 007-2025).

Laboratory chemical analysis and feed formulation

Raw soybeans were roasted in a metal pan over a wood fire and considered adequately processed when the beans reached the locally recognized golden-brown color. They were then cooled in open air at ambient temperature (23°C) for 24 hours before being ground for feed preparation. Dry matter (DM), crude fiber (CF), total ash (TA), ether extract (EE), and crude protein (CP) were determined according to (AOAC, 2000). The Chemical compositions of the FFSB and SBM are presented in Table

1. The metabolizable energy (ME) was calculated according to the study of Wiseman (1987).

$$ME \text{ (kcal/kgDM)} = 3951 + 54.4 EE - 88.7CF - 40.8 \text{ ash}$$

Based on the laboratory analytical data results, five experimental diets were formulated with graded inclusion levels of FFSB at 0%, 10%, 15%, 20%, and 25% FFSB. The diet containing 20% SBM (0% FFSB) was used as a positive control treatment. All the experimental diets were formulated containing a minimum of 15% CP based on the NRC (1994) nutrient requirements for laying hens Table 2. Lysine and methionine were added to all diets to maintain consistent and adequate levels of these limiting amino acids, independent of the varying crude protein content.

Table 1. Chemical composition and calculated metabolizable energy (ME) of soybean meal and full-fat soybean (as-DM basis)

Chemical composition	Soybean meal	Full-fat soybean
Dry matter (%)	89.6	91.21
Crude protein (%)	38.47	32.89
Crud fiber (%)	5.84	8.25
Ether extract (%)	5.27	11.62
Ash (%)	3.06	1.86
ME (Kcal/kg DM)	3,594.83	3,775.57

Study design and management of experimental hens

The experiment was conducted at the Bahir Dar University poultry farm, Ethiopia, using 120 Bovans Brown laying hens aged 34 weeks with an average body weight of approximately 1.44 ± 0.01 kg. The hens were randomly allocated in a completely randomized design into five treatment groups (24 hens per treatment), each subdivided into three replicates of eight hens, and housed in pens (2 m² per pen) bedded with 5 cm of teff (*Eragrostis tef*) straw. The hens were allowed a one-week adaptation period to the experimental diets before data collection, which was carried out over a 12-week period. Ambient temperature (T°) and relative humidity (RH) were recorded daily using a digital thermo-hygrometer (Graph 1), and weekly average temperature-humidity index (THI) values were calculated (Zulovich and DeShazer, 1990).

$$THI \text{ layers} = 0.60 T \text{ max} + 0.40 T \text{ min}$$

The experiment was conducted under natural daylight conditions without artificial lighting, with the open-sided house allowing adequate sunlight and ventilation throughout the day (approximately 12-13 hours of light). All hens were vaccinated at the rearing farm before arrival

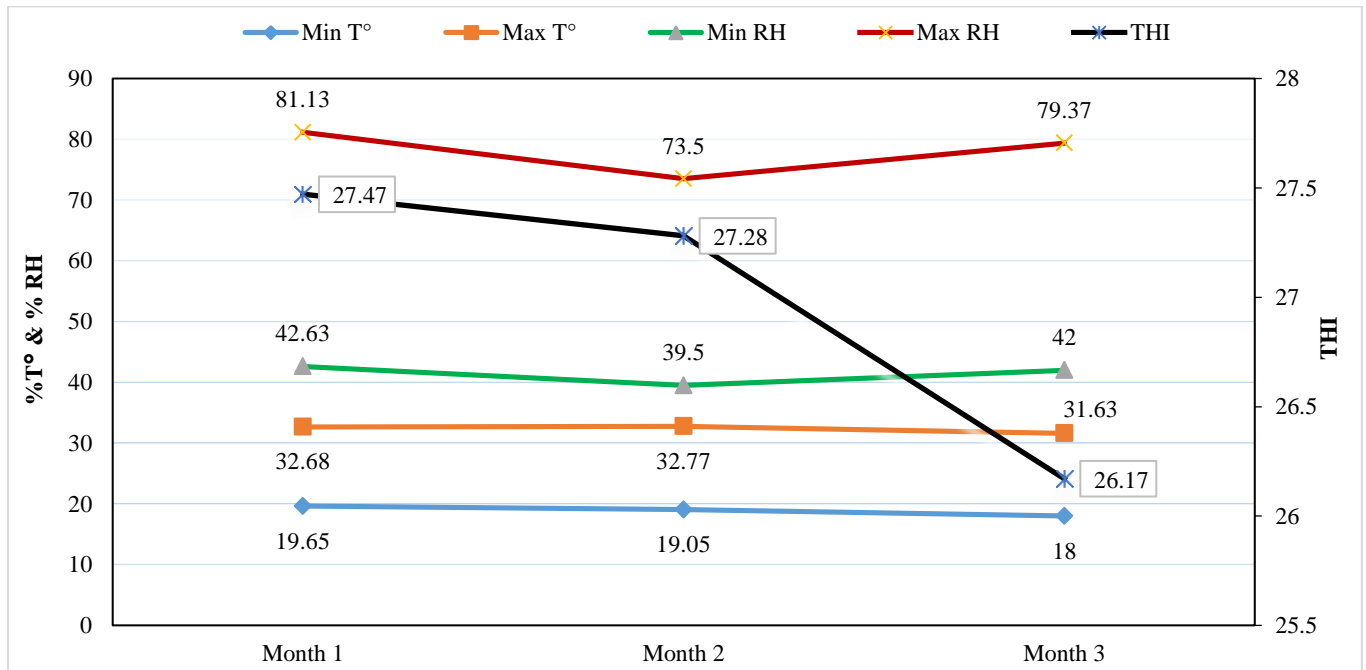
against common poultry diseases such as Newcastle disease, infectious bursal disease, and fowl pox; hence, no additional vaccinations were administered during the study. Layers were inspected upon arrival to confirm health status and were monitored daily for health and behavior under standard hygiene and biosecurity practices.

Feed and water were provided on a pen basis, with each pen equipped with its own feeder and drinker. Feed was offered twice daily (morning at 8:00 AM and afternoon at 2:00 PM), and refusals were collected and weighed the following day, while clean drinking water was supplied *ad libitum* throughout the experimental period.

Table 2. Ingredient proportions and chemical composition of dietary treatments for Bovans Brown layers (34-46 weeks of age)

Feed ingredient (%)	T1	T2	T3	T4	T5
White maize grain	54	55	50	50	50
Full-fat soybean	-	10	15	20	25
Soybean meal	20	-	-	-	-
Noug seed cake	11	20	20	15	10
Bone meal	4	4	4	4	4
Limestone	9	9	9	9	9
L-lysine-HCL	0.5	0.5	0.5	0.5	0.5
DL-Methionine	0.5	0.5	0.5	0.5	0.5
Vitamin and mineral Premix	0.5	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5	0.5
Analyzed nutrients					
Dry matter (%)	91.04	92.29	92.03	92.33	92.16
Crude protein (%)	16.03	15.05	15.42	15.59	16.29
Crud fiber (%)	5.06	7.61	8.46	6.75	6.06
Ether extract (%)	1.29	3.96	5.70	6.28	7.08
Ash (%)	11.07	10.83	11.58	12.85	12.86
ME (Kcal/kg DM)	3,120.25	3,049.87	3,038.40	3,169.94	3,275.12

HCl: Hydrochloride salt form; T1: 0% FFSB; T2:10% FFSB; T3: 15% FFSB; T4: 20% FFSB; T5: 25% FFSB. Per kilogram of premix: Vitamin A: 6,000,000 IU; Vitamins B1: 3000 mg; Vitamins B2: 4000 mg; Vitamins B6: 3000 mg; Vitamins B12: 8 mg; Vitamins E: 40,000 mg; Vitamins K: 1200 mg; Niacin: 30 mg; Folic acid: 1000 mg; Biotin: 300 mg; Ca-pantothenate: 10,000 mg; Iron: 25 000 mg; Copper: 1200 mg; Manganese: 4000 mg; Zinc: 4000 mg; Iodine: 400 mg; Selenium: 30 mg; Cobalt: 200 mg.



Graph 1. Average minimum and maximum temperature (T°), average minimum and maximum relative humidity (RH), and temperature-humidity index (THI) during the three experimental months.

Production performance parameters

Daily feed intake (FI) per replicate was calculated as feed offered minus refusal. Body weight (BW) was measured individually at trial start and end, with body weight change (BWC) per replicate derived from initial and final differences. Eggs were collected twice daily per pen to record production, and the hen-day egg production (HDEP) rate was calculated as the average percentage per replicate (Hunton, 1995).

$$\text{HDEP (\%)} = \frac{\text{No of eggs collected per day}}{\text{No of hens present that day}} \times 100$$

Daily egg weight per replicate was measured immediately after collection, with average egg weight (EW) calculated as total weight divided by egg count. Egg mass (EM) per pen was determined using the formula published by North (1984). $\text{EM} = \text{HDEP (\%)} \times \text{EW}$

Feed conversion ratio (FCR) was calculated per replicate as total feed intake divided by total egg mass produced.

Egg quality parameters

A total of 30 eggs (six per treatment, two per replication) were randomly sampled at two-week intervals for quality analysis, with replicate means calculated from sample averages for each parameter. Eggs were weighed individually, then carefully cracked onto a flat tray, and the shells were separated and weighed. Shell thickness was measured using a digital caliper after removing the shell membranes, by taking readings at three points on each egg at the air cell (broad end), equator (middle portion), and sharp end. The mean value of these three measurements was recorded as the average shell thickness for each egg. Yolk color was assessed using a Roche color fan, while albumen and yolk heights were determined with a tripod micrometer. The Haugh unit (HU) for each replicate was then calculated using the formula described by Haugh (1937), where h is albumen height (mm), and W is the weight of the egg (g)

$$\text{HU} = 100 \log_{10} (h + 7.57 - 1.7w^{0.37})$$

Measurements of blood parameters and economic analysis

At the end of the experiment, approximately 3 mL of blood was collected from the wing veins of 15 randomly selected hens (three hens per treatment). The hematology analysis was performed with an automated hematology analyzer (BC- 5800, Mindray, China) after blood samples were deposited in K3-EDTA heparinized tubes, and serum samples were analyzed for total cholesterol (COL), low-

density lipoprotein (LDL), and high-density lipoprotein (HDL) after being separated by centrifuging (3,000 rpm, 15 minutes).

The economic viability of incorporating graded levels of roasted full-fat soybean (FFSB) into the diets of Bovans Brown layers was evaluated by calculating the net return per hen. The analysis followed the method of Upton (1979) for partial budget analysis, using feed formulation costs and local market egg prices. Total feed intake was multiplied by the corresponding ingredient costs to calculate feed cost:

$$\text{Feed cost/hen} = \text{Total Feed Intake(kg)} \times \text{cost of diet (USD/kg)}$$

To calculate egg revenue, the total egg output per hen was multiplied by the local market price of eggs in Bahir Dar, Ethiopia, during the time of the study.

$$\text{Income/ hen} = \text{Total egg produced} \times \text{price per egg (USD)}$$

Subsequently, the net return per hen was derived as the total egg revenue minus the total feed cost:

$$\text{Net return/ hen} = \text{income per hen} - \text{feed cost per hen (USD)}$$

Statistical analysis

All data were analyzed by one-way analysis of variance (ANOVA) using the general linear model (GLM) procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC), where a significant treatment effect was identified ($p < 0.05$). Treatment means were separated using the least significant difference (LSD) test according to the following model: $Y_{ij} = \mu + T_i + e_{ij}$

Where Y_{ij} is the response variable, μ is the overall mean, T_i is the fixed (feed) effect of the i^{th} dietary treatment (level of FFSB), and e_{ij} is the random error associated with the j^{th} observation (individual hen) within the i^{th} treatment.

RESULTS

Egg production performance

Hens fed the control diet exhibited significantly higher HDEP, egg mass, and body weight change compared with those fed diets containing roasted FFSB (Table 3; $p < 0.05$). Among the FFSB-fed groups, no significant differences were observed in mean HDEP or body weight change ($p > 0.05$). However, hens fed the 10% roasted FFSB diet produced a higher egg mass than those receiving 15% and 25% inclusion levels ($p < 0.05$), while the 20% group showed intermediate values that were not significantly different from either. A significant difference in FCR was observed among the treatment groups ($p < 0.05$), with hens fed the control diet showing the lowest FCR. As the level of roasted FFSB increased, the FCR gradually rose, with the 25% FFSB group exhibiting a numerically higher FCR than the 10-20% FFSB groups.

Egg quality

No significant differences ($p > 0.05$) were observed in most external and internal egg quality parameters among the treatment groups, except for eggshell weight and yolk color (Table 4). Eggshell weight was significantly higher in hens fed the 10% roasted FFSB diet compared with those on the control, 15%, and 20% FFSB inclusion levels ($p < 0.05$), while a slight improvement in yolk color was noted at the 25% FFSB inclusion level.

Blood analysis

Similarly, no significant differences were found in hematological parameters across dietary treatments. This included all measured white blood cell (WBC) counts

(heterophils, lymphocytes, monocytes, eosinophils) and red blood cell (RBC) parameters (hemoglobin, hematocrit; $p > 0.05$). Total cholesterol, HDL, and LDL levels were also unaffected by increasing FFSB inclusion (Table 5; $p > 0.05$).

Economic analysis

The economic returns of FFSB inclusion in layer diets, evaluated using partial budget analysis based on egg sales and feed costs, are presented in Table 6. Hens fed the control diet achieved significantly higher net return compared with those fed diets containing roasted FFSB (Table 6; $p < 0.05$).

Table 3. Effect of full-fat soybean inclusion on egg production performance of Bovans Brown layer

Parameters	T1	T2	T3	T4	T5	SEM	P-value
HDEP (%)	89.72 ^a	63.57 ^b	57.03 ^b	57.27 ^b	57.81 ^b	3.451	<0.0001
Egg weight (g)	60.48 ^a	60.45 ^a	60.12 ^{ab}	60.52 ^a	57.43 ^b	0.454	0.1719
Egg mass (g/hen/day)	54.28 ^a	38.44 ^b	34.257 ^c	34.65 ^{bc}	33.14 ^c	2.157	<0.0001
Final BW (kg)	1.743 ^a	1.552 ^b	1.489 ^b	1.441 ^b	1.444 ^b	0.035	0.0107
Initial BW (kg)	1.429 ^a	1.446 ^a	1.439 ^a	1.437 ^a	1.427 ^a	0.012	0.9927
BWC (g)	314 ^a	106 ^b	49 ^b	4 ^b	17 ^b	0.036	0.0111
Feed intake (g/hen/day)	112.03 ^a	117.17 ^a	113.93 ^a	112.30 ^a	110.04 ^a	1.216	0.4789
FCR	2.06 ^b	3.07 ^a	3.34 ^a	3.27 ^a	3.37 ^a	0.139	0.0006

Means within the same rows with different ^{a-c} Superscripts are significantly different ($p < 0.05$). Whereas values with similar superscripts do not differ significantly ($p > 0.05$); T1: Control (20% SBM); T2: 10% FFSB; T3: 15% FFSB; T4: 20% FFSB; and T5: 25% FFSB inclusion level; SEM: Standard error of mean; HDEP: Hen-day egg production; BW: Body weight; BWC: Body weight change; FCR: Feed conversion ratio.

Table 4. Effect of full-fat soybean inclusion on egg quality parameters of Bovans Brown layer

Parameters	T1	T2	T3	T4	T5	SEM	P-value
Egg index	78.331 ^a	78.456 ^a	78.381 ^a	78.203 ^a	77.669 ^a	0.245	0.8554
Sample EW(g)	59.917 ^a	61.429 ^a	61.363 ^a	59.092 ^a	59.183 ^a	0.435	0.2964
Eggshell weight (g)	7.687 ^c	8.337 ^a	7.933 ^{bc}	7.787 ^{bc}	8.100 ^{ab}	0.075	0.0261
Eggshell thickness (mm)	0.357 ^a	0.360 ^a	0.352 ^a	0.352 ^a	0.362 ^a	0.003	0.6871
Albumen height (mm)	8.446 ^a	9.396 ^a	8.779 ^a	8.650 ^a	8.913 ^a	0.112	0.0965
Albumen weight (g)	36.754 ^a	38.613 ^a	38.558 ^a	36.858 ^a	36.613 ^a	0.374	0.2964
HU (%)	91.804 ^b	96.207 ^a	93.176 ^{ab}	93.072 ^{ab}	94.385 ^{ab}	0.537	0.1345
Yolk weight (g)	15.417 ^a	14.479 ^a	14.871 ^a	14.446 ^a	14.471 ^a	0.177	0.3380
Yolk index	0.462 ^a	0.472 ^a	0.479 ^a	0.469 ^a	0.472 ^a	0.003	0.3723
Yolk color	1.00 ^c	1.54 ^b	1.83 ^{ab}	1.83 ^{ab}	2.13 ^a	0.1205	0.0089

Means within the same rows with different ^{a-c} Superscripts are significantly different ($p < 0.05$). Whereas values with similar superscripts do not differ significantly ($p > 0.05$); T1: Control (20% SBM); T2: 10% FFSB; T3: 15% FFSB; T4: 20% FFSB; and T5: 25% FFSB inclusion level; SEM: Standard error of mean; EW: Egg weight; HU: Haugh unit.

Table 5. Effect of full-fat soybean inclusion on blood hematology and chemistry of Bovans Brown layer

Parameters	T1	T2	T3	T4	T5	SEM	P-value
WBC ($\times 10^3/\mu\text{L}$)	40.265 ^a	37.390 ^a	38.300 ^a	40.215 ^a	38.910 ^a	0.9058	0.9250
HT ($\times 10^3/\mu\text{L}$)	11.548 ^a	14.343 ^a	15.832 ^a	19.156 ^a	13.121 ^a	1.0682	0.2604
LYM ($\times 10^3/\mu\text{L}$)	26.114 ^a	20.017 ^a	20.392 ^a	19.631 ^a	23.791 ^a	1.2842	0.5736
MON ($\times 10^3/\mu\text{L}$)	0.4021 ^c	1.8707 ^a	1.3360 ^{ab}	0.6635 ^{bc}	0.9088 ^{bc}	0.1932	0.0533
EO ($\times 10^3/\mu\text{L}$)	2.201 ^a	1.122 ^a	0.741 ^a	0.764 ^a	1.089 ^a	0.3126	0.6783
RBC ($\times 10^6/\mu\text{L}$)	2.5700 ^a	2.4050 ^a	2.4950 ^a	2.5400 ^a	2.5650 ^a	0.0673	0.9763
HGB (g/dL)	8.850 ^a	8.400 ^a	8.150 ^a	8.750 ^a	8.450 ^a	0.3895	0.9927
HCT (%)	32.250 ^a	30.900 ^a	31.450 ^a	29.745 ^a	31.750 ^a	0.6878	0.8930
PLT ($\times 10^3/\mu\text{L}$)	5.500 ^a	7.500 ^a	5.500 ^a	8.000 ^a	11.500 ^a	1.3921	0.3906
HDL (mg/dL)	22.50 ^a	11.00 ^a	16.50 ^a	15.50 ^a	20.50 ^a	3.5895	0.9463
LDL (mg/dL)	33.50 ^a	23.00 ^a	28.50 ^a	39.50 ^a	40.50 ^a	6.2911	0.9175
COL (mg/dL)	131.00 ^{ab}	99.00 ^{ab}	76.50 ^b	134.50 ^a	100.00 ^{ab}	8.4719	0.1742

Means within the same rows with similar superscripts did not differ significantly ($P > 0.05$); T1: Control (20% SBM); T2: 10% FFSB; T3: 15% FFSB; T4: 20% FFSB; and T5: 25% FFSB inclusion level; SEM: Standard error of mean; WBC: White blood cell count; HT: Heterophil; LYM: Lymphocytes; MON: Monocytes; EO: Eosinophils; RBC: Red blood cell count; HGB: Hemoglobin; HCT: Hematocrit; PLT: Platelets; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; COL: Cholesterol.

Table 6. Economic return of Bovans Brown layers fed graded levels of full-fat soybean

Variable	T1	T2	T3	T4	T5	SEM	P-value
Total egg (number/hen)	80.87 ^a	56.99 ^b	51.31 ^{bc}	47.19 ^c	51.41 ^{bc}	3.321	<0.0001
Total feed consumed (kg/hen)	10.08 ^a	10.55 ^a	10.25 ^a	10.11 ^a	9.91 ^a	0.109	0.4789
Total feed cost per head (USD)	3.52 ^a	3.41 ^{ab}	3.24 ^{bc}	3.16 ^{bc}	3.06 ^c	0.055	0.0217
Total return (USD)	6.53 ^a	4.60 ^b	4.14 ^{bc}	3.81 ^c	4.15 ^{bc}	0.268	<0.0001
Net return (USD)	3.00 ^a	1.19 ^b	0.90 ^b	0.65 ^b	1.09 ^b	0.233	0.0001

Means within the same rows with different ^{a-c} Superscripts are significantly different ($p < 0.05$). Whereas values with similar superscripts do not differ significantly ($p > 0.05$); T1: Control (20% SBM); T2: 10% FFSB; T3: 15% FFSB; T4: 20% FFSB; and T5: 25% FFSB inclusion level; SEM: Standard error of mean.

DISCUSSION

The inclusion of FFSB in the layer diet decreased the HDEP compared to the control group, though egg weight remained unaffected. These results align with earlier findings by [de Faria et al. \(1995\)](#), who reported that substituting SBM with 5 to 10% roasted FFSB in layer rations reduced egg-laying rates during 33-50 weeks of age, without altering egg weight. [Karimi et al. \(2022\)](#) demonstrated that replacing SBM with 25-100% roasted or autoclaved FFSB had no significant impact on average egg weight.

The observed disparity in egg production and body weight change between hens fed the control diet and those receiving varying levels of FFSB may stem from differences in nutritional composition (Table 1) and

residual anti-nutritional factors (ANFs), particularly trypsin inhibitors (TI) that persist in FFSB even after processing. This interpretation is consistent with early findings by [Ari et al. \(2012\)](#) and [Erdaw et al. \(2016\)](#), who indicated that thermal processing methods significantly affect the nutritional quality and ANFs; roasting reduces TI content, yet residual TI levels remain higher than those in commercial SBM. Additionally, a recent large-scale analysis by [Schulz et al. \(2024\)](#), which evaluated 434 soybean meal samples globally, reported substantially higher TI levels in full-fat SBM (17.1 mg/g) compared to expeller (10.8 mg/g) and solvent-extracted SBM (5.1 mg/g), suggesting that incomplete TI inactivation during FFSB processing may impair nutrient utilization.

The inclusion of 10-25% roasted FFSB had no significant effect on egg production rate, consistent with

the findings of [Senkoylu et al. \(2005\)](#), who observed that increasing FFSB inclusion up to 22% in layer diets did not adversely affect egg production between 33 and 42 weeks of age. However, hens fed the 10% FFSB diet produced a higher egg mass than those receiving 15% and 25% inclusion levels, which may be attributed to the increased presence of anti-nutritional factors (ANFs) at higher FFSB levels. Although hens fed diets containing 10-25% FFSB exhibited a lower body weight change than the control group, they maintained their initial body weight throughout the study, with no evidence of weight loss even at the highest inclusion level (25%).

In the present study, the control group exhibited a significantly lower FCR, attributed to its superior egg production performance. This finding aligns with findings by [Ruiz et al. \(2004\)](#), who also reported that feed conversion was more efficient in the SBM treatment than FFSB. In the current study, a numerical increase in FCR was observed with increasingly higher FFSB inclusion levels, although these differences were not statistically significant. This trend agrees with the findings of [Karimi et al. \(2022\)](#), who reported that replacing soybean meal with 25-100% roasted FFSB increased the FCR of Bovans White laying hens. Similarly, [Senkoylu et al. \(2005\)](#) observed better FCR performance at a lower inclusion level (10% FFSB) compared to higher levels (22% FFSB), suggesting that excessive FFSB inclusion may reduce feed efficiency.

The internal and external qualities of eggs are important measures in the poultry industry because of food safety and economic implications. Full-fat soybean inclusion in layer diet at levels ranging from 10% to 25% demonstrated minimal impact on overall egg quality, with no significant alterations observed in key parameters such as albumen height, haugh units, or shell thickness. However, eggshell weight was highest in hens fed the 10% FFSB diet, attributed to their relatively greater egg weight and thicker eggshells. [Senkoylu et al. \(2005\)](#) also reported that increasing the level of dietary FFSB inclusion up to 22% did not alter the external egg quality.

This study used blood tests to check the overall health of the hens. While both hematological and blood chemistry values were in normal range across the different diets, WBC counts exceeded the reference ranges of $12-30 \times 10^3/\mu\text{L}$ ([Bounous and Stedman, 2000](#)). This elevation likely reflects the hen's exposure to tropical heat stress and may represent a compensatory response to counteract pathogen invasion, which is exacerbated by heat-induced compromised immunity ([Hassan et al., 2023](#)).

Feed costs per hen were lowest in the highest FFSB inclusion level (25%) and highest in the control group (20% SBM), reflecting that FFSB is a cheaper alternative ingredient in the layer diet. However, higher egg production in the control group resulted in the greatest total return (\$6.53) and net return(\$3.00), indicating that savings in feed cost in 15-25%FFSB inclusion levels did not compensate for reduced productivity. Overall, these results highlight that while feed cost reduction is possible, maintaining optimal nutrient density is critical for maximizing egg production and economic profitability.

CONCLUSION

The inclusion of roasted full-fat soybean (FFSB) in layer diets influenced productivity and efficiency, but its benefits were limited at higher inclusion levels. Although egg weight and quality parameters remained largely unaffected, increasing levels of FFSB led to a reduction in hen-day egg production and feed efficiency compared to the control diet. These outcomes likely stem from the presence of residual anti-nutritional factors, particularly trypsin inhibitors that persist despite thermal processing and may impair nutrient utilization. Hens fed diets containing up to 25% roasted FFSB maintained stable body weight and normal hematological and biochemical profiles, indicating no adverse health effects. Economically, although feed costs decreased with increasing FFSB inclusion, the reduction in egg production offset these savings, resulting in lower overall net returns. Consequently, even though FFSB may appear fairly similar to SBM as a layer feed ingredient, its full substitution for SBM proved financially unprofitable, highlighting SBM's continued dominance in cost-efficient formulation. It is suggested that future studies investigate alternative locally applicable processing methods, such as cooking or fermentation, to more effectively deactivate ANFs in FFSB and thereby minimize its performance gap compared to soybean meal.

DECLARATIONS

Acknowledgments

The authors would like to express their gratitude to Bahir Dar University for providing materials and experimental hens for this study. They also extend their appreciation to Andasa Livestock Research Center and the Amhara Regional Agricultural Research Institute for establishing the laboratory for egg quality analysis.

Authors' contributions

All authors contributed to the study conception and design. Yemsrach Yimer conducted material preparation, data collection, and analysis, and drafted the initial manuscript. Firew Tegegne and Solomon Demeke collaborated on the critical review and editing. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The data of the current study are available from the corresponding author upon reasonable request.

Funding

The authors declare that no funds, grants, or other support were received.

Ethical considerations

All authors have ensured compliance with ethical standards, including checks for plagiarism, publication consent, research misconduct, data fabrication or falsification, duplicate publication or submission, and redundancy. The authors confirmed they have not assisted the AI in conducting the present study and preparing the present article.

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Molecular Identification and Genetic Diversity of *Avibacterium paragallinarum* Isolated from Chickens with Infectious Coryza in Bogor, Indonesia

Nia Karunia¹ , Ryan Septa Kurnia² , Christian Marco Hadi Nugroho² , Muhammad Ade Putra² ,
Agustin Indrawati³ , and Safika^{3*} 

¹Study program of Animal Biomedical Sciences, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, 16680, Indonesia

²Indonesia Research and Diagnostic Unit, PT. Medika Satwa Laboratoris, Bogor, 16166, Indonesia

³Division of Medical Microbiology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, 16680, Indonesia

*Corresponding author's E-mail: safika@apps.ipb.ac.id

Received: September 24, 2025, Revised: October 28, 2025, Accepted: November 23, 2025, Published: December 25, 2025



ABSTRACT

Infectious coryza, a respiratory disease caused by *Avibacterium paragallinarum* (*A. paragallinarum*), poses a major threat to poultry health and productivity, particularly in tropical countries such as Indonesia. This study aimed to isolate, molecularly identify, and characterize the genetic diversity of *A. paragallinarum* from chickens exhibiting clinical symptoms of coryza. A total of 60 infraorbital sinus swab samples were aseptically collected from commercial layer and broiler chickens in Bogor Regency, West Java, Indonesia. The method consists of phenotypic identification (culture on Nicotinamide Adenine Dinucleotide [NAD], Gram staining, and biochemical tests) and molecular confirmation using Polymerase Chain Reaction (PCR) HPG-2, Enterobacterial Repetitive Intergenic Consensus (ERIC)-PCR, and *16S rRNA* sequencing. Eight field isolates were successfully cultured on NAD-supplemented blood agar, Gram-negative, catalase and oxidase-negative. Molecular confirmation was conducted using species-specific HPG-2, with all isolates amplifying the expected 500 bp product. The evaluation of genetic diversity was conducted through ERIC-PCR, which identified five unique clusters among the isolates, demonstrating considerable genomic variation. Furthermore, partial *16S rRNA* gene sequences were amplified and analyzed through phylogenetic tree construction and BLAST comparison. Sequence analysis revealed 95.7–99.2% homology with reference *A. paragallinarum* strains in GenBank, and 96.5–99.2% homology among the study isolates themselves. The phylogenetic tree highlighted a close relationship among the local isolates, yet also indicated a distinct genetic lineage from several international reference strains, suggesting possible regional specificity. This study provided the first detailed genetic characterization of *A. paragallinarum* field isolates in Indonesia using *16S rRNA* sequencing. These findings highlighted the need for continuous molecular surveillance to guide accurate diagnosis.

Keywords: *16S rRNA* sequencing, *Avibacterium paragallinarum*, Field isolate, Molecular identification

INTRODUCTION

Coryza, also known as snout, is one of the infectious diseases that affects the respiratory system in chickens. This disease may affect all varieties of chickens, including laying hens, broilers, and native breeds (El-Gazzar et al., 2025). Coryza is induced by the bacterium *Avibacterium paragallinarum*, which was originally classified as *Haemophilus paragallinarum* (Blackall et al., 2005). *Avibacterium paragallinarum* is classified as a Gram-negative bacterium and a member of the *Pasteurellaceae* family (Liu et al., 2023). Clinical symptoms in infected chickens include conjunctivitis, unilateral or bilateral ocular inflammation in acute conditions, and serous exudate leading to caseous sinusitis in chronic conditions

(Babazadeh and El-Ghany, 2023). Coryza has a significant economic impact on poultry farming.

The mortality rate in chickens is relatively low, around 1%-5% but may reach up to 30% under severe or uncontrolled conditions; however, the morbidity rate might reach 80%-100% (Kusumaningsih and Poernomo, 2000). In laying hens, the productivity decline might be as much as 10%-40% (Blackall and Soriano-Vargas, 2020). The eradication of coryza disease in developing countries is challenging due to multiple factors and the presence of mixed infections. This disease may rapidly spread from one chicken to another within a flock or across different flocks (Clothier et al., 2019). Direct transmission can occur between infected or carrier chickens and other susceptible chickens (Pierdon et al., 2025). Coryza may

infect, particularly during seasonal transitions or due to various types of stress, such as the cage environment, weather, nutrition, vaccination efforts, and diseases that reduce immunity (Moenek, 2016).

Diagnosis of coryza is acquired by visible clinical symptoms and confirmed by several tests, including bacterial isolation, biochemical characterization, and polymerase chain reaction (PCR) test; nevertheless, culturing *A. paragallinarum* bacteria faces significant challenges (Deresse et al., 2022). This bacterium has a slow growth rate, requires particular media and environmental conditions, and shows little reactivity in biochemical tests (Aker et al., 2016). This microorganism is frequently associated with co-infection involving other pathogens, such as *Escherichia coli*, *Pasteurella multocida*, *Bordetella avium*, and *Mycoplasma gallisepticum*, which also complicate the isolation of pure cultures and diagnosis of the disease (Sarika et al., 2019).

According to Page (1962), *A. paragallinarum* bacteria are categorized into three serogroups: A, B, and C. On the other hand, the Kume classification differentiates these bacteria into nine serovars: A-1, A-2, A-3, A-4, B-1, and C-1, C-2, C-3, C-4 (Kume et al., 1983; Buter et al., 2023). Both classifications derive from the results of the hemagglutination inhibition (HI) test. Subsequent development began using multiplex PCR for serotyping (Sakamoto et al., 2012). The amplification of *16S ribosomal ribonucleic acid (16S rRNA)* is often selected for genus and species identification due to its universal presence in all bacteria (Srinivasan et al., 2015). The *16S rRNA* gene serves as a conserved molecular marker widely employed for studying bacterial phylogeny and taxonomic relationships (Indraswari et al., 2021). Despite the economic burden caused by infectious coryza in Indonesia, studies into the molecular characteristics of *A. paragallinarum*, particularly its genetic diversity, remain limited. Notably, no studies have been published on the *16S rRNA* gene sequences of field isolates from Indonesia. This hinders the understanding of strain variation and its epidemiological consequences. The present study aimed to isolate, molecularly identify, and sequence the *16S rRNA* of *A. paragallinarum* bacteria from chickens exhibiting coryza symptoms.

MATERIALS AND METHODS

Ethical approval

All chicken samples and related procedures were ethically approved by the Animal Ethics Committee, School of Veterinary Medicine and Biomedical Sciences, Bogor, Indonesia, with approval number 293/KEH/SKE/II/2025.

Sample collection

A total of 60 swab samples were collected from four commercial farms located in Bogor Regency, West Java, Indonesia. Samples were taken from the layer and broiler

chickens that showed symptoms of nasal discharge, lacrimation, and head swelling. The exudate was aseptically collected from the infraorbital sinus using a sterile swab.

Isolation and identification

All the sample swabs were inoculated onto 5% chicken blood agar (CBA; Oxoid Ltd., UK) supplemented with nicotinamide adenine dinucleotide (NAD; Oxoid Ltd., UK), and incubated at 37°C for 24 hours. The growth on the plates was subsequently evaluated. Afterward, the colonies underwent Gram staining and testing, followed by the observation of specific characteristics, which included a lack of growth on catalase and being oxidase negative, along with indole production, H₂S production, and being non-hemolytic on CBA. The suspected colonies from each sample were then passaged onto new CBA to isolate pure suspected colonies of *A. paragallinarum* (Tangkonda et al., 2019; Fauziah et al., 2021).

DNA extraction

The DNA from bacteria was extracted using the boiling method. Around 2-3 well-separated colonies of *A. paragallinarum*, which corresponded to an estimated cell mass of about 10⁸ colony-forming units (CFU), were placed into 500 µL of nuclease-free water within an Eppendorf tube. The mixture was then heated to 98°C for 10 minutes using a dry bath incubator, followed by a freeze for 5 minutes. The tube was centrifuged at 10,000 rpm for one minute. After the DNA was extracted, 50 µL of the supernatant was collected and stored at -20°C until further analysis (Dashti et al., 2009).

Species-specific polymerase chain reaction (PCR)

The polymerase chain reaction test adheres to the method reported by Chen et al. (1996) and Putra et al. (2023) with some modifications. The final volume was 50 µL, which contained 25 µL MyTaq HS Red Mix, 2 µL of both forward and reverse HPG-2 primers (Table 1), 16 µL nuclease-free water, and 5 µL of DNA template. The PCR process entails pre-denaturation at 95°C for one minute, followed by denaturation, annealing, and extension at 95°C for 15 seconds, 55°C for 15 seconds, and 72°C for 10 seconds, for as many as 30 cycles using a thermal cycler. Amplicons were visualized using agarose gel electrophoresis with Ethidium bromide staining in 100 mL of 1 × TAE buffer.

Enterobacterial Repetitive Intergenic Consensus (ERIC)-PCR

The PCR kit utilized for ERIC-PCR was obtained from the KAPA2G Fast Hotstart Readymix PCR Kit (Kapa Biosystem, Wilmington, MA, USA). The amplification consists of three minutes at 95°C and 35 cycles each consisting of 95°C for one minute, annealing at 52°C for 30 seconds, and 72°C for 6 minutes, followed by a final extension step of five minutes at 72°C. The

amplified products were analyzed by electrophoresis on a 1.5% agarose gel stained with ethidium bromide. The fragment sizes were estimated with a 100 bp molecular weight marker (VC 100 bp Plus DNA Ladder, Vivantis, Selangor, Malaysia). Banding patterns were assessed using

PyElph software version 1.4 to create a binary matrix. Hierarchical clustering was then conducted employing the Ward's linkage method in Minitab version 18. The primer pair ERIC1R as reverse primer and ERIC2 as forward primer used is shown in Table 1.

Table 1. The PCR primers used in this present study for the identification of *Avibacterium paragallinarum*

Gene	Primer sequence	Reference
HPG-2	(F) 5'-TGAGGGTAGTCTTGCACGCGAAT-3'	Chen et al. (1996)
	(R) 5'-CAAGGTATCGATCGTCTCTCTACT-3'	
ERIC	(F) 5'-AAGTAAGTGACTGGGGTGAGCG-3'	Soriano et al. (2004)
	(R) 5'-ATGTAAGCTCCTGGGGATTAC-3'	
16S rRNA	(F) 5'-AGAGTTTGATCMTGGCTCAG-3'	Jeong et al. (2020)
	(R) 5'-TACGGYTACCTTGTTACGACTT-3'	

HPG-2: *Haemophilus paragallinarum*, ERIC: Enterobacterial Repetitive Intergenic Consensus, 16S rRNA: 16S ribosomal RNA

16S rRNA sequencing

The isolates that had been identified as *A. paragallinarum* were tested for the presence of the 16S rRNA gene using 16S rRNA primers (Table 1). The PCR process was conducted with MyTaq HS Red Mix (Bioline), comprising a total reaction volume of 50 µl and an annealing temperature set at 56°C. First BASE Laboratories, Malaysia, performed Sanger sequencing, which separated the PCR products using electrophoresis, and the target band was purified for sequencing. The nucleotide and amino acid sequences of a recently sequenced 16S rRNA gene were determined using Bioedit v.7 (<https://bioedit.software.informer.com/7.0/>), and ClustalW was used for alignment. A modern phylogenetic tree was constructed using MEGA v 7.0 (<https://www.megasoftware.net/>) with the neighbor-joining method and 1,000 bootstrap replicates. The phylogenetic tree was compared the the present study isolates and other prototype *A. paragallinarum* isolates from GenBank. The genetic distance between isolates and the topology of the phylogenetic tree were used to compare strains. The genetic similarity percentage among all study strains was calculated using the maximum composite likelihood model available in MEGA v 7.0.

Nucleotide sequence accession numbers

The partial coding sequence (CDS) of the 16S rRNA gene from all *A. paragallinarum* isolates examined in this study has been submitted to GenBank and assigned the accession numbers PV653197, PV653198, PV653199, PV653200, PV653201, PV653202, PV653203, and PV653204.

