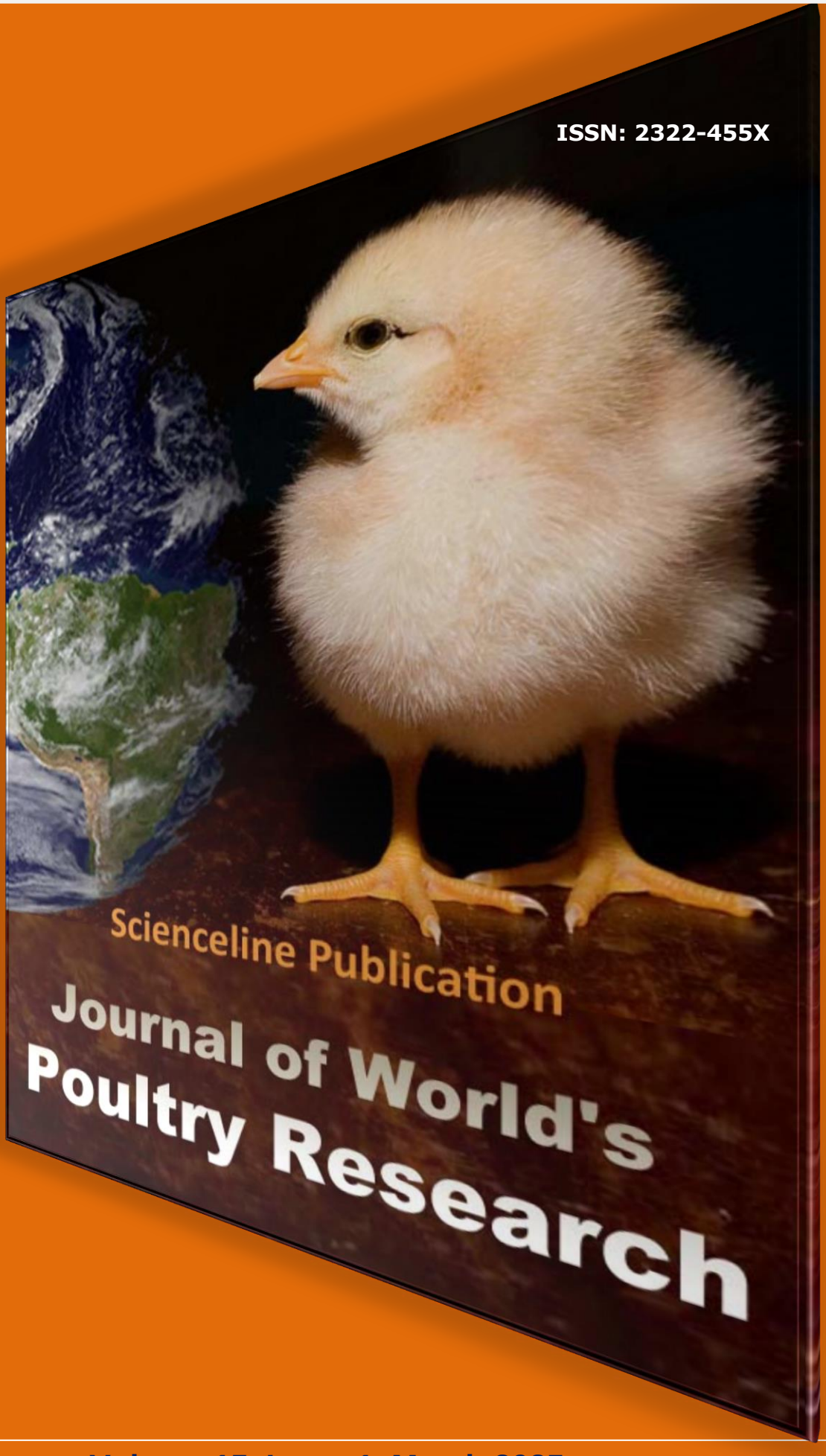
Journal of World's Poultry Research

BOOKLET



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Research Paper

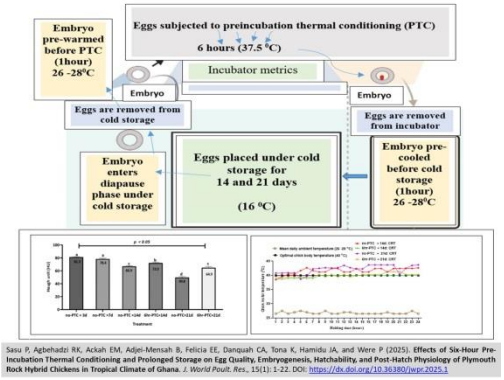
Effects of Six-Hour Pre-Incubation Thermal Conditioning and Prolonged Storage on Egg Quality, Embryogenesis, Hatchability, and Post-Hatch Physiology of Plymouth Rock Hybrid Chickens in Tropical Climate of Ghana

Sasu P, Agbehadzi RK, Ackah EM, Adjei-Mensah B, Felicia EE, Danquah CA, Tona K, Hamidu JA, and Were P.

J. World Poult. Res. 15(1): 1-22, 2025; pii: S2322455X2500001-15
DOI: https://dx.doi.org/10.36380/jwpr.2025.1

ABSTRACT: Prolonged storage negatively impacts incubation outcomes in commercial hatchery operations, highlighting the need for efficient storage strategies. This study assessed the impact of prolonged storage durations and six-hour pre-incubation thermal conditioning (PTC) on egg quality, embryonic development, hatchability, chick quality, blood profile, and thermoregulation. A total of 2,000 fertile eggs were collected from a flock of 72-week-old Plymouth Rock hybrid hens and subjected to a 2 × 2 factorial design, involving storage for either 14 or 21 days, with or without the application of 6-hour PTC. Following storage, the eggs were incubated in a Jamesway P5000 set at a temperature of 37.5°C and relative humidity of 56%, then transferred on incubation day 18 to a hatcher set at 36.5°C and 60% until hatching. Results revealed that prolonged egg storage without PTC significantly diminished egg protein while PTC effectively countered this decline, enhancing Haugh unit values and blastoderm diameter. Prolonged egg storage without PTC also resulted in increased relative egg weight loss (REWL), fluctuated daily eggshell temperature, and reduced embryonic growth during incubation while PTC significantly reduced these effects, with embryos demonstrating significantly enhanced growth. Additionally, while fertility rates remained stable across all treatments, PTC significantly reduced mortality and improved hatchability by 11.4% in 14-day stored eggs and 10.8% in 21-day stored eggs. It also shortened incubation time, increased post-hatch chick body weights and enhanced their hematological and serum profiles, including normalized thyroid hormone (T3 and T4) levels compared to the non-PTC (control) group. Pearson correlation showed that longer incubation time was positively correlated with higher rectal temperature, serum glucose, and thyroid hormones, but negatively correlated with hemoglobin, mean corpuscular hemoglobin, and total protein in non-PTC chicks. In conclusion, six-hour pre-incubation thermal conditioning mitigates the negative effects of prolonged egg storage and enhances embryogenesis, hatchability, chick quality, blood profile, and thermoregulation in Plymouth Rock hybrid chickens.

Keywords: Embryonic development, Extended egg storage, Plymouth rock hybrid chicken, Pre-incubation thermal conditioning, Thermoregulation



[Full text-PDF]

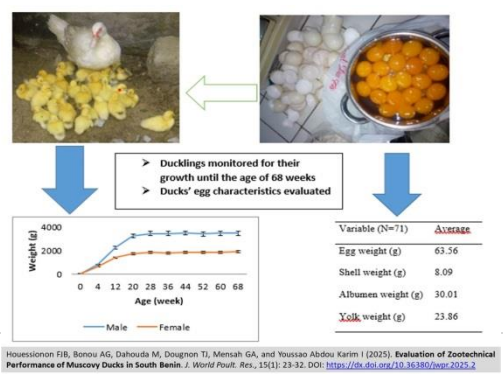
Research Paper

Evaluation of Zootechnical Performance of Muscovy Ducks in South Benin

Houessionon FJB, Bonou AG, Dahouda M, Dougnon TJ, Mensah GA, and Youssao Abdou Karim I.

J. World Poult. Res. 15(1): 23-32, 2025; pii: S2322455X2500002-15
DOI: https://dx.doi.org/10.36380/jwpr.2025.2

ABSTRACT: Studies on the zootechnical performance of Muscovy ducks are scarce in Benin. The current study aimed to evaluate the performance of these ducks in a controlled environment for a better valorization of potentialities. Data were collected from 193 ducks for growth performance, 30 ducks for egg-laying performance, and 71 eggs for egg characteristics of Muscovy ducks in South Benin. The ducks were raised in controlled conditions. At hatching, male and female ducks had similar weights and body measurements. From week 2 to week 68, males had significantly higher weight than females. Males had higher initial specific growth than females (0.52 vs 0.63 per week), while females were older than males regarding age inflection point (33.10 vs 25.98 weeks). In addition, males had longer bodies, wider thoraces, and longer tarsus than



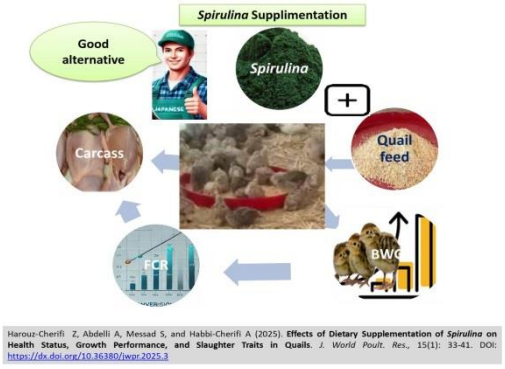
females. Regarding the wingspan, the difference between males and females was observed from week 8, with higher values in males. Individual feed intake and feed conversion ratio increased as the ducks grew older. In the first week, the individual feed intake was 20.08 g per day, and the feed conversion ratio was 1.51. After 20 weeks of age, Muscovy ducks consumed 136 g daily with a high feed conversion ratio of 26. The age of the first egg of Muscovy duck was 6.17 months, and the average number of eggs laid per brooding was 15.37 eggs. The brooding duration was 35.63 days, with a hatching rate of 73.06%. The duckling's survival rate at hatching was 95.28%, of which 97.47% were weaned. The average weight of a duck egg was 63.56 g, and that of the shell was 8 g, while albumen and yolk amounted to 30.01 g and 23.86 g, respectively. Duck eggshell dominant color was white (60.5%), followed by dirty white (26.31%), and finally brown (13.64%). These results on the zootechnical performance of the Muscovy duck can be considered a reliable basis for this species' potential improvement in South Benin.
Keywords: Average daily gain, Benin, Feed conversion ratio, Muscovy duck, Weight, Zootechnical performance

[Full text-PDF]

Research Paper

Effects of Dietary Supplementation of Spirulina on Health Status, Growth Performance, and Slaughter Traits in Quails

Harouz-Cherifi Z, Abdelli A, Messad S, and Habbi-Cherifi A.
J. World Poult. Res. 15(1): 33-41, 2025; pii: S2322455X2500003-15
DOI: https://dx.doi.org/10.36380/jwpr.2025.3



Harouz-Cherifi Z, Abdelli A, Messad S, and Habbi-Cherifi A (2025). **Effects of Dietary Supplementation of *Spirulina* on Health Status, Growth Performance, and Slaughter Traits in Quails**. *J. World Poult. Res.*, 15(1): 33-41. DOI: https://dx.doi.org/10.36380/jwpr.2025.3