RESULTS

Phenotypic identification

Only eight isolates were successfully cultured on agar media and subjected to further characterization. Every isolate demonstrated growth on NAD-CBA, and non-

hemolysis was observed (Figure 1A). The characteristics of the bacterial cells were Gram-negative with a coccoid bipolar structure (Figure 1B). Furthermore, all isolates were found to be negative for catalase and oxidase, indole negative, and none were able to produce H₂S (Table 2).

Molecular identification

Species-specific PCR was conducted on all samples to verify their identification as *A. paragallinarum*. All samples produced a 500 bp amplicon, confirming the presence of *A. paragallinarum* (Figure 2).

Avibacterium paragallinarum diversity based on Enterobacterial Repetitive Intergenic Consensus (ERIC)-PCR

Enterobacterial Repetitive Intergenic Consensus (ERIC-PCR) displayed differing patterns of DNA fragments across the eight *A. paragallinarum* isolates examined in this study. There were 3-8 bands generated in these fragment patterns, with sizes ranging from 300–2800 bp (Figure 3A). Hierarchical clustering analysis of DNA fragment patterns was conducted using the Dice similarity coefficient and Ward's linkage method, applying a 95% similarity threshold to define genetic clusters (Figure 3B). Based on this analysis, five clusters were identified: Cluster I (Apg-6), Cluster II (Apg-5 and Apg-8), Cluster III (Apg-2 and Apg-3), Cluster IV (Apg-1), and Cluster V (Apg-4 and Apg-7).

Homology and phylogenetic investigation

The amplification of the partial PCR product for 16S rRNA is illustrated in Figure 4A. Using the BLAST algorithm to analyze the nucleotide sequences of 16S rRNA indicated that the present *A. paragallinarum* isolates exhibit a high degree of sequence similarity, ranging from 95.7–99.2% with the NCBI database reference strains. When compared among the recent study isolates (Apg-1 to Apg-8), the sequences obtained from all eight isolates demonstrated a homology of 96.5-99.2% (Figure 4B). A phylogenetic analysis of 16S rRNA sequences,

incorporating 33 sequences, which included the eight isolates identified in this study and 25 sequences available in GenBank, representing strains isolated from various

geographical regions such as Japan, China, Mexico, and the United States. A phylogenetic tree created using the neighbor-joining method was shown in Figure 5.

Table 2. Biochemical and morphological characteristics of *Avibacterium paragallinarum* study isolates

Test Performed	Isolate code							
	Apg-1	Apg-2	Apg-3	Apg-4	Apg-5	Apg-6	Apg-7	Apg-8
Gram nature	Negative Rods	Negative Rods	Negative Rods	Negative Rods	Negative Rods	Negative Rods	Negative Rods	Negative Rods
Catalase	-	-	-	-	-	-	-	-
Oxidase	-	-	-	-	-	-	-	-
Indole production	-	-	-	-	-	-	-	-
H ₂ S production	-	-	-	-	-	-	-	-
Hemolysis on BA	-	-	-	-	-	-	-	-
NAD dependence	+	+	+	+	+	+	+	+

Apg: *Avibacterium paragallinarum* isolate, H₂S: Hydrogen Sulphide, BA: Blood agar, NAD: Nicotinamide adenine dinucleotide

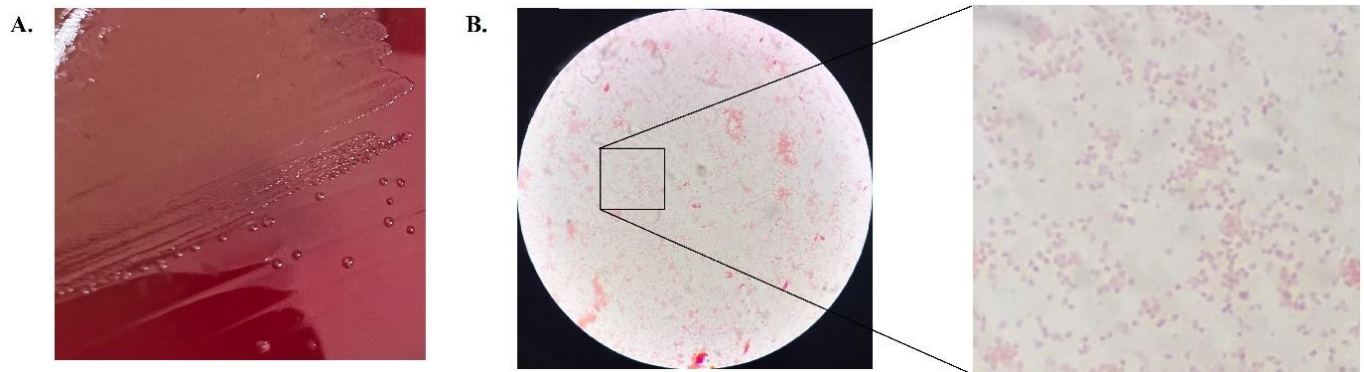


Figure 1. Satellite colonies of *Avibacterium paragallinarum* (A) and coccoid bipolar structure of gram-negative bacteria isolated from infraorbital sinus swabs of chickens in Indonesia (Gram staining, ×1000, B).

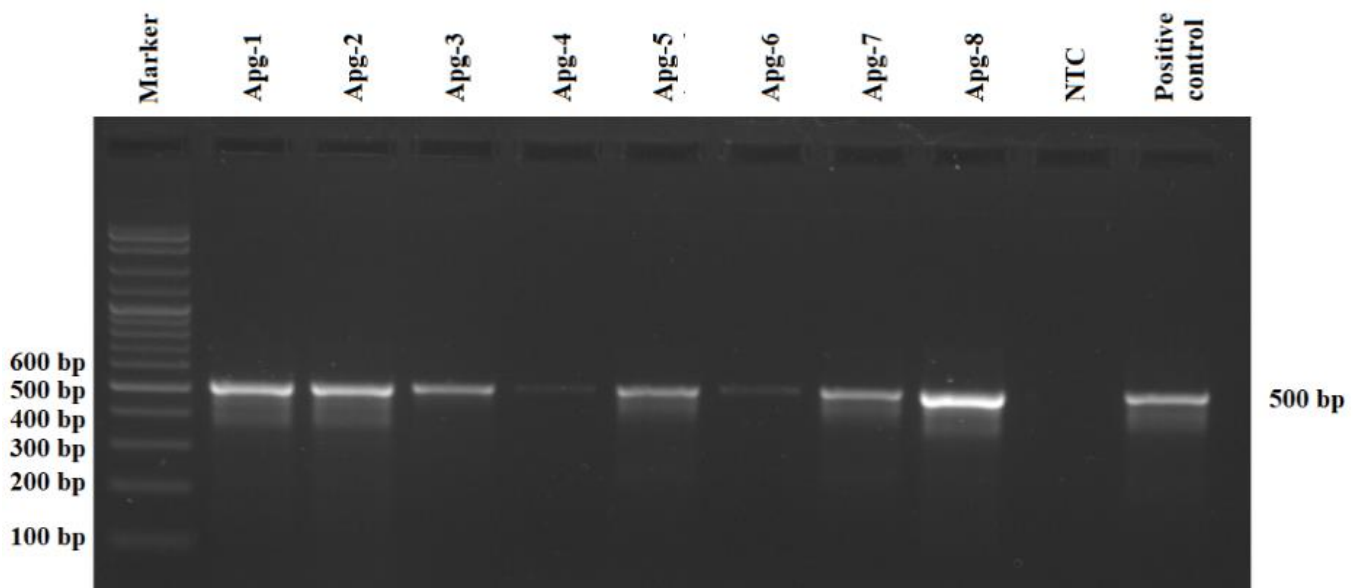


Figure 2. Molecular detection of HPG-2 PCR from Indonesian field isolates of *Avibacterium paragallinarum*

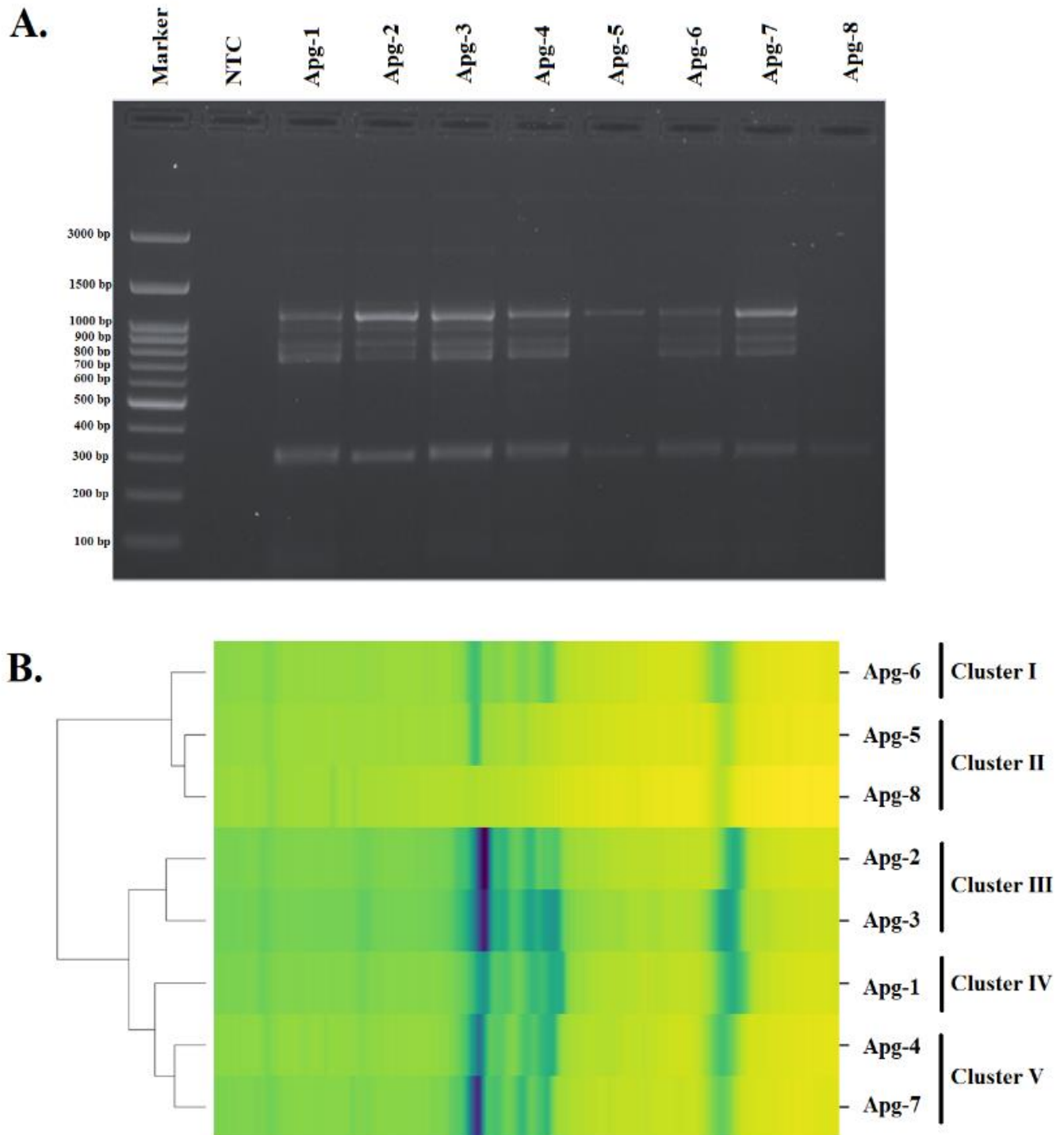
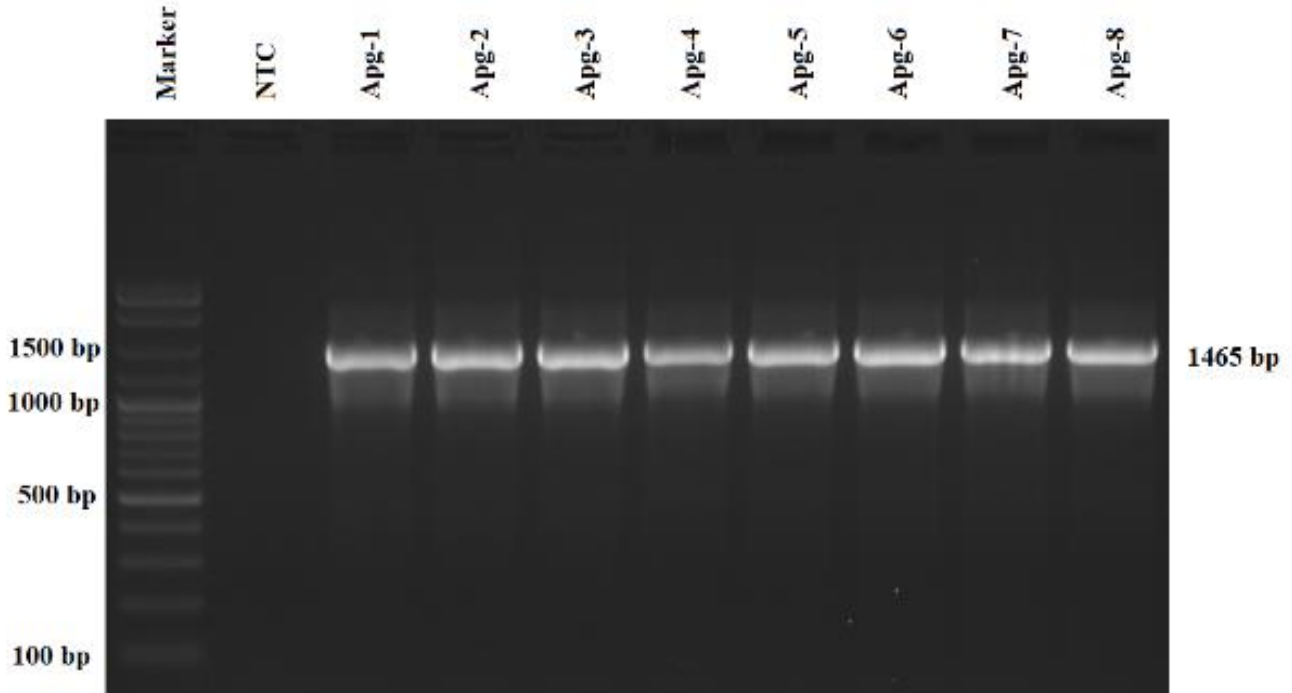


Figure 3. ERIC-PCR banding patterns obtained for eight *Avibacterium paragallinarum* field isolates collected from chickens in Indonesia (A), showing 3-8 DNA bands ranging from 300-2800 bp. The dendrogram generated using Ward's linkage method (B) revealed five distinct clusters, indicating genetic diversity among the isolates. NTC: No template control.

A.



B.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33			
1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	JN692516.1_Ambacterium_endocandidae_strain_UH15947	
2	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	M17500.1_Ambacterium_talarum_strain_NCTC_4101	
3	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	NR_029537.1_Ambacterium_olivetum_strain_NCTC_3438	
4	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	NR_115217.1_Ambacterium_olivetum_strain_CCUG_3743		
5	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	NR_046750.1_Ambacterium_satum_strain_NCTC_11297	
6	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	NR_116216.1_Ambacterium_satum_strain_CCUG_12833	
7	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	NR_118754.1_Ambacterium_gallinarum_strain_NCTC_11188	
8	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	JN692516.1_Ambacterium_sp._RB410	
9	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	JN455087.1_Haemophilus_paragallinarum_strain_Moderato	
10	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	JN455087.1_Haemophilus_paragallinarum_strain_SA7177	
11	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	GU737037.1_Ambacterium_paragallinarum_strain_SCUD_1	
12	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	GU951544.1_Ambacterium_paragallinarum_strain_HP105
13	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	GU951544.1_Ambacterium_paragallinarum_strain_HP105
14	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	KC951265.1_Ambacterium_paragallinarum_strain_BSV-129
15	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	KC951276.1_Ambacterium_paragallinarum_strain_HF60
16	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	KM354708.1_Ambacterium_paragallinarum_strain_FAREFR-107
17	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	KT371945.1_Ambacterium_paragallinarum_strain_ESV-142
18	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	KT371941.1_Ambacterium_paragallinarum_strain_ESV-184
19	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	KT370996.1_Ambacterium_paragallinarum_strain_ESV-244
20	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	KT370996.1_Ambacterium_paragallinarum_strain_ESV-346
21	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	KT380001.1_Ambacterium_paragallinarum_strain_Lavtec_02
22	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	KT22532.1_Ambacterium_paragallinarum_strain_IND102
23	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	M1705902.1_Ambacterium_paragallinarum_strain_PL_06
24	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	M1705902.1_Ambacterium_paragallinarum_strain_PL_08
25	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	KT371936.1_Ambacterium_paragallinarum_strain_ESV-68
26	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	Apg-1_PV6531917
27	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	Apg-2_PV6531918
28	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	Apg-3_PV6531919
29	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	Apg-4_PV6532000
30	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	Apg-5_PV6532001
31	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	Apg-6_PV6532002
32	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	Apg-7_PV6532003
33	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	Apg-8_PV6532004

Figure 4. The PCR amplification targeting the *16S rDNA* (A) and homology comparison of eight Indonesian field isolates with GenBank database reference strains of *A. paragallinarum* (B).

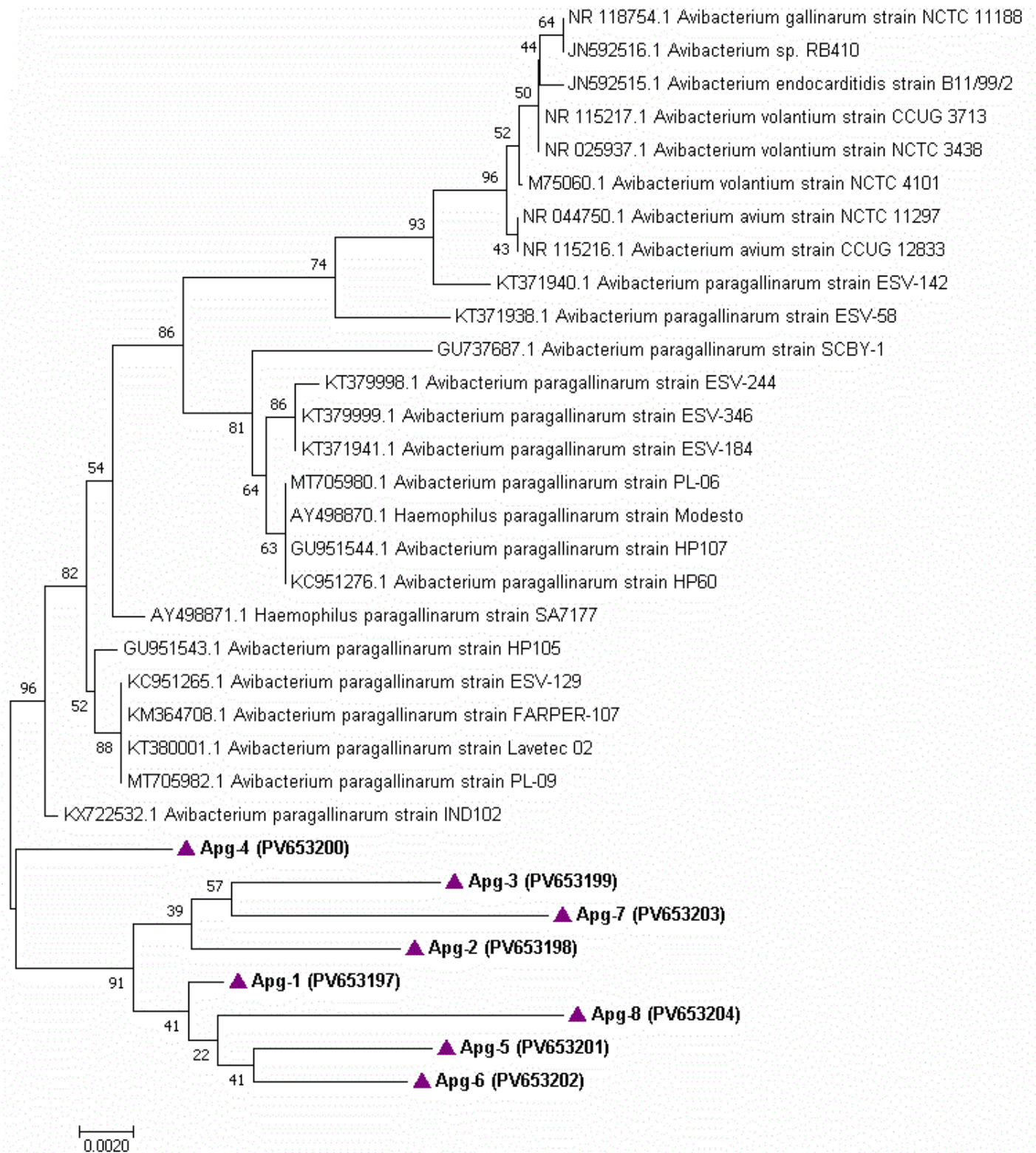


Figure 5. Phylogenetic tree based on the nucleotide sequences of the 16S rRNA. The tree includes eight *Avibacterium paragallinarum* isolates from this study (▲) and reference strains retrieved from GenBank. The local isolates clustered in a separate clade, indicating genetic divergence from several international reference strains.

DISCUSSION

Avibacterium paragallinarum is a bacterium that is challenging to cultivate in media. The growth of *A. paragallinarum* bacteria requires a temperature of 37°C under aerobic or anaerobic environments, together with elevated CO₂ pressure levels (5%-10%) for a duration of 24-48 hours (Akter et al., 2014). Because nicotinamide adenine dinucleotide (NAD; V-factor) is essential for *in vitro* growth, *Staphylococcus aureus* is also inoculated in the same medium to produce additional NAD (Akter et al., 2014; Deresse et al., 2022). Although, along with the advancement, it has been discovered that *A. paragallinarum* could multiply in the absence of NAD (Deshmukh, 2015). In this study, *A. paragallinarum* was successfully cultivated on blood agar supplemented with NAD and 5% chicken serum. The colonies that grew were recultured until pure colonies of *A. paragallinarum* were obtained. The study by Badr et al. (2022) used the infraorbital swab results to grow bacteria on blood agar medium containing 10% sheep blood and added *Staphylococcus aureus* as an NAD feeder. Satellite growth of bacterial colonies was also shown in the blood medium.

Avibacterium paragallinarum is classified as a Gram-negative bacterium, resulting in a red hue during Gram staining. The envelope of a Gram-negative cell is made up of three distinct layers, namely the outer membrane, the peptidoglycan cell wall, and the inner or cytoplasmic membrane (Fivenson et al., 2024). According to the cell wall permeability theory, Gram-negative bacteria possess thin cell membranes that allow the primary stain to be removed, enabling absorption of the counterstain (Vijayakumar et al., 2023). Meanwhile, the biochemical assays conducted were catalase, oxidase, and indole tests, revealing negative results. The catalase test identifies microorganisms that produce the enzyme catalase, which neutralizes hydrogen peroxide and forms bubbles in positive results (Chandra, 2023). The oxidase test determines the ability of bacteria to manufacture the enzyme (Hafezi and Khamar, 2024). The indole test demonstrated the ability of the microorganisms to produce the tryptophanase enzyme, while the bacteria neither produced Hydrogen Sulphide (H₂S) nor exhibited hemolytic activity (Roy et al., 2023). Previous studies have also reported Gram-negative results in the form of coccobacilli, biochemical tests showed negative results for catalase, oxidase, motility, urease, and indole, followed by positive results for lactose, maltose, mannitol, and sorbitol (Akter et al., 2016; Wahyuni et al., 2022).

Molecular analysis using PCR was performed on eight samples collected from an Indonesian field. All analyzed samples were declared positive for *A. paragallinarum*. According to Chen et al. (1996), HPG-2 PCR gave equivalent results as conventional culture techniques and was recommended as a confirmatory and species-specific test for *A. paragallinarum*. Polymerase chain reaction (PCR) detection in Indonesia is still limited

due to the difficulty in isolating this bacterium; nevertheless, a few published reports include Fauziah et al. (2021) and Putra et al. (2023), who successfully identified *A. paragallinarum* from swab samples of layer hens that had coryza symptoms in the Sleman district, Yogyakarta. Numerous further investigations across various countries have used HPG-2 PCR to detect this bacterium with a target band of 500 bp (Nabeel Muhammad and Sreedevi, 2015; Feberwee et al., 2019; Guo et al., 2022).

The Enterobacterial Repetitive Intergenic Consensus (ERIC)-PCR method has proven to be a valuable molecular tool for assessing genomic diversity among bacterial isolates, including *Avibacterium paragallinarum* (Hellmuth et al., 2017). In the present study, ERIC-PCR successfully differentiated eight *A. Paragallinarum* isolates obtained from chickens with infectious coryza in Indonesia. The banding patterns generated by ERIC-PCR revealed substantial variation, with each isolate displaying between 3 to 8 distinct bands ranging from 300 bp to 2,800 bp. This heterogeneity underscores the genomic diversity present among field strains of *A. paragallinarum*, which may contribute to differences in virulence, transmission dynamics, and vaccine responsiveness (Morales-Erasto et al., 2014).

The application of hierarchical clustering analysis with Ward's linkage approach divided the isolates into five separate clusters at a 95% similarity level. This finding is significant as it indicates that despite originating from a relatively narrow geographical area, the *A. paragallinarum* population in the study site exhibits considerable genotypic variability. Such clustering patterns reflect the existence of multiple circulating strains or subpopulations within a region, which may arise from genetic recombination, mutation, or evolutionary pressure exerted by antimicrobial usage and host immune responses.

The presence of several unique ERIC profiles among the isolates supports the notion that *A. paragallinarum* possesses a dynamic genome, likely influenced by mobile genetic elements such as plasmids or phages that are commonly targeted by ERIC primers. This aligns with previous studies that have demonstrated the discriminatory power of ERIC-PCR in distinguishing between bacterial strains with high resolution, making it a useful method for epidemiological surveillance and outbreak investigations (Bakhshi et al., 2018). Moreover, the clustering patterns observed may have epidemiological implications. For example, isolates that grouped closely within the same cluster could represent transmission events within or between locations, whereas isolates in separate clusters may indicate multiple sources of infection (Aljindan et al., 2018). The diversity revealed by ERIC-PCR should be considered when designing control strategies, including the development of autogenous vaccines tailored to the circulating strains.

According to Li et al. (2024), *16S rRNA* gene sequencing is a more sensitive method for detecting

pathogens and adequate to identify novel bacteria not only in human and animal diseases. Similarity tests from sequencing results showed that the eight samples were *A. paragallinarum* bacteria. Sequence analysis revealed 95.7-99.2% homology with reference *A. paragallinarum* strains in GenBank, and 96.5-99.2% homology among the study isolates themselves. The test sequence may be categorized as having similarities with the sequence in GenBank if its query cover and percent identity values are above 96% or as having a higher similarity if it approaches 100% (Bahri et al., 2023). The study conducted by Patil et al. (2017) employed *16S rRNA* sequencing to detect *A. paragallinarum* isolated from Indian field conditions, and the BLAST analysis demonstrated 96% to 99% similarity with the reference strain *A. paragallinarum*. The *16S rRNA* is located in bacterial ribosomes with a total length of approximately 1540 nucleotides (Yang et al., 2024). This gene is remarkably conserved in structure and function, making it often used to reflect phylogenetic relationships between bacteria (Idris et al., 2020).

The findings of *16S rRNA* sequencing in this study were used to generate a phylogenetic tree. All eight local isolates clustered within the same clade, indicating a close genetic relationship among them. However, these isolates formed a separate clade from the reference strains, suggesting the presence of distinct genetic diversity between the local Indonesian isolates and the reference *A. paragallinarum* strains. This result is contrary to the phylogenetic tree derived from *16S rRNA* sequencing of isolates from chickens in Korea between 2011-2016, which formed several clades sharing the same branch as the reference isolate (Jeong et al., 2020). However, the phylogenetic cluster of several *A. Paragallinarum* samples isolated from China displayed a distant genetic relationship, indicating significant geographical specificity (Cui et al., 2025). The observed genetic variation may result from factors such as genetic mutations, international transmission, or inherent local diversity, which necessitate further epidemiological analysis and tracing the origin strain is required (Guo et al., 2024).

CONCLUSION

Based on the findings of this study, *Avibacterium paragallinarum* was successfully isolated and identified from chickens showing clinical symptoms of infectious coryza in Indonesia. Phenotypic characterization and species-specific PCR confirmed all eight field isolates as *A. paragallinarum*. ERIC-PCR analysis revealed considerable genetic diversity among the isolates, indicating the presence of multiple circulating strains. The *16S rRNA* sequencing further confirmed the species identity and demonstrated high homology with global reference strains, while also showing distinct phylogenetic clustering. The present results underscored the importance of molecular surveillance for accurate diagnosis and effective control of infectious coryza in poultry flocks.

Future studies should consider larger sample sizes across different regions in Indonesia and incorporate antimicrobial resistance profiling to enhance disease control strategies.

DECLARATIONS

Acknowledgments

The authors would like to acknowledge and thank the Ministry of Higher Education, Science, and Technology (Kemdiktisaintek), through the research grant with number 23333/IT3.D10/PT.01.03/P/B/2025.

Author's contributions

Nia Karunia, Ryan Septa Kurnia, Muhammad Ade Putra, Christian Marco Hadi Nugroho, Agustin Indrawati, and Safika contributed to writing the original text, the experiment, carried out the experiments, and conducted the statistical analysis. Nia Karunia, Christian Marco Hadi Nugroho, Agustin Indrawati, and Safika analyzed and reviewed the results and then composed the final essay. All authors have reviewed and approved the final version of the manuscript for publication.

Funding

The present study was financially supported by the Ministry of Higher Education, Science, and Technology (Kemdiktisaintek), through the research grant with number 23333/IT3.D10/PT.01.03/P/B/2025.

Competing interests

The authors declared that there are no competing interests.

Ethical considerations

The authors declare that this manuscript is original and is not being considered elsewhere for publication. The authors have reviewed additional ethical concerns, such as research misconduct, data fabrication, and redundancy. The authors confirmed they have not assisted the AI in conducting and preparing the present study.

Availability of data and materials

All data from the current study are available upon reasonable requests from the authors.

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Impacts of Nicotinamide Adenine Dinucleotide Supplementation on Longevity, Egg Quality, and Immunity in Laying Hens

Bamboo XZ Yan¹ , Xiaoxuan Zhang² , and Ramsha Shahbaz^{3*} 

¹Department of Molecular Bioscience, Australian Institute of Integrative Medicine, New South Wales, Australia

²PWI Research and Technology Pty Ltd, Somersby, Australia

³M.Phil. University of Lahore, Institute of Molecular Biology and Biotechnology (IMBB), Lahore, Pakistan

*Corresponding author's E-mail: madpudding33@gmail.com

Received: October 06, 2025, Revised: November 11, 2025, Accepted: December 04, 2025, Published: December 25, 2025



ABSTRACT

The productivity and health of laying hens are major challenges for the global poultry industry, especially as flocks age and egg production declines. The present study aimed to evaluate the effects of nicotinamide riboside chloride (NRCl), a bioavailable precursor of nicotinamide adenine dinucleotide (NAD⁺), on longevity, egg quality, and immune performance in laying hens. A total of 180 Hy-Line Brown hens, 24-week-old, were randomly assigned to three dietary groups for 24 weeks, including a control group receiving standard layer feed, a low-dose NRCl group receiving 25 mg NR/kg feed, and a high-dose NRCl group receiving 50 mg NR/kg feed. The present study assessed survival rate, daily egg production, egg weight, shell strength, yolk nutrient composition, and immune parameters, including white blood cell (WBC) count, immunoglobulins (IgA and IgG), and cytokines (IL-6 and IFN- γ). The present findings indicated that supplementation with NRCl significantly increased the survival rate, resulting in 100% survival in the high-dose group compared to 93.3% in the control group. Egg production and feed conversion ratio were also enhanced in NRCl groups, indicating improved physiological resilience. The NRCl groups demonstrated significant improvements in egg quality, including stronger, thicker shells and enhanced nutritional content such as higher yolk protein, vitamin D3, and omega-3 fatty acid levels. Furthermore, immune markers were markedly elevated, reflecting enhanced immune modulation and potential disease resistance. The NRCl supplementation effectively supported metabolic efficiency, immune competence, and egg quality in laying hens. The current findings highlighted the potential of NAD⁺ precursor supplementation as a nutritional strategy to prolong the productive lifespan of commercial layers while promoting sustainable poultry production.

Keywords: Egg quality, Immune response, Laying hens, Longevity, Nicotinamide adenine dinucleotide supplementation, Poultry feed

INTRODUCTION

Egg production plays an essential role in global food security, providing an affordable and high-quality source of protein worldwide (Supriya et al., 2023). Poultry farming is vital to the rural livelihoods in many developing countries, providing direct household consumption and income through poultry-based activities. However, the poultry industry faces several challenges, particularly the ageing of laying hens, which leads to a gradual decrease in egg production after approximately 72 weeks of age (Li et al., 2021).

In many regions, including the United States and Australia, increasing feed and transportation costs, along with recurrent disease outbreaks such as avian influenza, have intensified these challenges (Rehman et al., 2023). The progressive decline in productivity associated with hen ageing is closely linked to metabolic and cellular changes, including reductions in nicotinamide adenine dinucleotide (NAD⁺) levels, which are known to influence energy metabolism, DNA repair, and mitochondrial function (Covarrubias et al., 2021). Therefore, strategies that restore or maintain NAD⁺ balance may help mitigate

the effects of ageing and improve the longevity and productivity of laying hens.

In recent years, advances in molecular biology have highlighted NAD⁺ as a crucial regulator in cellular energy metabolism, aging, and immune function (Zhang et al., 2025). The NAD⁺ plays an essential role in redox reactions, mitochondrial activity, and DNA repair, all of which depend on adequate intracellular NAD⁺ levels (Braidy et al., 2019). Supplementing the diet with NAD⁺ precursors such as nicotinamide riboside chloride (NRCl) in mammals has been demonstrated to extend lifespan, enhance metabolic efficiency, and improve immune performance (Elhassan et al., 2019). These findings suggested exploring the potential use of NAD⁺ supplementation in non-mammalian species, particularly in agricultural settings such as poultry production (Wang et al., 2022). However, there are a limited number of studies that investigated the avian use of NAD⁺ and its physiological effects in laying hens (Xie et al., 2020).

The present study aimed to evaluate the effects of NRCl on the longevity, egg quality, immune function, and overall productivity in ageing laying hens.

METHODS AND MATERIALS

Ethical approval

All experimental procedures were conducted in accordance with national and institutional ethical standards for animal welfare. Approval for the present study was obtained from the Institutional Animal Care and Use Committee (IACUC) of PWI Research and Technology Pty Ltd, Somersby, New South Wales, Australia. Humane euthanasia, performed according to the American Veterinary Medical Association (AVMA) guidelines, was conducted in cases of severe mortality.

Nicotinamide adenine dinucleotide formulation

During the present study, a stable and bioavailable NAD⁺ precursor formulation was developed in collaboration with a certified poultry feed manufacturer. The NAD⁺ precursor was NRCl, which was purchased from Sigma-Aldrich, USA, with a stated purity of $\geq 99\%$. The NRCl was selected because it is highly bioavailable and has been shown to increase NAD⁺ levels in mammalian models, *in vivo* (Yang et al., 2019). The NRCl powder was mixed into the basal feed by simple physical mixing, rather than encapsulation, using 0.05% w/w of maltodextrin and microcrystalline cellulose as carrier materials. This method was selected to ensure a homogeneous distribution of the supplement while

maintaining its stability during feed manufacturing. The NRCl remains stable at moderate temperatures (25-30°C) and humidity levels typical in poultry feed processing environments (Elhassan et al., 2019).

The toxicity and efficacy of NAD⁺ precursor supplementation in poultry were initially assessed in a small-scale pilot trial involving 20 Hy-Line Brown hens, allocated to two NRCl dosages of 25 mg/kg and 50 mg/kg feed, for four weeks, as recommended by Elsharif et al. (2019). No adverse clinical signs, mortality, or reductions in feed intake were observed at either dose. However, hens receiving 50 mg/kg of NRCl exhibited improved activity, normal droppings, and a slight increase in egg production compared to the supplemented controls. Based on these preliminary observations, 50 mg NRCl/kg feed was selected as a safe and effective concentration for the experiment (Maynard et al., 2024). A carrier ratio of 1:10 (NRCl: carrier) was used to ensure even dispersion in the feed, and the mixture was processed with a horizontal feed mixer for 10 minutes to achieve uniform blending. The prepared feed was then stored in opaque, airtight containers at approximately 25°C to minimize light- and oxygen-induced degradation. The NAD⁺ stability in the feed was verified through periodic sampling. The NAD⁺ concentrations were quantified using high-performance liquid chromatography (HPLC) according to the method described by Jing et al. (2022).

Study design

The present experiment was conducted at a certified poultry research facility located in Punjab Province, Pakistan, under ambient temperatures of 24-27°C and relative humidity of 55-65%. Each hen was individually housed in a wire-floored cage (40 × 45 × 45 cm; 2 poultry /m² stocking density) within an environmentally controlled house. Hens were maintained on a 16-hour light: 8-hour dark photoperiod and had *ad libitum* access to feed and fresh water. The experimental period lasted 24 weeks.

Animals

A total of 180 Hy-Line Brown laying hens, 24 weeks of age and with an average initial body weight of 1.75 ± 0.05 kg, were selected based on the uniform health status and laying performance, according to the Hy-Line brown hen conventional system (2022) and Zhao et al. (2022). Baseline data on body weight and egg production were collected before treatment allocation and confirmed to have no significant differences across groups. Unpublished pre-test data showed that the average body weight (Control: X kg; NR-5: Y kg; NR-10: Z kg) and hen day

egg production (Control: A; NR-5: B; NR-10: C) were similar across all groups. The hens were divided into three dietary groups. The control group was fed only the standard commercial layer diet (Group A). The second group received a low dose of nicotinamide riboside (NR), which was included in the basal diet containing 25 mg of NR per kilogram of feed (Group B), and the third group received a high dose of NR, fed with a basal diet containing 50 mg of NR per kilogram of feed (Group C).

Diet compositions

A lower NR inclusion rate (25 mg/kg) was expected to support metabolic balance, whereas a higher rate (50 mg/kg) was anticipated to exhibit a stronger physiological response without adverse effects. The expected physiological effects included biological responses at each NR inclusion level, supported by previous mammalian and initial avian studies on NRs. The basal diet was formulated

according to [NRC \(1994\)](#) nutrient recommendations for layers and contained approximately 17.5% of crude protein, 11.3 MJ/kg of metabolizable energy, 3.5% of calcium, 0.45 % of available phosphorus, 0.35% of methionine, and 0.75% lysine, with vitamin-mineral premixes meeting or exceeding standard requirements. The metabolizable energy of the basal diet was 11.3 MJ/kg, meaning it provided 11.3 megajoules of usable energy per kilogram.

Premix composition of vitamin-mineral

The premix contained vitamins A, D3, E, K3, B-complex, and essential trace minerals such as zinc (Zn), manganese (Mn), copper (Cu), iron (Fe), iodine (I), and selenium (Se), and required electrolytes. The vitamin-mineral premix was added at levels that meet or exceed the nutrient requirements of the laying hen as specified by the [NRC \(1994; Table 1\)](#).

Table 1. Inclusion levels of nicotinamide riboside and anticipated physiological consequences, and the basal diet composition used in the experiment

NR Inclusion Level	Justification	Expected physiological effect
25 mg/kg feed (Low dose)	Supported by prior mammalian and preliminary avian findings showing dose-dependent mitochondrial and antioxidant benefits	Supports metabolic balance without excessive stimulation
50 mg/kg feed (High dose)	Higher doses have been shown to enhance mitochondrial activity and antioxidant capacity	Stronger physiological response with no observed adverse effects
Basal diet composition (as-fed basis)		
Nutrient	Level	
Crude protein	17.5%	
Metabolizable energy	11.3 MJ/kg	
Calcium	3.5%	
Available phosphorus	0.45%	
Methionine	0.35%	
Lysine	0.75%	
Vitamin–mineral premix	Met or exceeded NRC (1994) requirements (Dale, 1994)	

NR: Nicotinamide riboside.

Data and sample collection

Throughout the experiment, trained personnel closely monitored the hens to ensure their well-being and health status. Veterinary staff inspected the poultry daily for any signs of disorder and administered appropriate interventions when required. Several performance parameters evaluated included hen longevity, daily egg production, egg weight, shell strength and thickness, egg nutrient composition, and immune parameters. Digital egg counters and radio frequency identification tracking systems were used to record individual egg production daily. Each week, egg quality traits, including shell strength and shell thickness, were measured using a

breaking force tester (Model EFR-01, Imada Co., Japan) and micrometer calipers (Mitutoyo Corp., Japan). Calibrated digital weighing scales were used to accurately measure egg weight.

Egg nutrient composition was analyzed for protein, lipid profile, vitamins, and mineral content. Protein and lipid contents were determined using the Kjeldahl and Soxhlet extraction methods, respectively, while vitamins were quantified using HPLC (Agilent Technologies, USA). Mineral concentrations were assessed through atomic absorption spectroscopy (AAS; PerkinElmer, USA) following the method described by [Jiang et al. \(2023\)](#).

Sampling procedures were kept to a minimum to reduce stress and minimize blood collection. A subgroup of ten hens per group was sampled monthly via wing vein puncture, with approximately 3 mL of blood collected from each hen. White blood cell (WBC) counts, immunoglobulin levels, such as IgA and IgG, cytokine concentrations, including interleukin-6 (IL-6) and interferon-gamma (IFN- γ), were measured. Samples were analyzed using an automated hematology analyzer (Mindray BC-2800Vet, China) and enzyme-linked immunosorbent assay (ELISA) kits (Cusabio Biotech, Wuhan, China) according to the manufacturer's instructions. The ELISA method ensured consistent and repeatable data collection for both production and immune performance parameters.

Statistical analysis

The data were analyzed using Statistical Package for the Social Sciences (SPSS) version 26.0 (IBM Corp., USA). A one-way analysis of variance (ANOVA) was performed to determine significant differences among the three treatment groups, followed by Tukey's post hoc test for multiple comparisons. For repeated measures, such as egg production over time, a mixed-model ANOVA was applied. A significance level of $p < 0.05$ was used for all statistical analyses. Data visualization was conducted using GraphPad Prism software (version 9.0; GraphPad Software, San Diego, CA, USA) to generate line and bar graphs representing mean values and variations. Results are expressed as mean \pm standard deviation (SD).

RESULTS

Hen longevity and egg production metrics

Hen survival and egg production were consistently monitored over the 24-week experimental period in all treatment groups. The present results indicated a significant difference in survival rates among the control group and both NR-supplemented groups ($p < 0.05$; Table 2). Group A demonstrated a survival rate of 93.3%, whereas Group B recorded 96.7%, and Group C exhibited 100% survival. The few deaths observed in the control group occurred between weeks 18 and 20, most likely due to age-related physiological decline. No necropsy was performed, so the precise cause of death could not be pathologically confirmed. These findings suggested that NR supplementation, particularly at the higher level, enhanced longevity and reduced mortality during the late laying period. These improvements might be associated

with greater general health and physiological resilience in the supplemented hens.

The average daily egg production rate differed significantly among the treatment groups ($p < 0.05$). Group C indicated the highest production at 0.93 ± 0.01 eggs/hen/day, which was significantly greater than that of the control group (0.84 ± 0.02). Group B produced at an intermediate rate of 0.89 ± 0.01 . The higher egg production in Group C indicated that NR supplementation helped maintain productivity as hens aged. By week 20, the control group exhibited a statistically significant 12% reduction in egg production, compared to 6% in Group B and 3% in Group C ($p < 0.05$). No significant differences were observed in body weight and egg production at the start of the study among groups ($p > 0.05$). Therefore, the enhanced performance observed in the NR-supplemented groups likely resulted from treatment rather than pre-existing health or physiological differences.

Furthermore, the slightly higher average egg weight in the supplemented groups might have contributed to the apparent improvement in shell strength, as larger eggs generally require stronger shells to maintain integrity. The present results demonstrated that NR supplementation improved survival rate and egg production in ageing hens during the experiment (Figure 1).

Table 2. Hy-Line Brown laying hens' survival and egg production among treatment groups

Group	Treatment (mg NR/kg feed)	Survival (%)	Avg. Egg Production (Eggs per hen per day \pm SD)
A	0 (Control)	93.3 ± 1.0	0.84 ± 0.02^a
B	25	96.7 ± 0.6	0.89 ± 0.01^b
C	50	100 ± 0.0	0.93 ± 0.01^b

Avg: Average, SD: Standard deviation, ^{a, b} Different superscript letters in a column indicate significant differences ($p < 0.05$).

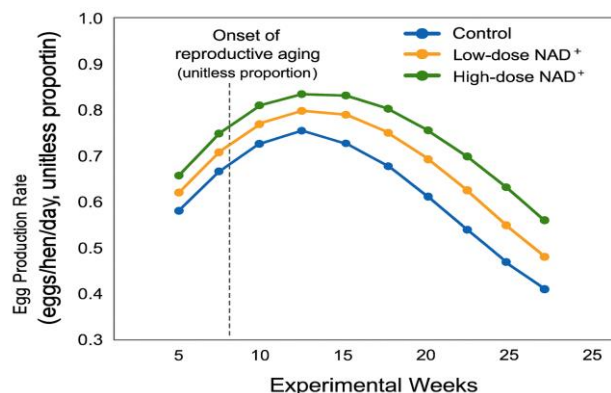


Figure 1. The y-axis is the egg production rate (eggs/hen/day, unitless proportion), whereas the x-axis is experimental duration (weeks). The study showed that high-dose NR supplementation (50 mg/kg feed) resulted in a high laying rate and retarded the onset of reproductive aging in high-dose supplementation as opposed to the control and low-dose groups. The dotted line represents the estimated age of reproductive aging towards Week 9.

Egg quality and nutrient analysis

The average egg weight across all groups increased gradually over 24 weeks. In week 24, the mean egg weights were 60.2 ± 1.5 g for Group A, 62.4 ± 1.2 g for Group B, and 63.8 ± 1.0 g for Group C. Group C exhibited the highest increase in egg weight among all groups, with statistically significant differences observed between Group A and Group C ($p < 0.05$; Table 3). Regarding shell quality, Group C produced significantly thicker shells (0.38 mm) compared to Group A (0.32 mm; $p < 0.05$). Likewise, shell strength was higher in Group C (3.5 kg) than in Group A (2.9 kg), indicating that NR supplementation improved shell thickness and strength. These findings suggested that NR supplementation enhanced the structural integrity and durability of eggshells, which are desirable characteristics in commercial egg production. The protein and vitamin levels in eggs differed significantly among treatment groups ($p < 0.05$). Eggs from Group C contained a higher level of protein (13.4%) and vitamin D₃ (3.8 µg/egg), compared to Group A (12.2%; 2.6 µg/egg, respectively). Similarly, the omega-3 fatty acid level was higher in Group C (230 mg/egg) than in Group A (180 mg/egg). These enhancements in egg nutrient content could benefit consumer health and the egg market value (Table 4).

Immune response and antiviral testing

The WBC count and immunoglobulin levels in the NR-supplemented groups were significantly higher than those in the control group ($p < 0.05$). The WBC count in Group A was 17,200 WBC/µL, in Group B was 19,400 WBC/µL, and in Group C was 20,100 WBC/µL; therefore, increasing NR supplementation was associated with a

greater immune response in the treated groups. Similarly, IgA concentrations were significantly higher in Group C (4.1 mg/mL) compared to Group A (2.8 mg/mL; $p < 0.05$), suggesting that NR supplementation might enhance mucosal immunity by stimulating antibody production. The IFN-γ and IL-6 levels were significantly elevated in groups B and C ($p < 0.05$), particularly in Group C, indicating a stronger activation of immune regulatory pathways (Table 5). These findings demonstrated that NR supplementation improved immune competence, supporting humoral and cellular immune responses in laying hens during the experiment.

Observational data (behavioral health, feeding habits)

Improved overall health and well-being were observed in the NR-supplemented groups. The chickens in the control group demonstrated reduced activity and inconsistent feed intake compared to Group C, which exhibited consistent feeding behavior and remained more alert throughout the study period. Compared to the control group, hens in the NR-supplemented groups exhibited fewer signs of stress or nutrient deficiency, such as feather pecking and feather loss, thereby indicating improved welfare and physiological stability. In the NR-supplemented groups, the feed conversion ratio (FCR) improved, indicating greater feed efficiency. Group C had the most efficient FCR at 1.90, followed by Group B (1.98) and Group A (2.10; Table 6). These observations demonstrated that NR supplementation not only enhanced overall health and behavior but also enhanced feed utilization efficiency, thereby supporting improved performance in laying hens throughout the study.

Table 3. Effect of dietary supplementation of Nicotinamide Riboside on egg weight and shell quality of laying hens

Group	Egg weight (g ± SD)	Shell thickness (mm ± SD)	Shell strength (kg ± SD)
A (Control)	60.2 ± 1.5^a	0.32 ± 0.01^a	2.9 ± 0.1^a
B (25 mg/kg)	62.4 ± 1.2^b	0.35 ± 0.01^b	3.2 ± 0.1^b
C (50 mg/kg)	63.8 ± 1.0^b	0.38 ± 0.01^b	3.5 ± 0.1^b

SD: Standard deviation, ^{a, b} Different superscript letters in a column indicate significant differences ($p < 0.05$).

Table 4. Yolk nutrient composition of eggs from different dietary treatments in laying hens

Group	Protein (%)	Vitamin D ₃ (µg/egg)	Omega-3 fatty acids (mg/egg)
A (Control)	12.2 ± 0.3^a	2.6 ± 0.1^a	180 ± 5^a
B (25 mg/kg)	12.8 ± 0.2^{ab}	3.2 ± 0.2^b	205 ± 4^b
C (50 mg/kg)	13.4 ± 0.2^b	3.8 ± 0.2^b	230 ± 6^b

^{a, b} Different superscript letters in a column indicate significant differences ($p < 0.05$).

Table 5. Immune indicators of laying hens fed Nicotinamide Riboside-supplemented diets

Group	WBC ($\times 10^3$ cells / μL)	IgA (mg/mL)	IgG (mg/mL)	IL-6 (pg/mL)	IFN- γ (pg/mL)
A (Control)	17.2 \pm 0.3 ^a	2.8 \pm 0.2 ^a	3.6 \pm 0.2 ^a	21.4 \pm 0.8 ^a	19.8 \pm 0.7 ^a
B (25 mg/kg)	19.4 \pm 0.4 ^b	3.6 \pm 0.3 ^b	4.0 \pm 0.3 ^b	25.1 \pm 0.9 ^b	23.2 \pm 0.6 ^b
C (50 mg/kg)	20.1 \pm 0.3 ^b	4.1 \pm 0.2 ^b	4.5 \pm 0.3 ^b	28.3 \pm 1.0 ^b	25.7 \pm 0.7 ^b

^{a, b} Different superscript letters in a column indicate significant differences ($p < 0.05$).

Table 6. Feed conversion ratio of laying hens during the experiment

Group	FCR (feed intake/egg mass)
A (Control)	2.10 \pm 0.04 ^a
B (25 mg/kg)	1.98 \pm 0.03 ^b
C (50 mg/kg)	1.90 \pm 0.03 ^b

FCR: Feed conversion ratio, ^{a, b} Different superscript letters indicate significant differences ($p < 0.05$).

DISCUSSION

The current findings supported the hypothesis that NR improved physiological strength in laying hens and prolonged their productive lifespan, consistent with previous studies on other avian and mammalian species (Braidy et al., 2019; Elhassan et al., 2019). The improvement in egg production, particularly in group C, suggested that NR supplementation might delay reproductive ageing and promote sustained metabolic activity. This was likely due to the key role of NAD⁺ in maintaining mitochondrial energy metabolism and cellular repair mechanisms.

The observed increase in eggshell thickness might be linked to enhanced calcium metabolism driven by improved mitochondrial efficiency in shell gland cells, resulting from elevated intracellular NAD⁺ levels. Similarly, the improved egg nutrient composition, including higher levels of protein, vitamin D₃, and omega-3 fatty acids, indicated increased metabolic turnover and more efficient nutrient deposition due to enhanced redox status balance. Furthermore, the higher FCR efficiency observed in NR-supplemented groups could be associated with improved energy utilization and immune status. Enhanced NAD⁺ metabolism is known to support avian immunometabolism by activating sirtuin pathways and improving cytokine regulation, thereby strengthening immune resilience and reducing physiological stress (Fang et al., 2023). These findings indicated that NR supplementation supported metabolic and immune homeostasis in laying hens, thereby enhancing productivity and product quality. This result is particularly relevant to commercial poultry production, where ageing

hens and disease remain persistent challenges (Rehman et al., 2023).

Implications of increased egg weight, shell strength, and nutrient enrichment in eggs from NR-supplemented groups generally resulted in enhanced egg quality, which is essential for the poultry industry and consumer health. Therefore, eggs from supplemented groups with higher levels of omega-3 fatty acids and vitamin D₃ are marketed as premium eggs, meeting the growing consumer demand for value-added functional foods (Papanikolaou and Fulgoni, 2021; Palmieri et al., 2022). Moreover, elevated WBC and IgA levels indicated improved immune function following NR supplementation, which can serve as a natural strategy to enhance disease resistance, reduce antibiotic use, and strengthen biosecurity measures on poultry farms (Elhassan et al., 2019; Maynard et al., 2024). During the present study, NR supplementation resulted not only in behavioral improvements but also in enhanced hen activity and greater feed conversion efficiency. NR supplementation represented a strategy to improve the sustainability of poultry farming. Enhancing hen health and metabolic efficiency can potentially lower feed costs and increase the economic viability.

CONCLUSION

Supplementation with NR, particularly at higher inclusion levels, effectively enhanced egg production, eggshell quality, and immune function, thereby extending hens' lifespan. These findings suggested that NR can be considered a promising feed additive to support long-term poultry performance and welfare. To strengthen these findings, future investigations should focus on optimizing

NR dosage and evaluating its physiological impacts under different production and environmental conditions. This would help assess NR's full potential within the commercial poultry sector.

DECLARATIONS

Acknowledgments

The authors expressed their gratitude to the staff of the poultry research facility at PWI Research and Technology Pty Ltd, Somersby, New South Wales, Australia, for their technical assistance, and to the Institutional Animal Care and Use Committee (IACUC) of PWI Research and Technology Pty Ltd, Somersby, Australia, for their support during the experimental procedures.

Authors' contributions

Bamboo Xiu Zhu Yan conceived and designed the study, monitored experimental implementation, and drafted the manuscript. Xiaoxuan Zhang performed data collection and laboratory analyses and assisted with the interpretation of results. Ramsha Shahbaz contributed to writing the manuscript, statistical analysis, and data interpretation. All authors read and approved the final edition of the manuscript prior to submission.

Competing interests

The authors declare that there are no financial or personal conflicts of interest.

Funding

The present study received funding from the PWI research institute, as the chief funding organization for the conduct of research and thesis fees.

Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Ethical considerations

All the authors confirmed that ethical concerns, such as plagiarism, permission to publish, research misconduct, data fabrication or falsification, duplicate submissions, and redundant publication, have been thoroughly reviewed. The authors disclose that a generative artificial intelligence tool application was involved in the language editing and removing grammatical errors. Apart from that, no AI tool was utilized in data generation, statistical analysis,

interpretation of results, or conclusion. The authors checked and approved the originality of the scientific content, data analyses, and interpretations very carefully before publication.

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Analysis of Poultry Product Market Integration at the Producer, Wholesaler, and Retail Levels in Jambi Province, Indonesia

Firmansyah^{1*}, Fachroerrozi Hoesni², Afriani Harahap¹, and Lailal Gusri³

¹Department of Animal Husbandry Socio-Economics, Faculty of Animal Husbandry, Jambi University, Indonesia

²Department of Animal Production, Faculty of Animal Husbandry, Jambi University, Indonesia

³Faculty of Science and Technology, Jambi University, Indonesia

*Corresponding author's E-mail: firmarysah_fapet@unja.ac.id

Received: September 21, 2025, Revised: October 26, 2025, Accepted: November 23, 2025, Published: December 25, 2025



ABSTRACT

Price increases in poultry products remain inevitable, with a significant price disparity persisting between consumer and producer levels. This condition results from an unhealthy oligopolistic livestock market, leading to low purchase prices for farmers. The present study aimed to analyze market integration at the producer, wholesale, and retail levels for poultry products (chicken meat and eggs) and to develop a price-forecasting model for these products at the producer, wholesale, and retail levels for the future. A quantitative approach was used to analyze secondary data on average weekly prices for chicken meat and eggs at producer, wholesale, and retail levels in Jambi Province, Indonesia. The present data were obtained from the Center for Strategic Food Price Information from January 2020 to July 2023. The present results indicated a long-term cointegration relationship among chicken meat and egg prices at producer, wholesale, and retail levels in Jambi Province, Indonesia. The prices of chicken meat and eggs at the producer, wholesale, and retail levels influence each other not only in the current period but also concerning prices over the previous 2-6 weeks. Wholesale and retail chicken prices affect producer prices, and retail prices affect wholesale prices. For eggs, wholesale prices affect producer prices, and retail prices also affect producer and wholesale prices. The Vector Error Correction forecasting model for chicken and egg prices at producer, wholesale, and retail levels in Jambi Province demonstrated strong forecasting performance. Market integration occurs across different market levels, producer, wholesale, and retail, for chicken meat and eggs, both in the short and long term. This integration contributed to improving the efficiency of poultry marketing in Jambi Province, Indonesia.

Keywords: Market integration, Producer, Retail, Wholesaler

INTRODUCTION

The stability of poultry supply in Indonesia is influenced by its diverse farming structure, where small-scale farms, which often lack advanced biosecurity and climate control, contribute 40% of the national production (Sumiati et al., 2025). However, price increases for poultry products remain unavoidable, and a wide price gap persists between consumer and producer levels. This condition results from the oligopolistic nature of the poultry market, which is considered inefficient and uncompetitive. Profit distribution along the marketing chain, among producers, wholesalers, and retailers, is perceived as unfair, as

farmers often receive disproportionately low purchase prices (Fitriani et al., 2014). Under these conditions, wholesalers hold market power over retailers, as retail prices generally respond more rapidly to positive shocks than to negative ones in the long-run retail-wholesale equilibrium. Conversely, wholesale prices tend to adjust more slowly in response to negative deviations from the long-run equilibrium between wholesale and farmgate markets (Zamani et al., 2021). This asymmetric price adjustment pattern, frequently described as the rockets and feathers effect, has been widely documented in both agricultural and other markets (Peltzman, 2000; Loy et al., 2014; Surathkal and Chung, 2017). Additionally, the

rockets and feathers phenomenon is consistent with evidence on price dynamics in agricultural products in Los Angeles and Iran (Hassouneh et al., 2012; Richards et al., 2014). Such price disparity creates price inconsistency from producer to wholesaler to retailer and reduces the efficiency of the marketing system (Suganda et al., 2024). Imperfect price transmission in the poultry industry results from limited transportation, product perishability, and an imbalance of market power (Karim et al., 2017). Differences in price movements at each market level (producer, wholesaler, and retailer) may create negative effects for market participants. Market participants with greater power tend to control the supply chain, price, and product availability, to increase their profitability (Bakucs et al., 2013). However, wholesalers, who possess greater market power, often set the prices of chicken meat and eggs at the expense of other market participants, namely, producers and retailers, or limit their flexibility to set prices (MacDonald and Key, 2015).

An efficient market is where price signals are transmitted vertically and symmetrically throughout the meat value chain (Pozo et al., 2021). Market integration generally leads to more efficient outcomes, yet many real-world markets fail to integrate (Pukthuanthong and Roll, 2009). Additionally, market integration may negatively affect many traders (Kumar et al., 2022). Market integration competition results can increase market dominance by large participants functioning within an oligopoly (Hou and Song, 2021). A non-integrated market leads to suboptimal marketing efficiency due to slow information transfer, inadequate physical infrastructure, and the transmission of inaccurate information, which collectively distort producer decisions and the flow of livestock commodities (Nugroho, 2021; Evalia et al., 2022). Moreover, transportation and communication systems require time to achieve full price integration (Mohapatra and Singh, 2021). Transportation costs between producers and wholesalers are a vital part of expenses in the poultry supply chain (Zamani et al., 2021). In the poultry value chain, such as chicken meat and eggs, profits are often distributed unevenly among producers, wholesalers, and retailers. Wholesalers tend to capture a larger profit margin due to their greater control over selling prices, particularly during periods of market price fluctuations. Producers and retailers might be forced to sell livestock products at lower prices, so consumers encounter higher prices than expected.

Analyzing and comparing price transmission patterns improves the understanding of market integration in Indonesia (Jojo et al., 2021). In Indonesia, the poultry

industry faces significant challenges, particularly volatile input and output prices. Producers perceive their influence over pricing as limited and are consequently required to comply with dominant market trends in the absence of external support mechanisms (Chapot et al., 2014).

Market integration has received considerable attention from academics and policymakers over the past few decades (Roman, 2020). Economists studying markets have also shown interest in the issue of market integration (Lence et al., 2017; Pan and Li, 2019; Popat et al., 2022). Market integration continues to be a significant area of interest for those focusing on the dynamics and performance of agricultural markets (von Cramon-Taubadel, 2017). Having access to price information can assist in predicting profits and planning effective policies (Hoshino et al., 2021). Therefore, effective and integrated markets are crucial for traded products and services domestically.

Assessing market integration at the producer, wholesale, and retail levels to determine the marketing efficiency of livestock products in Jambi Province, Indonesia, has become increasingly important. Moreover, consumers, producers, wholesalers, and retailers are experiencing increasingly unstable livestock product prices, with greater irregularities in price patterns. These conditions have been exacerbated by the post-COVID-19 economic recovery challenges and the ongoing global food crisis. The present study aimed to analyze market integration across producer, wholesale, and retail levels for poultry products (chicken meat and eggs) and to develop a price-forecasting model for chicken meat and eggs at the producer, wholesale, and retail levels for the future.

MATERIALS AND METHODS

A quantitative approach was employed to analyze secondary data from Jambi Province, Indonesia. The present study utilized 558 weekly average price data for chicken meat and 558 price data for chicken eggs, covering three levels of the market structure, namely producers (186 data), wholesalers (186 data), and retailers (186 data). The data were obtained from the Center of Strategic Food Price Information (PIHPS) from January 2020 to July 2023. The first step was to test whether the chicken meat and egg price data at the producer, wholesaler, and retailer levels had unit roots, so a stationarity test was applied. The Augmented Dickey-Fuller (ADF) test was used to compare the ADF statistic with the 1% Mackinnon critical value using Formula 1 (Dickey and Fuller, 1981).

$$\Delta P_t = \alpha_0 + \gamma P_{t-1} + \beta_i \sum_{j=1}^m \Delta P_{t-j} + \varepsilon_t \quad (\text{Formula 1})$$

ΔP_t represent the first difference of the variable P_t , capturing short-run changes in price. α_0 is the constant term, while P_{t-1} is the lagged level of the variable, capturing long-run adjustment effects. The coefficients β_i measures the short-run dynamic effects of past changes in P_t , and ε_t is the white noise with zero mean and constant variance.

The determination of optimal lag length is important because lag length has a significant effect in time series analysis. The optimal lag of poultry product prices at the producer, wholesaler, and retailer levels was determined by several criteria. The present study determined the optimal model specification by applying several statistical selection measures, namely Akaike information criterion (AIC), Schwarz information criterion (SC), Hannan-Quinn criterion (HQ), likelihood ratio (LR), and final prediction error (FPE). To determine long-term market integration for poultry products at the producer, wholesaler, and retailer levels, a cointegration test was employed. The cointegration testing method was [Johansen's \(1988\)](#), using the following formula.

$$\Delta Y_t = K_0 + \Pi_1 \Delta Y_{t-1} + \Pi_{p-1} \Delta Y_{t-p+1} + \Pi Y_{t-1} + \mu_t \quad (\text{Formula 2})$$

ΔY_t represents the first difference of the dependent variable, capturing short-run changes. K_0 is the constant term, while Π_1, \dots, Π_{p-1} is the coefficients of lagged differences, reflecting short-run dynamics. The term ΠY_{t-1} is the long-run equilibrium component (error correction term), indicating adjustment toward equilibrium. μ_t is the stochastic error term, assumed to be the white noise.

The Johansen cointegration approach is a standard method for identifying long-run equilibrium linkages among variables that are nonstationary over time. This method can detect multiple cointegration relationships simultaneously and is suitable for multivariate analysis ([Fernández-Polanco and Llorente, 2019](#)). Cointegration is a statistical property observed in time series data, based on the stationarity and integration levels of the involved variables. Cointegration occurs among two or more non-stationary time series when they have the same order of integration and a linear combination (weighted average) of these series is stationary ([Ankamah-Yeboah and Bronnmann, 2018](#)).

Granger causality testing determines the cause-and-effect relationship between two time series variables by analyzing whether one variable can predict the future values of the other ([Granger, 1969](#)). The causality test in

the vector autoregression (VAR) model is usually conducted using the Granger causality test, which compares an unrestricted model with a restricted one ([von Cramon-Taubadel, 2017](#)).

Unrestricted equation is;

$$Y_t = \sum_{i=1}^m \alpha_1 Y_{t-i} + \sum_{i=1}^m \beta_1 X_{t-i} + \varepsilon_{1t} \quad (\text{Formula 3})$$

Y_t is the dependent variable and X_t is the explanatory variable. The coefficients α_1 captures the influence of the lagged values of Y_t (the autoregressive component). β_1 measures the effect of the lagged values of X_t on the current value of Y_t . The error term ε_{1t} means the stochastic disturbance, assumed to be white noise with zero mean and constant variance

The restricted equation is;

$$Y_t = \sum_{i=1}^m \gamma_1 X_{t-i} + \varepsilon_{2t} \quad (\text{Formula 4})$$

Y_t is the dependent variable and X_{t-i} represents the lagged values of the explanatory variable. The coefficients γ_1 captures the dynamic effects of past values of X_t on the current value of Y_t , reflecting how changes in X influence Y over time. The term ε_{2t} is the stochastic disturbance, assumed to be the white noise with zero mean and constant variance.

The estimation process for the VAR/VECM model ([von Cramon-Taubadel, 2017](#); [Popat et al., 2022](#)) entails constructing a VAR framework, assessing variable stationarity, verifying cointegration relationships, determining the optimal lag length, and evaluating variable suitability for inclusion in the model. The VECM is a restricted version of VAR because additional restrictions are required when data are non-stationary at the level but have cointegration relationships ([von Cramon-Taubadel, 2017](#)).

The general model of VAR with lag 1 was;

$$Y_t = \alpha_{1i} + \sum \beta_{1i} Y_{t-1} + \sum \gamma_{1i} X_{t-1} + \varepsilon_t \quad (\text{Formula 5})$$

Y_t is the dependent variable and X_{t-1} denotes the lagged explanatory variable. The constant term α_{1i} captures the intercept of the relationship, while the coefficients β_{1i} measures the influence of the lagged values of Y_t on its current level (the autoregressive component). The parameters γ_{1i} indicate the impact of the lagged values of X_t on Y_t , reflecting the short-run and possibly long-run dynamic relationship between the two variables. The disturbance term ε_t represents the random error.

$$X_t = \alpha_{2i} + \sum \beta_{2i} Y_{t-1} + \sum \gamma_{2i} X_{t-1} + \varepsilon_t \quad (\text{Formula 6})$$

X_t is the dependent variable and Y_{t-1} denotes the lagged explanatory variable. The constant term α_{2i} captures the intercept. The coefficients β_{2i} measures

the effect of lagged values of Y_t on X_t . The parameters γ_{2i} reflect the influence of lagged values of X_t on its current level, capturing the autoregressive component of the model. The error term ε_t represents the stochastic disturbance.

VECM models;

$$\Delta Y_t = \varphi_1 + \delta_{1t} + \lambda_{1e_{t-1}} + \gamma_{11}\Delta Y_{t-1} + \dots + \gamma_{1p}\Delta Y_{t-p} + \omega_{11}\Delta X_{t-1} + \dots + \omega_{1q}\Delta X_{t-q} + \varepsilon_{1t} \quad (\text{Formula 7})$$

ΔY_t denotes the first difference of the dependent variable, capturing short-run dynamics. The constant term φ_1 and trend component δ_{1t} represent deterministic elements in the model. The term e_{t-1} is the error correction term, and its coefficient λ_1 measures the speed of adjustment toward the long-run equilibrium following a short-run deviation. The parameters γ_{11} capture the short-run effects of past changes in Y_t . ω_{11} represent the short-run impacts of changes in X_t . The disturbance term ε_{1t} is the stochastic error.

$$\Delta X_t = \varphi_2 + \delta_{2t} + \lambda_{2e_{t-1}} + \gamma_{21}\Delta X_{t-1} + \dots + \gamma_{2p}\Delta X_{t-p} + \omega_{21}\Delta Y_{t-1} + \dots + \omega_{2q}\Delta Y_{t-q} + \varepsilon_{2t} \quad (\text{Formula 8})$$

ΔX_t denotes the first difference of variable X_t , capturing short-run dynamics. The constant term φ_2 and deterministic trend δ_{2t} represent the intercept and time trend components. The error correction term e_{t-1} , with coefficient λ_2 , measures the speed of adjustment toward long-run equilibrium after short-run disequilibrium. The parameters γ_{21} capture the short-run effects of past changes in X_t . ω_{21} represents the short-run influence of changes in Y_t . The stochastic disturbance term is ε_{2t} .

Johansen cointegration testing uses two statistics, the trace statistic and the maximum eigenvalue. The testing procedure compares the calculated statistic with the critical value. The trace statistic or maximum eigenvalue exceeds the critical value, indicating a cointegration relationship. After the Johansen cointegration test confirmed a long-term relationship among the variables, the vector error correction model (VECM) can be used to identify that relationship (Johansen, 1988). In general, the VECM model can be written as follows.

$$\Delta R_t = \gamma_1 + \sum_{i=1}^k \alpha_{wi} \Delta W_{t-1} + \sum_{j=1}^l \alpha_{rj} \Delta R_{t-j} + \beta Z_{t-1} + \varepsilon_{t1} \quad (\text{Formula 9})$$

ΔR_t denotes the first difference of the dependent variable R_t , capturing short-run adjustments. The constant term γ_1 represents the intercept, while α_{wi} and α_{rj} are coefficients that measure the short-run effects of lagged changes in W_t and R_t , respectively. The term Z_{t-1} is the error correction component, and its coefficient β indicates the speed at which deviations from the long-run equilibrium are corrected. The disturbance term ε_{t1} represents the stochastic error.

The error correction model (ECM) is widely used in econometric studies because it explains short-term dynamics between variables and shows the speed of adjustment toward long-term equilibrium (Granger, 1969). The final analysis in the present study was the impulse response function (IRF) and variance decomposition, which measure the response of variables to shocks and assess the contribution of other variables to the variation of a variable in the system (Pang et al., 2023). The accuracy of the poultry product price forecasting models was evaluated using the root mean square error (RMSE), mean absolute error (MAE), mean absolute percentage error (MAPE), and Theil's inequality coefficient.

Data analysis

In the first stage, the data was analyzed using descriptive statistics, including the mean, median, maximum, minimum, standard deviation, skewness, kurtosis, Jarque-Bera, and probability values. In the second stage, a stationarity test was conducted using the ADF method by comparing the ADF statistic value to the MacKinnon critical value at the 1% significance level. After that, the optimal lag length in the VAR model was determined based on the AIC, SC, HQ, LR, and FPE. Long-term market integration was analyzed using a cointegration test, where the null hypothesis (H_0) was rejected if the trace statistic surpassed the critical value, indicating the existence of cointegration. If no cointegration was found among variables, the VARD (VAR in difference) model was applied; otherwise, the VECM was employed. In the fifth stage, the existence of a significant causal relationship was tested using the pairwise Granger causality test at a 5% significance level based on the F-statistic ($p \leq 0.05$). The sixth stage involved estimating the VAR/VECM model using coefficients, standard errors, t-statistics, F-statistics, Akaike AIC, and Schwarz SC values. Then the IRF was analyzed, which illustrated the response of one variable to a shock in another variable over time using coefficients and standard errors. The eighth stage involved variance decomposition analysis, which provided information on the proportion of the movement in a variable explained by shocks to itself and to other variables in both the current and future periods. The final stage involved forecasting, which was achieved by projecting the current and future values of all variables using historical data. Forecast accuracy was evaluated using the RMSE, MAE, MAPE, mean squared error (MSE), and Theil's inequality coefficient.

RESULTS AND DISCUSSION

On average, the chicken meat price at the producer level was 1.13 USD/kg, which was below the reference price of 1.27-1.39 USD/kg. At the retail level, the average price was 1.95 USD/kg, also below the reference price of 2.23 USD/kg. Both prices remained below the sales reference prices set by the National Food Agency of the Republic of Indonesia Regulation Number 5 in 2022, except for the maximum prices of chicken meat at the producer and retail levels (1.42 USD/kg and 2.54 USD/kg), which were higher than the established reference prices (1.27-1.39 USD/kg and 2.23 USD/kg). Likewise, the average egg prices at the producer and retail levels were 0.95 USD/kg and 1.43 USD/kg, respectively (Table 1), which were below the reference prices, except that the maximum price of eggs at the retail level exceeded the reference price. For the producer level, it was 1.33-1.45 USD/kg, and for the retail level, it was 1.64 USD/kg. Currently, no reference price exists for selling chicken meat and eggs at the wholesale level. This fact forces the Indonesian Government, particularly the National Food Agency, to establish reference prices at the wholesale level, particularly for poultry products. On average, chicken

meat and egg prices at the producer and retail levels were lower than the reference prices set in the National Food Agency Regulation No. 5 in 2022, reflecting an overall stable market condition and suggesting that the government's price stabilization policy remained effective during the study period. The stable market condition indicated that supply and demand for these products were generally well-balanced, and that producers were able to maintain production levels sufficient to meet market needs without causing significant price surges. However, the occurrence of maximum prices for chicken meat at both the producer and retail levels, as well as for eggs at the retail level, exceeding the reference prices, revealed the presence of temporary market pressures. These pressures align with the findings of [Selmi et al. \(2023\)](#), who identify key drivers such as seasonal demand increases during religious holidays, rising feed costs, and disruptions in distribution channels, all of which can limit supply and elevate prices. Such dynamics highlighted the sensitivity of poultry and egg markets to short-term shocks, emphasizing the importance of continuous monitoring and adaptive policy responses to maintain price stability and protect both producers and consumers.

Table 1. Chicken meat and egg prices at producer, wholesale, and retail levels from January 2020 to July 2023 in Jambi Province, Indonesia

Descriptive statistics (USD/kg)	Chicken Meat			Egg		
	Producer	Wholesaler	Retail	Producer	Wholesaler	Retail
Mean	1.13	1.53	1.95	0.95	1.27	1.43
Median	1.15	1.52	1.95	0.95	1.26	1.38
Maximum	1.42	2.13	2.54	0.98	1.57	1.76
Minimum	0.72	1.06	1.30	0.88	0.99	1.13
Standard deviation	0.19	0.19	0.24	0.13	0.14	0.16
Skewness	-0.38	0.25	0.23	-3.38	0.17	0.39
Kurtosis	2.32	2.71	3.04	19.99	2.27	2.99
Jarque-Bera	8.09	2.10	1.66	25.90	5.05	12.60
Probability	0.02	0.35	0.43	0.00	0.08	0.00

Chicken meat prices indicated low variability, with standard deviations of 0.19 USD/kg at both the producer and wholesaler levels and 0.24 USD/kg at the retail level. Similarly, egg prices exhibited low fluctuations, with standard deviations of 0.13 USD/kg, 0.14 USD/kg, and 0.16 USD/kg at the producer, wholesaler, and retail levels, respectively. The skewness values of chicken meat prices were -0.38, 0.25, and 0.23 at the producer, wholesaler, and retail levels, respectively, indicating near-symmetrical distributions. In contrast, egg prices demonstrated skewness values of -3.38, 0.17, and 0.39, reflecting a

strong left skew at the producer level and slight right skewness at the wholesale and retail levels.

The kurtosis values of chicken meat prices were 2.32, 2.71, and 3.04 at the producer, wholesaler, and retail levels, respectively, indicating distributions close to normal. In contrast, egg prices indicated kurtosis values of 19.99, 2.27, and 2.99, suggesting extreme price fluctuations at the producer level and more stable distributions at the wholesale and retail levels.

The Jarque-Bera values for chicken meat prices were 8.09, 2.10, and 1.66 at the producer, wholesaler, and retail levels, respectively, indicating near-normal distributions

except for a slight deviation at the producer level. In contrast, egg prices recorded values of 25.90, 5.05, and 12.60, showing stronger deviations from normality, particularly at the producer level.

Based on the unit root test results for poultry products, the chicken meat price data at the producer, wholesale, and retail levels were found to be stationary at their level form. Meanwhile, egg price data at the wholesale and retail levels were stationary at the first difference, except at the producer level, which remained stationary at the level. The optimal lag length for the chicken price VAR model was 2, indicating that prices at the producer, wholesale, and retail levels were interdependent and influenced by prices from the previous two weeks. In contrast, the optimal lag length for the egg price VAR model was 6, suggesting a more prolonged dynamics where prices at all three levels influence each other relative to prices from the previous six weeks. Meanwhile, egg price data at the wholesale and retail levels were stationary at the first difference, except at the producer level, which was stationary at the level. The cointegration test for chicken prices at the producer, wholesale, and retail levels revealed significant results ($p \leq 0.05$) for both the trace statistic ($63.41872 > 24.27596$) and the Max-Eigen statistic ($34.74923 >$

17.79730), indicating the existence of a long-run cointegration relationship among these market levels. Similarly, the cointegration test for egg prices at the producer, wholesale, and retail levels indicated significant trace ($81.91906 > 24.27596$) and Max-Eigen statistics ($48.02391 > 17.79730$) with $p \leq 0.05$, confirming the presence of a long-term equilibrium relationship among the respective price series. These findings indicated a long-term cointegration relationship among egg prices at the producer, wholesale, and retail levels, suggesting that price movements across market levels are interconnected over time. This result is consistent with those of Putri et al. (2022), who also reported strong long-run price linkages within the poultry market chain.