ABSTRACT: The supplementation of sustainable alternative sources such as nutrient-rich algae, especially rich in proteins, in animal feed is a promising and innovative strategy to improve feed autonomy, especially in poultry diets. This study evaluated the effect of Spirulina platensis (SP) supplementation on growth performance and slaughter characteristics in Japanese quails (Coturnix japonica). A total of 180 unsexed, 2-day-old quail chicks with a mean body weight of 9 ± 1.42 g were randomly assigned to three dietary groups, each containing 60 quails. Each group was divided into 4 subgroups, with 15 quails in each (4 repetitions per group). Three groups were provided with commercial diets (starter, grower, and finisher) for five weeks. These diets were supplemented with Spirulina at concentrations of 0.5 g/kg (SP0.5), and 1 g/kg (SP1), while the control group (SP0) received no Spirulina supplementation. Growth performance was monitored, and at the end of the trial (35 d), 60 quails (20 per group) were slaughtered for carcass evaluation including hot and cold carcass weight and liver weight. Results showed that Spirulina supplementation at 1 g/kg (SP1) significantly increased feed intake and weight gain compared to the control and SP0.5 groups. Significant differences in growth performance and feed intake were observed between the Spirulina-supplemented groups (0.5 and 1 g/kg) and the control group. Carcass characteristics, including hot carcass yield and liver weight, were significantly higher in the SP0.5 and SP1 groups compared to the control group (SP0). In conclusion, supplementing quail diets with 0.5 and 1 g/kg Spirulina improved growth performance and carcass quality without negative effects on overall performance. This supplementation can be considered as a cost-effective diet ingredient for enhancing meat quality in quail production.
Keywords: Carcass trait, Growth, Japanese quail, Performance, Spirulina platensis

[Full text-PDF]

Research Paper

Effect of Cassava Silage Diet on Performance and Internal Organs of Male Ducks

Sandi S., Sahara E., Laconi E.B., Sudarman A., Wiryawan K.G., and Mangunwijaja D.
J. World Poult. Res. 15(1): 42-52, 2025; pii: S2322455X2500004-15
DOI: https://dx.doi.org/10.36380/jwpr.2025.4

Sandi S., Sahara E., Laconi E.B., Sudarman A., Wiryawan K.G., and Mangunwijaja D. (2025). **Effect of Cassava Silage Diet on Performance and Internal Organs of Male Ducks**. *J. World Poult. Res.*, 15(1): 42-52. DOI: https://dx.doi.org/10.36380/jwpr.2025.4

ABSTRACT: It would be beneficial to consider supplementary feeding of livestock as a means of increasing production, although this may be constrained by the residues produced. Silage is one of the forage feed conservation techniques that has also been the subject of interest in recent years concerning poultry feed. The present study aimed to gain insight into the potential impact of feeding cassava-based silage (CS) on the internal organs and performance of male ducks. The study was conducted using 200 male local ducks aged one day, which were then reared in cages for 10 weeks. The research design was based on a completely randomized design (CRD) with five treatments and four replications. The treatments were arranged based on the amount/percentage of silage used in the basal ration and were as follows S0 (silage ration 0% CS/control), S25 (silage ration 25% CS), S50 (silage ration 50% CS), S75 (silage ration 75% CS), and S100 (silage ration 100% CS). In further observations, several variables were considered, including body weight gain (BWG), ration consumption, ration

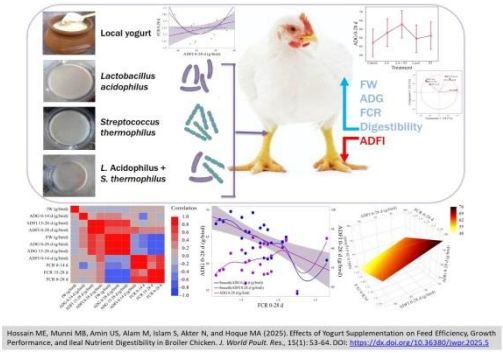
conversion, abdominal fat percentage, spleen percentage, liver percentage, kidney percentage, heart percentage, gizzard percentage, pancreas percentage, thyroid percentage, serum thiocyanate levels, and mortality, as well as serum thiocyanate. The results indicated a notable decline in performance ($p < 0.05$) in BWG observations when CS was provided in amounts exceeding 25% and consumption exceeded 50%. Furthermore, there was a notable increase in the weight of internal organs, which appeared to coincide with an increase in the level of use of cassava-based silage in duck rations. Based on the results of the study the use of cassava-based silage could be considered as a potential alternative or replacement for up to 50% of basal rations, without necessarily resulting in significant changes in the performance and internal organs of livestock.
Keywords: Body weight, Complete ration, Cassava, Silage, Internal organ, Male duck.

[Full text-PDF]

Research Paper

Effects of Yogurt Supplementation on Feed Efficiency, Growth Performance, and Ileal Nutrient Digestibility in Broiler Chicken

Hossain ME, Munni MB, Amin US, Alam M, Islam S, Akter N, and Hoque MA.
J. World Poult. Res. 15(1): 53-64, 2025; pii: S2322455X2500005-15
DOI: https://dx.doi.org/10.36380/jwpr.2025.5



Hossain ME, Munni MB, Amin US, Alam M, Islam S, Akter N, and Hoque MA (2025). Effects of Yogurt Supplementation on Feed Efficiency, Growth Performance, and Ileal Nutrient Digestibility in Broiler Chicken. *J. World Poult. Res.*, 15(1): 53-64. DOI: https://dx.doi.org/10.36380/jwpr.2025.5

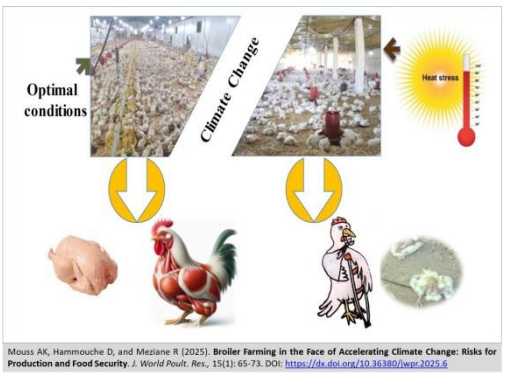
ABSTRACT: The use of probiotics, particularly fermented yogurt, in poultry diets has gained substantial interest due to their capacity to enhance growth performance, feed conversion efficiency, and nutrient absorption in broiler chickens. This study evaluated the effects of yogurt supplementation on broiler performance and nutrient utilization. Two hundred one-day-old Ross-308 male broiler chicks were randomly assigned to five dietary treatments using a completely randomized design. Each treatment group included five replicates with eight chicks per replicate. The dietary treatments consisted of a control diet (without yogurt), locally prepared yogurt (5 mL/L in drinking water), yogurt fermented with Lactobacillus acidophilus (LA, 5 mL/L), yogurt fermented with Streptococcus thermophilus (ST, 5 mL/L), and yogurt co-fermented with L. acidophilus and S. thermophilus (LA+ST, 5 mL/L). The performance and ileal digestibility of nutrients were measured. Results indicated that the average daily feed intake (ADFI) significantly decreased in the LA+ST group at 0-14 days, with an 11.7% reduction compared to the control. Broilers receiving yogurt demonstrated a higher average daily gain (ADG) at 0-14 days, with the LA+ST group showing an 8% improvement over the control. At 0-28 days, the LA+ST group maintained the highest ADG, 6.8% higher than the control. The feed conversion ratio (FCR) significantly improved with yogurt supplementation at 0-14 days. Compared to the control, FCR improved by 3.6%, 7.9%, 5.7%, and 15.7% in the Local, LA, ST, and LA+ST groups, respectively. Additionally, yogurt fermented with specific lactic acid bacteria (LAB) significantly enhanced the ileal digestibility of dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), ether extract (EE), and total ash (TA). These findings highlight the efficacy of yogurt fermented with L. acidophilus and S. thermophilus as a dietary supplement to enhance growth performance and nutrient utilization in broiler chickens.
Keywords: Broiler, Daily gain, Feed conversion ratio, Feed intake, Yogurt

[Full text-PDF]

Research Paper

Broiler Farming in the Face of Accelerating Climate Change: Risks for Production and Food Security

Mouss AK, Hammouche D, and Meziane R.
J. World Poult. Res. 15(1): 65-73, 2025; pii: S2322455X2500006-15
DOI: https://dx.doi.org/10.36380/jwpr.2025.6



Mouss AK, Hammouche D, and Meziane R (2025). **Broiler Farming in the Face of Accelerating Climate Change: Risks for Production and Food Security**. *J. World Poult. Res.*, 15(1): 65-73. DOI: https://dx.doi.org/10.36380/jwpr.2025.6

ABSTRACT: Climate change poses significant challenges to poultry farming, particularly when broiler farms rear chickens in suboptimal housing conditions. The objective of the present study was to examine the impact of climate change, expressed through the Temperature Humidity Index (THI), on quantitative (carcass yields, pectoral muscles, thighs and drumsticks, and abdominal fat rate) and qualitative production parameters (composition of muscles in dry matter, mineral matter, crude proteins, and fat). The study was conducted in two separate poultry buildings over 45 days in northern Algeria. A total of 300 one-day-old unsexed chicks were randomly allocated into three replicates of 50 broilers each per building. The conditions of temperature and relative humidity were strictly regulated in control group but it was

unregulated, exposing birds to natural climate variations in the experimental group. The impact of climate change, represented by the Temperature Humidity Index (THI), on carcass yield, pectoralis major and minor (pectoral muscles), sartorius and gastrocnemius (thigh and drumstick muscles), as well as abdominal fat content were evaluated. The results revealed that the control group was exposed to THIs of 30.88, 20.45, and 19.19, while the experimental group was subjected to THIs of 33.07, 31.48, and 30.87 for the three growth phases. The increase in THI resulted in significant proportional deteriorations in the experimental group compared to the control group, for all the parameters under study, particularly at the end of breeding. There were reductions in yields of -6.12% for eviscerated carcasses, -8.16% for thighs and drumsticks, and -9.28% for pectoral muscles. Furthermore, the abdominal fat rate increased by +21.03%. The nutritional composition of pectoral muscles showed that chickens in the experimental group had +6.17% dry matter, +13.23% fat, -13.88% mineral matter, and -8.78% crude proteins. A similar trend was observed for thigh and drumstick muscles, with +6.10% dry matter, +14.39% fat, -12.28% mineral matter, and -12.50% crude proteins. The study highlighted the impact of climate change on poultry farming, which potentially affects production and threatens food security.

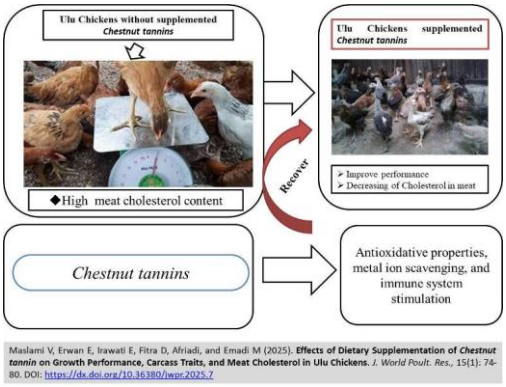
Keywords: Broiler chicken, Carcass, Climate change, Food security, Muscle, Nutritional quality, Yield

[Full text-PDF]

Research Paper

Effects of Dietary Supplementation of Chestnut tannin on Growth Performance, Carcass Traits, and Meat Cholesterol in Ulu Chickens

Maslami V, Erwan E, Irawati E, Fitra D, Afriadi, and Emadi M.
J. World Poult. Res. 15(1): 74-80, 2025; pii: S2322455X2500007-15
DOI: https://dx.doi.org/10.36380/jwpr.2025.7



Maslami V, Erwan E, Irawati E, Fitra D, Afriadi, and Emadi M (2025). **Effects of Dietary Supplementation of *Chestnut tannin* on Growth Performance, Carcass Traits, and Meat Cholesterol in Ulu Chickens**. *J. World Poult. Res.*, 15(1): 74-80. DOI: https://dx.doi.org/10.36380/jwpr.2025.7

ABSTRACT: Tannin from chestnuts has garnered interest in poultry nutrition due to its potential impact on meat quality. The current study investigated the effects of Chestnut tannins (CT) which were derived from natural chestnut wood, on poultry health and meat characteristics. The primary objective was to determine the effects of CT supplementation in commercial feed on performance, carcass, and meat cholesterol in Ulu chickens. A total of 48 one-day-old Ulu chickens were divided randomly based on a completely randomized design into four treatment groups, with four replications each, and raised until 63 days of age. The treatments consisted of varying doses of CT (0.1%, 0.2%, and 0.3%) supplemented with a commercial diet. The parameters measured were performance, carcass traits, and meat cholesterol. The results showed that the supplementation of different levels of CT did not significantly alter performance, carcass traits, and meat cholesterol in ulu chickens. However, correlation and trend analysis indicated that the 0.3% CT treatment yielded the best growth performance, with a body weight gain of 934.85 g and a feed conversion ratio of 2.53, respectively. Conversely, the best treatment for reducing meat cholesterol was 0.2% of CT. It can be concluded that while CT supplementation did not influence the performance and carcass characteristics, it was effective in reducing meat cholesterol levels in Ulu chickens.

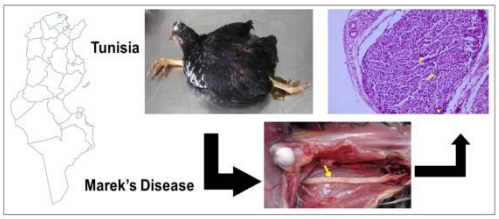
Keywords: Carcass, Cholesterol, Performance, Chestnut tannins, Ulu chicken

[Full text-PDF]

Research Paper

Prevalence, Gross Pathology, and Histopathology of Marek's Disease in Backyard Chickens in Northeastern Tunisia

Kaboudi K, Mkadem E, and Amara A.
J. World Poult. Res. 15(1): 81-91, 2025; pii: S2322455X2500008-15
DOI: https://dx.doi.org/10.36380/jwpr.2025.8



Kaboudi K, Mkadem E, and Amara A (2025). **Prevalence, Gross Pathology, and Histopathology of Marek's Disease in Backyard Chickens in Northeastern Tunisia**. *J. World Poult. Res.*, 15(1): 81-91. DOI: https://dx.doi.org/10.36380/jwpr.2025.8

ABSTRACT: Marek's disease (MD) is a common worldwide lymphomatous and neuropathic disease of chickens. Infection can cause significant losses in chicken production due to high mortality and morbidity. The present study aimed to determine the prevalence of MD in backyard flocks in the Grand-Tunis region of northeastern Tunisia and to analyze clinical cases over an eight-year and three-month period, from September 2012 to December 2020. A total of 798 cases were received for necropsy examination in the avian clinic of the National School of Veterinary Medicine of Sidi Thabet, Tunisia. Among these, chicks suspected of having MD underwent clinical observation, postmortem examination, and histopathological analysis. The results showed that 61 chickens (7.64%) were suspected to have MD. Clinical and postmortem examinations revealed different forms of MD including visceral (31 cases), mixed (20 cases), and nervous forms (10 cases). Postmortem examinations showed two types of lesions including hypertrophy and lymphomatous tumors. The highest frequencies of lesions were noted in the liver (74%), spleen (62%),

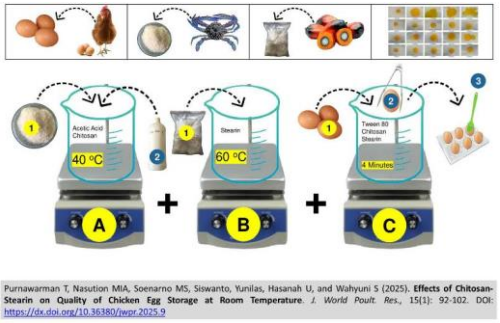
sciatic nerves (48%), lungs (36%), and kidneys (31%). Hypertrophy predominated in the spleen (49%), sciatic nerves (48%), liver (28%), kidneys (25%), lungs (21%), proventriculus (18%), and gonads (17%). Conversely, lymphomatous tumors were more frequently observed in the liver (46%), heart (23%), lungs (15%), and spleen (13%). Histopathological investigations revealed pleomorphic infiltrations with lymphocytes and plasmocytes in visceral organs, sciatic nerves, and the skin. High histological scores were recorded in the liver, spleen, lungs, kidneys, and heart. The current study confirmed endemic MD in backyard chicken populations in Grand-Tunis région and confirmed that it can be a serious threat to poultry health in the study area.
Keywords: Backyard chickens, Clinical examination, Histopathology, Lymphoma, Marek's disease, Postmortem examination

[Full text-PDF]

Research Paper

Effects of Chitosan-Stearin on Quality of Chicken Egg Storage at Room Temperature

Purnawarman T, Nasution MIA, Soenarno MS, Siswanto, Yunilas, Hasanah U, and Wahyuni S.
J. World Poult. Res. 15(1): 92-102, 2025; pii: S2322455X2500009-15
DOI: https://dx.doi.org/10.36380/jwpr.2025.9



Purnawarman T, Nasution MIA, Soenarno MS, Siswanto, Yunilas, Hasanah U, and Wahyuni S (2025). **Effects of Chitosan-Stearin on Quality of Chicken Egg Storage at Room Temperature**. *J. World Poult. Res.*, 15(1): 92-102. DOI: https://dx.doi.org/10.36380/jwpr.2025.9

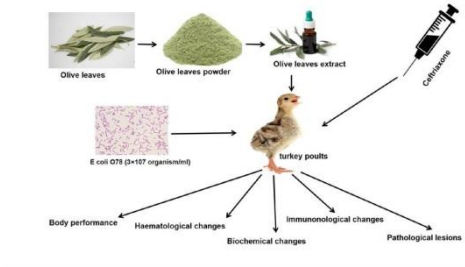
ABSTRACT: Consumption of chicken eggs has perishable properties, the quality of eggs declines faster and the shelf life of eggs is considerably short at room temperature compared to cold temperatures. The present study aimed to evaluate the application of chitosan-stearin as a coating on the quality of chicken egg storage at room temperature. The present study used a Completely Randomized Design (CRD) 4 x 5 factorial pattern with three replications. Each replicate consisted of six fresh chicken eggs, resulting in 360 eggs. The groups included Without Coating (FD0), Virgin Coconut Oil (FD1), 1.5% Chitosan + 1% Stearin (FD2), and 3% Chitosan + 1% Stearin (FD3). The second effective variable in grouping was storage time 0 Days (ST0), 14 Days (ST14), 28 Days (ST28), 42 Days (ST42), and 56 Days (ST56). The current results indicated that the storage time and the formula dosage had a notable effect on haugh unit, yolk index, and albumen index, but no significant effect on the pH of the albumen. Formula dosage had no significant effect, but storage time had a significant effect on yolk pH and color, and weight loss. There was an interaction between formula dosage and storage time on haugh unit, albumen index, and yolk index, but there was no interaction on albumen pH, weight loss, yolk pH, and yolk color. The Chitosan-Stearin coating can maintain the quality of chicken eggs during storage for up to 56 days. The use of 3% Chitosan + 1% Stearin as a coating formula indicated the best results in maintaining the quality of chicken eggs during storage time at room temperature.
Keywords: Chicken egg, Chitosan, Coating, Room temperature, Stearin

[Full text-PDF]

Research Paper

Effects of Olive Leaf Extract on Growth Performance and Immunobiochemical Parameters in Turkey Poults

El Khder GM, Mostafa DIA, Sarhan MM, Abd El Kader SA, Ewis HA, Abd El Wahab SA, and Kassem M.
J. World Poult. Res. 15(1): 103-117, 2025; pii: S2322455X2500010-15
DOI: https://dx.doi.org/10.36380/jwpr.2025.10



El Khder GM, Mostafa DIA, Sarhan MM, Abd El Kader SA, Ewis HA, Abd El Wahab SA, and Kassem M (2025). **Effects of Olive Leaf Extract on Growth Performance and Immunobiochemical Parameters in Turkey Poults.** *J. World Poult. Res.*, 15(1): 103-117. DOI: https://dx.doi.org/10.36380/jwpr.2025.10

ABSTRACT: Olive leaf extract (OLE) is known to have numerous bioactivities attributed to its high phenolic compound content. This study aimed to investigate the impact of OLE and Ceftriaxone on Escherichia coli (E. coli) in turkey poults. A total of 150 cloacal swabs were taken from turkey poults for isolation and identification of E. coli. Fifty-one-day-old turkey poults were divided into five equal groups. The first group served as the control, and the second group orally received 400 mg/kg body weight OLE daily for 35 days. The third, fourth, and fifth groups were infected with a culture suspension of E. coli O78 (0.3 ml, 3×107 organism/ml) via the nasal route. The third group was infected untreated. The fourth group was treated with 50 mg/Kg body weight of Ceftriaxone for 5 consecutive days. The fifth group received 400 mg/kg body weight of OLE from day to day 35 of age. Bacteriological examination revealed positive swabs in 18.18%, 46.67%, and 53.33% of healthy, diseased, and recently deceased poults, respectively. Serological identification of E. coli isolates included O157 (2), O78 (2), and O11 (1). Poults of the third group showed typical clinical signs, gross pathological changes such as congestion in various organs, and a 30% mortality rate. Additionally, significant reductions in body weight, weight gain, catalase (CAT), and superoxide dismutase (SOD) were observed, alongside anemia, hypoproteinemia, and hypoalbuminemia. Conversely,

significant increases were noted in the phagocytic index, killing percentage, total globulin, immunoglobulins, and the albumin/globulin ratio. Furthermore, significant increases were observed in FCR, leukocytic counts, lysosome, tumor necrosis factor α (TNF-α), interleukin-10, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), uric acid, creatinine, and malondialdehyde (MDA) levels. Poults in the fourth and fifth groups showed fewer clinical signs, lower lesion scores, and reduced mortality rates. Additionally, there was a decrease in E. coli re-isolation, modulation of altered parameters, and improvement in pathological lesions compared to the infected, untreated poults. Both OLE and Ceftriaxone were found to modulate the haematological, biochemical, and immunological parameters, as well as mitigate performance changes and pathological lesions induced by E. coli infection in turkey poults.
Keywords: Blood parameter, Ceftriaxone, E. coli, Olive leaves extract, Performance, Turkey poult

[Full text-PDF]

Research Paper

Impacts of Zinc, Selenium, and Vitamin E Supplementation on Growth Performance, Hematological and Biochemical Parameters of Blood in Broiler Chickens

Hossain J, Hossain ME, and Ahammed M.
J. World Poult. Res. 15(1): 118-127, 2025; pii: S2322455X2500011-15
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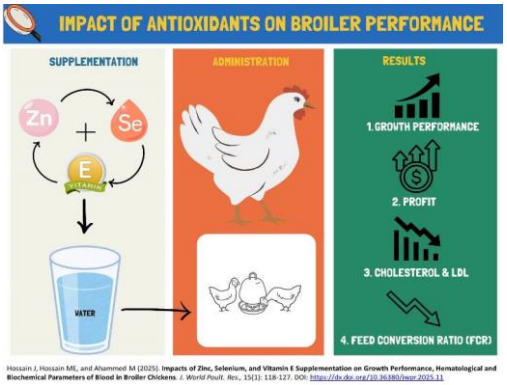