The cointegration of poultry product prices at the producer, wholesale, and retail levels indicated that, in the long term, the market was integrated. However, in the short term, integration might not exist. Therefore, further analysis using the VECM was required to examine short-term integration of the poultry market at the producer, wholesale, and retail levels. Table 2 shows a significant error correction parameter, indicating a mechanism for adjusting chicken prices from the short term to the long term, with a value of -0.282 at the producer level.

Table 2. Vector error correction model estimation results for chicken meat prices in producer, wholesale, and retail levels from January 2020 to July 2023 in Jambi Province, Indonesia

Error correction	D (Producer)			D (Wholesale)			D (Retail)		
	SE	T-statistics		SE	T-statistics		SE	T-statistics	
CointEq1	-0.282192*	(0.04562)	[-6.18535]	0.010537	(0.04025)	[0.26177]	0.056695	(0.07224)	[0.78477]
D Producer (-1)	-0.034416	(0.06703)	[-0.51346]	-0.057798	(0.05914)	[-0.97732]	-0.211041	(0.10614)	[-1.98836]
D Producer (-2)	-0.002550	(0.06683)	[-0.03815]	-0.012834	(0.05897)	[-0.21763]	-0.119525	(0.10583)	[-1.12939]
D Wholesale (-1)	-0.127694	(0.10966)	[-1.16449]	-0.580782*	(0.09675)	[-6.00273]	-0.311502	(0.17364)	[-1.79392]
D Wholesale (-2)	0.067912	(0.08399)	[0.80854]	-0.120925	(0.07411)	[-1.63172]	0.080368	(0.13300)	[0.60426]
D Retail (-1)	-0.021631	(0.06525)	[-0.33152]	0.745166*	(0.05757)	[12.9437]	0.317191*	(0.10332)	[3.06995]
D Retail (-2)	-0.043127	(0.08355)	[-0.51615]	0.215082*	(0.07372)	[2.91746]	-0.141069	(0.13231)	[-1.06620]
C	-3.742058	(112,557)	[-0.03325]	-8.657082	(99.3115)	[-0.08717]	26.63930	(178,235)	[0.14946]

SE: Standard errors, C: The constant term (intercept), which represents the average weekly change in the price at that market level when all other explanatory variables in the equation are zero, D: The first difference, which denotes the weekly change in price. *Indicates significance difference in a column ($p \leq 0.05$).

The present findings suggested that price shocks at the wholesale level were temporary. The market adjusts after a large price fluctuation the next week, offsetting approximately 58% of the prior week's changes. This VAR analysis revealed that a 1 USD/kg increase in the retail price predicted a further 0.32 USD/kg increase the following week. Retail prices exhibited a short-term corrective response by decreasing 0.21 USD/kg for every 1 USD/kg increase in the prior week's wholesale price, and the markets were tightly integrated, with a 1 USD/kg

change in retail price associated with a 0.75 USD/kg change in wholesale price the following week. The cointegration test results indicated that the CointEq1 variable in D (Producer) was statistically significant ($p \leq 0.05$), suggesting the presence of an error correction mechanism at the producer level that drives adjustment toward long-run equilibrium at a speed of 28.2% per period. The D (Wholesale) was significant ($p \leq 0.05$) for D Wholesale (-1), D Retail (-1) and D Retail (-2), while the D (Retail) was significant ($p \leq 0.05$) only for D Retail (-1),

suggesting the long-run equilibrium was maintained primarily through adjustments in producer prices, not wholesale or retail prices. The estimated constant (C) in the producer price equation was not statistically significant (t-stat: -0.033), indicating that there was no consistent drift or average change in the producer price regardless of the other variables.

The cointegration test results (Table 3) revealed that CointEq1 in D (Wholesale, 2) and D (Retail, 2) were significant ($p \leq 0.05$), indicating the presence of an error-correction mechanism at the wholesale and retail levels, the D (Producer) was significant ($p \leq 0.05$) for D Producer (-4), while the D (Retail) was significant ($p \leq 0.05$) for D Wholesale (-1, 2), D Wholesale (-2, 2), D Wholesale (-3, 2) and D Wholesale (-5, 2). The estimated constant (C) in the producer price equation was statistically insignificant (t-stat: 0.734) and could not be interpreted as a meaningful economic adjustment.

The subsequent analysis employed a VECM to investigate short-term dynamics in the egg market. The current results indicated a complex adjustment process. The error-correction mechanism exhibited that deviations from the long-run equilibrium were corrected at a rate of 17.3% per period at the retail level. However, the positive and significant coefficient for the wholesale level (14.8%) indicated a destabilizing effect, as disequilibrium caused wholesale prices to move further from their equilibrium. This finding indicated that the primary adjustment effort was placed on the retail sector, while wholesale prices exhibited potentially volatile patterns. Short-term dynamics revealed significant self-correcting behavior and cross-market influences ($p \leq 0.05$). At the producer level, a 1.00 USD/kg increase in price four weeks prior was associated with a 0.44 USD/kg decrease in the current price, indicating strong negative feedback. Furthermore, retail prices were highly sensitive to the past wholesale price accelerations. For instance, a 1 USD/kg acceleration in wholesale price growth one week prior was associated with a 1.03 USD/kg decrease in the current retail price, with this influence persisting over several weeks (coefficients of -0.91, -0.72, -0.48, and -0.42 for lags two through five).

The observed destabilizing wholesale adjustment and the multi-week lagged effects aligned with findings of [Penone and Trestini \(2022\)](#) that futures markets can influence agricultural prices and exhibit complex temporal dynamics. The segmentation and incompleteness of price adjustments align with the patterns observed in the study conducted by [Noda and Kyo \(2023\)](#) in meat markets. Most importantly, the evidence of a malfunctioning error correction mechanism at the wholesale level strongly supported the theory that market power can distort and weaken price transmission ([Deb et al., 2022](#)), preventing the market from efficiently self-correcting. The Granger causality test revealed distinct market structures for chicken meat and eggs (Table 4). In the chicken meat market, the relationship was hierarchical, with both

wholesale and retail prices unidirectionally influencing producer prices ($p \leq 0.05$), indicating that downstream market levels determine producer prices. Furthermore, a strong bidirectional relationship existed between wholesale and retail prices ($p \leq 0.05$), confirming their tight mutual adjustment. In contrast, the egg market exhibited a different dynamic. Retail prices unidirectionally influence wholesale prices ($p \leq 0.05$). However, producer prices were independent, showing no significant relationship with either wholesale or retail prices ($p > 0.05$). This suggested a disconnect in which consumer price signals did not fully transmit back to egg producers.

This evidence of asymmetric price transmission, where producer prices did not respond to market signals, was consistent with findings of [Seok et al. \(2018\)](#) and [Deb et al. \(2022\)](#) that retailers and wholesalers can use market power to influence pricing dynamics. Following the onset of COVID-19, prices for poultry products fell sharply across farm, wholesale, and retail markets for the first three months, subsequently rising by roughly 40% for chicken meat and over 30% for eggs ([Amin et al., 2023](#)). The supply chain was disrupted because the distribution system was blocked, causing production to accumulate at the producer level and prices to fall. At the same time, demand also declined due to reduced purchasing power during COVID-19 ([Surni et al., 2020](#)).

The IRF analysis revealed a distinct hierarchical, lagged structure in the price transmission of chicken meat in Jambi, Indonesia, quantifying how shocks propagate across market levels (Table 5). A shock originating at the retail level, indicative of a change in demand, is the most potent driver of the system. The shock elicited an immediate and substantial response across all levels, with prices increasing by 1,692.28 at retail ($p \leq 0.05$), 1,496.88 at wholesale ($p \leq 0.05$), and a significant 438.32 at the producer level ($p \leq 0.05$) within the first period. This final figure was 4.7 times higher than the producer's response to a wholesale shock, showing that consumer signals reach farmers much more effectively than intermediary ones. In contrast, a shock at the producer level, indicating a cost push, resulted in a delayed pass-through. This shock at the producer level caused a large initial increase in its own price (1,507.27), but its effect on downstream markets only peaked after a considerable delay, reaching a maximum of 766.91 at wholesale and 745.64 at retail in the fifth period. Meanwhile, a wholesale price shock indicated strong, immediate integration with the retail sector, eliciting a 1,299.61 response. Still, the effect had a weak, short-lived effect on producers, turning negative after the third period. This evidence collectively painted a picture of a retail-driven market in which downstream demand shocks prompt rapid upstream adjustments, whereas upstream cost shocks were absorbed and transmitted to consumers only after a considerable delay, with the wholesale level acting as a primary link to retailers but a weak link to producers.

Table 3. Vector error correction model estimation results for egg prices in producer, wholesale, and retail levels from January 2020 to July 2023 in Jambi Province, Indonesia

Error correction	D (Producer)			D (Wholesale, 2)			D (Retail, 2)		
	SE	T-statistics		SE	T-statistics		SE	T-statistics	
CointEq1	-0.009614	(0.00834)	[-1.15222]	-0.014805*	(0.00529)	[2.79813]	-0.017291*	(0.00498)	[-3.46913]
D Producer (-1)	0.028346	(0.07988)	[0.35488]	0.099750	(0.05065)	[0.01969]	-0.249165	(0.47715)	[-0.52220]
D Producer (-2)	0.008928	(0.07992)	[0.11172]	-0.520516	(0.50679)	[-1.02708]	-0.183504	(0.47740)	[-0.38439]
D Producer (-3)	-0.022331	(0.07067)	[-0.31602]	-0.977809*	(0.44812)	[-2.18202]	-0.672969	(0.42213)	[-1.59423]
D Producer (-4)	-0.441462*	(0.07082)	[-6.23315]	0.249017	(0.44914)	[0.55444]	0.113150	(0.42308)	[0.26744]
D Producer (-5)	0.015876	(0.07899)	[0.20098]	-0.027545	(0.50092)	[-0.05499]	-0.183812	(0.47187)	[-0.38954]
D Producer (-6)	0.009995	(0.07808)	[0.12800]	-0.533817	(0.49517)	[-1.07804]	-0.132049	(0.46645)	[-0.28309]
D Wholesale (-1, 2)	-0.069795	(0.05266)	[-1.32531]	-0.101628	(0.33396)	[-0.30431]	-1.033752*	(0.31459)	[-3.28604]
D Wholesale (-2, 2)	-0.036093	(0.04777)	[-0.75564]	-0.218612	(0.30290)	[-0.72172]	-0.917867*	(0.28533)	[-3.21682]
D Wholesale (-3, 2)	-0.028129	(0.04067)	[-0.69164]	-0.277792	(0.25791)	[-1.07710]	-0.718272*	(0.24295)	[-2.95650]
D Wholesale (-4, 2)	-0.027741	(0.03218)	[-0.86196]	-0.125102	(0.20409)	[-0.61297]	-0.483273*	(0.19225)	[-2.51373]
D Wholesale (-5, 2)	0.002045	(0.02361)	[0.08663]	-0.017076	(0.14969)	[-0.11407]	-0.419705*	(0.14101)	[-2.97639]
D Wholesale (-6, 2)	0.000200	(0.01503)	[0.01330]	0.064975	(0.09532)	[0.68166]	-0.055314	(0.08979)	[-0.61604]
D Retail (-1, 2)	0.081356	(0.05691)	[1.42962]	-0.379166	(0.36088)	[-1.05067]	0.642659	(0.33995)	[1.89047]
D Retail (-2, 2)	0.049314	(0.05176)	[0.95278]	-0.236109	(0.32822)	[-0.71935]	0.509019	(0.30918)	[1.64633]
D Retail (-3, 2)	0.032501	(0.04442)	[0.73169]	-0.105462	(0.28168)	[-0.37440]	0.362593	(0.26534)	[1.36650]
D Retail (-4, 2)	0.044726	(0.03531)	[1.26682]	0.035969	(0.22389)	[0.16065]	0.317596	(0.21090)	[1.50588]
D Retail (-5, 2)	0.020112	(0.02671)	[0.75284]	-0.108482	(0.16941)	[-0.64035]	0.159474	(0.15958)	[0.99931]
D Retail (-6, 2)	0.013248	(0.01817)	[0.72893]	-0.171677	(0.11525)	[-1.48954]	-0.121483	(0.10857)	[-1.11894]
C	6.765237	(9.21229)	[0.73437]	5.722529	(58.4196)	[0.09796]	10.983379	(55.0309)	[0.19959]

SE: Standard errors, C: The constant term (intercept), which represents the average weekly change in the price when all other explanatory variables are zero, D: The first difference, which denotes the simple weekly change for producer and for wholesale and retail prices, it denotes the appropriate differenced series used to achieve stationarity. * Indicates significant differences in a column ($p \leq 0.05$)

Table 4. Granger causality test results for poultry product prices in producer, wholesale, and retail levels from January 2020 to July 2023 in Jambi Province, Indonesia

Chicken Meat	F-Statistics	Probability value	Egg	F-Statistics	Prob.
Wholesale - Producer	20.6705*	8.E-09	Wholesale -----> Producer	2.00268	0.0680
Producer - Wholesaler	1.36264	0.2586	Producer -----> Wholesaler	0.34233	0.9135
Retail - Producer	20.1798*	1.E-08	Retail -----> Producer	1.99925	0.0685
Producer - Retail	0.17566	0.8391	Producer -----> Retail	0.45678	0.8394
Retail - Wholesale	109.725*	8.E-32	Retail -----> Wholesale	10.3460*	1.E-09
Wholesale - Retail	5.62176*	0.0043	Wholesale -----> Retail	0.81481	0.5598

F-Statistics: * Indicates significant differences in a row ($p \leq 0.05$).

Table 5. The impulse response function of chicken's meat price from January 2020 to July 2023 in Jambi Province, Indonesia

Period	Response of producer			Response of wholesaler			Response of retail		
	Producer	Wholesaler	Retail	Producer	Wholesaler	Retail	Producer	Wholesaler	Retail
1	1507.271* (78.5719)	-	-	92.13679 (92.5068)	1253.131* (65.3240)	-	438.3294 (168.119)	1496.885 (147.150)	1692.289* (88.2166)
2	1077.787* (128.389)	215.3716 (105.271)	236.4793 (109.811)	292.3961 (173.847)	1481.956* (150.166)	1299.610 (113.419)	244.9584 (248.063)	1283.554 (227.936)	2041.168* (197.687)
3	764.9420* (120.200)	531.4367 (125.627)	414.0491 (131.720)	76.81566 (194.434)	1032.163* (192.291)	1196.625 (179.052)	-32.82334 (229.289)	876.8867 (248.494)	1256.646* (243.946)
4	556.8817* (133.380)	731.8545 (146.717)	656.1556 (144.777)	-59.28651 (171.387)	730.4505* (196.227)	714.0961 (201.167)	-96.41490 (200.870)	711.7966 (241.415)	763.9416* (254.725)
5	374.1701* (147.176)	766.9092 (163.717)	745.6371 (154.639)	-72.86634 (157.955)	585.5645* (192.878)	502.4680 (197.290)	-90.83263 (189.396)	583.4699 (235.115)	588.1081* (235.304)
6	228.5340 (151.881)	718.9354 (171.023)	700.9001 (161.645)	-71.50221 (149.322)	449.6264* (182.638)	405.5632 (179.213)	-95.88382 (176.770)	427.8945 (214.094)	440.7079* (208.018)
7	127.5990 (149.780)	640.0202 (174.310)	613.9962 (165.348)	-76.45083 (137.651)	317.0927 (166.174)	294.8205 (158.824)	-97.80534 (158.983)	292.4627 (193.355)	290.7657 (182.849)
8	60.08912 (143.892)	545.5968 (173.421)	519.3146 (164.473)	-74.32502 (121.207)	214.5404 (151.878)	192.8388 (142.693)	-88.04149 (135.651)	193.7833 (177.128)	180.6274 (165.920)
9	15.39921 (135.094)	446.2916 (168.012)	422.6194 (158.814)	-64.97440 (101.561)	140.8141 (139.474)	121.3887 (130.334)	-73.70023 (110.472)	121.5938 (162.175)	108.5903 (151.402)
10	-12.28219 (123.528)	352.3430 (159.443)	330.8200 (150.040)	-53.99870 (81.7453)	86.62581 (127.424)	72.70204 (118.700)	-59.83145 (86.7678)	68.39915 (147.418)	58.66981 (137.331)

Standard errors are presented in parentheses. * Indicates significant differences ($p \leq 0.05$).

The IRF for egg prices revealed a market with weak integration and significant volatility, particularly in response to wholesale shocks (Table 6). A shock at the producer level had a positive but gradually decaying effect on its own price, decreasing from 116.56 to 13.6 and remaining significant ($p \leq 0.05$) from period 1 to 4. In contrast, its impact on wholesale and retail prices was minimal and statistically insignificant across all 10 periods ($p > 0.05$). A shock at the wholesale level exerted a positive and significant effect ($p \leq 0.05$) on its own price (720.73) and a strong negative effect on producer prices (-63.44), which further intensified to -105.37 by period 4. This indicates that cost pressures at the wholesale level are transmitted upstream to producers, resulting in sustained price suppression. Meanwhile, a shock at the retail level had a positive effect (577.43) on its own price and was significant ($p \leq 0.05$) in periods 1, 2, 4, 7, and 9. Finally, a shock at the retail level also exerted an immediate negative influence on producer prices (-83.63) and created fluctuating, often negative, responses in wholesale prices. The most striking feature was the consistent negative response of producer prices to both wholesale and retail shocks, demonstrating that producers experienced market disruptions more. Additionally, the retail market exhibited wide and unpredictable fluctuations, failing to settle into a stable equilibrium within the observed timeframe.

The variance decomposition analysis indicated which shocks at the producer, wholesale, and retail levels contributed to fluctuations in poultry prices (chicken meat and eggs). In the first period, 100% of the variation was caused by shocks at the producer level. This self-influence gradually decreases over time, falling to 44.60% by the

tenth period. Concurrently, the influence of shocks from the wholesale level increased to 29.47%, and from the retail level to 25.93% (Table 7). Initially, wholesale price variance was entirely (99.46%) explained by its own shocks, with only 0.54% from the producer level. Over time, the contribution of wholesale price shocks declined to 58.45%, while shocks originating from the retail level became a major factor, accounting for 40.27% of the variation by the tenth period. The influence from the producer level remains small, increasing slightly to 1.27%. The retail level was influenced by all three levels from the start. In the first period, 54.07% of its variation was due to its own shocks, 42.30% from wholesale shocks, and 3.63% from producer shocks. This dynamic stabilizes, with retail's own shocks becoming the dominant factor (61.65%), followed by wholesale shocks (36.43%), while producer-level influence remained minimal (1.91%).

At the producer level, the standard error of chicken meat prices increased gradually from 0.091 USD/kg in the first period to 0.193 USD/kg in the tenth period, indicating a moderate increase in forecast uncertainty as the prediction horizon extended. Similarly, the standard error values at the wholesale level increased from 0.076 USD/kg to 0.196 USD/kg, and at the retail level from 0.139 USD/kg to 0.242 USD/kg (Table 7). Such progressive increases in standard error are typical in variance decomposition analysis, as forecast errors tend to accumulate over longer horizons. Overall, the relatively low standard error magnitudes across all market levels suggested that the model produced stable and reliable estimates of chicken meat price dynamics at the producer, wholesale, and retail levels.

Table 6. Impulse response function results of egg prices from January 2020 to July 2023 in Jambi Province, Indonesia

Period	Response of producer			Response of wholesaler			Response of retail		
	Producer	Wholesaler	Retail	Producer	Wholesaler	Retail	Producer	Wholesaler	Retail
1	116.5565* (6.16020)	-	-	-63.44108 (53.9740)	720.7286* (38.0917)	-	-83.62848 (50.3873)	342.8415 (46.8084)	577.4281* (30.5180)
2	109.1291* (10.8666)	2.126972 (9.17199)	9.382338 (8.70667)	-14.61331 (62.4544)	69.10047 (62.1328)	334.4932 (56.7804)	-53.16382 (55.1416)	131.3594 (54.4209)	130.1406* (50.9352)
3	96.67954* (13.3822)	17.29681 (12.4178)	-5.009949 (11.9700)	-55.75431 (59.7262)	-33.75448 (62.4713)	83.06692 (59.1944)	-17.81089 (52.1349)	13.05960 (54.5712)	-54.60292 (52.1004)
4	83.30814* (15.1199)	14.93264 (14.6132)	-5.627737 (13.9485)	-105.3729 (59.0117)	-46.96195 (62.4504)	-52.55257 (59.3263)	-68.68930 (52.5935)	12.16472 (55.1844)	-115.8414* (52.2412)
5	19.56857 (15.9150)	16.43882 (16.0600)	8.863565 (14.9513)	-10.80488 (59.4433)	104.1179 (62.9056)	-76.53270 (59.5409)	-20.76690 (52.4106)	60.73543 (55.0019)	-55.37512 (52.4912)
6	12.64758 (15.9341)	27.98823 (16.1069)	-0.572234 (14.5544)	-9.460613 (58.6101)	17.21420 (62.8600)	-97.21674 (59.1538)	4.731710 (51.3345)	-61.82128 (54.5580)	-48.59772 (51.8344)
7	6.624650 (15.6581)	17.20979 (15.3800)	10.43083 (14.3471)	11.00733 (46.3593)	-94.34024 (59.6550)	-95.85121 (59.4779)	9.744696 (43.5274)	45.56801 (52.2972)	-198.3516* (53.0655)
8	0.848460 (15.5662)	17.50656 (12.9231)	4.550301 (11.3497)	18.10395 (44.2605)	-11.19106 (40.6764)	-94.24168 (46.8414)	35.24793 (43.7882)	-22.00622 (35.7454)	18.09749 (43.0518)
9	18.24547 (15.5770)	9.021906 (10.3384)	0.288201 (8.99511)	-6.980016 (40.3891)	-3.334132 (33.6756)	57.82960 (40.5545)	2.462929 (39.7226)	-23.10982 (30.5635)	90.04003* (39.3580)
10	13.62375 (15.5158)	0.985953 (8.23325)	1.909630 (7.33287)	6.540386 (36.0771)	-4.929292 (31.0867)	56.22133 (38.1191)	-11.24682 (36.6791)	-40.14171 (28.2672)	53.58497 (36.7495)

Standard errors are presented in parentheses. * Indicates significant differences ($p \leq 0.05$).

Table 7. The variance decompositions of chicken meat prices from January 2020 to July 2023 in Jambi Province, Indonesia

Period	Variance decomposition of the producer				Variance decomposition of wholesale				Variance decomposition of retail			
	Producer	Wholesaler	Retail	SE	Producer	Wholesaler	Retail	SE	Producer	Wholesaler	Retail	SE
1	100.00	-	-	0.091	0.54	99.46	-	0.076	3.63	42.30	54.07	0.139
2	97.11	1.31	1.58	0.114	1.69	67.87	30.43	0.143	2.26	34.81	62.93	0.203
3	87.84	7.19	4.97	0.130	1.24	60.00	38.76	0.172	1.87	34.45	63.68	0.223
4	73.98	14.77	11.24	0.147	1.14	58.96	39.90	0.183	1.80	35.32	62.88	0.232
5	62.63	20.36	17.01	0.162	1.12	58.85	40.03	0.189	1.77	35.94	62.29	0.237
6	55.16	24.03	20.81	0.174	1.13	58.68	40.19	0.192	1.78	36.22	61.99	0.240
7	50.42	26.44	23.14	0.182	1.17	58.56	40.28	0.194	1.82	36.35	61.82	0.242
8	47.45	27.97	24.58	0.187	1.21	58.50	40.29	0.195	1.86	36.41	61.73	0.242
9	45.65	28.91	25.44	0.191	1.24	58.47	40.28	0.195	1.89	36.43	61.68	0.242
10	44.60	29.47	25.93	0.193	1.27	58.45	40.27	0.196	1.91	36.43	61.65	0.242

SE: Standard error

Producer prices primarily caused the fluctuations and shocks. This effect began at 100% in the first period and remained very high, decreasing only slightly to 94.24% by the tenth period. Shocks from the wholesale level had a minor influence (4.98%), while retail-level shocks were negligible (0.78%). In the second to tenth periods, the impact of shocks to producer egg prices decreased from 99.64% to 94.24%, while the impacts of shocks to wholesale prices and retail prices were 4.98% and 0.78%, respectively. The wholesale level was primarily influenced by its own shocks, which accounted for 99.23% of the initial variation. Over time, this self-influence declined to 75.20%, while shocks from the retail level became significant ($p \leq 0.05$), explaining 22.16% of the variation by the tenth period. The influence of producer prices remains small, increasing to 2.63%. The retail level was dominated by its own shocks from the outset (72.81%), with shocks from the wholesale level explaining most of the remaining variation (25.67%). The influence from the producer level was minimal, starting at 1.53% and increasing slightly to 2.86% by the tenth period (Table 8).

Fluctuations in retail prices were moderately affected by shocks originating from the wholesale market. The contribution of wholesale price shocks accounted for 25.67% in the first period, increased slightly to 27.23% in the second period (Table 8), and then remained relatively stable at around 25-26% throughout the subsequent periods. This pattern suggested that wholesale prices play a consistent and moderate role in shaping retail price variability over the forecast horizon. In comparison, producer-level shocks contributed only about 2-3%, whereas the largest portion of retail price fluctuations (approximately 71-73%) was attributed to shocks within the retail market itself, highlighting the dominance of internal market dynamics at the retail level.

At the producer level, the standard error of egg prices indicated a slight increase from 0.007 USD/kg in the first

period to 0.013 USD/kg in the tenth period, reflecting minimal forecast uncertainty over the estimation horizon. Similarly, the standard error at the wholesale level increased modestly from 0.044 USD/kg to 0.052 USD/kg, while at the retail level, the standard error increased from 0.041 USD/kg to 0.046 USD/kg. Such minor and gradual increases in standard error values were characteristic of variance decomposition analysis, as forecast errors naturally accumulate over extended horizons. Overall, the consistently low standard error values across all market levels indicated that the model provided robust, stable, and reliable estimates of egg price dynamics at the producer, wholesale, and retail levels.

The present findings are consistent with those of [Tomycho et al. \(2023\)](#), who examined price transmission in the Indonesian egg market using cointegration and VECM analysis. [Tomycho et al. \(2023\)](#) found that market integration between areas with oversupply and areas of shortage existed before the COVID-19 pandemic but declined significantly during it. The variance decomposition results indicated that own-price effects for chicken parts, legs, breasts, and wings account for more than 80% of the variation after nine months. In contrast, for whole chickens, the decomposition became nearly evenly distributed between whole chicken and breast prices after approximately seven months ([McKenzie et al., 2009](#)). The results of the forecast error variance decomposition indicated that the broiler's own price consistently served as the primary determinant of price variability across all subperiods ([Duangnate et al., 2025](#)). In the Danish markets, shocks in the chicken market exhibited both own-market and cross-market effects that manifest quickly and intensely ([Andersen et al., 2007](#)). The present results are in line with the findings of [Dietrich et al. \(2021\)](#), who indicated that the effects of increasing food prices seemed to be caused by interconnected markets that relied more on trade with other markets in

low- and middle-income countries, such as Indonesia. In a well-functioning market, competition ensures that the impact of a shock on the price of a product in one location spreads to other locations, allowing producers and consumers to take advantage of opportunities (Iregui and Otero, 2017).

This finding in Jambi Province was in line with the study conducted by Setiadi (2022), which revealed a positive correlation between chicken egg prices in Kendal Regency and Semarang City, Indonesia. Overall, egg prices across Semarang City, Kendal Regency, and Semarang Regency in Central Java Province exhibited a symmetric relationship. In traditional markets in Jambi Province, chicken meat prices tended to experience substantial fluctuations, whereas in modern markets, chicken meat prices indicated considerable variation. In contrast, egg prices in modern markets were generally more stable, although price instability demonstrated a noticeable increase. In urban areas in Jambi Province, Indonesia, chicken meat prices exhibited pronounced volatility, while egg prices maintained relatively low

levels of fluctuation (Firmansyah and Harahap, 2024). The present results were not consistent with the findings of Firmansyah and Harahap (2024), who reported that chicken meat prices in urban markets were volatile, whereas egg prices remained relatively stable. Additionally, Liu and Tang (2023) examined poultry market integration in China, finding that it was stronger in major production provinces but weaker in main consumption provinces.

Market integration and the application of poultry price forecasting at the producer, wholesale, and retail levels play a crucial role in stabilizing prices by reducing market imperfections, such as monopoly and monopsony practices, while also improving efficiency. The comparison of forecasting methods uses MAPE to identify the most accurate forecasting model (Sukiyono et al., 2021). Table 9 provides the assessment outcomes of the poultry price forecasting models for the producer, wholesale, and retail market levels. The present findings indicated that the forecasting models performed too optimally across all three market levels.

Table 8. The variance decompositions of egg prices from January 2020 to July 2023 in Jambi Province, Indonesia

Period	Variance decomposition of the producer				Variance decomposition of D(Wholesale)				Variance decomposition of D (Retail)			
	Producer	D (Wholesale)	D (Retail)	SE	Producer	D (Wholesale)	D (Retail)	SE	Producer	D (Wholesale)	D (Retail)	SE
1	100.00	-	-	0.007	0.77	99.23	-	0.044	1.53	25.67	72.81	0.041
2	99.64	0.02	0.34	0.010	0.66	81.86	17.47	0.048	1.98	27.23	70.78	0.043
3	98.82	0.86	0.32	0.011	1.13	80.64	18.23	0.049	2.03	27.08	70.89	0.043
4	98.42	1.24	0.34	0.012	2.76	79.03	18.21	0.050	2.87	26.15	70.98	0.044
5	97.64	1.84	0.52	0.013	2.71	78.67	18.62	0.050	2.92	26.49	70.59	0.044
6	95.91	3.58	0.51	0.013	2.69	77.60	19.71	0.050	2.89	26.90	70.21	0.044
7	95.04	4.21	0.75	0.013	2.64	76.86	20.50	0.051	2.69	25.31	71.99	0.046
8	94.35	4.86	0.79	0.013	2.65	75.89	21.46	0.051	2.90	25.31	71.79	0.046
9	94.22	4.99	0.78	0.013	2.64	75.53	21.82	0.052	2.86	25.02	72.12	0.046
10	94.24	4.98	0.78	0.013	2.63	75.20	22.16	0.052	2.86	25.10	72.04	0.046

SE: Standard error, D: The first difference price series at the wholesale and retail levels.

Table 9. The forecasting models for poultry product price from January 2020 to July 2023 in Jambi Province, Indonesia

Poultry products	Market levels	Root mean square errors	Mean absolute errors	Mean absolute percentage error	Theil inequality coefficient
Chickens' meat	Producer	1232.381	954.6619	3.829234	0.024290
	Wholesaler	1478.322	971.9190	5.437471	0.039325
	Retail	2257.241	1719.889	5.310742	0.034835
Egg	Producer	684.0396	500.9430	2.432937	0.016141
	Wholesaler	110.1971	46.64723	0.298112	0.003500
	Retail	639.8025	454.8876	1.951737	0.013442

For chicken meat, the MAPE model performed best at the producer level (3.82%), with slightly higher errors at the wholesale (5.43%) and retail levels (5.31%). For eggs, the MAPE forecasts were more accurate overall, particularly at the wholesale level (0.29%), followed by

the retail (1.95%) and producer levels (2.43%). The Theil inequality coefficients, all below 0.04, indicated excellent predictive accuracy and confirmed that the models effectively captured short-term price movements across poultry markets in Jambi Province, Indonesia.

CONCLUSION

On average, chicken prices at both the producer and retail levels remained below their normal levels, not reaching the target sales prices. Similarly, average egg prices at the producer and retail levels were below normal. There was a long-term cointegration relationship among chicken and egg prices across producer, wholesale, and retail markets in Jambi Province, Indonesia. Prices of chicken and eggs at all market levels affect one another not only in the current period but also with respect to prices observed two and six weeks prior. Wholesale and retail chicken prices influence producer prices, retail prices affect wholesale prices, and wholesale prices influence retail prices. Wholesale egg prices influence producer prices, retail prices influence producer prices, and retail egg prices influence wholesale prices. The vector error correction model used to forecast chicken and egg prices at the producer, wholesale, and retail levels in Jambi Province, Indonesia, demonstrated outstanding predictive performance. There was no reference price for chicken and eggs at the wholesale level; this has led the government of Indonesia, specifically the National Food Agency and the Ministry of Trade, to establish reference prices at the wholesale level, especially for poultry products (chicken and eggs), to prevent large price fluctuations. Future studies are recommended to analyze market integration across different market levels (producer, wholesale, and retail) for chicken meat and eggs in Indonesia, encompassing both producing and consuming provinces, such as West Java, Central Java, and East Java, and consuming provinces (Jakarta and Banten) in Indonesia.

DECLARATIONS

Acknowledgments

The authors gratefully acknowledge the Rector of Jambi University, Indonesia, for facilitating the opportunity to undertake the present study and for providing financial support.

Funding

The present study was funded by the Faculty Flagship Applied Research Scheme under the DIPA PNB of the Faculty of Animal Science, Jambi University, Indonesia, Fiscal Year 2023 (Grant No. SP DIPA-023.17.2.677565/2023, issued on November 30, 2022), in accordance with the Research Contract Agreement No. 350/UN21.11/PT.01.05/SPK/2023, dated April 17, 2023, with a total funding amount of USD 3,030.

Availability of data and materials

The datasets generated or analyzed in the present study can be obtained from the corresponding author upon reasonable request

Authors' contributions

Firmansyah contributed to the conceptualization, methodology, and formal analysis. Fachroerrozi Hoesni assisted in drafting the manuscript. Afriani Harahah carried out the data analysis and interpretation. Lailal Gusri was involved in the investigation and project administration. All authors have read and approved the final edition of the manuscript.

Competing interests

The authors declare that there are no conflicts of interest.

Ethical considerations

Ethical considerations, including plagiarism, consent for publication, research misconduct, data fabrication or falsification, duplicate publication or submission, and redundancy, have been reviewed and confirmed by all authors. Additionally, the authors have not assisted artificial intelligence during the conduct of the present study and preparing the manuscript.

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Effects of Replacing Duckweed with Red Corn Meal and Rice Bran on Growth Performance of Local Chicken in Semi-Confined and Confined Systems

Chiv Phiny*^{}, Tum Saravuth^{}, Sin Puteasath^{}, Srey Lida^{}, Lay Sokphin^{}, and Phuon Phou^{}

Research and Development Office, Svay Rieng University, Cambodia

*Corresponding author's E-mail: chiv.phiny@mail.sru.edu.kh

Received: October 03, 2025, Revised: November 08, 2025, Accepted: December 05, 2025, Published: December 25, 2025



ABSTRACT

Incorporating duckweed into poultry diets can improve feed efficiency and growth rate. However, its effectiveness as a replacement for traditional feeds such as red corn meal or rice bran remains less explored. The present study aimed to evaluate the feed intake, average daily gain (ADG), and feed conversion ratio (FCR) in local chickens reared under confined and semi-confined systems. The experiment was conducted at the campus of Svay Rieng University, Svay Rieng Province, Cambodia, from June to August 2024. The present study consisted of two factors. The first factor was the rearing system, including semi-confined and confined systems, and the second factor was the diet, with four treatments. A total of 120 local chickens, 35 days old, with an average initial body weight of 300 grams, were arranged in a 2×4 factorial layout using a completely randomized design within eight treatments and three replications. The first group included 5% duckweed, 22% commercial feed, 20% red corn meal, 52.5% rice bran, and 0.5% premix/salt (T1). The second group consisted of 10% duckweed, 19% commercial feed, 20% red corn meal, 50.5% rice bran, and 0.5% premix/salt (T2). The third group had 15% duckweed, 16% commercial feed, 20% red corn meal, 48.5% rice bran, and 0.5% premix/salt (T3), and the fourth group included 20% duckweed, 13% commercial feed, 20% red corn meal, 46.5% rice bran, and 0.5% premix/salt (T4). There were no significant interactions between four diets and two raising systems for feed intake, ADG, and FCR in the chickens, except for the crude fiber intake. However, significant differences were observed in T1 and T2 compared to T4 regarding feed intake and ADG, with a tendency toward improved FCR for chickens fed T3 compared to T4 and those raised under the confined system. The growth performance in chickens raised under the confined system was higher than that of chickens raised under the semi-confined system. The present results indicated that using duckweed at 15% mixed with other ingredients led to higher feed intake, higher ADG, and improved FCR, even when chickens were raised in a confined or semi-confined system.

Keywords: Commercial feed, Confined system, Duckweed, Performance, Red corn

INTRODUCTION

Poultry production plays an important role in the agricultural sector across Cambodia, particularly backyard chicken rearing, a common practice among rural households. Approximately 32.4%-67.6% of rural farmers rear local chickens, with flock sizes ranging from 31 to 42 chickens per household (Yitayih et al., 2023). Backyard or village chickens contribute significantly to poverty reduction and food security in developing countries such as Vietnam, Laos, and Cambodia (Saroeun et al., 2010). However, improving poultry growth performance requires providing nutritionally balanced feeds that supply the

essential nutrients for growth, egg production, and meat development (Saroeun et al., 2010). The free-range production system alone often fails to meet these nutritional requirements. Therefore, it is recommended to supplement the diet of the free-range chickens with protein-rich feeds to provide affordable, sustainable sources of protein and vitamins (Saroeun et al., 2010). The forages can partially replace conventional protein ingredients such as fish meal or soybean meal, making them useful feed alternatives for smallholder and household-level poultry production (Vlaicu et al., 2024). Duckweed is a rich protein source, especially a superior source of the essential amino acids compared to most

vegetable proteins, and is very useful as a protein source for animal feed (Demann et al., 2022). Duckweed represents a valuable source of high-quality protein, suitable for utilization in household backyard animals, particularly for the cultivation of pigs and poultry production (Hang, 1998; Samnang, 1999; Khang and Brian, 2004a), and is a suitable source of vitamins and minerals for growing ducks (Men et al., 1995). To date, only a limited number of studies have examined the protein requirements in local chicken breeds raised under confined or semi-confined systems in Cambodia, even though numerous investigations have focused on the nutrient needs of hybrid or exotic chickens. Johnson (1998) reported that the crude protein (CP) content of duckweed varied widely from 7% to 40%, depending on its growth medium, with experimentally measured values ranging from 8.7% to 24.9%. Additionally, the lysine content of duckweed ranges from 0.40% to 1.13%. In a duckweed feed containing 29%-31.82% of CP, the methionine content is 0.18%, and the lysine content is 10.9% (Yilmaz et al., 2004).

Corn is considered the principal energy source in poultry diets and is an important cereal grain for human consumption (Ojediran and Olorunlowu, 2025). Recently, scientists have been exploring alternative feedstuffs that could partially or fully replace corn in poultry diets due to its increasing cost and competition with human food needs (Agwunobi, 1999). Corn is regarded as an excellent grain for poultry feed because it is low in fiber and provides a well-balanced nutrient profile. Its chemical composition includes 86% of dry matter (DM), metabolizable energy at 3373 kcal/kg, CP at 7.5%, methionine at 0.18%, cysteine at 0.18%, tryptophan at 0.07%, tannin at 0.29%, crude fat at 3.5%, crude fiber (CF) at 1.9%, ash at 1.1%, calcium at 0.01%, and phosphorus at 0.12% (Jacob, 2016).

Rice bran is a rich source of nutrients and is currently used in animal diets (Tahira and Butt, 2007). According to Sivala et al. (1991), rice bran is high in energy and fat and contains essential vitamins, particularly vitamins B and E, as well as important minerals. Jacob (2016) reported that the nutrient composition of rice bran includes 91% of DM, digestible energy at 2040 kcal/kg, 13.5% of CP, methionine at 0.17%, cysteine at 0.10%, lysine at 0.50%, tryptophan at 0.10%, threonine at 0.40%, crude fat at 5.9%, 13.0% of CF, ash at 11.0%, calcium at 0.10%, and phosphorus at 1.70%.

The present study aimed to evaluate the effects of replacing duckweed with a commercial feed containing red corn meal and rice bran on feed intake, growth rate,

and feed conversion ratio (FCR) in local chickens reared under confined and semi-confined systems.

MATERIALS AND METHODS

Ethical approval

The chickens were treated in accordance with the animal welfare guidelines of the Animal Health and Production Office in Svay Rieng province, Cambodia. The experiment received ethical approval from Svay Rieng University, as documented in the reference letter dated September 9, 2024.

Study area

The experiment was conducted from June 1, 2024, to August 31, 2024, at the research station of Svay Rieng University on National Road 1, in Sangkat Chek, Svay Rieng City, Svay Rieng Province, Cambodia. The temperature of the environmental house during the experimental period was maintained at $27^{\circ}\text{C} \pm 2$.

Experimental design

A total of 120 local chickens (60 males and 60 females), aged 35 days, with an average initial body weight of 300 g, were selected and arranged in a 2×4 factorial arrangement using a completely randomized design with eight treatments and three replications. The chickens were weighed at the beginning of the experiment (June 1) and every 10 days thereafter until the end of the experiment on August 31, 2024.

Experimental factors

The present study included two factors. The first was the rearing system, which consisted of semi-confined (SC) and confined (C) systems, and the second was the diet, with four treatments. The dietary treatments were formulated based on the book Animal Nutrition (McDonald et al., 2011) and the nutrient requirements of poultry (NRC, 1994). The first group contained 5% duckweed, 22% commercial feed, 20% red corn meal, 52.5% rice bran, and 0.5% premix/salt (T1), the second group contained 10% duckweed, 19% commercial feed, 20% red corn meal, 50.5% rice bran, and 0.5% premix/salt (T2), the third group contained 15% duckweed, 16% commercial feed, 20% red corn meal, 48.5% rice bran, and 0.5% premix/salt (T3), and the fourth group contained 20% duckweed, 13% commercial feed, 20% red corn meal, 46.5% rice bran, and 0.5% premix/salt (T4; Table 1).

Table 1. Ingredients and chemical composition of the experimental diets for local chickens during the experiment period

Diet ingredients	Group	T1	T2	T3	T4
Ingredients (%)					
Commercial feed		22	19	16	13
Duckweed		5	10	15	20
Red corn meal		20	20	20	20
Rice bran		52.5	50.5	48.5	46.5
Premix/salt		0.5	0.5	0.5	0.5
Chemical composition (% on dry weight basis)					
Dry matter ¹		85.1	81.1	77.1	73.1
Crude protein ²		19.9	20.0	20.2	20.3
Crude fibre ²		4.50	4.81	5.13	5.44
Organic matter ²		88.2	88.6	89.1	89.6

¹: Analysis results of the animal feed laboratory of Svay Rieng University, Cambodia, ²: Analysis of results of the Laboratory at the Center for Livestock and Agriculture Development, Cambodia.

Housing

There were a total of 24 pens, each measuring 2 meters in length, 1 meter in width, and 1.5 meters in height. The pens were constructed from steel frames and plastic netting and housed in an open-sided shed with a roof made of a steel structure and zinc sheets. Each pen had a drinker and a feeder individually. A total of 120 local chickens were housed, five heads per pen. A routine preventive vaccination program was implemented. Chickens were vaccinated at three, six, and eight weeks of age against Newcastle disease, Fowl Pox, and Fowl Cholera. This approach aligns with standard poultry health management principles, where vaccination is customized to local disease risks and administered on a sequential schedule to develop effective immunity (Marangon and Busani, 2006; Cserep, 2009). The vaccines used were produced in Vietnam and imported by the Green Feed company, Cambodia (Islam et al., 2008; Hossain et al., 2025). The chickens were acclimated to the feed and pen conditions for seven days before the experiment. The housing temperature was maintained at $27^{\circ}\text{C} \pm 2$, while the relative humidity ranged from 70% to 80% during the experiment.

Experimental feeds

Duckweeds were grown in the big pond in the research station of Svay Rieng University, Cambodia, and were collected from the pond daily in the morning. For the commercial feed, red corn and rice bran were purchased from the local market and from Green Feed Company, Cambodia.

The feed amounts provided were based on a dry-matter intake equivalent to 10% of each chicken's body weight, as determined during the adaptation period. Feed offered during the collection period was adjusted according to the actual intake recorded during adaptation.

Water was available *ad libitum* through a drinker placed in each pen (Dukhta et al., 2018).

Duckweeds were dried under a shaded roof for one hour before mixing with relevant ingredients. The duckweed, commercial feed, red corn, rice barn, and premix were weighed and well mixed, and then fed to local chickens three times per day at 7 am, 11 am, and 4 pm according to the advice by the Green Feed and CP companies, Cambodia. There were no refusals of any feed during the experimental period.

Sample collection

The chickens were weighed in the morning before feeding, at the beginning of the experiment, and every 10 days thereafter. Feed offered was collected and weighed daily. Both the offered feed and the feed residues were placed in plastic bags and stored at -20°C until further analysis. At the end of each 10-day experimental period, the collected feed offered and the residues were thoroughly mixed by hand, then homogenized in a coffee grinder before laboratory analysis (Phiny et al., 2008).

Chemical analysis

Chemical analysis of the feed ingredients, diets, and feed offers was carried out according to the procedures of AOAC (1990) for determining ash, nitrogen, and CF. Dry matter content was determined using the microwave method described by Undersander et al. (1993). All analyses of the samples were conducted in duplicate in order to avoid different errors.

Statistical analysis

The data for feed intake, FCR, growth rate, and standard error of the mean (SEM) were analyzed using Analysis of Variance (ANOVA) via the General Linear Model (GLM) procedure in Minitab version 16.

Significant differences among treatment means were compared using Tukey’s Honestly Significant Difference (HSD) test at a significance level of p value less than 5% ($p < 0.05$). The source of variation in the data was attributed to the effects of diet, raising system, their interaction, and the residual error.

RESULTS

Chemical characteristics

The feed ingredients, such as red corn meal, commercial feed, and rice bran, had relatively high DM contents of 92.1%, 89.7%, and 87.6%, respectively, compared to duckweed, which had a DM content of 9.12%. However, duckweed had a higher CP content of 34.5% on a dry-matter basis than red corn meal (12.2%) and rice bran (10.8%), which were primarily used as energy sources. Additionally, red corn meal had a higher CF content (28.7%) and a higher organic matter (OM) content (98.7%) on a dry-matter basis (Table 2).

Feed intake

Notable effects on the chicken’s consumption were that DM intake in T1 (80.7 g/day) and T2 (80.3 g/day) were significantly higher than the DM intake in T4 (73.5 g/day; $p < 0.05$). However, DM intake in T1 was not significantly different compared to T2 (80.3 g/day) and T3

(78.1 g/day; $p > 0.05$; Table 3; Figure 1). The DM intake in the chickens reared under the confined system was significantly higher than in the semi-confined system ($p < 0.05$; Table 3). The dietary consumption in chickens indicated that CF (61.1 g/day) and OM (181 g/day) intakes in T4 were significantly higher than those in T1 (6.95 g/day of CF, 107 g/day of OM), T2 (10.4 g/day of CF, 136 g/day of OM), and T3 (13.5 g/day of CF, 162 g/day of OM; $p < 0.05$). Additionally, CP intake in T3 and T4 was not significant ($p > 0.05$). There were no significant interactions between diet and rearing system for CP (Figure 2) and OM intakes ($p > 0.05$; Figure 3). However, a significant interaction between diet and rearing system was observed for CF intake ($p < 0.01$; Figure 4). The dietary intake curves illustrating the relationship between feed intake and days of the experiment demonstrated that as the experiment duration increased, feed intake of the chickens rose across both dietary regimens and rearing systems. There was a significant increase in feed intake in T1 and T2 compared to T3 and T4 ($p < 0.05$; Figure 5). Moreover, chickens reared under the confined system demonstrated a greater increase in DM intake than those reared under the semi-confined system ($p < 0.05$). This was likely because the DM intake in chickens between days 20 and 60 of the experiment increased in a mostly linear pattern (Figure 6).

Table 2. Chemical composition of different feed ingredients for local chickens before starting the experiment

Ingredients	As a percentage of DM ²			
	DM (%) ¹	CP (%)	CF (%)	OM (%)
Commercial feed	89.7%	45.7%	3.1%	76.7%
Duckweed	9.12%	34.5%	10.2%	91.2%
Red corn meal	92.1%	12.2%	28.7%	98.2%
Rice bran	87.6%	10.8%	5.21%	89.7%
Premix/salt	98.3%	0%	0%	0%

DM: Dry matter, CP: Crude protein, CF: Crude fiber, and OM: Organic matter. ¹: Analysis results of the animal feed laboratory of Svay Rieng University, Cambodia. ²: Analysis of results of the Laboratory at the Center for Livestock and Agriculture Development, Cambodia.

Table 3. Feed intake in local chickens using duckweed to replace commercial feed mixed with red corn meal and rice bran

Parameters	System (S)			Diet (D)					
	Confined	Semi-confined	SEM	T1	T2	T3	T4	SEM	S*D
DM intake (g/d)	86.7 ^a	69.6 ^b	0.97	80.7 ^a	80.3 ^a	78.1 ^{ab}	73.5 ^b	1.37	ns
CP intake (g/d)	52.1 ^a	41.5 ^b	0.54	32.4 ^d	43.1 ^c	52.3 ^{ab}	59.6 ^a	0.76	ns
CF intake (g/d)	13.1 ^a	10.4 ^b	0.13	6.95 ^d	10.4 ^c	13.5 ^b	16.1 ^a	0.19	S
OM intake (g/d)	163 ^a	130 ^b	1.67	107 ^d	136 ^c	162 ^b	181 ^a	2.36	ns

DM: Dry matter, CP: Crude protein, CF: Crude fiber, and OM: Organic matter. T1: 5% duckweed + 22% commercial feed + 20% red corn meal + 52% rice bran + 0.5% premix/salt, T2: 10% duckweed + 19% commercial feed + 20% red corn meal + 50.5% rice bran + 0.5% premix/salt, T3: 15% duckweed + 16% commercial feed + 20% red corn meal + 48.5% rice bran + 0.5% premix/salt, T4: 20% duckweed + 13% commercial feed + 20% red corn meal + 46.5% rice bran + 0.5% premix/salt. ^{a, b, c, and d} Means within rows with different superscript letters are significantly different at $p < 0.05$. ns: not significant. S: significant

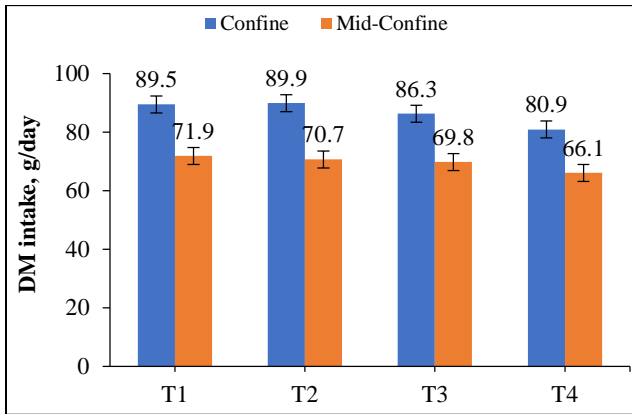


Figure 1. Interaction between dry matter intake and raising systems in 18-week-old local chickens fed duckweed to replace commercial feed mixed with red corn meal and rice bran

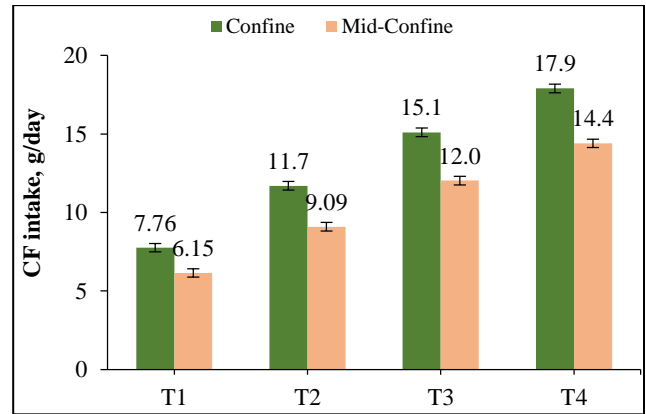


Figure 4. Interaction between crude fiber intake and raising systems in 18-week-old local chickens fed duckweed to replace commercial feed mixed with red corn meal and rice bran

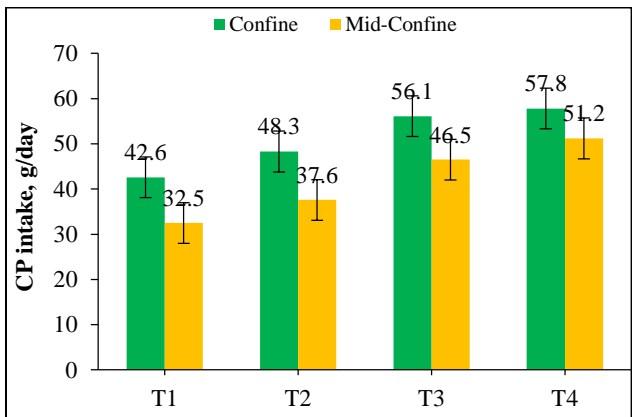


Figure 2. Interaction between crude protein intake and raising systems in 18-week-old local chickens fed duckweed to replace commercial feed mixed with red corn meal and rice bran

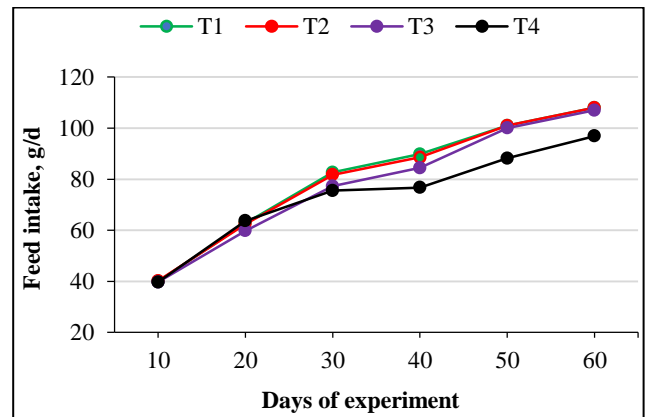


Figure 5. Feed intake curves in 18-week-old local chickens with different diets fed duckweed to replace commercial feed mixed with red corn meal and rice bran

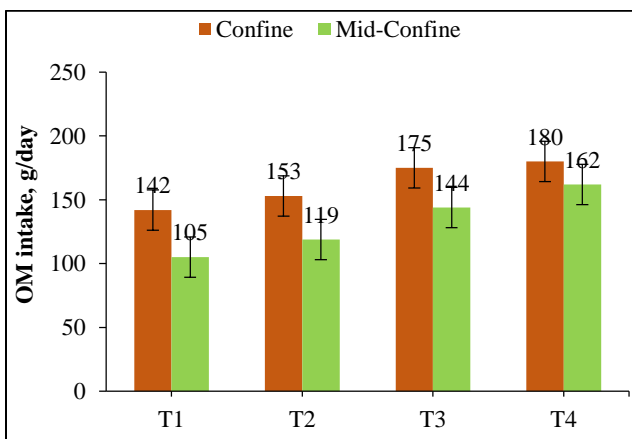


Figure 3. Interaction between organic matter intake and raising systems in 18-week-old local chickens fed duckweed to replace commercial feed mixed with red corn meal and rice bran

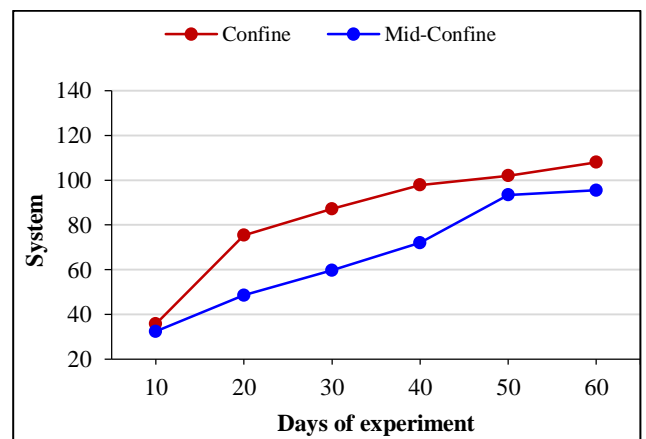


Figure 6. Feed intake curves for 60 days in local chickens with different rearing systems fed duckweed to replace commercial feed mixed with red corn meal and rice bran

Growth performance and feed conversion ratio

Considerable changes in average daily gain (ADG) and FCR were observed in the chickens' performance. Group T3 indicated the highest ADG (18.1 g/day; Figure 7). Additionally, T3 had the most improved FCR (4.29), compared to T4 (4.58), under both rearing systems. However, no significant differences in FCR were observed between T3 (4.29) and T1 (4.64) or T2 (4.50; $p > 0.05$; Table 4; Figure 8). The ADG and FCR of the chickens reared under the confined system were significantly higher than those reared under the semi-confined system ($p < 0.05$; Table 4). There were no significant interactions between diet and rearing system for ADG and FCR ($p >$

0.05). The best performance, including feed intake, ADG, and FRC, was observed in chickens reared under the confined system (Table 4). The growth curves illustrating the relationship between the ADG and the days of experiment indicated that the ADG of the chickens increased as the experimental period progressed. The increase of ADG was more pronounced in T3 compared to those in T1, T2, and T4 ($p < 0.05$; Figure 9). The growth curves in chickens reared under the confined system demonstrated a greater increase in ADG from 40 days onward compared to the chickens raised under the semi-confined system ($p < 0.05$; Figure 10).

Table 4. Average daily gain and feed conversion ratio in 18-week-old local chickens using duckweed to replace commercial feed mixed with red corn meal and rice bran

Parameters	System (S)			Diet (D)					
	Confined	Semi-Confined	SEM	T1	T2	T3	T4	SEM	S*D
Initial weight (g)	364	360	9.49	361	362	361	363	13.4	ns
Final weight (g)	1422	1362	25.3	1396	1412	1444	1316	35.8	ns
ADG (g/d)	17.6 ^a	16.7 ^b	0.32	17.3 ^{ab}	17.5 ^{ab}	18.1 ^a	15.9 ^b	0.45	ns
FCR	4.72 ^a	4.29 ^b	0.07	4.64	4.50	4.29	4.58	0.09	ns

ADG: Average daily gain, FRC: Feed conversion ratio. T1: 5% duckweed + 22% commercial feed + 20% red corn meal + 52% rice bran + 0.5% premix/salt, T2: 10% duckweed + 19% commercial feed + 20% red corn meal + 50.5% rice bran + 0.5% premix/salt, T3: 15% duckweed + 16% commercial feed + 20% red corn meal + 48.5% rice bran + 0.5% premix/salt, T4: 20% duckweed + 13% commercial feed + 20% red corn meal + 46.5% rice bran + 0.5% premix/salt. ^{a,b} Means within rows with different superscript letters are significantly different at $p < 0.05$. ns: Not significant.

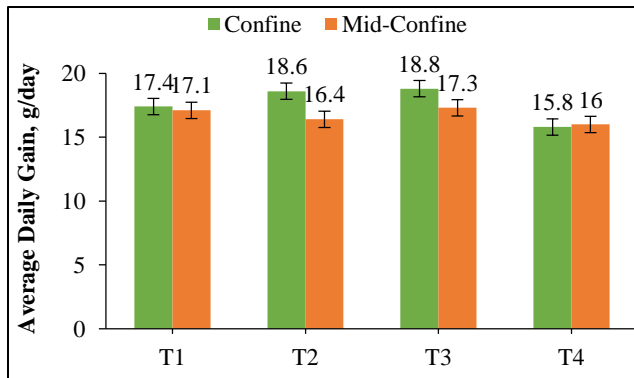


Figure 7. Mean values of live weight gain in 18-week-old local chickens fed duckweed to replace commercial feed mixed with red corn meal and rice bran

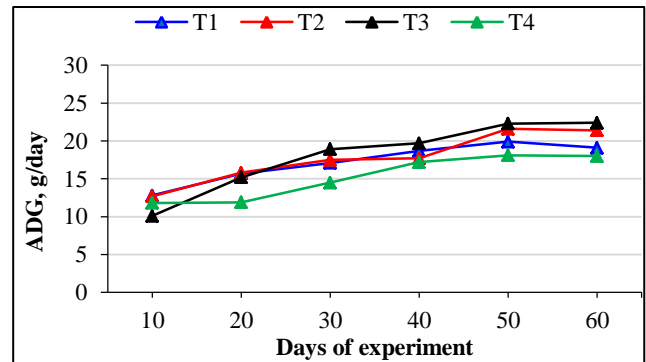


Figure 9. Growth curves for 60 days in local chickens with different diets included duckweed to replace commercial feed mixed with red corn meal and rice bran.

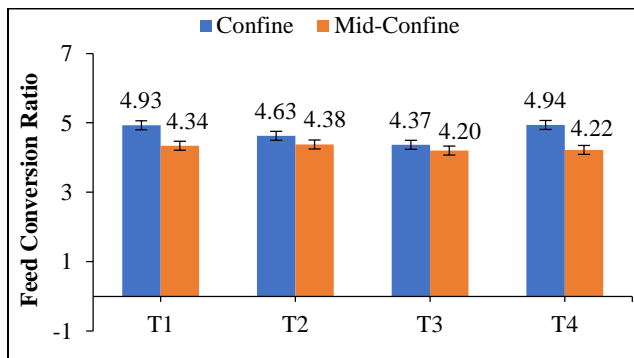


Figure 8. Feed conversion ratio in 18-week-old local chickens fed duckweed to replace commercial feed mixed with red corn meal and rice bran.

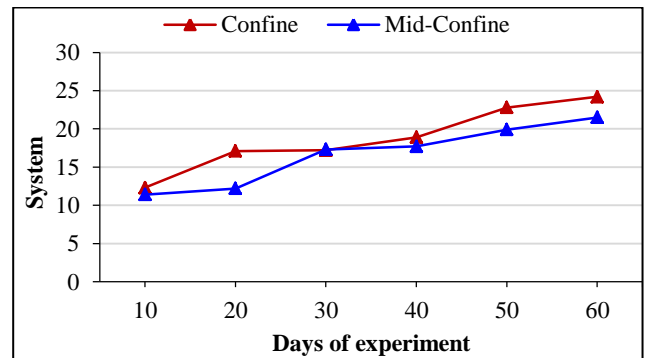


Figure 10. Growth curves in 60 days in local chickens with different rearing systems fed duckweed to replace commercial feed mixed with red corn meal and rice bran.

DISCUSSION

The present study exhibited higher DM, CF, and OM contents but lower CP levels, in contrast to the findings of Mwale and Gwaze (2013). The red corn meal byproduct analyzed in the present study had a DM content of 92.1%, aligned with the DM content (92%) reported for corn bran by Beck *et al.* (2024); however, its CP content was in contrast to that of the present study. The CF content of red corn meal (28.7%) in the present study was similar to the CF content (26.8%) reported for corn and cob meal (26.8%) by Ojediran and Olorunlowu (2025). However, the observed variation might be attributed to differences in red corn meal varieties and production locations in Cambodia. Similarly, the DM (87.6%) and OM (89.7%) contents of rice bran in the present study aligned with the findings of Shin *et al.* (2004).

The DM intake observed in the present study contrasted with the findings of Samnang (1999), who reported substantially lower DM intakes (30-40 g/day) in native chickens fed duckweed combined with broken rice under scavenging systems. Similarly, the present results were in contrast to those of Rodriguez and Preston (1999), who reported DM intakes ranging from 30 to 36 g/day when chickens were fed duckweed mixed with rice bran or duckweed alone as a supplement. Furthermore, the DM intake recorded in the present study was in contrast to the findings of Khang and Ogle (2004a), who reported a substantially lower DM intake of 38.3 g/day in chickens fed a concentrate diet supplemented with fresh duckweed in the confined system. This discrepancy in the results might be due to the *ad libitum* inclusion of fresh duckweed, which led to nutrient imbalances and potentially resulted in nutrient deficiencies and reduced feed intake. The CP and CF intakes in the present study were consistent with those reported by Khanum *et al.* (2005), who indicated that growing ducks were supplemented with duckweed mixed with other ingredients, including concentrate feed, soybean meal, maize, and wheat bran.

In the present study, the ADG of chickens fed a diet containing 15% duckweed mixed with red corn meal and rice bran was 18.1 g/day, higher than that of other diets. The current results were higher than those reported by Kingori *et al.* (2003), who fed a balanced diet containing 18% CP, maize, maize gluten meal, fish meal, and limestone, resulting in ADG of 12.2 g/day. This discrepancy might be attributed to the utilization of duckweed at different inclusion levels as a substitute for commercial feed within the diet. This substitution

potentially has influenced the chickens' preference for the experimental feed diet. Additionally, the ADG observed in the present study was in contrast to the higher ADG (20.4 g/day) reported by Thuy and Ogle (2005) for Loung Phuong chickens fed a nutritionally balanced mixed diet containing duckweed. Thuy and Ogle (2005) reported a superior performance, possibly due to a more balanced diet. Their diet included fish meal, soybean meal, broken rice, rice bran, and *ad libitum* duckweed, which probably led to higher feed intake and greater nitrogen retention than the mixed diet used in the present study. The lower growth performance observed in the present study contrasts with the findings of Khang and Ogle (2004a), who supplemented the chickens with duckweed *ad libitum*. The FCR measures the efficiency of chicken performance by comparing feed utilization to live weight gain. In the present study, local chickens fed the diet containing 15% duckweed exhibited an FCR of 4.29, representing the greatest improvement among the dietary treatments. This result was in alignment with the findings of Nakkazi *et al.* (2015), who reported FCR values ranging from 2.7 to 4.9 in local chickens fed diets containing maize, fish meal, and sunflower (formulated to 20-23% CP) under an intensive feeding system during the 1-6-week growing period. The present findings were also in line with the results of Putra and Ritonga (2018), who fed native chickens conventional starter and finisher diets containing 5-20% duckweed meal and reported FCR values ranging from 3.96 to 4.18 over a 13-week growing period. Additionally, the present results are consistent with those of Khang and Ogle (2004b), who reported a slight improvement in daily weight gain and FCR when fresh duckweed was provided *ad libitum* alongside a concentrate diet. These differences in results might be attributed to duckweed nutrient content, especially its carotenoid content, as well as differences in species, feeding system, and rearing conditions. The FCR observed in the present study was in contrast to that reported by Ma *et al.* (2021), who recorded an FCR of 5.23 in chickens fed diets containing yellow corn, fish meal, rice bran, molasses, and limestone with 15% duckweed. The difference in FCR may be attributed to the inclusion of 5% molasses as an energy source in their formulated diets, which likely reduced DM and CP intake during the experimental period.

CONCLUSION

Based on the present findings, duckweed can be included at 15% and mixed with other feed ingredients. Local

chickens consumed high amounts of DM content, exhibited increased live weight of 18.1 g/day, and demonstrated a greater FCR of 4.29. Moreover, the local chickens raised under the confined system had an enhanced ADG of 17.6 g/day compared to those raised under the semi-confined system. Future studies should investigate the nutritional digestibility, carcass quality, and economic efficiency of diets containing duckweed under different management systems.

DECLARATIONS

Availability of data and materials

The datasets produced and examined in the present study can be obtained from the corresponding author upon reasonable request.

Funding

The World Bank Group in Cambodia funded this study through the Ministry of Education, Youth, and Sport at the Svay Rieng University, Cambodia.

Authors' contributions

Chiv Phiny conceived, developed, and designed the present experiment. All data collection was carried out by Srey Lida, Lay Sophin, and Phuon Phou. Data analysis and the initial draft of the manuscript were completed by Chiv Phiny. Tum Saravuth and Sin Putheasath provided critical input and assistance in improving the English language and overall quality of the paper. All authors have read and approved the manuscript before its submission to the JWPR journal.

Ethical considerations

The authors conducted a comprehensive review of the manuscript to ensure adherence to ethical guidelines, including the prevention of plagiarism, proper attribution of authorship and consent to publish, avoidance of research misconduct, data fabrication, duplicate publication, and/or submission redundancy. All data presented in this study were collected and analyzed with integrity and transparency. The authors confirmed that the manuscript is original, has not been published elsewhere, and is not currently under consideration by other journal. The authors confirmed that they have not used AI tools in conducting the present study.

Competing interests

The authors declared that they have no known financial, personal, or professional conflicts of interest.

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Evaluation of Body Weight and Intestinal Health in Broiler Chickens Supplemented with Encapsulated *Morinda citrifolia* L. Extract, Zinc, and Copper

Linda Nur Baetavianti¹, Vitus Dwi Yunianto*¹, Mulyono¹, and Lilik Krismiyanto¹

Department of Animal Science, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Central Java, 50275, Indonesia

*Corresponding author's E-mail: vitus.dbi@gmail.com

Received: October 17, 2025, Revised: November 22, 2025, Accepted: December 12, 2025, Published: December 31, 2025



ABSTRACT

The broiler poultry sector is strategically important, serving as a major contributor to animal protein supply and an essential component of national food security. The present study aimed to examine the effects of dietary supplementation of encapsulated noni fruit (*Morinda citrifolia* L.) extract (ENFE) enriched with zinc (Zn) and copper (Cu) on intestinal health and body weight in broiler chickens. A total of 200 eight-day-old broiler chickens, with an initial weight of 233.69 ± 7.28 grams, were assigned to four treatments using a completely randomized design, with five replications per treatment. The treatment groups included a control group with a basal diet (T0), a basal diet with 0.06% ENFE, Zn at 40 ppm and Cu at 5 ppm (T1), a basal diet with 0.12% ENFE, Zn at 40 ppm and Cu at 5 ppm (T2), and a basal diet with 0.18% (T3) ENFE, Zn at 40 ppm and Cu at 5 ppm (T3). The body weight gain (BWG), counts of total lactic acid bacteria (LAB), coliform bacteria, pH value, villus height, crypt depth, relative length, and weight of each segment of the small intestine were assessed. The current results indicated that chickens in 0.12% (T2) and 0.18% (T3) significantly enhanced BWG, LAB populations, villus height, crypt depth, and length, and relative weight of the duodenum, jejunum, and ileum. Additionally, reduced duodenal and ileal pH, as well as coliform counts across all intestinal segments, were observed in 0.12% (T2) and 0.18% (T3). However, ENFE supplemented with Zn and Cu did not significantly affect jejunal pH. Administering ENFE at 0.12% supplemented with Zn and Cu represented the most optimal dose. Although the higher dose of 0.18% (T3) yielded statistically comparable results and was superior in some parameters, the 0.12% (T2) inclusion level is recommended as the most practical dose for enhancing BWG and intestinal health in broiler chickens.

Keywords: Body weight gain, Broiler chicken, Encapsulation, Intestinal morphology, *Morinda citrifolia*

INTRODUCTION

Poultry farming, especially broiler chicken production, is crucial for providing animal protein and supports national food security. Nonetheless, successful production depends heavily on health, genetics, management practices, and on maintaining low mortality rates (Khan et al., 2023). Meanwhile, susceptibility to diseases, particularly gastrointestinal infections caused by pathogenic bacteria, can decrease productivity (Hashem et al., 2022).

Providing high-quality feed and incorporating herbal supplements, such as phytobiotics, are strategies to maintain gut health. Phytobiotics possess antimicrobial properties that may prevent infection and enhance the efficiency of the digestive system (Chodkowska et al.,

2022). The mechanism of action of antimicrobials in phytobiotics involves disrupting the cell membranes of harmful bacteria, thereby stopping the spread of disease.

Noni fruit (*Morinda citrifolia* L.) has the potential to serve as a source of phytochemicals due to its flavonoid compounds, which possess antibacterial and antioxidant properties (Sunder et al., 2016). Flavonoids in noni fruit can improve intestinal morphology, maintain microbiota balance, and support nutrient absorption efficiency, all of which positively affect the growth of broiler chickens (Song et al., 2022). To obtain beneficial active compounds, such as flavonoids, and to reduce antinutritional factors in noni fruit, extraction is an effective method (Widjastuti et al., 2025). The active compounds in noni fruit are unstable and easily degraded

due to the presence of short-chain unsaturated organic acids. Therefore, the addition of microminerals such as zinc (Zn) and copper (Cu) is necessary to protect these active compounds and maintain their utilization, especially in broiler chickens' intestine (Widjastuti et al., 2023). On the other hand, the addition of Zn and Cu minerals to noni extract supplementation can enhance intestinal morphology and maintain a balanced microbiota. Zinc is known to improve the histomorphometry characteristics of the small intestine, such as villus height, indicating an increased capacity for nutrient absorption (Dogra et al., 2023). Meanwhile, Cu can enhance intestinal morphology by increasing the small intestine's weight and length and by improving the beneficial microbiota composition, thereby supporting greater nutrient absorption (Nguyen et al., 2022). Bioactive compounds with natural antioxidant activity, such as flavonoids in noni fruit, are generally unstable under heat and oxidative conditions (Tanwiriah et al., 2024). To overcome this instability, the noni fruit extract is prepared using an encapsulation technique supplemented with Zn and Cu. Encapsulation technology is a method of coating active compounds to increase stability and bioavailability, and controlled secretion in target tissues (Temiz and Öztürk, 2018). In the present study, maltodextrin was used as a coating material due to its hygroscopic nature, which allows for a gradual release of active compounds, thereby enhancing their absorption and effectiveness in broiler intestinal tracts (Febrianta et al., 2022). The present study aimed to evaluate the effects of supplementing broiler chickens' diet with encapsulated *Morinda citrifolia* L. extract, Zn, and Cu on body weight and intestinal health.

MATERIALS AND METHODS

Ethical approval

All procedures involving animals were approved ethically by the Faculty of Animal and Agricultural Science, Diponegoro University, Semarang, Indonesia (Approval No. 61-10/A-30/KEP-FPP).

Experimental design

The present study was conducted from August to September 2024 at the poultry farm of the Faculty of Animal and Agricultural Sciences, Diponegoro University, Indonesia. A total of 200 eight-day-old Ross 308 broiler chickens, with a mean weight of 233.69 ± 7.28 g, were used for the present study. The stocking density was 10 chickens/m², with each of the 20 pens (1 × 1 m²) housing 10 chickens. Temperature and humidity in the cages during the rearing period were between 28 and 32 °C, with humidity ranging from 46 to 59%. A completely

randomized design with four treatments was used. Each treatment was replicated five times, with ten broiler chickens per replicate. The treatment dose of noni fruit extract was determined according to the modified study by Krismiyanto et al. (2023), while the mineral doses for Zn and Cu were chosen based on the recommendations of the NRC (1994). The treatment groups included a control group receiving the basal diet (T0). The second group which received the basal diet supplementing with 0.06% noni fruit (*Morinda citrifolia* L.) extract (ENFE), Zn at 40 ppm and Cu at 5 ppm (T1), the third group was given 0.12% ENFE with Zn at 40 ppm and Cu at 5 ppm (T2), and the fourth group was administered 0.18% ENFE with Zn at 40 ppm and Cu at 5 ppm (T3). Following the method of Anjani et al. (2025), chickens were given adaptive feed until they reached 7 days old, comprising a mixture of commercial feed (B-11S, Charoen Pokhand, Indonesia) and experimental feed, with its composition gradually changed. The feed proportions on days 1-2 were 75% commercial feed and 25% experimental feed, on days 3-4, 50% commercial feed and 50% experimental feed, and on days 5-6, 25% commercial feed and 75% experimental feed. On day 7, all chickens were given the full treatment feed, and from day 8 to day 35, their diet was controlled as per the predetermined plan treatment. Vaccination was carried out on 3-day-old chickens using Newcastle Disease and Infectious Bronchitis vaccines (Medivac ND-IB, Medion, Bandung, Indonesia) administered via eye drops. Drinking water was given *ad libitum*. The composition of the diet ingredients is shown in Table 1.

Table 1. Composition of ingredients and nutritional content of diets for starter and finisher periods

Feed ingredients	Composition (%)	
	Starter (8-21 days)	Finisher (22-35 days)
Yellow corn	50.11	53.41
Rice bran	15.04	16.74
Soybean meal	24.00	19.00
Fish meal	10.00	10.00
Limestone	0.30	0.30
Premix	0.25	0.25
Lisin	0.10	0.10
Metionin	0.20	0.20
Total	100.00	100.00
Nutrient content		
Metabolic energy (kkal/g) ²	2993.57	3018.24
Crude protein (%) ¹	21.22	19.33
Crude fat (%) ¹	4.45	4.59
Crude fiber (%) ¹	5.24	5.51
Calcium (%) ¹	1.04	1.07
Phosphorus (%) ¹	0.61	0.74

Note: ¹Result of Analysis of Animal Nutrition and Feed Science Laboratory, Faculty of Animal and Agricultural Sciences, University Diponegoro, Semarang (2024). ²Based on the Bolton formula, Bolton (1967) as follows, $40.81 (0.87 [\text{crude protein} + 2.25 \times \text{crude fat} + \text{nitrogen-free extract}] + 2.5)$.

Treatment preparation

Noni fruit was collected from Semarang City, Central Java, Indonesia. Noni fruit was dried in an oven (Binder ED56, Germany) at 50°C and ground into a fine powder. The extraction procedure was carried out according to the guidelines of Gouda et al. (2021). Noni fruit flour was dissolved in 96% ethanol at a 1:10 (w/v) ratio and stirred until homogeneous. The noni fruit and ethanol mixture was sonicated for 60 minutes at a controlled temperature of 37°C using a sonicator (Power Sonic 405, South Korea) set at 50 Hz. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was then evaporated in a rotary evaporator (RV 10, Germany) to obtain a thick extract. This extract was supplemented with 40 ppm Zn and 5 ppm Cu, which are considered microminerals (Tanwiriah et al., 2024).