ABSTRACT: Metabolism, lipid synthesis, and reducing oxidative stress contribute to broiler chickens' growth and immunity. The current study examined how zinc, vitamin E, and selenium impact broiler growth, carcass characteristics, hematological and serum biochemical parameters, and profitability. There were 300-day-old straight-run chicks (Indian River) raised in a deep litter system until 28 days old. On day 7, the chicks were randomly divided into four groups of 75 chicks, each group replicated into 3 replications. Supplementation of zinc, selenium, and vitamin E through water was conducted from day 7 to day 28. This experiment was performed during the lifespan of chickens from 0 to 28 days of age. The treatment groups were control (drinking water with no supplementation), Zn (drinking water with 4 ml/L zinc), Se+Vit E (drinking water with 0.25 ml/L E-Sel), and Zn+Se+Vit E (drinking water with both 4 ml/L zinc and 0.25 ml/L E-Sel). The results indicated significant changes in growth and feed conversion ratio among Zn, Se, and Vit-E supplemented groups. Among the supplemented groups, the Zn+Seleium+VitaminE group exhibited higher growth performance, lower cholesterol, and lower production costs. The findings showed no significant changes in dressing characteristics and feed consumption among groups. The combined group of Zn, Se, and Vit-E had a lower abdominal fat content than other supplemented groups. Supplemented with Zn, Se, and Vit-E groups had lower cholesterol and LDL levels than the control group. Serum differential leukocyte count (eosinophils, lymphocytes, neutrophils, and monocytes) and liver and kidney function tests (ALT, AST, creatinine) showed no significant variations between the groups. Antioxidants increased profitability, with the Zn+Se+Vit E group having a higher profit per kg broiler and cost-benefit ratio. Broiler growth performance, dressing characteristics, biochemicals, and hematological indicators are associated with supplementation Zn, Se, and Vit- E. The addition of Zn (4 ml/L) and Se and Vit E solution (E-Sel) (0.25 ml/L) to drinking water could enhance broiler growth performance and reduce cholesterol and high-density lipoprotein (HDL) concentration.
Keywords: Broiler, Selenium, Vitamin E, Zinc, Performance

[Full text-PDF]

Review

Association Between Genetic Polymorphisms of Growth Hormone Gene and Egg Production Traits in Chickens: A Systematic Review

Rankotsane HV, Louis TT, and Abdulkareem AA.
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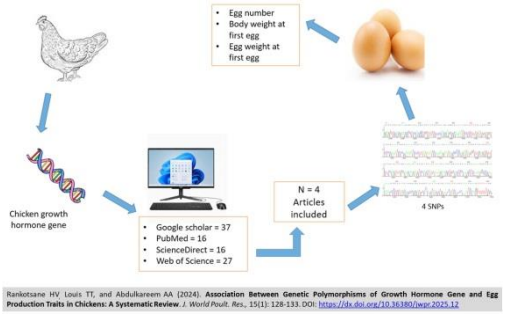

ABSTRACT: Chicken performance traits are affected by the chicken growth hormone (cGH) gene due to its essential part in metabolism and growth, and genetic polymorphisms may be useful as a genetic marker for growth traits. However, no comprehensive review provides information on the cGH polymorphisms and their correlation with egg production traits. The study systematically reviewed the single nucleotide polymorphisms (SNPs) of the growth hormone gene and their association with the chicken's egg production traits. Four databases, Google Scholar, ScienceDirect, PubMed, and Web of Science, were used to search the literature where the keywords 'growth hormone, single nucleotide

polymorphisms, genetic variations, genetic effects, egg production traits, and chickens were the keywords during the literature search. The outcomes revealed that four articles published in 2013, 2014, 2015, and 2018 were included. The results indicated that four SNPs (T185G, G662A, T3094C, and C3199T) were identified, with allelic frequencies ranging from 0.020 to 0.964 and genotypic frequencies ranging from 0.007 to 0.930. The findings indicated that some of the articles used more than one breed. The present reveiw revealed that egg number was found to be significantly associated with discovered genotypes six times, while body weight at first egg and egg weight at first egg were found to be significantly related to discovered genotypes four times. However, additional research is required to validate the identified SNPs. Furthermore, identified SNPs could serve as possible molecular markers to genetically improve egg production in chickens.
Keywords: Average egg weight, Body weight, Egg number, Egg weight, Genotype

[Full text-PDF]

Review

Roles of Environment, Nutrition, and Genetics in Woody Breast Condition in Chickens

Shakeri M.
J. World Poult. Res. 15(1): 134-138, 2025; pii: S2322455X2500013-15
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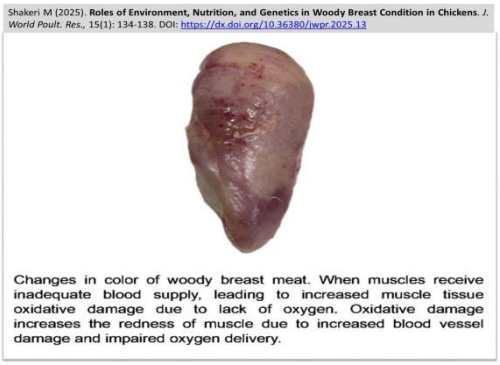

ABSTRACT: Woody breast (WB) condition is a muscle disease in broiler chickens that makes breast meat hard and rubbery, and it has a negative impact on the texture and appearance of fast-growing broiler chicken breast muscle. This condition is safe for consumers, but the meat generally goes under extra meat processing, such as making pet foods, because of lower consumer acceptance, which is an additional cost for the industry. The exact etiology of myopathy is unknown. Although there is no promising solution for the issue, several strategies, such as nutrition, have been introduced to reduce the WB rate. The present study reviewed the strategies that improved WB conditions, including genetics, nutritional, and environmental factors such as temperature and air quality.
Keywords: Broiler chicken, Environment, Genetic, Mitochondria, Nutrition, Woody breast

[Full text-PDF]

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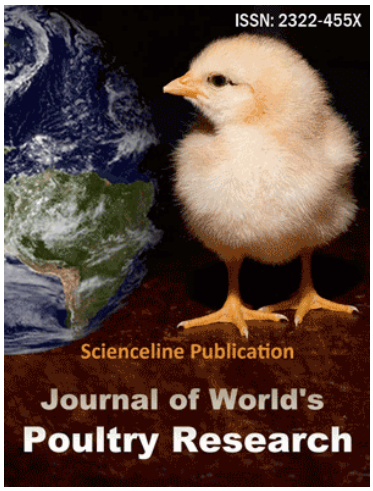

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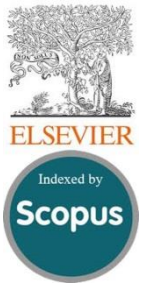

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Effects of Six-Hour Pre-Incubation Thermal Conditioning and Prolonged Storage on Egg Quality, Embryogenesis, Hatchability, and Post-Hatch Physiology of Plymouth Rock Hybrid Chickens in Tropical Climate of Ghana

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ABSTRACT

Prolonged storage negatively impacts incubation outcomes in commercial hatchery operations, highlighting the need for efficient storage strategies. This study assessed the impact of prolonged storage durations and six-hour pre-incubation thermal conditioning (PTC) on egg quality, embryonic development, hatchability, chick quality, blood profile, and thermoregulation. A total of 2,000 fertile eggs were collected from a flock of 72-week-old Plymouth Rock hybrid hens and subjected to a 2 × 2 factorial design, involving storage for either 14 or 21 days, with or without the application of 6-hour PTC. Following storage, the eggs were incubated in a Jamesway P5000 set at a temperature of 37.5°C and relative humidity of 56%, then transferred on incubation day 18 to a hatcher set at 36.5°C and 60% until hatching. Results revealed that prolonged egg storage without PTC significantly diminished egg protein while PTC effectively countered this decline, enhancing Haugh unit values and blastoderm diameter. Prolonged egg storage without PTC also resulted in increased relative egg weight loss (REWL), fluctuated daily eggshell temperature, and reduced embryonic growth during incubation while PTC significantly reduced these effects, with embryos demonstrating significantly enhanced growth. Additionally, while fertility rates remained stable across all treatments, PTC significantly reduced mortality and improved hatchability by 11.4% in 14-day stored eggs and 10.8% in 21-day stored eggs. It also shortened incubation time, increased post-hatch chick body weights and enhanced their hematological and serum profiles, including normalized thyroid hormone (T3 and T4) levels compared to the non-PTC (control) group. Pearson correlation showed that longer incubation time was positively correlated with higher rectal temperature, serum glucose, and thyroid hormones, but negatively correlated with hemoglobin, mean corpuscular hemoglobin, and total protein in non-PTC chicks. In conclusion, six-hour pre-incubation thermal conditioning mitigates the negative effects of prolonged egg storage and enhances embryogenesis, hatchability, chick quality, blood profile, and thermoregulation in Plymouth Rock hybrid chickens.

Keywords: Embryonic development, Extended egg storage, Plymouth rock hybrid chicken, Pre-incubation thermal conditioning, Thermoregulation

INTRODUCTION

Poultry production depends heavily on optimizing factors that influence embryogenesis and chick quality (Tona et al., 2003). Refining egg handling processes is essential to meet the growing demand for high-quality chicks, as they directly impact egg quality, hatchability, and post-hatch outcomes (Gharib, 2013). Proper storage is a critical component of egg handling, facilitating efficient egg collection and transportation coordination to ensure a consistent supply of day-old chicks (Underwood et al., 2021). Prolonged storage poses significant challenges,

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especially in tropical climates where high temperatures accelerate the natural deterioration of eggs (Adriaensen et al., 2022). This extended storage negatively affects embryonic viability, disrupts gas exchange (Reijrink et al., 2010), and leads to nutrient loss due to alterations in the eggshell (Özlü et al., 2018). The negative impacts of prolonged egg storage are intensified by the genetic variability among chicken breeds, which differ in their resilience to adverse conditions (Küçükyılmaz et al., 2012). This genetic diversity influences eggshell characteristics, nutritional content, and developmental potential, leading to breed-specific responses to prolonged storage (Scott and Silversides, 2001). Moreover, the interplay between genetic factors and environmental conditions, particularly in tropical climates, complicates the situation further (Zita et al., 2009). High temperatures typical of tropical environments can compromise eggshell integrity, disrupt gas exchange, and accelerate the breakdown of egg components, including albumen, reducing its viscosity and ability to support embryonic development (Fernandes et al., 2023). Elevated temperatures promote lipid oxidation in the yolk, reducing the nutritional quality and shelf life of stored eggs (Suresh et al., 2015). Consequently, chicken eggs stored under such conditions may lose up to 3.67% of their weight within just ten days (Jin et al., 2010; Wang et al., 2017) and experience a significant decline in hatchability (Chen et al., 2005). These issues are particularly concerning for breeds such as the Plymouth Rock hybrid chicken, known for its adaptability and genetic resilience (Kong et al., 2016), which is commonly raised in tropical climates, especially in Ghana (Guo et al., 2019). Despite their resilience, the heat and humidity fluctuations inherent to these regions adversely impact their physiological processes, accelerating egg degradation during storage (Varguez-Montero et al., 2012; Adegbenro, 2023) and further reducing chick viability (Yamak et al., 2020). Therefore, it is crucial to explore strategies to mitigate the negative effects of storage, particularly for these breeds that are commonly raised as commercial layers.

Given the unique challenges posed by elevated temperatures and prolonged egg storage in tropical climates, it is crucial to explore strategies that mitigate these negative effects, especially for breeds like the Plymouth Rock hybrid chicken, which is commonly raised as a commercial layer. One promising strategy to mitigate the negative effects of prolonged egg storage is the application of short incubation periods during storage, referred to as pre-incubation thermal conditioning (PTC) in the present study. This technique mimics the natural incubation behavior of brooding hens (Damaziak et al., 2018). By briefly exposing stored eggs to controlled warmth, PTC reactivates key metabolic processes, helping to counteract the detrimental effects of extended storage (Nicholson et al., 2013). Previous studies have demonstrated that PTC can enhance hatchability and chick quality by stimulating physiological processes that prepare eggs for incubation (Al-Samrai and Al-Dhanki, 2017; Areaaer and Ibrahim, 2019). However, most research on PTC has focused on broiler chickens under temperate conditions, with shorter exposure durations, typically around four hours, applied over shorter storage periods. This creates a gap in understanding its effectiveness in layer breeds like Plymouth Rock hybrids, which are commonly raised in tropical climates of Ghana. In this region, extended storage periods combined with higher temperatures and humidity further challenge egg quality, potentially altering the effectiveness of PTC compared to temperate conditions.