The encapsulation technique was based on the method suggested by Agusetyaningsih et al. (2022). Maltodextrin as a coating material was dissolved in distilled water at a 1:3 (w/v) ratio. The noni fruit extract, supplemented with 40 ppm Zn and 5 ppm Cu, was then added to the maltodextrin solution at a 1:5 (v/v) ratio. The mixture was dried using a freeze dryer (Sp VirTis, USA) at -50°C. This process produced a crystal powder containing the encapsulated noni fruit extract, Zn, and Cu. The encapsulation efficiency was evaluated *in vitro* using an antimicrobial inhibition assay and antioxidant assays to verify whether the noni fruit extract, Zn, and Cu were successfully incorporated into the encapsulated matrix.

Total intestinal bacteria and pH value

Total intestinal bacterial measurements involved collecting 2 cm of digesta from each small intestine segment, including the duodenum, jejunum, and ileum. The total counts of lactic acid bacteria (LAB) and coliform were determined using the total plate count method. Lactic acid bacteria were cultured on de Man Rogosa Sharpe agar (MRS), whereas coliform was cultured on MacConkey agar. Bacterial counts were determined after 48 hours of incubation at 44°C for LAB and 24 hours at 37°C for coliforms (Pratama et al., 2017). Results were expressed as log₁₀ colony-forming units (CFU) per gram. The pH was determined in three segments of the small intestine (duodenum, jejunum, and ileum) by using a pH meter (Eco Testr pH 1, Singapore).

Villi height and crypt depth

The duodenum, jejunum, and ileum were cut into 3 cm segments and immersed in 10% buffered formalin for 24 hours. Tissues were processed, stained with

hematoxylin and eosin, and examined under a microscope (Binokuler Leica DM500[®], Heerbrugg, Switzerland) using a 40x magnification objective lens connected to a screen and computer (Sohel et al., 2019).

Length and weight

The small intestine was separated into the duodenum, jejunum, and ileum segments. The length of each segment was measured using a measuring tape, while the weight of each segment was measured using an analytical scale with an accuracy of 0.0001 g. Then, the relative length was calculated using the formula by Abdel-Kafy et al. (2022), and the relative weight was calculated using the formula by Rotiah et al. (2018).

$$\text{Relative length (\%)} = \frac{\text{length of the small intestine (g)}}{\text{weight life (g)}} \times 100\%$$

$$\text{Relative weight (\%)} = \frac{\text{weight of the small intestine (g)}}{\text{weight life (g)}} \times 100\%$$

Body weight gain

Body weight gain (BWG) was determined by subtracting the initial body weight at 8 days of age and the final body weight, following the calculation method described by Jie et al. (2024).

$$\text{BWG} = \text{Final weight} - \text{initial weight}$$

Statistical analysis

Data were analyzed using SPSS version 22.0. The data were evaluated for normality and homogeneity and were then analyzed using analysis of variance (ANOVA). When the one-way ANOVA revealed significant differences ($p < 0.05$), the means were further compared using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Body weight

Encapsulation of ENFE supplemented with Zn and Cu had a significant effect on increasing the BWG in broiler chickens ($p < 0.05$). The 0.06% (T1) level of ENFE did not differ from the control group (T0), whereas both 0.12% (T2) and 0.18% (T3) levels significantly increased BWG compared to control group (T0) and 0.06% (T1; $P < 0.05$), with no significant difference between 0.12% (T2) and 0.18% (T3; $P > 0.05$; Table 2).

There was no significant difference between control group (T0) and 0.06% (T1) in BWG (Table 2), consistent with the gut microbiota response pattern at the 0.06% (T1) ENFE level (Table 3). In the segments of the duodenum, jejunum, and ileum, 0.06% (T1) demonstrated an increase

in LAB counts and a decrease in coliforms. However, the variations were not statistically significant enough to distinguish from the control group (T0; $p > 0.05$). The present finding indicated that the 0.06% (T1) ENFE level might not have been high enough to induce significant changes in the microbiota and intestinal environment that could impact growth performance. Furthermore, the intestinal pH in 0.06% (T1) was close to the control group (T0) pH value, suggesting that the modulation of the luminal environment had not yet been optimized at this dose. Conversely, levels of 0.12% (T2) and 0.18% (T3) provided a significant increase in BWG compared to control group (T0) and 0.06 (T1; $p < 0.05$). Although no significant difference was observed between 0.12% (T2) and 0.18% (T3; $p > 0.05$), BWG increased gradually with increasing ENFE inclusion levels. The mentioned pattern suggested a dose-responsive physiological adaptation rather than a strict threshold effect, with 0.18% (T3) demonstrating the highest numerical values. The observed improvement could result from the antioxidant, antimicrobial, and metabolic activities of noni fruit extract

bioactive compounds, especially flavonoids, interacting with Zn and Cu.

Flavonoids present in noni fruit extract were bioactive compounds with antioxidant and antimicrobial properties that might contribute to improved growth performance by enhancing gut health and nutrient utilization. According to Kouvedaki *et al.* (2024), the presence of natural antioxidants promotes healthier digestive processes, strengthens the immune system, and increases the efficiency of nutrient absorption from the feed. The antioxidant and antimicrobial components in the flavonoids might have contributed to greater growth performance in broiler chickens. Although other factors, such as initial weight, can also affect weight gain.

Zinc stimulates increased appetite by regulating hormones (El-Hack *et al.*, 2017). Meanwhile, Cu contributes to the antioxidant mechanism by activating the superoxide dismutase (SOD) enzyme, which protects cells from oxidative damage, thereby strengthening the immune system, enhancing nutrient digestion, and promoting growth (Sharif *et al.*, 2021).

Table 2. Body weight gain in broiler chickens fed diets with encapsulated noni fruit extract supplemented with zinc and copper for four weeks

Treatments	T0	T1	T2	T3	P-value
Initial weight	227.38 ± 3.39	228.22 ± 3.23	235.12 ± 1.02	244.02 ± 4.35	0.436
Final weight	1902.67 ± 37.09 ^b	1929.98 ± 52.47 ^b	2011.82 ± 41.21 ^a	2030.50 ± 67.39 ^a	0.002
Body weight gain	1675.29 ± 36.57 ^b	1701.76 ^b ± 49.95	1776.70 ± 41.00 ^a	1786.48 ± 69.40 ^a	0.007

Note: ^{a, b, and c} Different superscript letters in the same row significantly differ ($p < 0.05$). T0: Basal diet; T1: Basal diet + 0.06% ENFE supplemented with Zn, Cu; T2: Basal diet + 0.12% ENFE supplemented with Zn and Cu; T3: Basal diet + 0.18% ENFE supplemented with Zn and Cu.

Table 3. Total lactic acid bacteria, coliform, and pH value in intestinal segments of broiler chicken fed diets with encapsulated noni fruit extract supplemented with zinc and copper for four weeks

Treatments	T0	T1	T2	T3	P-value
Duodenum					
LAB (log cfu/g)	5.93 ± 0.45 ^b	6.09 ± 0.31 ^{ab}	6.50 ± 0.19 ^a	6.50 ± 0.21 ^a	0.021
Coliform (log cfu/g)	4.05 ± 0.40 ^b	3.91 ± 0.31 ^b	3.38 ± 0.27 ^a	3.37 ± 0.28 ^a	0.006
pH	6.21 ± 0.37 ^c	5.86 ± 0.04 ^b	5.59 ± 0.06 ^a	5.48 ± 0.05 ^a	< 0.001
Jejunum					
LAB (log cfu/g)	6.66 ± 0.40 ^b	6.83 ± 0.33 ^b	7.40 ± 0.35 ^a	7.47 ± 0.31 ^a	0.004
Coliform (log cfu/g)	4.83 ± 0.09 ^b	4.68 ± 0.13 ^b	4.05 ± 0.41 ^a	4.05 ± 0.47 ^a	0.002
pH	6.66 ± 0.30	6.43 ± 0.20	6.38 ± 0.17	6.38 ± 0.15	0.162
Ileum					
LAB (log cfu/g)	7.67 ± 0.22 ^b	7.96 ± 0.52 ^a	8.61 ± 0.42 ^a	9.05 ± 0.38 ^a	0.000
Coliform (log cfu/g)	4.88 ± 0.07 ^b	4.69 ± 0.24 ^b	4.54 ± 0.28 ^{ab}	4.33 ± 0.35 ^a	0.024
pH	6.92 ± 0.16 ^b	6.85 ± 0.04 ^{ab}	6.73 ± 0.17 ^a	6.70 ± 0.10 ^a	0.049

Note: ^{a, b, and c} Different superscript letters in the same row significantly differ ($p < 0.05$). T0: Basal diet; T1: Basal diet + 0.06% ENFE supplemented with Zn, Cu; T2: Basal diet + 0.12% ENFE supplemented with Zn and Cu; T3: Basal diet + 0.18% ENFE supplemented with Zn and Cu; LAB: Lactic Acid Bacteria.

Lactic acid bacteria, coliform, and potential hydrogen (pH)

Supplementing the broiler diet with ENFE, Zn, and Cu significantly affected LAB populations ($p < 0.05$). A consistent increase in LAB population was observed in 0.12% (T2) and 0.18% (T3) compared to 0.06% (T1) and control group (T0; Table 3), although no significant difference was observed between 0.12% (T2) and 0.18% (T3; $p > 0.05$). The mentioned response indicated that the inclusion levels of ENFE at 0.06% (T1) were correlated with a beneficial modulation of the intestinal microbiota population. In the ileum segment, an increase in LAB population was already evident at 0.06% (T1) ENFE, which was not significantly different from 0.12% (T2) and 0.18% (T3; $P > 0.05$). Nevertheless, a gradual increase with higher ENFE levels was observed, with 0.18% (T3) exhibiting the highest numerical value among treatments. The higher LAB population observed at increased ENFE levels was likely related to the selective antimicrobial activity of flavonoids, as reported in previous studies. Flavonoids can inhibit bacterial growth, including *Escherichia coli*, due to their antibacterial properties (Pan et al., 2023). Additionally, Manner et al. (2018) found that flavonoids can inhibit quorum sensing in pathogenic bacteria by interfering with their communication, while flavonoids do not affect the communication among beneficial bacteria. Flavonoids form complex compounds with the extracellular proteins of pathogenic bacteria, causing cell membrane damage and inhibiting DNA, RNA, and protein synthesis, thereby inhibiting pathogen growth (Rodríguez et al., 2023).

Total coliforms in each segment of the small intestine of broiler chickens significantly decreased due to the ENFE, Zn, and Cu supplementation ($p < 0.05$, Table 3). As shown in Table 3, a similar statistical pattern was observed across all intestinal segments. In the duodenum and jejunum, the coliform levels in 0.18% (T3) were not significantly different from those in 0.12% (T2; $P > 0.05$), although both were clearly lower than those in 0.06% (T1) and control group (T0). In the ileum, 0.18% (T3) demonstrated the lowest numerical values, followed by 0.12% (T2), whereas the highest coliform counts were recorded in the control group (T0). Although 0.18% (T3) was slightly lower than 0.12% (T2), these differences were not statistically significant ($P > 0.05$), suggesting that a plateau in the inhibitory response might have been reached at the 0.12% (T2) inclusion level.

The reduction in coliforms was accompanied by increases in LAB counts and decreases in intestinal pH

observed in the 0.12% (T2) and 0.18% (T3). The mentioned pattern appears to be consistent with the activity of LAB, which have been reported to ferment carbohydrates into lactic acid and other organic acids, thereby lowering luminal pH and creating an environment that selectively favors beneficial bacteria while limiting the growth of coliforms. The enhanced LAB proliferation in 0.12% (T2) and 0.18% (T3) likely contributed to the formation of a more acidic microenvironment, thereby reinforcing competitive exclusion and limiting coliform proliferation. In broiler chickens, pathogenic bacteria are negatively correlated with LAB in the small intestine. The negative correlation between coliforms and LAB was explained by ecological competition between them in the intestine. Furthermore, flavanol compounds from noni fruit, such as quercetin, have been reported to interact with phospholipids in bacterial cell membranes, increasing membrane permeability and leading to leakage of intracellular components, which may impair bacterial survival in the intestinal environment (Ren et al., 2024).

The pH values of the duodenum and ileum segments decreased significantly following the inclusion of ENFE supplemented with Zn and Cu in the broiler diet ($p < 0.05$). In the duodenum, a significant reduction ($p < 0.05$) was observed in, while 0.18% (T3) did not differ statistically from 0.12% (T2; $P > 0.05$) but still indicated a slightly lower numerical value than the other treatment groups (Table 3). The current findings indicated that the primary acidification response occurred at a 0.12% (T2) inclusion level, while the decrease at 0.18% (T3) was not statistically significant ($P > 0.05$). A non-significant reduction in ileal pH was observed at the 0.06% (T1) ENFE supplementation level compared to the control group (T0; $P > 0.05$; Table 3). Meanwhile, the jejunum did not exhibit a significant response to dietary supplementation with ENFE, Zn, and Cu ($p > 0.05$). The observed decrease in small intestine pH occurred concurrently with higher LAB counts (Table 3), indicating a close association between microbial dynamics and luminal activity. Consistent with the present findings, Dityana et al. (2024) reported that an increase in LAB population led to higher production of bacterial metabolites, such as organic acids, which contributed to a decrease in small intestinal pH.

Mechanistically, organic acids, such as short-chain fatty acids (SCFA), release positive hydrogen ions (H^+) that lower pH and make the digestive tract more acidic (Chukwudi et al., 2024).

Intestinal segment morphology

The villi height in broiler chickens was significantly affected by the addition of ENFE supplemented with Zn and Cu ($p < 0.05$). Villus height in the duodenum and ileum increased significantly in 0.12% (T2) and 0.18% (T3). Groups 0.12% (T2) and 0.18% (T3) demonstrated significantly higher villus height compared to 0.06% (T1) and control group (T0; $P < 0.05$), with no significant difference between 0.12% (T2) and 0.18% (T3; $P > 0.05$; Table 4). In the jejunum segment, 0.06% (T1) exhibited an increase in villus height, followed by 0.12% (T2), with the highest increase found in 0.18% (T3). Although no significant differences were detected between 0.12% (T2) and 0.18% (T3) across all intestinal segments ($P > 0.05$), the numerically highest villus height consistently appeared in 0.18% (T3). The present results indicated a dose-dependent increase, yet the effect at 0.18% (T3) was not biologically greater than lower doses. Despite statistical

equivalence between 0.12% (T2) and 0.18% (T3), the consistent improvement in villus morphology across the small intestine indicated that ENFE, supplemented with Zn and Cu, enhanced mucosal development. The antimicrobial properties of bioactive compounds in noni fruit extract, when combined with Zn and Cu, have been reported to suppress pathogenic bacteria, thereby reducing toxin production and mucosal irritation and creating ideal conditions for epithelial cell turnover and villus development. The present findings are in line with those of Prihambodo et al. (2021), who reported that flavonoids exhibited anti-inflammatory and antioxidant activities, neutralizing free radicals, minimizing cellular damage, and enhancing epithelial regeneration, thereby supporting villus development. Small intestinal villi are closely related to nutrient absorption capacity, where longer villi increase absorption efficiency (Abdellatif et al., 2022).

Table 4. Villus height, crypt depth, relative length, and weight of intestinal segments in broiler chicken fed diets with encapsulated noni fruit extract supplemented with zinc and copper for four weeks

Variables	Treatments	T0	T1	T2	T3	P-value
Duodenum						
Villus height (μm)		1297.01 \pm 100.76 ^b	1391.46 ^b \pm 109.75 ^b	1536.21 \pm 93.98 ^a	1557.28 \pm 51.21 ^a	< 0.001
Crypt depth (μm)		242.74 \pm 8.63 ^b	248.73 ^b \pm 3.35 ^b	276.37 \pm 21.55 ^a	280.48 \pm 12.42 ^a	< 0.001
Length (%)		1.12 \pm 0.36 ^b	1.15 ^a \pm 0.41 ^a	1.18 \pm 0.58 ^a	1.26 \pm 0.62 ^a	0.004
Weight (%)		0.34 \pm 0.10 ^b	0.50 ^a \pm 0.06 ^a	0.52 \pm 0.02 ^a	0.52 \pm 0.04 ^a	< 0.001
Jejunum						
Villus height (μm)		992.39 \pm 103.47 ^c	1157.18 \pm 43.09 ^b	1241.88 \pm 54.64 ^{ab}	1294.21 \pm 78.60 ^a	< 0.001
Crypt depth (μm)		201.92 \pm 11.31 ^b	225.12 \pm 13.21 ^a	241.52 \pm 5.12 ^a	246.07 \pm 24.13 ^a	< 0.001
Length (%)		3.62 \pm 0.16 ^c	3.92 \pm 0.12 ^b	3.92 \pm 0.18 ^b	4.15 \pm 0.07 ^a	< 0.001
Weight (%)		0.92 \pm 0.06 ^b	0.99 \pm 0.07 ^b	1.19 \pm 0.15 ^a	1.22 \pm 0.09 ^a	< 0.001
Ileum						
Villus height (μm)		471.33 \pm 79.94 ^b	496.58 \pm 75.83 ^b	826.71 \pm 63.77 ^a	830.59 \pm 121.64 ^a	< 0.001
Crypt depth (μm)		117.37 \pm 16.40 ^b	127.27 \pm 7.47 ^b	170.94 \pm 17.91 ^a	156.34 \pm 19.52 ^a	< 0.001
Length (%)		3.61 \pm 3.32 ^b	4.10 \pm 3.62 ^a	4.19 \pm 1.89 ^a	4.16 \pm 1.72 ^a	0.006
Weight (%)		0.77 \pm 0.03 ^c	0.95 \pm 0.06 ^b	1.06 \pm 0.06 ^a	1.13 \pm 0.04 ^a	< 0.001

Note: ^{a, b, and c} Different superscript letters in the same row significantly differ ($p < 0.05$). T0: Basal diet; T1: Basal diet + 0.06% ENFE supplemented with Zn, Cu; T2: Basal diet + 0.12% ENFE supplemented with Zn and Cu; T3: Basal diet + 0.18% ENFE supplemented with Zn and Cu.

The crypt depth of every segment of the small intestine was significantly affected by the addition of ENFE supplemented with Zn and Cu ($p < 0.05$). Crypt depth was significantly greater in the duodenum and ileum of 0.12% (T2; $p < 0.05$). In the jejunum, however, an increase in 0.06% (T1) was already observed compared with the control group (T0). The duodenal crypt depth in 0.18% (T3) did not differ from 0.12 (T2) but was significantly greater than in 0.06% (T1) and control group

(T0; $p < 0.05$, Table 4). In the jejunum, the crypt depth in 0.18% (T3), 0.12% (T2), and 0.06% (T1) was significantly greater than in the control group (T0; $P < 0.05$). Meanwhile, the ileal crypt depth in 0.18% (T3) was similar to that in 0.12% (T2), and both were significantly higher than in 0.06% (T1) and control group (T0; $p < 0.05$; Table 4). Although the jejunum demonstrated an additional significant response at 0.18% (T3), other critical morphological parameters, such as villus height and crypt

depth in the duodenum and ileum, stabilized at the 0.12% (T2) ENFE dosage.

The observed increase in crypt depth might be due to the biological activity of flavonoids, which have been reported to exert antimicrobial effects that suppress pathogenic bacteria, promote LAB populations, and enhance SCFA production. The SCFAs are known to interact with G protein-coupled receptors (GPCRs), potentially stimulating glucagon-like peptide-1 (GLP-1) secretion, thereby supporting epithelial cell growth at the base of the crypt (Zhang et al., 2019; Paredes-López et al., 2024). Crypt depth is often used as a marker of epithelial cell proliferation and is crucial for villus renewal. Therefore, deeper crypts along with taller villi indicate enhanced mucosal turnover and improved intestinal function, potentially boosting digestive efficiency and nutrient absorption in broiler chickens (El-Sabry and Yalcin, 2022).

The relative length and weight of each segment of the small intestine were significantly affected by the addition of ENFE supplemented with Zn and Cu ($p < 0.05$). A significant increase in the relative length of the duodenum, jejunum, and ileum was observed in 0.06% (T1; $P < 0.05$). Similarly, the duodenum and ileum weights increased in T1, while the jejunal weight increased by 0.12% (T2). The duodenum's weight and length were significantly higher in 0.06% (T1), 0.12% (T2), and 0.18% (T3) than in the control group (T0; $P < 0.05$). The jejunum length in 0.06% (T1), 0.12% (T2), and 0.18% (T3) was significantly higher than in the control group (T0; $P < 0.05$). The ileal length and weight in 0.06% (T1), 0.12% (T2), and 0.18% (T3) were significantly higher than in the control group (T0; $P < 0.05$). The ileum had the longest length and the highest weight in 0.12% (T2) and 0.18% (T3; Table 4).

The increase in the length and relative weight of the small intestine, particularly in 0.12% (T2) and 0.18% (T3), generally correlated with an increase in villi height. Villi height probably enhanced the absorption surface area, resulting in more nutrient uptake, as observed in the increased intestinal weight and length. Ravindran and Abdollahi (2021) highlighted that absorbed nutrients, especially proteins, are essential for cell formation and the regeneration of epithelial cells in the intestine and different body tissues. Improved intestinal microbial balance, as reflected by an increase in LAB and a decrease in coliforms, was likely related to increases in the length and weight of each intestinal segment. Flavonoids might influence the gut ecosystem by promoting the growth of LAB, possibly suppressing some harmful microbes, and helping sustain a healthier balance of gut microbiota

(Rodsatian et al., 2023). Increased LAB populations improve mucosal health by lowering pathogenic pressure and inflammation. This promotes epithelial proliferation, leading to enhanced villus development and an expanded absorptive surface (Luo et al., 2024). Thus, the enhanced absorptive function supports the growth and development of intestinal tissues, as reflected in the increased relative lengths and weights of the duodenum, jejunum, and ileum observed during the present study.

While the highest numerical values were occasionally observed in 0.18% (T3), 0.12% (T2) consistently demonstrated substantial, statistically significant improvements in intestinal structure, microbial balance, and organ metrics, similar to those observed in 0.18% (T3) across most parameters. Accordingly, the 0.12% (T2) supplementation level can be considered the practical and effective optimal dose for promoting intestinal development in broiler chickens.

CONCLUSION

A positive response was observed with the inclusion of encapsulated noni fruit extract (ENFE) combined with Zn and Cu at 0.12%. This indicated an optimal inclusion level that substantially improved BWG, gut microbiota balance, and intestinal morphology in broiler chickens. Additionally, the higher ENFE level at 0.18% (T3) yielded statistically comparable results. Therefore, 0.12% (T2) is recommended as the most optimal level for commercial broiler production. Future studies should evaluate the economic feasibility of ENFE supplementation under commercial production conditions to confirm its cost-effectiveness.

DECLARATIONS

Acknowledgments

Vitus Dwi Yudianto and Mulyono provided significant assistance to the authors of the present study, and the authors express their profound gratitude for their support.

Authors' contributions

The study was conceptualized by Linda Nur Baetavianti, who also carried out data collection, data analysis, and manuscript drafting. Vitus Dwi Yudianto supervised the research process. Under the supervision of Mulyono, a manuscript was drafted and reviewed. The initial text was prepared by Lilik Krismiyanto, who also originated the idea and design of the study. All authors have read and approved the statistical analysis as well as the final edition of the manuscript.

Ethical considerations

All authors have reviewed the study for potential ethical concerns, including duplicate publication, redundancy, data fabrication or falsification, misconduct, or plagiarism. The authors originally wrote the article without seeking any help from available AI software.

Funding

The present study was conducted without external financial support.

Availability of data

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

Competing interests

The authors have declared that no competing interest exists.

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Poultry Production in Afghanistan: Trends, Challenges, and Future Prospects

Noor Aminullah^{1*}, Mohammad Naeem Azizi¹, and Mohammad Dawood Bawer², Obaidullah Mahaq³, and Mahboobullah Ahmadi¹

¹Department of Pri-Clinic, Faculty of Veterinary Science, Afghanistan National Agricultural Sciences and Technology University (ANASTU), Kandahar 3801, Afghanistan

²Department of Para-Clinic, Faculty of Veterinary Science, Afghanistan National Agricultural Sciences and Technology University (ANASTU), Kandahar 3801, Afghanistan

³Department of Animal Nutrition and Production, Faculty of Agriculture, Afghan International Islamic University (AIU), Kabul 1004, Afghanistan

*Corresponding author's E-mail: aminullah.noor18@gmail.com

Received: September 26, 2025, Revised: October 27, 2025, Accepted: November 30, 2025, Published: December 31, 2025



ABSTRACT

The demand for poultry products is steadily increasing globally as well as in Afghanistan, driven primarily by human population growth, economic development, and urbanisation. A clear understanding of Afghanistan's poultry sector is crucial for policy direction towards food security and rural development. The present study aimed to evaluate the current status of poultry farming and highlight the key challenges and future view of poultry production in Afghanistan. The study was synthesized from the available literature, government reports, and international datasets to provide a coherent overview of Afghanistan's poultry sector. Particular attention was given to identifying data inconsistencies and research gaps to guide evidence-based policies. The trend of investment in Afghanistan's poultry sector increased from 0.5 billion AFN (71.42 million USD) in 1986 to 200 billion AFN (2.85 billion USD) in 2022. Currently, a total of approximately 11,000 commercial poultry farms are operating across the country, which can produce about 219,000 metric tons (MT) of meat, while the total market demand is estimated as 295,000 MT in 2024. The available poultry meat in Afghanistan covers only approximately 46% of the World Health Organization's recommended rate per capita. However, the sector development is hindered by different factors such as traditional practices, poor infrastructure, lack of technical expertise, disease outbreaks, uncontrolled use of antimicrobial agents, poor input quality, and end-product price variation. The poultry sector's average annual growth rate in Afghanistan is estimated as 13.8%, and the future production capacity is projected to reach 400,332 MT by 2030. Poultry production in Afghanistan is gradually increasing, driven by growing market demand. However, the current production level still cannot meet the market demands in the country. The present findings contribute to a clearer understanding of poultry development priorities for national food security and sustainable growth in Afghanistan.

Keywords: Chicken, Market, Meat, Poultry production

INTRODUCTION

Globally, the overall meat production from animal sources was reported to be 400 million metric tons (MT) in 2023, representing a significant increase from 179.45 million MT in 1990 (Ritchie et al., 2023). The trend in meat production has reported a total annual growth of 3.58%, whereas poultry meat consumption shows a 5.18% increase per year (Memon et al., 2021). Poultry products, including meat and eggs, play a significant role in providing essential nutrients, such as energy, protein, amino acids, and minerals for human nutrition (Farrell,

2013). The global human population is projected to exceed 9.7 billion by 2050 (Berners-Lee et al., 2018). This upward trend is a direct factor that drives the rising demand for poultry products in the market, leading to increased poultry production at the farm level. It is reported that poultry production is the fastest-growing sub-sector compared to any other agricultural segment, particularly in developing countries, such as Afghanistan (World Bank, 2015). The poultry sector offers comparative benefits, including easier management, greater productivity, and a quicker return on investment

compared to other livestock industries (Azizi et al., 2021). In smallholders as well as in both rural and urban areas, the poultry sector plays a vital role in alleviating poverty and contributes to the families' income and nutrition (Bist et al., 2024).

The agricultural and livestock sectors are the cornerstone of Afghanistan's economy and social framework. The livestock production sector makes a significant contribution to poverty reduction and livelihood sustainability, providing job opportunities, particularly in the rural regions of developing countries such as Afghanistan (Azizi, 2024). Historically, the poultry sector in Afghanistan has been more traditional, characterized by small-scale operational units (Emal and Muhsni, 2017). Land-limited, low-income households often raise small livestock such as sheep, goats, and poultry for commercial purposes, as they require minimal initial investment and low operational costs (Muradi and Boz, 2018). The production potential of poultry native breeds, such as Golden, Naked Neck, and Watani, in Afghanistan remains very poor and has not significantly improved compared to the 1960s (Emal and Muhsni, 2017). Reports indicated that before 1963, the native chicken growth performance was as slow as 0.769 kg body weight at the age of four months (Sahota and Bhatti, 2003a) and produced about 30 eggs per year (Sahota and Bhatti, 2003b). However, the poultry sector is gradually shifting from a traditional backyard production system to more intensive and commercialized practices worldwide, as well as in Afghanistan (Wilson, 2021). Factors such as an improved economy and increasing urbanization trends among people are driving growth in the poultry sector (Zahir et al., 2024). Consequently, a significant portion of the protein required by humans is derived from poultry sources (FAO, 2023). However, several challenges hinder the development of poultry production in Afghanistan, including inadequate infrastructure, limited access to veterinary services, feed shortages, and ongoing socio-economic instability (Azizi, 2024).

A critical epidemiological situation, characterized by frequent disease outbreaks of viral infections such as Newcastle disease, infectious Bursal disease, and infectious bronchitis, posed serious challenges to production efficiency and farm performance in the poultry sector (Hafez and Attia, 2020). Market instability, price variation, and sector resilience with the international market are other serious challenges that the poultry production sector is facing. Ultimately, as Afghanistan moves towards economic recovery and agricultural commercialization and modernization, the poultry sector

stands as a promising resource for development and investment (World Bank, 2015). The availability of poultry products per capita, production capacity, demand, types of challenges, and potential impacts on the poultry sector, as well as future prospects, are issues that remain unclear. Despite growing attention, there is a lack of a comprehensive and updated synthesis that critically analyses the trends, constraints, and future prospects of poultry production in Afghanistan. Existing data are fragmented across different reports, creating uncertainty in national estimates and limiting policy formulation. Therefore, the present study aimed to discuss available information to assess the current status of poultry production in Afghanistan, identify key challenges and limitations, and explore future opportunities and policy directions for the sector development.

METHODOLOGY

To complete the narrative review study, the most relevant available literature was collected from international academic journals indexed in online databases such as Web of Science, Scopus, and PubMed using keywords such as poultry rearing practices, poultry meat production and market demand, challenges and requirements, and trends of investment in the poultry production sector in Afghanistan. The data and information were also collected from accredited and the most relevant international organizations, such as the Food and Agriculture Organization (FAO), the World Health Organization (WHO), the World Bank, Our World in Data, and the website of the Ministry of Agriculture, Irrigation, and Livestock (MAIL) of Afghanistan. An inclusive approach was employed, prioritizing relevance over strict exclusion criteria, given the limited peer-reviewed literature available on Afghanistan's poultry sector. To complement the literature, qualitative observations were drawn from the authors' professional experiments recorded during veterinary and field services in Afghanistan from 2016 to August 2025. These observations served to illustrate prevalent, widely acknowledged practices and challenges in contexts where published data remains limited.

THE CURRENT TRENDS OF POULTRY PRODUCTION IN AFGHANISTAN

The livestock sector contributed approximately 14% to the overall agricultural industry and provided around 3.3 million job opportunities in Afghanistan (World Bank, 2016). Meanwhile, the poultry subsector accounts for

about 4.81% of the agricultural sector and 9.84% of the livestock sector in the country (Khatami *et al.*, 2022). Therefore, the poultry subsector provided about 380,000 job opportunities across Afghanistan. The MAIL of Afghanistan reported that approximately 120,000 direct and 300,000 indirect employment opportunities were provided across the country in the poultry sector (MAIL, 2024). In Afghanistan, poultry farms are operated under two major rearing practices, primarily backyard and commercial poultry production (FAO, 2008; Siddiky, 2017). In some areas, such as semi-urban regions, production systems are primarily divided into three major components, including backyard poultry, semi-commercial, and commercial poultry production (Khatami *et al.*, 2022). The free-range backyard poultry production system is primarily employed in rural areas and plays a crucial role in generating income, providing family nutrition, creating job opportunities, empowering women, and alleviating rural poverty in impoverished communities (Siddiky, 2017).

Indigenous poultry breeds, such as the Golden, Naked neck, and Kulangi in Afghanistan, are well-adapted to local environmental conditions and have demonstrated superior resistance to prevalent diseases and fluctuating environmental factors (Siddiky, 2017). Nonetheless, the introduction of improved poultry breeds and biosecurity measures has the potential to enhance productivity and sustainability within the poultry production sector, significantly contributing to food security and supporting the nutritional status of the country's population (Davis and White, 2020). The poultry sector in the country is currently unable to meet local market demand effectively. According to the available report, the poultry products import rate followed the increasing trends of market demand in Afghanistan from 2003 to 2012 (World Bank, 2014). The import value of poultry products was estimated at 100 million dollars annually from neighboring countries and from distant countries such as Brazil and the USA in 2014 (Siddiky, 2017).

BACKYARD POULTRY PRODUCTION

The indigenous chicken breeds, along with backyard production systems, play a crucial dynamic role in many rural, impoverished, and marginalized communities, providing both income and nutritional support through high-quality protein and essential amino acid sources (FAO, 2008). Additionally, poultry production practices provide nutritional support to families, offering always-available fresh eggs and meat, which are essential components of a balanced diet for children and the elderly

(Ramasawmy, 2017). In Afghanistan's challenging socio-economic context, backyard poultry production represents a valuable strategy for poverty alleviation and building resilience within communities. The backyard poultry production system primarily utilizes local indigenous chicken breeds such as Watani, Golden, and Naked neck, employing scavenging feeding practices with limited production potential. However, the free-range production system using traditional practices is associated with several constraints, including disease outbreaks, feed shortages, feed imbalances, inadequate housing, and ineffective management systems (Emal and Muhsni, 2017). The poultry flock size in a free-range rearing system is usually small and varies depending on the investment capability, infrastructure, and production facilities. In a study conducted in the Paghman district of Kabul province, Afghanistan, the flock size ranged from 3 to 50 chickens, with an average number of 26 chickens, and a mean of 117 eggs produced per flock per week, indicating an average of 65% egg production in the poultry backyard system (Nicnam MM and Ghafari, 2020). A study reported that out of 12.5 million poultry, chickens are under rearing practices across the country in Afghanistan; the majority (99%) were raised under the backyard poultry production system from 2002 to 2004, in which women played a significant role, with an average of 5.87 chickens owned per family (FAO, 2008; Samadi, 2025).

COMMERCIAL POULTRY PRODUCTION

Commercial poultry production was initiated in Afghanistan when a new hybrid strain of chicken was introduced to the Badam Bagh farm in Kabul in 1963 (Hussain *et al.*, 2015). Thereafter, due to the country's conflicts, all the systems and strategies were demolished. However, over the last two decades, the poultry production sector has transitioned from a backyard to a more commercialized pattern, introducing remarkable developments. The poultry sector has expanded over time with increased investment and the establishment of additional broiler chicken and layer-rearing farms, hatcheries, and feed manufacturing units in Afghanistan (Zrawar *et al.*, 2023). The commercial poultry production sector employed approximately 82,000 people directly in 2018-2019 (Moore-Afghanistan, 2019). It is also reported that the poultry sector contributes 4.81% to agricultural growth and 9.84% to livestock sector growth. The investment rate in the poultry production sector tends to increase over time. The initial investment in the commercial poultry production system was estimated to be

5.0 billion AFN (71.42 million USD) in 1986, 20.0 billion AFN (285.71 million USD) in 1992, 57.0 billion AFN (814.3 million USD) in 2001 (Moore-Afghanistan, 2019), and 200 billion AFN (2.85 billion USD) in 2022 (Khatami et al., 2022). The investment background in the poultry sector is illustrated in Figure 1.

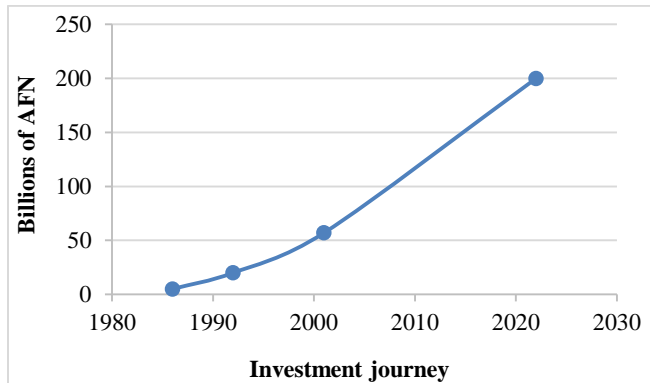


Figure 1. Investment trend in the poultry production sector in Afghanistan in local currency (Afghanis) during 1980-2025.

A total of 8,910 broiler chicken and 982-layer farms were actively operating across the country in 2015, in which about 56,155,778 chickens were estimated to be under rearing practices (CARD-F, 2016). The recent report indicated that a total of 11,000 broiler chicken and 233 layer poultry farms, 20 units of feed manufacturing plants, 28 units of hatcheries, and nine units of poultry meat processing plants are actively operating across the country in the poultry sector (Moore-Afghanistan, 2019). However, despite substantial investments in domestic poultry production, market demand remains unmet. The poultry sector was projected to meet approximately 70% of the country's meat and egg demand by 2024-2025, with an annual growth rate of 9-10% (CARD-F, 2016; Khatami et al., 2022). Comprehensive Agriculture and Rural Development-Facility (CARD-F) reported through an assessment that the market demand for poultry meat was 173,000 MT (11,529,276 chickens) per year in 2015 across Afghanistan. In contrast, Moore-Afghanistan (2019) reported a demand of 270,000 MT and 1.05 billion for poultry meat and eggs, respectively, in 2019, which was 57% higher than the estimate reported in 2015. The poultry meat production in the country was reported as 182,000 MT, and the total capacity was estimated to be 232,000 MT annually (Moore-Afghanistan, 2019), demonstrating the poultry sector's contribution of 67.4% to the overall poultry meat demand market. The future demand for poultry meat in the country was projected to cross 232,950 MT in 2024 (Moore-Afghanistan, 2019).

However, the recent production rate reported by MAIL (World Bank, 2016) of 219,000 MT was close to the value projected by CAED-F (CARD-F, 2016), whereas the total market demand was estimated to be 295,000 MT in 2024. Hence, the current production capacity can cover 74% of the country's poultry meat market demand, which is above the estimated 67.4% potential reported by Moore-Afghanistan (2019). It was reported that the poultry meat contribution to the total meat market in Afghanistan was 19% in 2018 and increased to 25.8% in 2022 (Khatami et al., 2022). The increasing demand for poultry meat is attributed to different factors, such as an improved economy, urbanization (WHO, 2020), reduced access to other animal protein sources, and a 2.7% annual human population growth in the country (World Bank, 2023). The total number of chickens under rearing practices, egg production, fertile eggs, day-old chicks (DoC), feed produced, market demand, and deficiency in number rate during 2014-2015 is presented in Table 1. The value of imported poultry meat increased from 1183 million AFN (16.9 million USD) to 5940 million AFN (85 million USD) from 2008 to 2012, while the value of imported eggs increased from 1225 million AFN (17.5 million USD) to 2072 million AFN (29.6 million USD; World Bank, 2023). The value of imported meat and eggs was reported to increase further to 183 USD million and 38 million USD, respectively, in 2019 (Moore-Afghanistan, 2019).

Table 1. Poultry under rearing practices, egg and day-old chickens' production, demand, and deficiency during 2014-2015, in Afghanistan

Component (Number/10,000)	National production	National demand	Deficiency
Broiler chickens	561.6	1,150.8	589.2
Table eggs	1,908.7	10,622.7	8,714.0
DoCs – broilers	122.4	1,211.4	1,089.0
DoCs -female layers	5.3	62.1	56.8
Fertile eggs -broiler	66.8	1,275.1	1,208.3
Fertile eggs - layer	5.8	130.8	125.0
Poultry feed- MT	1.3	6.7	5.4
Total fertile eggs	72.6	1,405.9	1,333.3
Total DoCs	127.7	1,273.5	1,145.8

Source: CARD-F (2016). DoCs: Day-old chicks, MT: Metric ton.

Despite the poultry production sector shifting from a backyard system to commercialization, as well as an increase in imported meat quantity over recent years, the availability of poultry meat and eggs per capita remains

deficient in Afghanistan. However, the average consumption of poultry meat is different among countries and nations. The global average consumption of poultry meat was estimated at 13.6 kg per capita, ranging from 1.77 kg in India to 43.81 kg in the United States in 2014 (Vaarst et al., 2015). In 2013, the average annual poultry meat and egg availability per capita in Pakistan was reported as 5 kg and 51 eggs, respectively (Hussain et al., 2015). In 2018, the average annual poultry meat consumption per capita in Afghanistan was reported to range from 8.79 kg to 6.5 kg (CARD-F, 2016; Our World in Data, 2018). The global meat consumption per capita was reported at 34.3 kg, of which poultry meat contributes 13.84 kg and is expected to increase to 14.69 kg by 2027 (Efremova, 2019). The data indicated that poultry meat could account for approximately 36% of the global meat market. According to the established WHO recommendation, a total of 27 g of protein per day per capita, equivalent to 102.3 g of fresh meat, is required to meet the optimum protein requirement in the human body (Hussain et al., 2015). Given Afghanistan's human population of 35 million, the total meat requirement was estimated at 1.306 million MT, of which 36% (470,477 MT) was derived from poultry meat (Bruinsma, 2017). The current poultry production in Afghanistan is estimated at 219,000 MT (MAIL, 2024), which can meet only 46.5% of the WHO-recommended standard requirement. Similar

data was presented by CARD-F (2016) and Moore-Afghanistan (2019) about poultry meat production in Afghanistan. Considering the poultry meat's contribution to the total meat market as 18.3% (FAO, 2024), the current poultry meat requirement can be calculated as 239,000 MT. In contrast, the total production reported by MAIL (MAIL, 2024) was 219,000 MT, which covered 92% of the country's requirement. No data is currently available regarding the value of poultry meat imports from the international market. The production level, market demand, and deficiency of poultry meat in Afghanistan are illustrated in Figure 2.

The available data on meat consumption per capita in Afghanistan varies. The FAO (2024) reported that the total annual meat consumption per capita in Afghanistan was 9.3 kg, out of which 1.7 kg is contributed from poultry meat. The data indicated that poultry meat occupies only 18.3% of total meat consumption. The availability of animal-origin protein in Afghanistan, per capita per day, was estimated at 10.2 g, while in Pakistan, the value was reported as 29.5 g, and in the USA, it was found to be 84.3 g (FAO, 2024). The majority of this protein can be sourced from meat, with a minor portion coming from eggs and milk. Therefore, the recommended consumption of red meat per capita would not be more than 98 g per week (WHO, 2023).

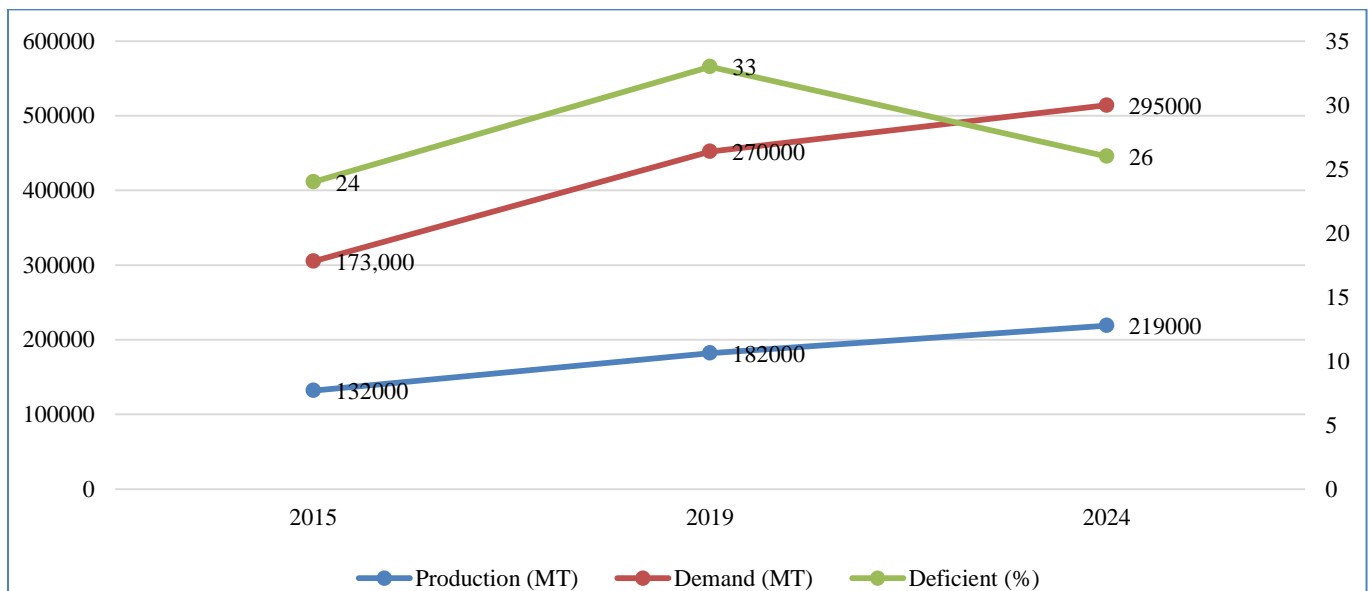


Figure 2. Production level, demand, and deficiency rate of poultry meat in Afghanistan from 2015 to 2019.

The primary challenge of the present study was the scarcity of reliable data on egg production and consumption in Afghanistan. However, the average egg

consumption per capita was reported to be 40 (number) per year (CARD-F, 2016), while this value in Pakistan was reported to be 70-80 eggs per capita. The volume imported

of eggs was reported as 812 million (in number) during the period of 2018-2019 (Moore-Afghanistan, 2019).

CHALLENGES

The poultry sector in Afghanistan is facing multiple challenges that hinder its development. The lack of sufficient literature and data on sectoral challenges poses a problem in preparing the current study. However, practical experience and observations from the field activities were minimally used here to enrich the study.

Traditional management practices

For preventing different infectious disease outbreaks, biosecurity practice is the most effective, economical, and applicable measure (Subasinghe et al., 2023). Despite this improvement, poultry raising practices in Afghanistan remain traditional, characterized by low management skills and limited knowledge, as reported by Abozar et al. (2021) and Samadi and Zadran (2023). The situation could directly affect poultry raising management, production performance, and the farming economy. A study conducted in Kabul province, Afghanistan, found that the majority of farmers were uneducated, and the biosecurity practices in most (50-70%) of the farming areas were evaluated as poor or very poor. According to the same report, approximately 27.66% of farmers have not implemented disinfection procedures for biosecurity. This lack of practice contributes to disease outbreaks, poor growth performance, higher mortality rates, and a weakened farming economy in poultry. The study conducted by Khatami et al. (2022) reported a higher mortality rate (11-20%) and economic losses during farming production cycles due to disease outbreaks, such as Newcastle disease, infectious bronchitis, and colibacillosis, as well as market price fluctuations in Afghanistan. This situation automatically leads to farmers' poor satisfaction with the farm's production performance (54%; Shaiq et al., 2024). Nonetheless, Faizy et al. (2022) and Samadi and Zadran (2023) have documented that poultry farms implement medium- to high-level biosecurity protocols, with approximately 98% engaging in vaccination practices, as evidenced throughout Kabul Province, Afghanistan. Additionally, Samadi and Zadran (2023) reported poor management practices, inadequate infrastructure, and suboptimal biosecurity measures in poultry farms in Kabul Province. Poor sanitation practices can have detrimental effects on meat and egg quality, potentially leading to the presence of foodborne pathogens such as *Clostridium perfringens*, *Salmonella enterica*, and

Escherichia coli, which significantly impact the poultry production sector in Afghanistan (Shaiq et al., 2024).

Poor infrastructure

The farm infrastructure plays a significant role in biosecurity and management practices, which can directly impact farm production performance and economy. Proper management and poultry welfare can be practiced only in a well-standardized infrastructure. Poor ventilation, which causes several health problems in poultry farms, has been reported in many farming systems due to inadequate infrastructure (Shaiq et al., 2024). Poor infrastructure was evident in several observations within the poultry production sector in Afghanistan (Nicnam and Ghafari, 2020). The poor infrastructure could directly impact the standardization of management in the farming system. A study conducted in Kabul province, Afghanistan, indicated that inappropriate farm structure and location lead to poor biosecurity measures, access of wild birds, availability of rodents, wild animals such as cats and dogs interfering with the red zone, and feed spoilage due to inappropriate storage and preservation in farming systems (Samadi and Zadran, 2023).

Lack of technical expertise

Field observations and practical experiences for the present study indicated that the poultry production sector was notably constrained by a lack of technical expertise, resulting in inadequate or poor-quality veterinary services, ineffective consultation, frequent disease outbreaks, and increased mortality rates. Consequently, these challenges contribute to higher veterinary input costs and negatively impact the overall economic viability of poultry farming. A study conducted in Baghlan province, Afghanistan, demonstrated that a lack of technical expertise, farmers' lower educational levels, poor ventilation provision, and local residency were recognized as highly important factors affecting farm development and sustainability (Shaiq et al., 2024). Zrawar et al. (2023) suggested that poor experience and low educational levels may be contributing factors to poor farming performance among farmers. The lack of testing laboratory facilities and diagnostic techniques contributes to the disease maintaining its epidemic flow.

Diseases outbreaks

According to the available literature and field experience, poultry diseases are the primary and most frequently occurring factor that severely hinders sector development in Afghanistan (Leslie et al., 2008; Sahab et

al., 2020; Kariithi *et al.*, 2021). Several reports indicated that some factors, such as rodents, wild birds, and poor biosecurity, are the key players contributing to infectious and zoonotic disease outbreaks among poultry and animals that can contaminate water and feed resources in farming systems (Meerburg and Kijlstra, 2007; Backhans and Fellström, 2012; Samadi and Zadran, 2023). Based on the clinical experiences and direct observations from the field during the present study, the frequently occurring viral diseases were infectious bursal disease, infectious bronchitis, Newcastle disease, and infectious laryngotracheitis. Bacterial infections, such as Colibacillosis, Salmonellosis, Enterobacteriosis, and Campylobacteriosis, commonly occur in broiler farms. *Mycoplasma* infections, such as chronic respiratory disease (CRD) and coccidiosis, are also primary and clinically important diseases (Sangary and Mohmand, 2020; Bawer *et al.*, 2025). All the diseases could maintain their epidemiological status for a long period and significantly affect farming performance.

Uncontrolled use of antimicrobial agents

Antimicrobials are primarily used for therapeutic purposes, as well as at subtherapeutic doses, as growth-promoting agents in the livestock and poultry production sector (Azizi *et al.*, 2024; Aminullah *et al.*, 2025). The continuous and uncontrolled use of antibiotics, particularly as growth-promoting agents, results in the development of antimicrobial resistance, which poses significant risks to both animal and human health (Mulchandani *et al.*, 2023; Aminullah *et al.*, 2025). A study conducted in 2024 by Danish (2024) evaluated antibiotic use in commercial broiler chicken farms in Kandahar Province, Afghanistan. Danish (2024) demonstrated that a total of 19 types of antibiotics are used in commercial poultry farms for three primary purposes, including disease treatment, prevention, and growth promotion in broiler chickens. The antibiotics that were most commonly used were colistin (78%), amoxicillin (97%), tylosin (83%), and enrofloxacin (100%). Additionally, Danish (2024) reported a total of 4.2 g of antibiotics per chicken from day one to market supply, without consulting a veterinary expert. This phenomenon can lead to the development of microbial-resistant strains, posing another challenge in the poultry production sector. Evidence from clinical experience during the present study demonstrated that most antibiotics at the established recommended dosage were insufficient to control the bacterial infection, indicating microbial resistance. Apart from antimicrobials, the use of minerals as growth promoters, such as copper, in large quantities in

poultry production and their excretion, which is highly toxic, poses another global challenge to animal and human health and to the environment (Aminullah *et al.*, 2022; 2023; Noor *et al.*, 2023).

Quality issues in feed and veterinary products

The quality of poultry feed and veterinary products in Afghanistan has been a significant concern, adversely impacting the poultry production sector. Inadequate regulations and limited access to high-quality, certified ingredient sources have led to substandard feed formulations, compromising poultry productivity (Faizy *et al.*, 2022). The challenge of poultry feed and veterinary products quality in Afghanistan was reported through an importance-performance analysis study conducted in Baghlan province, Afghanistan (Abozar *et al.*, 2021). Additionally, the improper and unsafe storage of feed and feed ingredients leads to fungal infestation and mycotoxin production (Ochieng *et al.*, 2021). Poor-quality veterinary input can further exacerbate the situation, leading to inappropriate treatments, increased disease susceptibility, and reduced productivity of the chickens (Sherman, 2005). The unavailability of technical experts, research and testing laboratories, and infrastructure are challenging factors that influence the quality evaluation of feed, as well as medicines and vaccines. A study conducted by Abozar *et al.* (2021) in Kunduz, Afghanistan, revealed significantly lower productive performance in hens fed a diet produced by local companies compared to those fed imported diets from international markets.

Price variations of final products

Poultry products, particularly meat, are highly susceptible to market price fluctuations compared to other commodities and agricultural products. Factors such as fluctuations in poultry production, seasonal and climatic variations, and human behaviors may account for the fluctuations observed in market prices (Upton, 2007). For instance, the price of DoC during the hot season, from May to August, is the lowest. During these months, the environmental temperature in Afghanistan is higher, particularly in the western and southern regions of the country. The environmental temperature in the southern region from May to August ranges from 40 to 48 °C, which is extremely hot and can significantly affect the poultry production performance (Wasti *et al.*, 2020). During May to August, an increase in the outbreak of infectious diseases leads to higher mortality rates among chickens, while the demand for poultry meat in the market has also decreased. In the central, eastern, and northern

regions of Afghanistan, the cold season typically begins in September and lasts until May. During the cold months, particularly in winter, chicken brooding costs increase due to the need for farm temperature maintenance, resulting in reduced poultry production levels. The increased production cost may be attributed to the lack of or unavailability of energy sources to maintain the farms' temperature at an optimum level. Other factors include variations in the poultry meat consumption ratio compared to other meats, which depend on seasonal and social behaviors, as well as insufficient domestic supply and reliance on the international market, which can lead to price variations in the poultry meat market. The Pakistani market plays a significant role in the poultry market in Afghanistan, which is also prone to seasonal price fluctuations (Hussain et al., 2015); therefore, these variations directly impact poultry market prices in Afghanistan.

Farming production performance

Poultry farming production performance can be influenced by different factors, including management style, animal welfare, nutrition, and technology (Ben Sassi et al., 2016). There is no reputable published literature available to address the most predominant factors and their impact on poultry farming. A study conducted by Zrawar et al. (2023) in Parwan Province, Afghanistan, reported a feed conversion ratio of 1.83, with 90.23% livability, an average market weight of 1.9 kg, and a mortality rate of 9.77%. From the production economy perspective, feed, DoC, and veterinary services expenses accounted for 70.45%, 6.98%, and 3.44%, respectively, of the total production cost (Zrawar et al., 2023). The cost-benefit ratio was reported to be 1:0.07, while Samadi and Zadran (2023) reported a value of 1:0.48.

FUTURE PROSPECTS

The demand for animal-origin protein is increasing, driving rapid growth in the global agriculture sector. It has been indicated that demand is shifting from red to white sources (lower cholesterol), such as poultry meat (Our World in Data, 2018). On the other hand, poultry production is a result of the expansion of fast-food chains, consumer preferences, competitiveness, and concerns over the safety of red meat (Charles, 2013). Globally, the religious motivation for consuming poultry meat is another factor driving higher demand in the market. The increasing demand for poultry products (Meat and eggs) in Afghanistan was being regulated by the growing

population (World Bank, 2023), rising household incomes, and urbanization (WHO, 2020). This increased demand for poultry products was attributed to the rising trend, from an initial investment of 5.0 billion AFN (71.42 million USD) in 1986 to 200 billion AFN (2.85 billion USD) in 2022 (Khatami et al., 2022). According to the World Bank (2023), the Afghan human population is growing at a rate of 2.7% per year, while the poultry sector was estimated to grow between 15% and 20% (Hafez and Attia, 2020). Another study reported a 400% increase in poultry growth rate from 1990 to 2019, with an average annual growth rate of 13.8% (OECD, 2017). As the current poultry meat production was reported to be 219,000 MT (MAIL, 2024), with an average annual growth rate of 13.8%, the projected production was calculated to be 400,332 MT by 2030.

Field studies and lectures indicated that Afghanistan has potential sources for poultry production, such as natural resources and growing demand for poultry products within its borders (Samadi, 2025). As Afghanistan continues to navigate its socio-economic challenges, the poultry sector presents an opportunity to enhance food security, generate employment opportunities, play a crucial role in the national economy, and foster rural development (Wong et al., 2017). In the current context of the country, particularly for the poultry backyard system in both rural and urban areas, poultry is a major asset and key to providing food security, economic income, poverty alleviation, and market participation (Mottet and Tempio, 2017). Furthermore, Afghanistan's diverse climate and agricultural landscape provided suitable conditions for poultry farming. The country's vast rural areas are conducive to both small-scale and commercial poultry operations (Samadi, 2025). The available resources not only preserve local biodiversity but also empower farmers through improved methodology practices and quality services. Investment in education and training for farmers is critical to the success of the poultry production sector.

In addition, collaboration with international organizations and agricultural experts can facilitate the transfer of knowledge and improved access to high-quality feed and veterinary services (Sennuga et al., 2022). A similar strategy can also be adopted in the Afghan circumstance. With the right investments in infrastructure and technology, the poultry industry not only meets national market demand but also can be exported to neighboring countries. By aligning investment strategies with local requirements and establishing a robust support system for farmers,

Afghanistan can transform its poultry industry into a sustainable source of nutrition and economic opportunity (Azizi, 2024). The adoption of modern feed strategies, including postbiotics and par-probiotics, could further improve poultry health and productivity (Danladi *et al.*, 2022a, 2022b). As Afghanistan strives for stability and prosperity, the advancement of poultry production stands out as a pivotal component of its agricultural strategy, as revealed by the Afghanistan National Development Strategy (ANDS, 2005). The development of the poultry sector has a significant impact on the environment and is a large consumer of natural resources.

CONCLUSION

The present study is among the first to comprehensively document and contextualize the poultry production sector in Afghanistan, providing an evidence-based study of its current status, key challenges, and potential future opportunities. The demand for poultry products in Afghanistan continues to increase, primarily due to factors such as economic growth and urbanization. The investment rate in the commercial poultry production system was estimated as 200 billion AFN (2.85 billion USD) in 2022. There are over 11,000 broiler poultry farms actively operating across the country, supplying approximately 219,000 MT of meat to the market, while the total demand was estimated at 295,000 MT in 2024. The available consumption per capita in Afghanistan is estimated to be 6.5 kg, which meets only 46% of the WHO recommendations. Different factors, including traditional management practices, inadequate infrastructure, a lack of technical expertise, disease outbreaks, misuse of antimicrobials, poor-quality veterinary input, and fluctuating end-product prices, hinder the sector's development and production performance. Strengthening the poultry sector with modern technologies and professional farming practices is essential for building long-term resilience in Afghanistan. With strategic interventions, the country has the potential to achieve an estimated poultry meat production capacity of 400,332 MT by 2030. Moreover, enhancing farmers' capacities in key aspects such as biosecurity, nutrition, and veterinary management plays a critical role in minimizing disease outbreaks and improving overall production efficiency. Future investigations should focus on refining knowledge of production dynamics, health management practices, and market performance across Afghanistan's poultry industry.

DECLARATIONS

Acknowledgments

The authors express their appreciation to the Faculty of Veterinary Science at Afghanistan National Agricultural Science and Technology University (ANASTU), Afghanistan, for their authorized and ethical assistance in conducting the present study.

Authors' contributions

Aminullah Noor and Mohammad Naeem Azizi conceptualized and drafted the study. Aminullah Noor, Mohammad Naeem Azizi, Mohammad Dawood Bawer, Obaidullah Mahaq, and Mahboobullah Ahmadi contributed to the review and editing of the manuscript. All authors reviewed and approved the final edition of the manuscript.

Competing of interests

The authors declared 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. The authors confirmed they have not assisted the AI in conducting the present study.

Availability of data and materials

No new data were generated or analyzed in the present study. All information presented was based on previously published sources, which are cited accordingly.

Funding

The authors received no funding for the present study.

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The Integration of Artificial Intelligence and Internet of Things in Ventilation Systems of Closed Houses in Broiler Chicken Farms of Indonesia: A Literature Review

Muhammad Irfan Maulana*  and Indrawati Yudha Asmara 

Faculty of Animal Husbandry, Padjadjaran University, Sumedang, 54363, Indonesia

*Corresponding author's E-mail: muhammad20125@mail.unpad.ac.id

Received: September 28, 2025, Revised: October 26, 2025, Accepted: November 22, 2025, Published: December 31, 2025



ABSTRACT

The closed house system has been widely adopted in Indonesia due to its ability to control the microclimate; however, its implementation still faces several challenges, including high investment costs, limited monitoring, and maintenance management that is not yet adaptive. This literature review aimed to analyse the design of Artificial Intelligence (AI) and Internet of Things (IoT) integration in the automatic ventilation control system of broiler chicken closed houses in Indonesia. The method employed is a systematic review of relevant international and national articles. The literature review followed PRISMA guidelines, identifying 28,827 publications on AI and IoT, filtered to 636 studies on poultry and 335 studies on AI-IoT-based monitoring systems. Ultimately, 98 articles met the inclusion criteria, including 20 studies specifically focused on studies in Indonesia. The findings indicated that AI-IoT integration has the potential to improve energy efficiency, optimize the microclimate, such as temperature, humidity, velocity, and support broiler chicken welfare through data-driven monitoring and automated decision-making systems. Nevertheless, the adoption of this technology continues to face challenges such as high initial costs, limited energy and internet infrastructure, and the digital skills gap among farmers.

Keywords: Adaptive ventilation, Energy-efficient, Microclimate, Poultry production, Smart farming, Tropical climate

INTRODUCTION

Broiler chicken production plays a vital role in ensuring global food security, contributing more than one-third of total meat consumption worldwide due to its efficiency and affordability. In Indonesia, poultry meat, particularly broilers, is the main source of animal protein, with production exceeding 717 thousand tons per year ([Center for Agricultural Data and Information System, 2024](#)). The industry's rapid growth reflects both global and domestic demand trends, yet maintaining productivity and welfare under Indonesia's tropical conditions remains a significant challenge. Consequently, technological innovation in climate control and housing systems has become essential to sustain growth and competitiveness.

The modern broiler farming industry increasingly adopts closed house systems, which minimize adverse environmental effects and climate fluctuations, creating a

thermoneutral zone optimal for growth and health ([Pakage et al., 2020](#)). This system automatically regulates temperature, humidity, air velocity, and air quality (O₂, CO₂, NH₃) according to the broiler chicken requirements ([Syahririni et al., 2020](#)). Compared with open houses, closed houses reduce disease risks, improve growth efficiency, and enhance feed conversion ([Abd-El Hamed et al., 2025](#)).

Broiler chickens require proper environmental conditions throughout growth phases, making ventilation management crucial ([Saner and Shekhawat, 2022](#)). Poor ventilation causes stress, reduced feed intake, growth issues, and even mortality ([Tainika et al., 2023](#)). Most ventilation systems regulated by climate control in closed houses primarily focus on desired temperature and humidity levels ([Setiadi et al. 2018](#)). However, to create more appropriate conditions, other factors should also be considered, such as air velocity, external temperature,

humidity, and ventilation needs based on the actual condition of the chickens, including age, stocking density, body weight, harmful gas concentrations, and microclimate distribution within the house (Curi et al., 2017; Syahririni et al., 2020). Thus, recalibration of systems is needed to align microclimates with animal comfort (Detsch et al., 2018). In this context, automation becomes essential for precise regulation and quick adaptation to environmental changes (Detsch et al., 2018).

Technological advances have introduced the Internet of Things (IoT) in livestock housing for real-time condition monitoring (Umaphathi et al., 2025). The IoT enables data collection from sensors tracking temperature, humidity, and air quality, accessible to farmers for enhanced decisions (Debauche, 2020; Jebari et al., 2023). Integration with Artificial Intelligence (AI) allows analysis of complex datasets, pattern recognition, and automated decision-making for ventilation (Yang et al., 2019; Debauche, 2020). Combined AI and IoT can optimize closed house management in real time, improving both productivity and animal welfare; however, adoption in Indonesia faces challenges. The tropical climate with high temperatures, unpredictable weather, and regional variation limits ventilation system effectiveness (Oke et al., 2024). Economic and infrastructural factors also play major roles in modern farming. Modern closed houses require large investments, rural areas often lack stable electricity and internet, and many farmers lack skills in operating automated systems.

Although studies on closed houses, IoT, and AI exist, most emphasize condition monitoring, fuzzy logic-based temperature–humidity control, or mathematical ventilation modelling (Husein and Kharisma, 2020; Saner and Shekhawat, 2022). Few studies have examined the comprehensive integration of AI and IoT for adaptive climate control systems specifically designed for Indonesia's tropical conditions, particularly regarding environmental fluctuations, microclimate distribution, infrastructure limitations, and farmer capacity gaps. Therefore, this article aimed to present an automated ventilation control design integrating climate control with AI and IoT in broiler chicken farms of Indonesia.

MATERIALS AND METHODS

This study employed a systematic literature review method to examine the integration of AI and IoT in ventilation systems of closed-house broiler farms in Indonesia. Literature was collected from international and national databases, including Scopus, ScienceDirect, Google

Scholar, and SINTA, using keywords such as “Closed House Poultry Ventilation”, “Artificial Intelligence in Broiler Farming”, “Internet of Things (IoT) in Poultry”, “Smart Poultry Farming”, and “Climate Control” in Tropical Poultry Houses.

The review process followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines to ensure transparency and reproducibility. An initial search identified 28,827 publications related to AI and IoT applications. After applying domain filters, 636 studies focused on poultry, and 335 studies discussed monitoring systems using AI–IoT integration. Following screening based on relevance, duplication, and data completeness, 98 studies were deemed eligible for inclusion in this review, and 20 very specific studies were relevant to the application of AI and IoT in Indonesia.

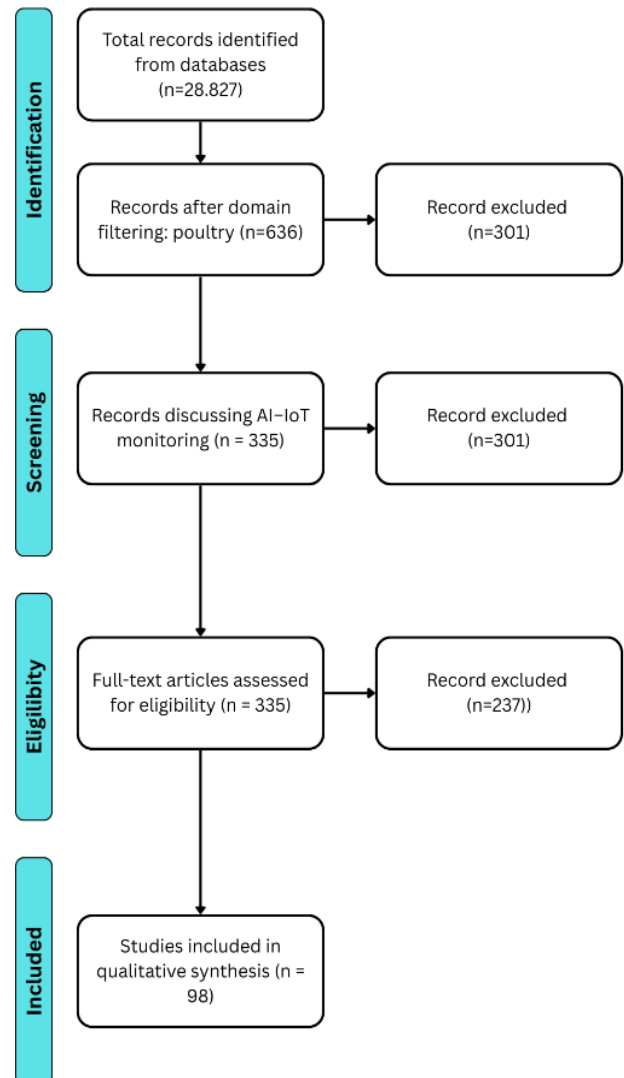


Figure 1. The PRISMA flow diagram for choosing the articles in this study

Inclusion criteria comprised peer-reviewed articles, conference proceedings, and reports published between 2014 and 2024 that addressed AI–IoT integration, ventilation, or microclimate control in broiler production. Exclusion criteria removed non-scientific publications, popular articles, and studies outside the poultry domain. Screening was performed in three stages: title review, abstract review, and full-text assessment.

The selected articles were analysed qualitatively by extracting information on objectives, methods, findings, and limitations. Following the screening process, the selected studies were systematically analysed and grouped into several thematic domains related to closed-house ventilation systems, IoT integration, AI applications, tropical climate challenges, and implementation perspectives in Indonesia. During this stage, findings from each theme were qualitatively synthesized to identify prevailing study trends, existing knowledge gaps, and potential innovation opportunities for the development of AI–IoT-based adaptive ventilation systems in Indonesian broiler production.

OVERVIEW OF LITERATURE SELECTION

Among the 98 studies included, 20 were identified as highly relevant to the application of AI and IoT in Indonesia's closed-house broiler systems. Overall, these studies highlight that integrating both technologies enhances energy efficiency, stabilizes the microclimate, and improves broiler welfare through real-time, data-driven automation.

Early Indonesian studies applied IoT-based temperature and humidity sensors (DHT22) integrated with ESP32 or Raspberry Pi microcontrollers for microclimate monitoring (Fathurohman *et al.*, 2023; Tambunan and Apryanto, 2024). These systems achieved over 90% accuracy at approximately 10–15% of the cost of commercial systems. Nalendra and Waspada (2021) added automatic fan speed control using Pulse Width Modulation (PWM), while Utomo *et al.* (2019) emphasized the need to integrate ventilation with automated feeding systems.

A study by Husein and Kharisma (2020) and Rosmasari *et al.* (2025) utilized AI-based fuzzy logic and naïve Bayes Gaussian algorithms for real-time classification of housing conditions. These models detected environmental anomalies and adjusted ventilation automatically. Fahrurrozi *et al.* (2024) and Safputra *et al.* (2023) integrated load-cell sensors and IoT-based feeder controls, improving feed efficiency and reducing mortality rates. From a networking perspective, Fathurohman *et al.*

(2023) and Hambali *et al.* (2020) demonstrated the importance of wireless sensor networks for reliable data transmission. Wicaksono *et al.* (2017) pioneered the use of wireless sensor-based temperature control, while Liani *et al.* (2021) combined LoRaWAN and fuzzy logic to dynamically adjust ventilation according to broiler growth stages.

In terms of AI, Ribeiro *et al.* (2019), Kiruthika *et al.* (2024), and Reddy *et al.* (2024) reported that Artificial Neural Network (ANN) and Support Vector Machine (SVM) models were most effective for temperature–humidity prediction, achieving less than 5% error rates. Barsagadea *et al.* (2024) and Bharanishree *et al.* (2025) developed machine learning-based climate control systems that reduced heat stress by up to 25%. Collectively, these findings suggest that AI–IoT adoption in Indonesia has advanced to experimental and semi-commercial stages, emphasizing tropical adaptation, cost efficiency, and infrastructure constraints. The main challenges include unstable electricity and internet access in rural areas and limited digital literacy among farmers. Nonetheless, current study directions point toward machine-learning-driven adaptive ventilation systems capable of adjusting to environmental and behavioral variations in real time.

THEMATIC SYNTHESIS OF FINDINGS

Closed house systems and implementation challenges

In closed house systems, two main ventilation types are used, such as cross ventilation and tunnel ventilation (Figure 2). The types of ventilation differ in airflow direction and distribution, which influence microclimate control. Cross ventilation moves air horizontally through sidewalls, using large exhaust fans on one side and window-like inlets on the opposite (Ghasemi *et al.*, 2025). This negative-pressure design distributes air across the house and works well in small to medium houses, but in longer houses it creates circulation inefficiencies or “dead zones” (Bustamante *et al.*, 2013; Ghasemi *et al.*, 2025). Cross ventilation also produces relatively low air velocity (0.60 ± 0.56 m/s to 0.64 ± 0.54 m/s), making it less effective against heat stress in summer (Wheeler *et al.*, 2003; Bustamante *et al.*, 2013). Tunnel ventilation moves air longitudinally from front to back under negative pressure created by high-capacity exhaust fans. It is characteristic design includes multiple exhaust fans arranged at one end of the house, while the opposite end is typically equipped with cooling pads serving as inlets (Du

et al., 2019). This setup draws cool air through the pads, distributing it rapidly along the house. Tunnel ventilation significantly reduces heat stress by increasing air velocity, which enhances sensible heat loss from the birds (Dozier et al., 2006).

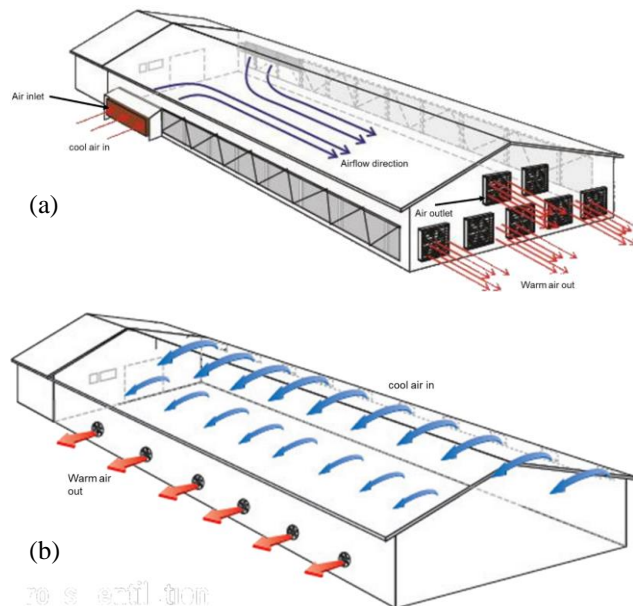


Figure 2. Schematic comparison of tunnel (a) and cross (b) ventilation in closed house systems. Source: Jongbo (2020).

In advanced broiler farming, closed house ventilation emphasizes energy efficiency, productivity, and animal welfare (Sans et al., 2021). Subtropical climates with sharp diurnal and seasonal variations require precise microclimate control (Al-Chalabi et al., 2017). Studies showed negative-pressure systems can regulate temperature and humidity in winter, though uneven heat distribution persists (Al-Chalabi et al., 2017). These systems are designed to expel harmful gases like ammonia and carbon dioxide, maintaining concentrations below harmful thresholds (Costantino et al., 2020).

Ventilation in subtropical countries faces seasonal challenges, such as winter, which must increase heating needs, and while summer demands efficient cooling to maintain the performance of the broiler. Developed countries have applied automation integrating exhaust fans, heaters, and cooling pads, digitally managed via IoT platforms for real-time monitoring and adjustments, improving stability and energy efficiency year-round (Oliveira et al., 2024). Additional innovations include thermal insulation, Computational Fluid Dynamics (CFD) supported airflow design, and improved tunnel/sidewall management (Saraz et al., 2017; Küçüktopçu et al., 2024).

Subtropical regions require dynamic strategies to handle moderate but fluctuating climates (Afeez et al., 2019).

In contrast to tropical systems, subtropical systems focus on mitigating variable heat stress with proactive control. IoT applications typically employ fewer but strategically placed sensors, while CFD modelling plays a central role in optimizing airflow, ventilation efficiency, and sensor placement (Chen et al., 2021). Techniques such as Renormalization Group k-epsilon (RNG k-ε) and Large Eddy Simulation (LES) improve air exchange, reduce blind spots, and guide optimal fan and cooling pad layouts to address seasonal variability (Lee et al., 2007; Pourvosoghi et al., 2018).

Closed house systems in Indonesia

In Indonesia, closed house systems typically use tunnel ventilation combined with evaporative cooling pads (Lillahulhaq et al., 2024). These pads lower incoming air temperature through water evaporation, highly relevant to the hot tropical climate. Studies showed this combination maintains indoor temperatures 3–5°C below the outside environment while keeping humidity at safe levels (Xin et al., 2017; Saner and Shekhawat, 2022). The working mechanism of evaporative cooling pads is illustrated in Figure 3.

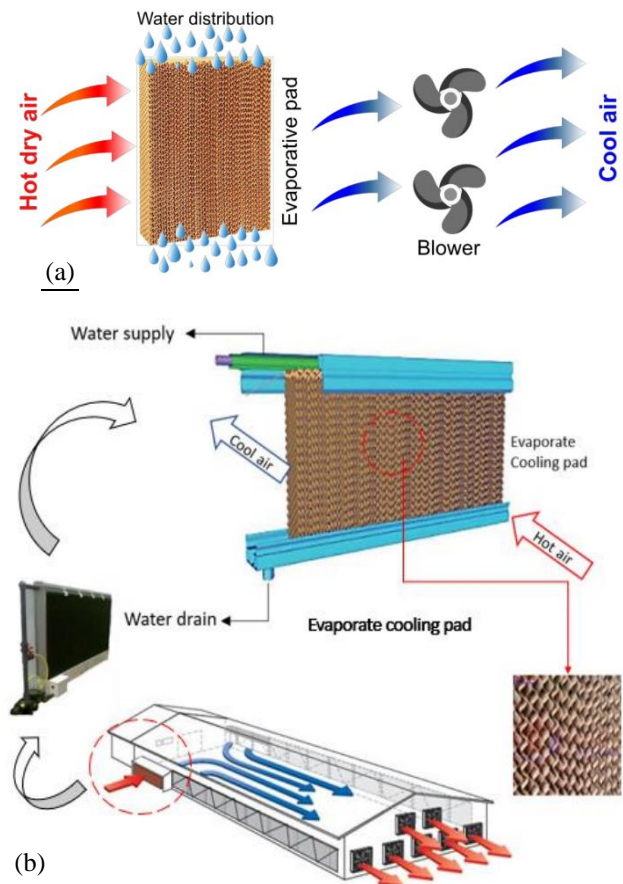


Figure 3. Working mechanism of evaporative cooling pads in closed house systems. Sources: (a) Shahzad et al., 2021; (b) Lillahulhaq et al., 2024.