To address this gap, the current study extended the application of PTC from the typical four hours to six hours, with storage periods prolonged up to 21 days. The objective was to assess its impact on egg quality, embryonic development, hatchability, chick quality, blood profile, and post-hatch thermoregulation in Plymouth Rock hybrid chickens. This is particularly relevant as no previous studies have explored the efficacy of longer PTC durations in this breed under tropical conditions. The six-hour PTC was chosen to provide a more sustained thermal activation that counteracts the accelerated egg deterioration seen in tropical climates, where higher temperatures and longer storage periods reduce hatchability.

MATERIALS AND METHODS

Study area and ethical considerations

The experiment was conducted at the Olympio Hatchery in the Department of Animal Science, Kwame Nkrumah University of Science and Technology (KNUST) in Kumasi, Ghana, a facility equipped for avian research. All experimental procedures adhered to the ethical guidelines approved by the Animal Research Ethics Committee (AREC) of KNUST, ensuring that animal welfare was prioritized throughout the study (Quality Assurance and Planning Unit, POLICY 0016, 2018). This included monitoring the handling of eggs and ensuring that all protocols were designed to minimize stress and discomfort to the experimental chicks involved.

Experimental design and treatment allocation

A 2 × 2 factorial design was applied in a completely randomized framework. The factors included two PTC levels (6-hour PTC versus non-PTC) and two storage durations (14 versus 21 days), resulting in four experimental groups: 6hr-PTC × 14d, non-PTC × 14d, 6hr-PTC × 21d, and non-PTC × 21d. Two additional control groups (3 and 7 days of storage without PTC) served as industry baselines, based on prior research (Ansah et al., 2023) in Black-tailed hybrid chickens.

Egg collection and cold storage conditions

A total of 2,000 fertile eggs were collected from 72-week-old Plymouth Rock hybrid hens at Baffour Farms, Kumasi, Ghana. Eggs were randomly selected within a weight range of ± 0.5 g to maintain uniformity and collected early in the morning to reduce handling time. To align with the designated storage durations, 800 eggs were collected first for the 21-day group, followed by another 800 eggs for the 14-day group one week later. Additionally, 200 eggs were collected each for the 7-day and 3-day groups. For the two longer storage periods, the eggs were equally divided into two groups, one for pre-incubation thermal conditioning and the other as a control group. All eggs were stored under controlled conditions at 16°C and 75% relative humidity.

Pre-incubation thermal conditioning procedure

The pre-incubation thermal conditioning (PTC) process involved several key steps, as illustrated in the schematic flow chart in Figure 1. Eggs were first retrieved from storage conditions (16°C and 75% humidity) and pre-warmed in the incubation corridor at ambient temperatures (26°C-28°C) for 1 hour. They were then placed in a setter incubator at 37.5°C and 56% humidity for 6-hour thermal conditioning. After the thermal conditioning, the eggs were pre-cooled in the incubation corridor for 1 hour before being returned to the cold storage room. Eggs designated for the 14-day storage group received a single PTC treatment on day 10, while the 21-day storage group received two PTC sessions on days 10 and 14. Control eggs remained in a cold storage room without undergoing any thermal conditioning.

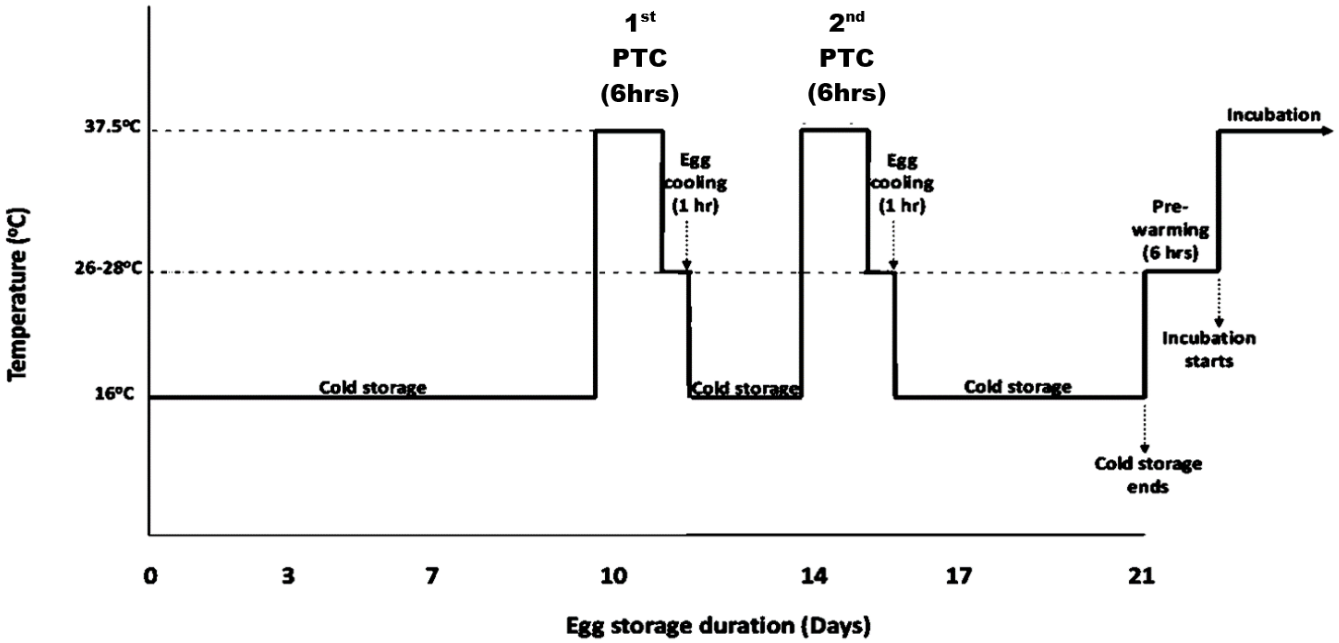

Figure 1. Schematic flow chart of pre-incubation thermal conditioning procedure. Adopted and modified from the earlier procedure. Source: Ansah et al. (2023).

Basic egg quality assessment after egg storage

At the end of the storage and PTC periods, a comprehensive quality assessment of the eggs was conducted before incubation. Each egg was weighed initially and again after the respective storage durations using a precision electronic balance (Model: Ohaus Navigator, Ohaus Corp., USA). The difference between these two weights was expressed as a percentage and recorded as relative egg weight loss during storage. To further assess egg quality parameters, five eggs were randomly selected from each treatment group and carefully cracked open onto a flat surface. The contents were separated into yolk, albumen, and shell, and each component was weighed individually. Eggshell thickness

was measured using a digital micrometer screw gauge (Model: Mitutoyo 293-240, Japan), while the height of the thick albumen surrounding the yolk was measured with the depth gauge of a digital Vernier caliper (Model: Mitutoyo 500-196-30, Japan). The fresh eggshells were weighed, recorded as wet eggshell weight, then washed under running water to remove albumin residues, air-dried for 24 hours, and reweighed to obtain the dry eggshell weight. The fresh yolks were weighed and noted as wet yolk weight, placed in pre-weighed aluminum foil, and dried in a laboratory oven (Model: Memmert UN55, Memmert GmbH + Co. KG, Germany) set at 60°C for four days to facilitate moisture evaporation. After drying, they were reweighed and recorded as "dry yolk weight." The Haugh unit (HU), an established measure of egg protein quality (Haugh, 1937), was calculated using the albumen height and initial egg weight, as described in the Formula 1.

$$Haugh\ unit = 100 \times log(h - 1.7\,w^{0.37} + 7.6) \quad (1)$$

Where h represents the albumen height and w is the egg weight.

Incubation process

Following the respective storage durations and the application of PTC, only the eggs stored for 14 and 21 days were incubated. Before incubation, the eggs were prewarmed for six hours in the incubation corridor, where ambient temperatures ranged from 26°C to 28°C. After prewarming, the eggs were divided into two groups of 1,000 eggs each and placed onto egg trays. These trays were then randomly assigned to two separate setter incubators (Model: P5000, Jamesway Chick Master Ltd, USA), which served as replicated experimental facilities. The incubation process was carried out under controlled conditions of 37.5°C and 56% relative humidity. The incubators were equipped with automatic turning mechanisms that rotated the eggs every hour to ensure even temperature distribution across all surfaces.

Egg weight loss, embryo growth, and metabolic heat production assessments during incubation

The egg weight loss was calculated by weighing two randomly selected egg trays both at the start of incubation (W_0) and on incubation day 18 (W_{18}). The difference in the two weights was used to calculate relative egg weight loss (REWL), expressed as a percentage of the initial weight, using the following formula 2. The external eggshell temperatures were monitored from incubation days 1 to 18 (ID1-18) using a digital infrared thermometer (Model: YI-400, Wenzhou Yosun Medical Technology Co., Ltd, China). The thermometer was calibrated for accuracy and aimed at the external eggshells within the incubator, allowing non-contact temperature measurements that reflected the internal metabolic heat production of the developing embryos (Agyekum et al., 2022). To ensure accuracy and consistency, two representative egg trays from each treatment group were randomly selected from the two separate setter incubators and tagged for all subsequent temperature measurements. Additionally, the temperature within the setter incubators was monitored and recorded daily. The embryo growth assessment was specifically assessed on IDs 4, 7, 11, 14, and 18 as described by Willemsen et al. (2011). Five eggs from each treatment group were randomly selected and cracked open. The embryos were carefully separated from the yolk, weighed, and recorded as “wet embryo weight”. Embryo lengths were measured by extending the embryo from the tip of the beak to the tip of the middle toes using a divider, and the lengths were transferred to a laboratory ruler (Model: 300 mm Stainless Steel Ruler, Mitutoyo Ltd, Japan). After measurements were taken, embryos were oven-dried at 60°C following the procedure described above to remove moisture and subsequently weighed to determine their dry weights.

$$REWL = \frac{(W_0 - W_{18})}{W_0} \times 100 \quad (2)$$

Hatching process and performance assessments

After the embryo assessments, the hourly turning of the eggs continued until day 18, at which point it was discontinued to allow for candling and transfer into the hatcher incubators, preparing the embryos for hatching. To evaluate hatching performance, the fertility rate, embryonic mortality, and hatchability were assessed. The first candling was conducted on day 10 of incubation to differentiate fertile eggs from those experiencing early embryonic death. This process enabled the reliable identification of infertile eggs by visualizing the development of embryonic membranes or color changes in the yolk due to embryonic activity. Infertile eggs were opened to confirm the absence of development. A second candling was performed on day 18 of incubation to assess embryo viability; eggs showing no signs of live embryos were opened to confirm embryo death. Viable eggs were then transferred into hatching baskets and placed in two separate hatcher incubators (Jamesway P5000, USA), set at 36.5°C and 60% relative humidity, to complete the hatching process. On day 22, all hatched chicks were removed from the hatcher, and unhatched eggs were opened to confirm embryo death. The incubation duration,

fertility rate, hatchability, and embryo mortality rates for each treatment group were calculated using the following formulas.

$$Fertility\ rate\ (\%) = \frac{Number\ of\ fertile\ eggs\ on\ day\ 10}{Total\ number\ of\ eggs\ set} \times 100 \quad (3)$$

$$Incubation\ Duration\ (hours) = Day\ of\ Hatch - Day\ of\ Set \quad (4)$$

$$Embro\ mortality\ (\%) = \frac{Number\ of\ dead\ embryos}{Total\ number\ of\ fertile\ eggs} \times 100 \quad (5)$$

$$Hatchability\ (\%) = \frac{Number\ of\ chicks\ hatched}{Total\ number\ of\ fertile\ eggs} \times 100 \quad (6)$$

Post-hatch chick quality assessments

Immediately after hatching, the chicks were gathered and transferred to a designated holding room, where they were organized into their respective treatment groups for a series of quality assessments focusing on critical aspects of body and skeletal development, as well as yolk absorption and navel quality. These assessments were repeated on the seventh-day post-hatch. On both assessment days, five chicks from each treatment group were randomly selected, and their body weights, lengths, and shank lengths were measured. Navel quality was evaluated using the PASCAR scoring system, as described by Rocha et al. (2013) and Yeboah et al. (2019). This system assesses factors such as the presence of navel strings, buttons, and the overall healing process. Chicks with fully closed and clean navels received a score of 1, while those with discolored navels, openings larger than 2 mm, leaking, or attached navel strings were assigned a score of 2. The number of chicks in each category was tallied and expressed as a percentage of the total number of hatched chicks.