This system uses porous pads continuously supplied with water, ensuring they remain moist (Shahzad *et al.*, 2021). When hot external air is drawn by exhaust fans through the wet pad surface, water flows downward via gravity and capillary action (Laknizi *et al.*, 2019). As the warm air passes through, part of its heat evaporates the water. Continuous evaporation (adiabatic process) produces a cooling effect until saturation, where air enthalpy remains constant while humidity increases (Mahmood *et al.*, 2016). This system uses exhaust fans to create a negative pressure inside the broiler house, which pulls cooler air from outside into the house. This method is effective in distributing air evenly and maintaining a comfortable temperature for the broilers (Setiadi *et al.*, 2018).

Despite its effectiveness, Indonesia's tropical climate presents challenges. During the rainy season, humidity often exceeds 80%, reducing ventilation efficiency and degrading litter quality. Wet litter encourages bacterial and fungal growth, increasing risks of respiratory diseases and skin problems such as dermatitis and hock burns (Kaukonen *et al.*, 2016). High humidity also accelerates the increase in ammonia (NH₃) levels, damaging the respiratory tract, lowering feed intake, and impairing growth (Beker *et al.*, 2004; Swelum, 2021). The negative effects of this situation can be minimized by integrating IoT into the cage ventilation system. Harrouz *et al.* (2021) note that in hot, humid climates, hybrid systems combining evaporative cooling with IoT-controlled dehumidifiers are more effective. Conversely, in the dry season in Indonesia, daytime temperatures often exceed 34–36°C, causing heat stress manifested as panting, reduced feed intake, and acid–base imbalance (Wasti *et al.*, 2020). Prolonged stress worsens feed conversion, weakens immunity, and increases mortality (Abo-Al-Ela *et al.*, 2021). Internet of Things (IoT) integrated ventilation systems can minimize hot ambient temperatures to enhance climate control, automating fans and heaters for optimal conditions (Afeeze *et al.*, 2019).

These challenges showed that closed houses require smarter, more adaptive technologies. Integrating AI with IoT offers a promising solution through real-time climate monitoring, AI-based pattern recognition, and automatic ventilation adjustments based on environmental conditions and actual broiler chicken needs (Debauche *et al.*, 2020). International studies confirm AI–IoT integration improves energy efficiency, reduces heat stress, and enhances welfare via precise ventilation control (Jabade *et al.*, 2024; Chen *et al.*, 2021).

Internet of things and artificial intelligence

The Internet of Things integrates physical components and sensing devices into an internet-based network that supports real-time data gathering and analytical processing (Nuanmeesri and Poomhiran, 2020). It is defined as a system of interdependent devices, objects, and individuals

with unique identifiers that transfer data across networks without requiring direct human interaction (Jebari, 2023). The IoT has significantly impacted livestock farming, especially broiler production, by improving resource management and environmental monitoring (Teng, 2015; Adli *et al.*, 2025).

Internet of Things applications in poultry farming allow farmers to collect data from various sensors installed in the house, including temperature, humidity, microclimate components, as well as animal health and welfare status (Jabade *et al.*, 2024). These sensors can measure parameters such as air quality, temperature, humidity, and animal activity (Yang, 2019). The data collected are then transmitted to an internet-connected digital platform, where farmers can access and analyse information in real time (Husein and Kharisma, 2020). Several studies have been conducted on IoT integration in broiler farming, as summarized in Table 1.

Integrated IoT–AI microclimate monitoring systems on closed house broiler cages are designed to stabilize environmental parameters. Sensor networks typically include temperature–humidity devices (DHT11, DHT22, DS18B20, BME280), gas sensors (MQ-135, MQ-137), and photodiode-based light sensors (Pereira *et al.*, 2020; Fathurohman *et al.*, 2023). These are connected to microcontrollers such as ESP8266 or Raspberry Pi for cost-effective monitoring. IoT prototypes achieve correlations above 0.90 with commercial devices at ~13% of the cost (Pereira *et al.*, 2020; Tambunan and Apryanto, 2024).

Sensor placement is guided by Computational Fluid Dynamics (CFD) simulations to map airflow, temperature, and humidity, identifying heat or moisture accumulation zones that could induce heat stress in broilers (Drewry *et al.*, 2017). Sensors are then positioned at floor, midsection, and ceiling levels (Saraz *et al.*, 2017; Faridah *et al.*, 2021; Küçüktopçu *et al.*, 2024). Field validation is conducted to ensure consistency between CFD models and real conditions (Heymsfield *et al.*, 2018). Advanced methods such as clustering, Standardized Euclidean Distance (SED), and Geographic Information System (GIS) optimize spatial distribution to detect microclimate heterogeneity (Trane *et al.*, 2023; Zanchi *et al.*, 2024).

Collected data undergo multi-sensor fusion using methods such as the Kalman Filter for dynamic integration and convex optimization to exclude faulty data (Zhang *et al.*, 2014; Sarbishei *et al.*, 2013). AI models such as Artificial Neural Networks (ANN) and Support Vector Machines (SVM) estimate gas concentrations, enhancing air quality assessments (De Vito *et al.*, 2007). Edge

computing devices (Raspberry Pi, Jetson Nano, ESP32) locally process data to reduce latency, lower cloud costs, and maintain reliability during internet disruptions (Li et al., 2021; Priyanka et al., 2023). Edge computing is

supported by microservices architecture with containerization, enabling lightweight, flexible, and scalable AI model deployment (Al-Doghman et al., 2023; Araújo et al., 2024).

Table 1. Studies on Internet of Things and artificial intelligence integration in broiler chicken farming in Indonesia

Author(s)	Year	Technologies and tools	Parameters observed	Key findings
Wicaksono et al.	(2017)	IoT, WSN, Xbee	Temperature, Humidity	The system maintained a heat index of 25°C
Hambali et al.	(2020)	IoT, WSN	Temperature, Humidity, Air Quality, Feed Feeder	The prototype reduced the mortality rate, provided automatic notifications
Revanth et al.	(2021)	IoT, Temperature, Humidity, Air Quality, Light Intensity, Litter Moisture Sensors	Temperature, Humidity, NH ₃ , Air Quality, Light Intensity, Litter Moisture	IoT-based poultry house environmental monitoring system
Liani et al.	(2021)	IoT, LoRaWAN, DHT22, Fuzzy Logic	Temperature, Humidity	Monitoring system adjusted to broiler growth stages
Ibrahima and Cissé	(2022)	IoT, DHT22, MQ137, Arduino Nano IoT 33	Temperature, Humidity, NH ₃	Data stored and displayed on a cloud-based dashboard
Safputra et al.	(2023)	ESP32, DHT22, RTC Module, Load Cell Sensor	Temperature, Humidity, Feed	Accurate monitoring and parameter control with set points
Fathurohman et al.	(2023)	IoT, DHT22, MQ-135, Anemometer, NRF24L01, ESP32, ATmega328P	Temperature, Humidity, NH ₃ , Wind Speed	Wireless transmission of microclimate data in real-time
Jebari et al.	(2023)	IoT, AI, Edge Computing, E-GRU	Temperature, Humidity, NH ₃ , CO, CO ₂ , CH ₄ , H ₂ S	Modular system enabling accurate data collection and environmental prediction
Sukri et al.	(2023)	IoT, K-Nearest Neighbour, Temperature and Humidity Sensors	Temperature, Humidity	The system effectively measured temperature, humidity, and distance for feed efficiency
Lashari et al.	(2023)	IoT, Temperature, Humidity, O ₂ , CO ₂ , CO, NH ₃ Sensors	Temperature, Humidity, O ₂ , CO ₂ , CO, NH ₃	Successfully maintained optimal climate and monitored harmful gases
Ghandi	(2023)	IoT, ARM Cortex M3 – LPC1769, LoRa, Jetson Nano	Temperature, Humidity	Achieved 99.72% accuracy in environmental condition classification
Jabade et al.	(2024)	IoT, DHT11	Temperature, Humidity	Alerts are sent to the smartphone when parameters exceed thresholds
Kiruthika	(2024)	IoT, SVM	Temperature, Humidity, Feeding, Disease	The SVM model predicted broiler growth with 90% accuracy
Reddy et al.	(2024)	IoT, CNN, Temperature, Humidity, Air Quality Sensors	Temperature, Humidity, Air Quality	Real-time optimization of poultry house environment using deep learning
Gowri et al.	(2024)	IoT, WSN, DenseNet	Temperature, Humidity, Air Quality, Feed	The prototype reduced mortality through automated corrective actions
Fahrurrozi et al.	(2024)	IoT, Random Forest ML	Temperature, Humidity, NH ₃	The prediction model achieved 96.67% accuracy for poultry house conditions
Barsagadea et al.	(2024)	IoT, Temperature, Humidity, NH ₃ , Light Intensity Sensors	Temperature, Humidity, NH ₃ , Light Intensity	Real-time monitoring system for poultry house conditions
Da Silva et al.	(2025)	IoT, Fuzzy Logic	Temperature, Humidity	The climate control system achieved 98% validation accuracy
Rosmasari et al.	(2025)	IoT, BME-680, MICS-5524	Temperature, Humidity, NH ₃	The monitoring system reached 82.03% accuracy in recording microclimate conditions
Bharanishree et al.	(2025)	IoT, DHT11, OpenCV, CNN, ThingSpeak	Temperature, Humidity, Broiler Health	The system predicted health issues and provided corrective recommendations

Note: IoT: Internet of things, WSN: Wireless sensor network, LoRaWAN: Long range wide area network, DHT: Digital humidity and temperature sensor, MQ: Metal oxide gas sensor, ESP: Espressif microcontroller, RTC: Real time clock, ANN: Artificial neural network, SVM: Support vector machine, CNN: Convolutional neural network, GRU: Gated recurrent unit, NH₃: Ammonia, CO₂: Carbon dioxide, CO: Carbon monoxide, CH₄: Methane, H₂S: Hydrogen sulfide.

During data processing, AI algorithms are applied to predict and control microclimate conditions in real time (Morozova, 2024). The Gated Recurrent Unit (GRU) model is preferred due to its computational efficiency and fewer parameters, making it suitable for edge devices with limited resources (Yang et al., 2020; Zarzycki and Ławryńczuk, 2021). Models based on Long Short-Term Memory (LSTM) and its bidirectional variant (BiLSTM) achieve superior predictive performance on long-sequence datasets but demand higher computational power (Yan et al., 2024; Saifullah, 2025). Convolutional Neural Networks (CNN) combined with attention mechanisms are also employed to extract spatio-temporal features from sensor data, enhancing the prediction accuracy of microclimate dynamics inside the house (Jia et al., 2024; Suresh et al., 2025).

Artificial Intelligence (AI)-driven ventilation systems often employ Artificial Neural Networks (ANN) to regulate water, feed, ventilation, temperature, and humidity (Morozova, 2024). Artificial Neural Network (ANN) models learn continuously by updating parameters and using classification errors to improve accuracy (Azadeh et al., 2014). Other studies also highlight the use of perceptron models, a simple form of ANN and a deep learning method, commonly applied to regression and classification tasks (Perrota, 2020). Figure 4 illustrates the learning model structure of neural networks. Hybrid control systems combine interpretable rule-based control (RBC) with adaptive machine learning, ensuring safety during critical events while enabling flexible responses to dynamic environments (Drgoňa et al., 2018; Aksjonov and Kyrki, 2023).

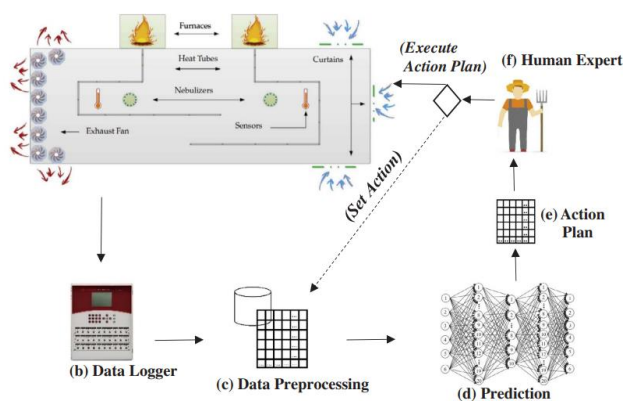


Figure 4. The learning model structure of neural networks. Source: Ribeiro (2019).

Beyond environmental parameters, AI integrates microclimate data with computer vision to monitor broiler

chickens' behaviour and welfare. Gated Recurrent Unit (GRU), Support Vector Machine (SVM), Random Forest, and Computer Neural Network (CNN) models detect diseases, stress, or abnormal behaviours (Ahmed et al., 2024; Taleb et al., 2025). Computer vision technologies such as YOLOv5 can recognize chicken behaviours in real time, supporting the detection of early disease symptoms or reduced productivity (Guo et al., 2025). These systems include mobile notifications to alert farmers for timely interventions (Elango et al., 2024).

In Indonesian applications, AI algorithms are commonly integrated with Arduino-based IoT systems. Sensors such as DHT22 monitor temperature and humidity (Fathurrohman et al., 2023), and blower fan speed control using Pulse Width Modulation (PWM) methods (Nalendra, 2021). Air quality monitoring employs oxygen, CO₂, and NH₃ sensors like NDIR CO₂, MQ135, MQ7, and MCIS-6814 (Fathurrohman et al., 2023; Mulling et al., 2023). Data processed via embedded AI algorithms generates control signals to automatically regulate poultry house equipment.

Challenges in implementing closed-house systems

One of the primary challenges in adopting IoT–AI technologies in closed-house poultry systems is the high initial investment cost. Farmers must install integrated infrastructure, including temperature, humidity, and ammonia sensors; actuators such as fans, cooling pads, and heaters; and hardware components like data loggers, edge devices, and cloud servers. These devices are costly, particularly for small- and medium-scale farmers (Alkhafaji et al., 2024; Wah, 2025). High upfront costs increase production expenses, potentially reducing broiler competitiveness unless efficiency gains offset the investment.

Beyond costs, reliable devices are essential. Sensors must withstand extreme barn conditions, high temperature, humidity, dust, and ammonia, yet low-cost sensors are often inaccurate or unreliable, while high-quality ones remain expensive (Chojer et al., 2022). The AI applications also demand advanced computing infrastructure, whether through edge devices or stable cloud services (Prangon and Wu, 2024). Consequently, IoT–AI systems are mostly deployed in pilot projects or by large integrators, while adoption among independent farmers remains limited (Abiri, 2023). Additional concerns include technical complexity, data privacy, and cybersecurity risks. Solutions such as federated learning reduce data transmission requirements, while blockchain ensures transparency and security (Rahaman et al., 2024;

Cai et al., 2025; Potdukhe et al., 2025). Cost-efficient modular approaches based on LoRaWAN and open-source platforms are also being developed to support smallholder farms (Finistrosa et al., 2025).

Internet connectivity poses another critical barrier. Internet of Things and AI on closed-house systems rely on continuous real-time data transmission to function effectively (Haseeb et al., 2017). However, many Indonesian broiler farms are located in rural areas with poor internet infrastructure (Ullah, 2024). Disruptions in connectivity compromise monitoring systems and increase farmers' workload, undermining the potential benefits of automation.

Farmer management practices also affect implementation. Many Indonesian farmers continue to follow generalized Standard Operating Procedures (SOPs) provided by companies rather than adjusting ventilation to actual broiler chicken responses. Ventilation equipment fans, pads, and exhausts are often operated on fixed schedules rather than real-time behavioural cues, even though chickens are sensitive bioindicators of microclimate changes (Sohsuebgarm et al., 2019; Belykh et al., 2021). Reliance on subjective observations, such as smell or visual inspection, often delays responses to issues like high humidity or ammonia buildup. The inability to adapt ventilation promptly results in mismatched environmental conditions, reducing efficiency (George and Hovan, 2023).

Another challenge is the digital skills gap. Most Indonesian broiler farmers rely on experience-based management and are unfamiliar with digital tools for microclimate monitoring or data analysis. Many lack the ability to interpret sensor outputs, understand AI models, or utilize cloud-based applications effectively (Alkhafaji et al., 2024). This gap is linked to limited education and insufficient technical training from government institutions (Slayi et al., 2023). Consequently, even when devices are installed, usage often remains suboptimal.

Resistance to new technology further slows adoption. Farmers frequently perceive IoT–AI systems as costly, complex, and uncertain in terms of profitability (Vuka and Wu, 2024). Early negative experiences, such as frequent breakdowns or difficulties operating automated systems, reinforce this skepticism (Postolache et al., 2025). Cultural reliance on intuition and generational practices also contributes to reluctance (Sädeharju, 2025). Many farmers prefer intuitive decision-making based on experience rather than formal decision-support tools informed by data.

Addressing these challenges requires integrated government and private sector involvement. Financial incentives such as subsidies can reduce high upfront costs, while training programs help bridge the digital skills gap (Chen et al., 2023). Public–private partnerships involving government, industry, and educational institutions are essential to provide infrastructure, technical support, and knowledge transfer. Additionally, developing affordable, context-specific IoT–AI models tailored to Indonesian infrastructure limitations is crucial to foster widespread adoption in poultry production.

CONCLUSION

The combination of IoT sensors with AI algorithms enables early detection of environmental changes, real-time ventilation adjustments, and more precise climate control. However, implementation at the farm level remains limited due to high construction costs, inadequate electricity and internet infrastructure in rural areas, and farmers' limited technical skills. Therefore, feasible solutions should focus on developing cost-effective and tropical-resistant technologies, improving access to digital infrastructure, and implementing training programs to strengthen human resource capacity.

DECLARATIONS

Acknowledgements

The authors would like to express their sincere gratitude to the Rector of Universitas Padjadjaran for providing financial support through the Padjadjaran Excellence Fast Track Scholarship (Beasiswa Unggulan Pascasarjana Padjadjaran).

Authors' contributions

Muhammad Irfan Maulana conducted the study, collected and analysed the data, and drafted the manuscript. Indrawati Yudha Asmara reviewed and edited the manuscript. All authors contributed to drafting, reviewing, and approving the final manuscript.

Funding

Funding for this literature review was supported by Padjadjaran University through the Padjadjaran Excellence Fast Track Scholarship (Beasiswa Unggulan Pascasarjana Padjadjaran).

Competing interests

The authors declare that they have no competing interests.

Ethical considerations

All ethical issues, including plagiarism, consent to publish, study misconduct, data fabrication or falsification, duplicate publication or submission, and redundancy, have been carefully checked and confirmed by all authors prior to submission of this manuscript. The authors declare that no artificial intelligence (AI) tools were used for data analysis, study selection, interpretation of results, or scientific decision-making in this study. All processes, including literature screening, data extraction, thematic synthesis, and manuscript preparation, were conducted manually by the authors.

Availability of data and materials

All data and materials used in this study are derived from publicly accessible academic databases (Scopus, ScienceDirect, Google Scholar, and SINTA). No primary or proprietary data were collected.

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Impacts of Restricting Ribonucleotide Reductase on Performance, Meat Quality, and Intestinal Health in Broiler Chickens Evaluated by Evans Blue Dye

Majid Shakeri^{1*} , Hamid Reza Rafieian-Naeini² , and Hassan Khanaki³ 

¹USDA-ARS, U.S. National Poultry Research Center, Athens, Georgia, United States

²Department of Poultry Science, University of Georgia, Athens, Georgia, United States

³School of Agriculture, Food and Ecosystem Sciences, Faculty of Science, The University of Melbourne, Dookie College, Victoria, Australia

*Corresponding author's E-mail: majid.shakeri.phd@gmail.com

Received: October 07, 2025, Revised: November 11, 2025, Accepted: December 09, 2025, Published: December 31, 2025



ABSTRACT

Ribonucleotide reductase (RNR) enzyme is essential for DNA synthesis and overall cellular health. Its inhibition interferes with metabolic pathways and mitochondrial function, leading to increased oxidative damage. Evans blue dye evaluates oxidative damage in tissues by penetrating through ruptured or destabilized membranes, thereby serving as an indicator of cell viability. The present study aimed to investigate the effects of restricting RNR on growth performance, meat quality, and intestinal health in broiler chickens after intestinal development was mainly completed. Twenty male broiler chickens, aged 14 days and weighing 445 ± 5 grams, were randomly divided into two equal groups: a control group and an experimental group, which received an RNR inhibitor (RR) at 20 mg/kg body weight/day for two weeks. Half of the chickens in both groups (5 broiler/group) were injected with Evans blue dye (EB) on the last day of the study. The oxidative damage was measured at the end of the study. The present results indicated that growth performance and feed intake were unchanged during the study. The RNR enzyme increased meat drip loss and lightness while reducing ultimate pH (pHu) compared to the control group. Evans blue concentration increased in the muscle, duodenum, jejunum, and ileum of the experimental group compared to the control group. Although RNR inhibition did not affect growth performance, it led to reduced meat quality and increased oxidative damage. These findings were evidenced by elevated drip loss and increased EB concentration in muscle and in all segments of the small intestine. The present results highlighted the essential role of RNR in maintaining meat quality and tissue integrity of broiler chickens.

Keywords: Evans blue dye, Intestine, Meat quality, Ribonucleotide reductase

INTRODUCTION

Ribonucleotide reductase (RNR) plays an essential role in DNA synthesis, a vital process for cell proliferation and the overall growth (Liew et al., 2016). As the enzyme responsible for converting ribonucleotides into deoxyribonucleotides, RNR ensures a steady supply for DNA synthesis (Liew et al., 2016). Disruption or inhibition of RNR activity can impair cellular metabolism and replication, resulting in developmental delays and reduced physiological function (Liew et al., 2016). In poultry, reduced RNR expression has been associated with

the incidence of muscle-related disorders (Shakeri et al., 2024; 2025).

Quantifying tissue damage is challenging, but this issue can be partially addressed by using markers such as Evans blue dye (EB; Shakeri et al., 2019). Evans blue dye binds tightly to plasma albumin and is used as an exogenous marker of plasma volume (Crooke and Morris, 1942). Following tissue injury, EB extravasates into the surrounding tissue, where it may be quantified as a marker of tissue damage (Radu and Chernoff, 2013).

Previous studies indicated that broiler chickens with woody breast muscle had reduced RNR activity and impaired meat quality parameters (Shakeri et al., 2024; 2025). Nevertheless, factors other than RNR may also influence the occurrence of woody breast (Hisasaga and Makagon, 2024). To investigate RNR's specific role in tissue health, its activity was artificially reduced using a targeted inhibitor during the present study. Therefore, the present study aimed to investigate the adverse effects of RNR inhibition on growth performance and meat quality, with an additional focus on measuring tissue damage using the EB marker.

MATERIALS AND METHODS

Ethical approval

All procedures used in the present study followed the guidelines of the Institutional Animal Care and Use Committee of the University of Melbourne, Melbourne, Australia.

Study design

In total, twenty 14-day-old male broiler Ross-308 chickens with an average body weight of 445 ± 5 g were randomly assigned to 10 equally sized pens. Body weight, feed intake, and feed conversion ratio (FCR) were recorded weekly. Feed for the grower phase (days 14-28), with a CP of 27.69% and ME of 3020 kcal/kg, was provided *ad libitum*, along with water, throughout the trial. The temperature was maintained at 21°C throughout the experiment. Humidity was maintained at $60 \pm 5\%$. Light was provided 24 hours/day throughout the study. The treatment groups included a control group and an experimental group receiving the RNR inhibitor (RR, Sigma-Aldrich USA, Hydroxyurea) at 20 mg/kg body weight/day administered orally for two weeks. The inhibitor dose was determined based on *in vitro* experiments and a study in other species (Geetha, 2018; Shakeri et al., 2025). Each treatment consisted of 10 broiler chickens, with two per pen. On day 28, all broiler chickens were slaughtered using the cervical dislocation or cutting the jugular vein for EB dye analysis. Samples from the duodenum, jejunum, and ileum, and breast muscle were collected for meat quality measurements. The experiment was limited to the growing phase, as this period focuses on supporting rapid muscle growth and skeletal development. The growing phase is crucial for setting a healthy growth and efficient feed conversion during the finishing phase of broiler chickens.

Meat quality assessment

A total of 10 broiler chickens from each group were used to measure the parameters. Approximately 10 g of breast muscle was suspended on a wire surface in a sealed box at 4°C to measure drip loss. To measure drip loss, initial weight (< 15 minutes) and 24-hour weights were recorded to calculate the drip loss percentage. The pH was measured at < 15 minutes using a pH meter (HannaInst, Australia). Then, the muscles were kept at 4°C in sealed plastic bags for measuring color (Minolta, USA) and pHu at 24 hours post-mortem.

Evans blue dye injection, extraction, and qualification

Five chicks per group (total 10 chicks) were injected with 10 mg/kg body weight of EB in 0.9% saline (Sigma Aldrich, USA) into the brachial vein at the end of the experiment. After 1 hour, broiler chickens were bled, and samples were collected from the ileum, jejunum, duodenum, and muscle tissue. All tissues were dried in an oven at 70°C for 48 hours, then EB was extracted from 100 mg of dried tissues with 500 µL formamide (Sigma Aldrich, USA) and incubated at 55°C for 24 hours. Samples were then centrifuged for 15 minutes at 14,000× g and 4°C. Finally, 200 µL of supernatant was loaded in duplicate against standards at 610 nm. The results were expressed as ng EB per mg of dry tissue weight.

Statistical analysis

Data was analyzed using a t-test (GraphPad Prism, version 10.2.2). The present results were considered significant at a p-value less than 5% ($p < 0.05$) and trending towards significance at $p = 0.05-0.1$. Mean values are given as standard error of the mean (SEM).

RESULTS

The current findings on performance and meat quality are presented in Table 1. There were no significant differences between the two groups in growth ($P = 0.37$), feed intake ($P = 0.57$), and FCR ($P = 0.82$) during the two-week experiment. Inhibition of ribonucleotide reductase lowered pHu ($P = 0.005$), increased drip loss, and decreased meat lightness (both $P = 0.03$) compared to the control group. Evans blue concentration was increased in the duodenum, ileum, and muscle of the experimental group ($P < 0.0001$, $P = 0.01$, and $P = 0.01$, respectively) compared to the control group, indicating possible greater damage to these tissues when RNR activity was restricted (Figure 1). Evans blue concentration tended to be higher in the jejunum of the experimental group than in the control group ($P = 0.11$).

Table 1. Growth, meat quality, and distribution of Evans blue in tissues of broiler chickens aged 42 days for two weeks with administration of ribonucleotide reductase inhibitor

Parameters	Control	SEM	RNR ¹ inhibitor	SEM	P-value*
Weight gain(g)²					
Day 14-21	343.4	2.03	341.1	3.99	0.61
Day 22-28	584.9	4.02	582.0	5.10	0.66
Day 14-28	928.3	4.09	923.1	4.07	0.37
Final body weight	1374.2	3.95	1367.1	4.18	0.24
Feed intake (g)					
Day 14-21	579.9	2.66	581.7	2.34	0.61
Day 22-28	1028.0	6.64	1020.1	5.86	0.40
Day 14-28	1608.1	6.92	1602.2	7.41	0.57
Feed conversion ratio					
Day 14-21	1.68	0.01	1.70	0.02	0.46
Day 22-28	1.75	0.01	1.75	0.02	0.90
Day 14-28	1.73	0.01	1.74	0.02	0.82
Meat quality measurements					
Drip loss (%)	1.76	0.16	4.22	0.97	0.03
pH (< 15 minutes)	6.64	0.04	6.61	0.16	0.87
pHu (24 hours)	5.60	0.06	5.22	0.07	0.005
Color (24 hours) ³					
Redness	0.69	0.28	1.01	0.34	0.52
Yellowness	11.15	0.98	12.75	1.05	0.29
Lightness	55.07	1.26	59.95	1.50	0.03
Evans blue (ng/mg)					
Muscle	47.8	10.78	187.0	41.9	0.01
Duodenum	145.1	25.60	499.9	16.50	< 0.0001
Jejunum	334.2	28.53	458.2	64.46	0.11
Ileum	315.2	37.62	492.2	44.01	0.01

¹RNR: Ribonucleotide reductase, SEM: Standard error of mean, ²Initial body weight for each broiler: 445 ± 5g, ³Skin side, *Data are compared within a row.

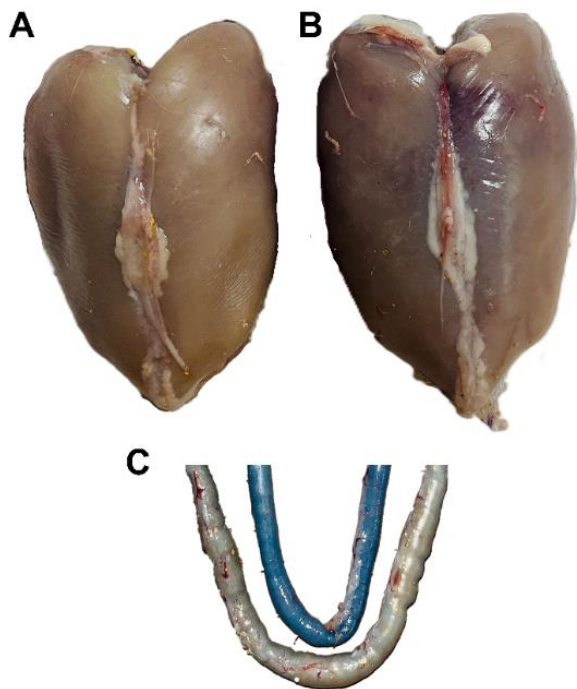


Figure 1. Breast muscle and duodenum samples from untreated broiler chickens or treated with Hydroxyurea at 20 mg/kg body weight for 14 days. Evans blue dye was administered to evaluate tissue damage in muscle from untreated (A), treated with Hydroxyurea (B), and duodenum from treated (inner loop) and untreated (outer loop) broiler chickens (C).

DISCUSSION

The current findings indicated that although growth performance remained unaffected by RNR restriction, meat quality parameters were compromised in the experimental group. Furthermore, the experimental group exhibited elevated EB concentration in muscle and intestinal tissues, indicating more severe tissue damage. This increase in EB aligns partially with previous findings of Shakeri et al. (2025a;b) in broiler chickens with woody breasts, in which reduced RNR activity was linked to poorer meat quality.

The RNR is the key enzyme that synthesizes deoxyribonucleotides, the major factor for DNA synthesis and cell proliferation (Liew et al., 2016). Additionally, RNR impacts the production of reactive oxygen species (ROS) and is itself influenced by the oxidative stress process. The RNR does not directly produce ROS, but its activity affects the cell's redox balance, and inhibiting RNR can increase ROS levels and cause oxidative stress (Andrs et al., 2023). Although the present findings demonstrated negligible changes in body weight gain, feed intake, and FCR in both groups, adding the RNR inhibitor negatively affected meat quality parameters, increasing

drip loss and meat lightness. The minimal effects on growth performance after treatment with RNR inhibitors might have resulted from compensatory metabolic changes, such as shifting metabolism to promote fat storage or altering the energy-use balance. The negative impacts on meat quality were associated with more severe oxidative tissue damage as EB concentration increased in broiler chickens treated with the RNR inhibitor. It has been shown that serious oxidative damage negatively impacts meat pHu and drip loss in broiler chickens (Chen et al., 2022).

CONCLUSION

Inhibiting RNR activity negatively affected meat quality parameters and intestinal health during the growing stage of broiler chickens, as evidenced by increased drip loss and elevated EB concentrations in tissues. The current findings might be beneficial in confirming RNR's direct role in the development of woody breast myopathy and related abnormalities. Further studies are warranted to explore different dosages of RNR inhibitors and assess their influence on growth performance, meat quality parameters, and the regulation of alternative growth-related pathways.

DECLARATIONS

Ethical considerations

The authors affirmed that this manuscript is original and has not been submitted for publication elsewhere. The authors assured that the data included in this manuscript are truthful and have not been manipulated. No AI-assisted technologies were used in the generation of this manuscript.

Availability of data and materials

All available data within the manuscript can be provided upon reasonable request from the corresponding author.

Acknowledgements

The authors conducted the study independently, with no external contributions received.

Authors' contributions

Majid Shakeri contributed to the conceptualization of the study, performed the formal data analysis and statistical evaluations, drafted the original manuscript, and managed the overall project. Hamid Reza Rafieian-Naeini supported the development of the methodology and contributed to the review and editing of the manuscript. Hassan Khanaki conducted the execution of the

experiment and the collection of experimental data and contributed to the review and editing of the manuscript. All authors have read and approved the final version of the manuscript before publication in the present journal.

Funding

The present study received no external funding.

Competing interests

The authors declared no conflict of interest.

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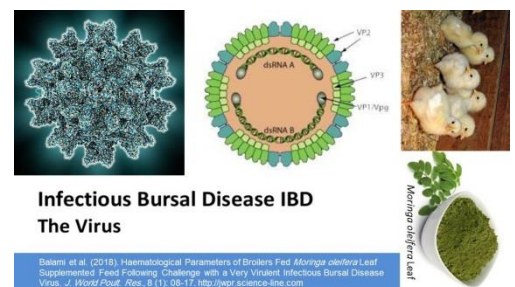
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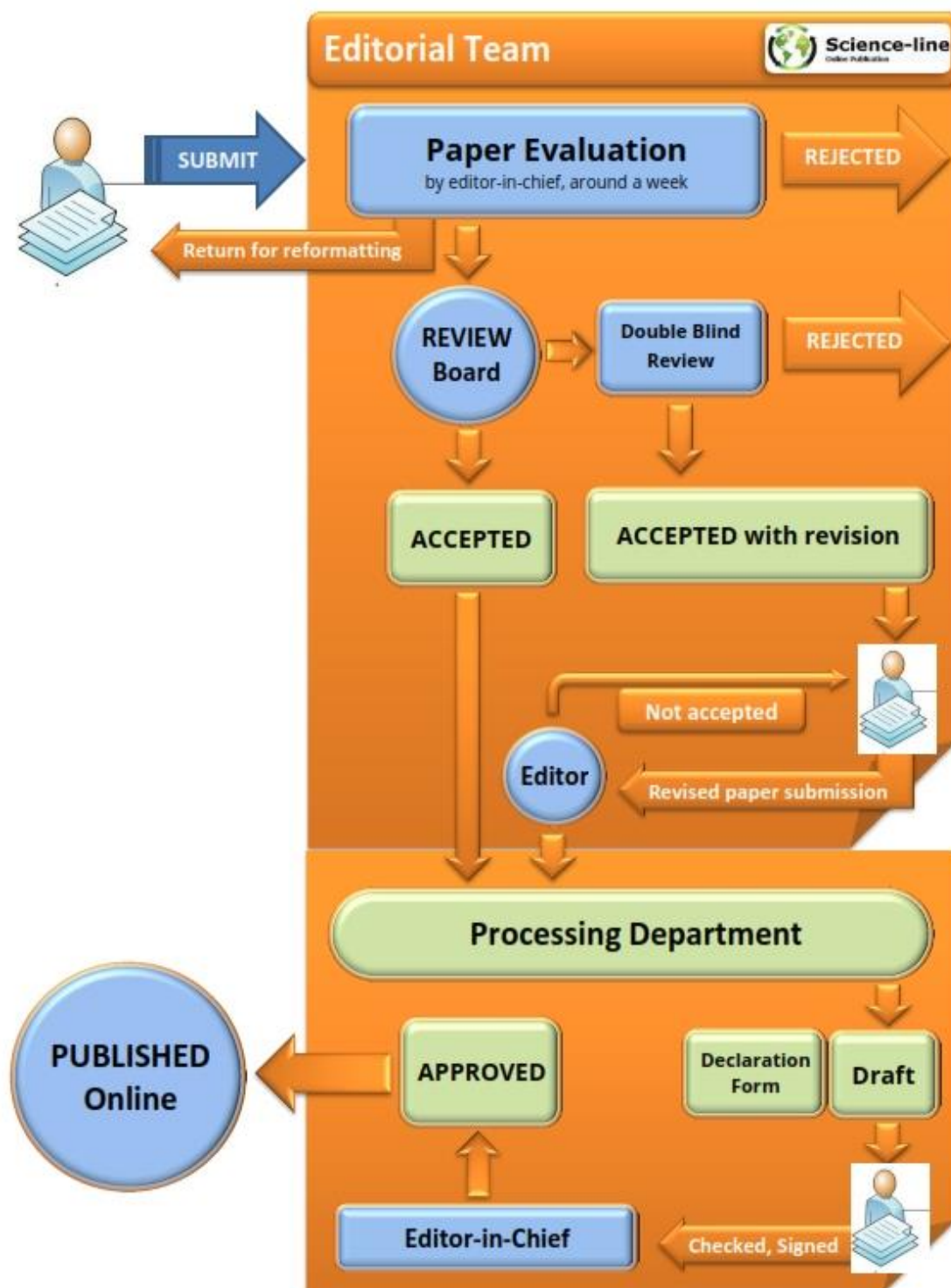
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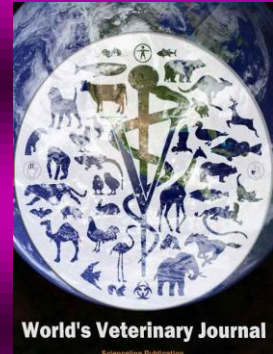
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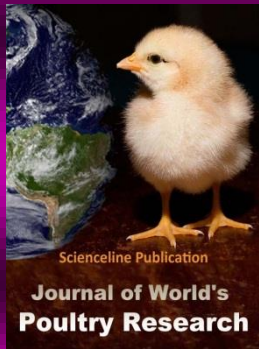
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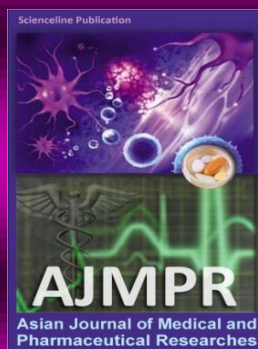
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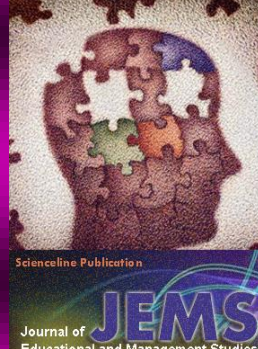
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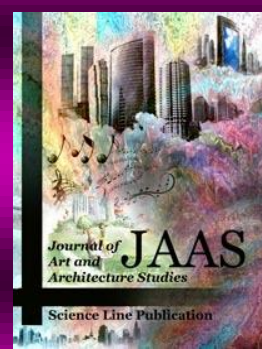
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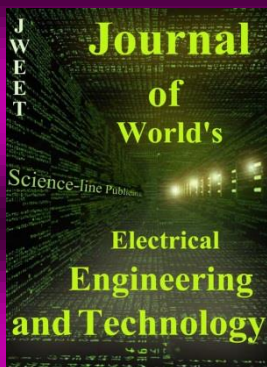
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