Post-hatch physiological assessments

Before and during the quality assessments, the chicks underwent a series of physiological evaluations, which included measurements of rectal temperature, hematological analyses, serum biochemistry, and thyroid hormone assessments, as described below.

Rectal temperature assessments

The rectal temperature of the chicks was measured immediately upon their arrival in the room and subsequently recorded hourly for the first 24 hours. Five chicks from each treatment group were randomly selected for this assessment, with their rectal temperatures recorded using a digital cloacal thermometer (Model: Omron Flex Temp Smart, Omron Healthcare, Japan). Each chick was gently restrained in an upright position with minimal force, ensuring both wings and legs were secured to prevent movement, following the method described by Agyekum et al. (2022). This assessment was conducted to evaluate their thermoregulation capacity and ability to adapt to ambient temperature variations within the holding room.

Blood sampling and laboratory assessments

Blood samples were taken for hematological and serum biochemical analyses by adhering to protocols established by Maxine (1961) and Alonge (2017). Hematological analysis provided insights into oxygen transport, immune function, and overall health. Serum biochemistry evaluated organ function, protein metabolism, and energy utilization, with optimal levels signifying effective nutrient processing crucial for post-hatch development. For both hematology and serum analyses, five chicks from each treatment group were randomly selected. Approximately 5 mL of blood was drawn from 5 chicks in each treatment group. The samples were split, with half placed in EDTA tubes for hematological analysis and the other half in plain red-top tubes for serum biochemistry analysis. All samples were appropriately labeled and transported in a cooler with ice packs to the Main Research Laboratory of Ghana Veterinary Services Directorate, Kumasi-Amakom Division, in line with the transport guidelines outlined by Barde (2022). In the lab, total red blood cell (RBC) was analyzed using a hemocytometer, as reported by Campbell (1995). The packed cell volume (PCV) was determined using the microhematocrit technique following the procedure of Oguntoye (2018). Haemoglobin (Hb) concentrations were analyzed using the cyanmethemoglobin method, as detailed by Simaraks (2004). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), were calculated based on the RBC, PCV, and Hb values, as described by Ritchie et al. (1994). For serum analysis, blood samples were centrifuged at 3,000 rpm for 15 minutes and the resultant serum was further analyzed. Serum total protein and albumin were quantified as described by Varley et al. (1980) and Doumas et al. (1971) respectively. Globulin was calculated by subtracting albumin from total protein as suggested by Barde (2022). Serum total cholesterol was evaluated using the enzymatic end-point method according to Roschlau et al. (1974), and triglycerides were measured using the colorimetric technique described by Bowers and Wong (1980). Glucose concentrations were assessed using the Glucose Oxidase/Peroxidase-Aminoantipyrine-Phenol (GOD/PAP) reagent method as reported by Trinder (1969). Finally, two key thyroid hormones, Tri-iodothyronine (T3) and Thyroxine (T4) concentrations were assessed using the Vitek Immuno Diagnostic Assay System (VIDAS), applying the enzyme-linked fluorescent assay (ELFA) technique described by Favresse et al. (2018). All samples were analyzed within a single assay batch to ensure consistency.

Statistical analysis

For the basic egg quality assessment data collected after storage, a one-way ANOVA was performed while data collected during incubation and post-hatch were analyzed using a two-way ANOVA. All data were analyzed using the generalized linear model (GLM) procedure in SAS version 9.4. Treatment means were compared using the Student Newman-Keuls (SNK) test, with significance determined at $p < 0.05$. The statistical model used to analyze the data was $Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha \times \beta)_{ij} + \varepsilon_{ijkY}$. Where *Yijk* represents the measured response (parameters) for either egg or chick. The term μ refers to the overall mean of all observations, providing a baseline reference for comparison. The term αi refers to the effect of egg storage duration, where *i* is either one of the two levels (14 or 21 days). β_j represents the effect of preincubation thermal conditioning (PTC), where *j* is either one of the two levels (no-PTC or 6-hour PTC). The interaction term, $(\alpha \times \beta)_{ij}$ captures how egg storage duration and PTC interact. Finally, ε_{ijk} captures the residual error term, which accounts for the variability in the data that could not be explained by the fixed effects (storage duration, PTC, or their interaction). Furthermore, Pearson correlation analysis was employed to examine relationships between incubation duration and key physiological parameters such as rectal temperature, hematology, and serum metabolites, with statistical significance set at $p < 0.05$.

RESULTS AND DISCUSSION

Effect of prolonged storage and pre-incubation thermal conditioning on egg quality

Table 1 presents the effects of prolonged egg storage and pre-incubation thermal conditioning (PTC) on basic egg quality parameters after storage. The results indicate no significant differences in final egg weight, egg weight loss, yolk weight, shell weight, albumen weight, or shell thickness ($p > 0.05$), suggesting that neither prolonged storage nor PTC significantly influenced these attributes. This finding contrasts with the studies by Fasenko et al. (2001), Addo et al. (2018), and Abioja et al. (2021), which reported that prolonged storage adversely affects these quality parameters. However, as illustrated in Figure 2, the Haugh unit (HU), a critical indicator of egg protein quality, confirms that prolonged storage duration negatively impacted egg quality. Specifically, eggs stored for both 14 and 21 days showed a significant decline in protein quality, with HU values of 68 and 49 compared to 81 and 78 for eggs stored for shorter durations of 3 and 7 days, respectively ($p < 0.05$). These results are consistent with previous research, which demonstrates that prolonged storage negatively impacts egg protein quality (Scott and Silversides, 2001; Sekeroglu et al., 2008; Akyurek and Okur, 2009; Chung and Lee, 2014; Adeoye et al., 2023). A key finding in this study is that pre-incubation thermal conditioning (PTC) helped mitigate the deterioration of protein quality caused by prolonged storage. For eggs stored for 14 days, PTC significantly improved the Haugh Unit (HU) to 72, compared to 68 in the non-PTC control group ($p < 0.05$).

This effect was even more pronounced in eggs stored for 21 days, where PTC-treated eggs maintained an HU of 65, while the control group dropped to 49 ($p < 0.05$). Furthermore, PTC significantly increased blastoderm diameter, a critical marker of early embryogenesis compared to the control groups ($p < 0.05$), confirming the findings that PTC not only preserves egg protein integrity but also enhances early embryogenesis during extended storage (Romão et al., 2008).

Table 1. Effects of prolonged storage and pre-incubation thermal conditioning on egg quality after storage in Plymouth rock hybrid chickens

Treatment	IEW (g)	FEW (g)	% EWL	WY-Wt (g)	DY-Wt (g)	WS-Wt (g)	DS-Wt (g)	ALB-Wt (g)	WST (cm)	DST (cm)	BD (cm)
non-PTC×3d	55.70	55.50	9.15	26.30	25.1	13.1	10.58	63.60	0.57	0.55	0.31^{c}
non-PTC×7d	55.40	55.20	9.17	27.60	26.4	13.8	10.30	62.50	0.55	0.50	0.32^{c}
non-PTC×14d	55.10	49.70	9.80	23.70	23.6	11.8	10.20	62.30	0.53	0.45	0.26^{d}
6hr-PTC×14d	55.10	50.00	9.25	24.10	24.5	11.5	10.10	62.30	0.54	0.47	0.36^{b}
non-PTC×21d	55.10	49.90	9.45	23.90	23.6	12.0	10.40	61.00	0.50	0.42	0.27^{d}
6hr-PTC-21d	55.10	50.20	9.00	24.90	24.6	12.6	10.40	61.80	0.52	0.44	0.41^{a}
SEM	0.321	0.732	1.27	0.430	0.421	0.220	0.330	0.523	0.432	0.221	0.002
P-value	0.624	0.124	0.637	0.157	0.157	0.157	0.129	0.221	0.070	0.060	<0.001

Means of different superscripts ($^{a, b, c, d}$) within a column differ significantly at $p < 0.05$. SD: Storage duration; PTC: 6-hour preincubation thermal conditioning; non-PTC×3d: Fresh eggs stored for 3 days without PTC (control), non-PTC×7d: Fresh eggs stored for 7 days without PTC (control), non-PTC×14d: Fresh eggs stored for 14 days without PTC (control), 6hr-PTC×14d: Fresh eggs stored for 14 days with PTC non-PTC×21d: Fresh eggs stored for 21 days without PTC (control), 6hr-PTC-21d: Fresh eggs stored for 21 days with PTC, EW: Initial egg weight (g), FEW: Final egg weight (g), %EWL: Egg weight loss, WY-Wt: Wet yolk weight (g), DY-Wt: Dry yolk weight (g), WS-Wt: Wet shell weight (g), DS-Wt: Dry shell weight (g), ALB-Wt: Albumen weight (g), WST: Wet shell thickness (cm), DST: Dry shell thickness (cm), BD: Blastoderm diameter (cm). SEM: Pooled standard error of means. P-value: probability value.

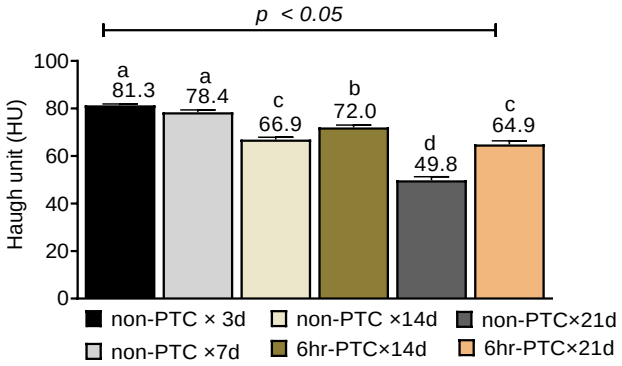

Figure 2. Effects of prolonged storage and pre-incubation thermal conditioning on Haugh unit as a measure of egg protein quality in Plymouth Rock hybrid chickens. non-PTC × 3d: Fresh eggs stored for 3 days without PTC (control), non-PTC×7d: Fresh eggs stored for 7 days without PTC (control), non-PTC×14d: Fresh eggs stored for 14 days without PTC (control), 6hr-PTC×14d: Fresh eggs stored for 14 days with 6hr-PTC, non-PTC×21d: Fresh eggs stored for 21 days without PTC (control), 6hr-PTC×21d: Fresh eggs stored for 21 days with PTC.

Effect of prolonged storage and pre-incubation thermal conditioning on embryo metabolic heat production during incubation

Table 2 shows the impact of storage duration and pre-incubation thermal conditioning (PTC) on the average eggshell temperature (AEST), which reflects the net metabolic heat production of embryos from incubation day 1 to 18 (ID1-18). No significant differences in AEST were observed between eggs stored for 14 and 21 days ($p > 0.05$). However, eggs subjected to PTC exhibited significantly higher AEST compared to the non-PTC (control) group for both storage durations ($p < 0.05$). As illustrated in Figure 3, the daily eggshell temperature (DEST) trends demonstrate that eggs stored for 14 days and treated with PTC maintained a more stable temperature, peaking at 38.5°C by ID18, compared to a fluctuating peak of 38.3°C in the non-PTC group. A similar pattern was observed in eggs stored for 21 days, where PTC-treated eggs reached a peak DEST of 38.6°C, while the control group exhibited a less stable rise, peaking at 38.0°C. These stable and higher DEST patterns in PTC-treated embryos suggest improved metabolic efficiency, as eggshell temperature serves as a key indicator of embryonic metabolic activity (Agyekum et al., 2022). Moreover, PTC may have activated pro-survival mechanisms, such as the upregulation of heat shock protein 70 (Hsp70), which is known to enhance embryonic metabolic activity and promote stress resilience (Jiang et al., 2011; Gan et al., 2015; Brady et al., 2022). This upregulation could explain the more stable and relatively higher eggshell temperatures observed in thermally conditioned embryos.

Table 2. Effects of prolonged storage and pre-incubation thermal conditioning on average eggshell temperature during incubation in Plymouth rock hybrid chickens

Factor	Eggshell temperature (°C)
SD	
14	37.94
21	37.89
SEM	0.057
P-value	0.536
PTC	
non-PTC	37.81[b]
6hr-PTC	38.02[a]
SEM	0.057
P-value	0.009
PTC × SD	
non-PTC × 14d	37.87
6hr-PTC × 14d	38.01
non-PTC × 21d	37.74
6hr-PTC × 21d	38.03
SEM	0.080
P-value	0.372

Means of different superscripts ([a, b]) within a column differ significantly at $p < 0.05$. SD: Storage duration; PTC: 6-hour preincubation thermal conditioning, non-PTC: Eggs that did not receive any PTC (control); 6hr-PTC: Eggs that received 6-hour PTC, PTC × SD: Non-PTC × 14d: Eggs stored for 14 days and did not receive PTC (control), 6hr-PTC × 14d: Eggs stored for 14 days and received PTC for 6 hours, non-PTC ×21d: Eggs stored for 21 days and did not receive PTC (control), 6hr-PTC × 21d: Eggs stored for 21 days and received PTC for 6 hours. SEM: Pooled standard error of means. P-value: Probability value.

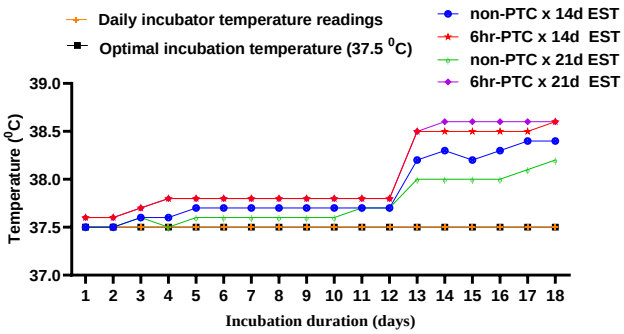

Figure 3. The pattern of daily eggshell temperature (DEST) and daily incubation temperature measured during the incubation period for Plymouth Rock Hybrid Chickens. The data points represent the average temperature measurements pooled from two separate incubators. EST: Eggshell temperature, non-PTC × 14d: Eggs stored for 14 days and did not receive PTC (control), 6hr-PTC × 14d: Eggs stored for 14 days and received PTC for 6 hours, non-PTC × 21d: Eggs stored for 21 days and did not receive PTC (control), 6hr-PTC × 21d: Eggs stored for 21 days and received PTC for 6 hours.

Effect of prolonged storage and pre-incubation thermal conditioning on relative egg weight loss during incubation

Table 3 presents the impact of storage duration and PTC on relative egg weight loss (REWL) during incubation. Extended storage increased REWL significantly, with eggs stored for 21 days losing 5.8% of their weight, compared to only 2.8% for those stored for 14 days ($p < 0.05$), as noted by Ruiz and Lunam (2002) and Khan et al. (2013). Pre-incubation thermal conditioning effectively reduced REWL across both storage periods. Specifically, PTC-treated eggs stored for 14 days lost only 4.0% of their weight compared to 5.6% in the control group. For eggs stored for 21 days, PTC-treated eggs showed an even lower weight loss of 1.5% while the control group lost 8.5% ($p < 0.05$). The increased REWL in the control group is likely due to albumen degradation and water loss (Hamidu et al., 2010). Overall, PTC demonstrated greater effectiveness in reducing weight loss, especially with prolonged storage, consistent with findings from Areaaer and Ibrahim (2019), Ansah et al. (2023), and Okasha et al. (2023).

Effect of prolonged storage and pre-incubation thermal conditioning on embryo growth dynamics during incubation

Table 4 shows the effects of prolonged storage and PTC on embryo growth. The results indicate that embryos from eggs stored for 14 days had significantly higher body weights by incubation day 18 (ID18) compared to those from the eggs stored for 21 days ($p < 0.05$). Notably, the application of PTC mitigated this negative impact and enhanced embryo growth compared to the non-PTC control group ($p < 0.05$). For eggs stored for 14 days, PTC-treated embryos had significantly higher body measurements by ID18, with wet weights of 23.1 g, dry weights of 8.2 g, and body lengths of 82.3 cm. In contrast, the control group recorded 22.7 g, 8.1 g, and 80.8 cm, respectively ($p < 0.05$). Similarly, for eggs stored for 21 days, PTC-treated embryos achieved 24.2 g wet weight, 9.7 g dry weight, and 97.3 cm in length, compared to 20.7 g, 7.2 g, and 71.8 cm for the control group ($p < 0.05$). These results affirm PTC's ability to mitigate the negative effects of prolonged storage, consistent with findings by Elmenawey (2019). Furthermore, Bakst et al. (2016) and Hemida et al. (2023) suggest that PTC upregulates heat shock protein 70 (Hsp70), enhancing metabolic processes and stress resilience while regulating anti-apoptotic proteins like B-cell lymphoma 2 (Bcl-2). This regulation likely inhibited early programmed cell death during prolonged storage, promoting cell survival and functionality, and ensuring effective cellular proliferation and differentiation, accounting for better growth outcomes observed in the PTC embryos (Kong et al., 2016).

Effect of prolonged storage and pre-incubation thermal conditioning on hatch outcomes

Figure 4 illustrates the effects of prolonged storage and PTC on hatch outcomes. Fertility rates remained consistent across all treatment groups, indicating that neither prolonged storage nor PTC adversely affected egg fertility. However, hatchability declined with longer storage periods, supporting findings from Ruiz and Lunam (2002), Mahmud et al. (2011), and Khan et al. (2013). Notably, PTC-treated eggs stored for 14 days achieved the highest hatchability rate of 76.4%, with a corresponding mortality rate of 23.6% and an incubation duration of 492 hours. In contrast, the non-PTC control group showed a hatchability rate of 65%, a mortality rate of 35%, and a longer incubation duration of 518.4 hours. For eggs stored for 21 days, PTC-treated embryos had a hatchability of 72.1%, with a mortality rate of 27.9% and an incubation duration of 496.8 hours, compared to a hatchability of 61.3%, a mortality rate of 38.7%, and an incubation duration of 530.4 hours in the control group. Overall, PTC improved hatchability by 11.4% and reduced incubation time by 26.4 hours for eggs stored for 14 days, increased hatchability by 10.8%, and reduced incubation duration by 33.6 hours for eggs stored for 21 days. These findings align with previous studies (Nicholson et al., 2013; Al-Samrai and Al-Dhanki, 2017; Areaaer and Ibrahim, 2019; Özlü et al., 2021; Abdel-Fattah et al., 2024), which emphasize PTC's role in enhancing hatchability and reducing incubation time. A shorter incubation duration is particularly beneficial, as prolonged incubation has been linked to reduced yolk sac absorption at the late stage of incubation (Özlü et al., 2021).

Effect of prolonged storage and pre-incubation thermal conditioning on post-hatch chick quality

Table 5 presents the impact of prolonged storage and PTC on post-hatch chick quality. Chicks hatched from eggs stored for 14 days had superior quality compared to those stored for 21 days ($p < 0.05$). The application of PTC significantly improved chick quality attributes compared to the control group ($p < 0.05$). For example, PTC-treated chicks from 14-day storage weighed 62.7 g, measured 146 mm in length, had a shank length of 27.3 mm, a residual yolk sac of 0.34 g, and a PASCAR score of 94.3%. In contrast, control chicks weighed 60.0 g, measured 115 mm, had a shank length of 25.9 mm, a residual yolk sac of 0.57 g, and a PASCAR score of 92.2%

($p < 0.05$). Similarly, for eggs stored for 21 days, PTC-treated chicks weighed 69.1 g, measured 138 mm, had a shank length of 22.2 mm, a residual yolk sac of 0.25 g, and a PASCAR score of 95.0%. The control group had significantly lower values: 47.5 g, 127 mm, 31.1 mm, 0.67 g, and a PASCAR score of 79.4% ($p < 0.05$). The improvements in body weight, PASCAR scores, and reduced residual yolk sac in PTC chicks support the findings of El-Garhy (2021) and Maman and Yildirim (2022). The reduced residual yolk sac in PTC-treated chicks indicates that PTC enhances yolk sac absorption, aligning with findings by Ebeid et al. (2017) and Damaziak et al. (2018) but contrasting those of Ansah et al. (2023). This improvement is crucial as embryos primarily depend on lipids from the yolk sac for energy during the final stages of incubation (Khosravinia, 2015).

Effect of prolonged storage and pre-incubation thermal conditioning on post-hatch chick thermoregulation capacity

Table 6 summarises the effects of prolonged egg storage and pre-incubation thermal conditioning (PTC) on chick rectal temperature (CRT) within the first 24 hours post-hatching. Chicks hatched from eggs stored for 21 days exhibited significantly higher CRTs compared to those stored for 14 days ($p < 0.05$), indicating a heightened metabolic rate and potential stress from compromised heat dissipation (Maman et al., 2019). However, PTC was effective in reducing CRT for both storage durations, helping maintain body temperature within a stable range. For instance, chicks from 14-day stored eggs with PTC showed a CRT of around 40.0°C, aligning with normal thermoregulation, whereas non-PTC counterparts recorded a higher CRT of 41.58°C ($p < 0.05$). Similarly, for 21-day stored eggs, PTC-treated chicks displayed a CRT of 40.07°C, notably lower than the 42.25°C observed in non-PTC chicks ($p < 0.05$). Figure 5 complements this by showing the CRT patterns across the 24 hours post-hatching, where PTC-treated chicks exhibited more stable and moderate temperatures. This stabilization suggests that PTC may enhance thermoregulatory capacity, potentially by supporting the development of key hormonal pathways, such as the hypothalamic-pituitary-thyroid and hypothalamic-pituitary-adrenal axes, which are essential for thermoregulation in homoeothermic animals (Debonne et al., 2008). These hormonal adaptations are likely to improve metabolic efficiency, allowing chicks to better regulate body temperature, an advantageous trait, especially for tropical breeds like Plymouth Rock (Ouchi et al., 2022). These findings align with previous studies (Nilsson and Persson, 2004; Page et al., 2022; El-Prollosy et al., 2024) suggesting that pre-incubation thermal conditioning can positively influence post-hatch thermoregulation and mitigate metabolic stress, especially for chicks hatched from eggs stored for extended periods. In contrast, the higher and more variable CRT in non-PTC chicks implies a less developed thermoregulatory response, potentially making them more susceptible to heat stress and dehydration, factors that could elevate early mortality risks (Romijn, 1954; Dunnington and Siegel, 1984; Decuypere et al., 2001).

Effect of prolonged storage and pre-incubation thermal conditioning on chick haematology

Table 7 highlights the effects of prolonged storage and PTC treatment on chick hematology, revealing that prolonged storage significantly impacted key parameters ($p < 0.05$). For eggs stored for 14 days, the non-PTC group exhibited a red blood cell (RBC) count of 2.10×10^{12}/L, significantly lower ($p < 0.05$) than the normal range of $2.2–4.0 \times 10^{12}$/L (Joshua et al., 2022), indicating reduced oxygen-carrying capacity. In contrast, the PTC group had a count of 2.37×10^{12}/L, which is within the normal range. The hemoglobin (HGB) level for the non-PTC group was 6.08 g/dL, significantly lower ($p < 0.05$) than the normal range of 7.0–11.0 g/dL (Benjamin, 1985), reflecting a potential risk of anemia, while the PTC group had a higher HGB of 8.67 g/dL, within normal limits. Likewise, for eggs stored for 21 days, the non-PTC group showed a significantly reduced RBC count of 1.65×10^{12}/L and HGB of 7.66 g/dL, both significantly lower ($p < 0.05$) than normal ranges, indicating severe anemia. Conversely, the PTC group maintained levels of 2.21×10^{12}/L and 9.20 g/dL, respectively, both within normal ranges (Merck veterinary manual, 2023). Haematocrit (HCT) also followed this trend, with the PTC group showing significantly higher values (32.9% versus 27.8% for non-PTC), indicating stable blood volume and improved health. Additionally, the PTC group had significantly higher mean corpuscular volume (136 versus134 fL), mean corpuscular hemoglobin (29.9 versus 28.7 pg), and mean corpuscular hemoglobin concentration (255 versus 247 g/dL, $p < 0.05$), reflecting enhanced blood oxygenation. Platelet counts were within normal ranges (Joshua et al., 2022) across all treatments, with the PTC group showing numerically higher counts, indicating better clotting ability. Overall, PTC treatment effectively mitigates the adverse effects of prolonged storage, promoting optimal growth and reducing the risk of anemia in Plymouth rock hybrid chickens.

Table 3. Effects of prolonged storage and pre-incubation thermal conditioning on relative egg weight loss during incubation in Plymouth rock hybrid chickens

Factor	Initial egg weight (g)	Final egg weight (g)	Relative egg weight loss (%)
SD			
14	50.14	48.75a	2.77[b]
21	50.21	47.29b	5.82[a]
SEM	0.032	0.730	1.270
P-value	0.066	0.017	0.029
PTC			
non-PTC	50.37	47.18[b]	6.33[a]
6hr-PTC	50.44	48.85[a]	3.15[b]
SEM	0.032	0.730	1.270
P-value	0.054	0.012	0.043
PTC × SD			
non-PTC × 14d	50.69	46.39[c]	5.55[b]
6hr-PTC × 14d	50.21	48.19[ab]	4.02[b]
non-PTC × 21d	50.80	47.98[b]	8.48[a]
6hr-PTC × 21d	50.26	49.52[a]	1.47[c]
SEM	0.045	1.030	1.800
P-value	0.090	< 0.001	< 0.001

Means of different superscripts ([a, b, c]) within a column differ significantly at $p < 0.05$. SD: Storage duration; PTC: 6-hour preincubation thermal conditioning, non-PTC: Eggs that did not receive any PTC (control); 6hr-PTC: Eggs that received 6-hour PTC, PTC × SD: non-PTC × 14d: Eggs stored for 14 days and did not receive PTC (control), 6hr-PTC × 14d: Eggs stored for 14 days and received PTC for 6 hours, non-PTC ×21d: Eggs stored for 21 days and did not receive PTC (control), 6hr-PTC × 21d: Eggs stored for 21 days and received PTC for 6 hours. SEM: Pooled standard error of means. P-value: Probability value.

Table 4. Effects of prolonged storage and pre-incubation thermal conditioning on embryo growth during incubation in Plymouth rock hybrid chickens

	Wet embryo weight (g)					Dry embryo weight (g)					Embryo length (cm)				
Factor	**4**	**7**	**11**	**14**	**18**	**4**	**7**	**11**	**14**	**18**	**4**	**7**	**11**	**14**	**18**
SD															
14	0.19^{a}	0.94^{a}	4.21^{a}	7.98^{a}	24.40^{a}	0.065	0.26^{a}	2.16^{a}	7.68^{a}	10.16^{a}	19.8^{a}	26.8^{a}	42.1^{a}	79.8^{a}	84.6^{a}
21	0.18^{b}	0. 69^{b}	3.62^{b}	6.96^{b}	22.20^{b}	0.080	0.20^{b}	2.01^{b}	6.48^{b}	8.14^{b}	18.1^{b}	20.3^{b}	36.2^{b}	69.6^{b}	81.6^{b}
SEM	0.002	0.021	0.021	0.006	0.510	0.012	0.003	0.040	0.091	0.350	0.580	0.320	1.55	0.870	2.54
P-value	0.009	< 0.001	<0.001	< 0.001	0.017	0.374	< 0.001	0.018	< 0.001	0.636	0.048	< 0.001	0.016	< 0.001	0.041
PTC															
non-PTC	0.19	0.20^{b}	3.24^{b}	6.91^{b}	21.7^{b}	0.057	0.57^{b}	1.78^{b}	5.50^{b}	5.63^{b}	18.1	20.1^{b}	32.4^{b}	69.1^{b}	76.3^{b}
6hr-PTC	0.18	0.27^{a}	4.58^{a}	8.03^{a}	23.2^{a}	0.088	1.10^{a}	2.39^{a}	6.66^{a}	8.98^{a}	19.8	27.1^{a}	45.8^{a}	80.3^{a}	89.8^{a}
SEM	0.002	0.003	0.021	0.006	0.510	0.012	0.021	0.040	0.091	0.35	0.58	0.32	1.55	0.87	2.54
P-value	0.940	< 0.001	< 0.001	<0.001	0.040	0.089	< 0.001	< 0.001	<0.001	0.020	0.763	< 0.001	< 0.001	< 0.001	0.002
PTC× SD															
non-PTC × 14d	1.93	0.158^{c}	3.88^{a}	6.28^{c}	22.72^{b}	0.040	0.41^{d}	2.03^{b}	4.62^{d}	8.08^{b}	18.3	25.3^{b}	26.1^{b}	76.3^{b}	80.8^{b}
6hr-PTC × 14d	2.03	0.26^{b}	4.50^{a}	8.33	23.10^{ab}	0.090	0.97^{b}	2.29^{a}	7.83^{b}	8.23^{b}	19.8	25.8^{b}	45.3^{a}	77.3^{b}	82.3^{b}
non-PTC × 21d	1.88	0.25^{b}	2.61^{b}	7.53^{b}	20.7^{b}	0.075	0.74^{c}	1.52^{c}	6.37^{c}	7.18^{b}	17.3	14.8^{c}	38.8^{b}	62.8^{c}	71.8^{b}
6hr-PTC × 21d	1.73	0.28^{a}	4.63^{a}	8.43^{a}	24.2^{a}	0.085	1.15^{a}	2.50^{a}	9.00^{a}	9.73^{a}	20.3	28.3^{a}	46.3^{a}	84.3^{a}	97.3^{a}
SEM	0.003	0.004	0.031	0.009	0.720	0.017	0.030	0.056	0.130	0.490	0.820	0.450	2.19	1.23	3.59
P-value	0.612	0.028	0.292	< 0.001	0.010	0.243	0.023	< 0.001	0.001	0.036	0.145	< 0.001	0.006	0.036	0.004

Means of different superscripts ($^{a, b, c}$) within a column differ significantly at $p < 0.05$. SD: Storage duration; PTC: 6-hour preincubation thermal conditioning, non-PTC: Eggs that did not receive any PTC (control); 6hr-PTC: Eggs that received 6-hour PTC, PTC × SD: Non-PTC × 14d: Eggs stored for 14 days and did not receive PTC (control), 6hr-PTC × 14d: Eggs stored for 14 days and received PTC for 6 hours, non-PTC ×21d: Eggs stored for 21 days and did not receive PTC (control), 6hr-PTC × 21d: Eggs stored for 21 days and received PTC for 6 hours. SEM: Pooled standard error of means. P-value: Probability value.

Table 5. Effects of prolonged storage and pre-incubation thermal conditioning on post-hatch chick quality in Plymouth Rock hybrid chickens

	Chick weight (g)		Chick length (mm)		Shank length (mm)		Residual yolk (g)		PASCAR
Factor	**Day 1**	**Day 7**	**Day 1**	**Day 7**	**Day 1**	**Day 7**	**Day 1**	**Day 7**	**Score (%)**
SD									
14	42.4^{a}	61.3^{a}	114^{a}	132^{a}	24.2^{a}	28.6^{a}	3.62^{b}	0.30^{b}	94.1^{a}
21	38.5^{b}	58.3^{b}	109^{b}	130^{b}	22.0^{b}	26.6^{b}	5.62^{a}	0.70^{a}	84.7^{b}
SEM	0.660	0.940	0.990	1.350	1.300	1.490	0.190	0.024	1.570
P-value	< 0.001	0.027	0.006	0.031	0.023	0.045	0.001	0.001	<0.001
PTC									
non-PTC	36.4^{b}	53.7^{b}	104^{b}	121^{b}	25.5^{a}	29.5^{a}	6.12^{a}	0.77^{a}	86.8^{b}
6hr-PTC	44.5^{a}	65.9^{a}	119^{a}	142^{a}	20.8^{b}	24.7^{b}	5.12^{b}	0.64^{b}	92.0^{a}
SEM	0.660	0.940	0.990	1.35	1.30	1.49	0.190	0.024	1.57
P-value	< 0.001	< 0.001	< 0.001	< 0.001	0.015	0.030	0.001	0.001	0.024
PTC × SD									
non-PTC × 14d	40.0^{b}	60.0^{b}	104^{c}	115^{d}	25.4^{a}	27.9^{ab}	6.12	0.77	92.2^{b}
6hr-PTC × 14d	44.8^{a}	62.7^{b}	123^{a}	146^{a}	23.0a^{b}	27.3^{ab}	5.12	0.64	94.3^{a}
non-PTC × 21d	32.7^{c}	47.5^{c}	104^{c}	127^{c}	25.5^{a}	31.1^{a}	6.12	0.77	79.4^{c}
6hr-PTC × 21d	44.3^{a}	69.1^{a}	115^{b}	138^{b}	18.5^{b}	22.2^{b}	5.12	0.64	95.0^{a}
SEM	0.930	1.330	1.400	1.910	1.840	2.110	0.270	0.033	2.210
P-value	0.001	< 0.0001	0.008	< 0.0001	0.021	0.041	1.000	1.000	0.020

Means of different superscripts ($^{a, b, c, d}$) within a column differ significantly at $p < 0.05$. SD: Storage duration; PTC: 6-hour preincubation thermal conditioning, non-PTC: Eggs that did not receive any PTC (control); 6hr-PTC: Eggs that received 6-hour PTC, PTC × SD: non-PTC × 14d: Eggs stored for 14 days and did not receive PTC (control), 6hr-PTC × 14d: Eggs stored for 14 days and received PTC for 6 hours, non-PTC × 21d: Eggs stored for 21 days and did not receive PTC (control), 6hr-PTC × 21d: Eggs stored for 21 days and received PTC for 6 hours. SEM: Pooled standard error of means. P-value: Probability value.

Table 6. Effects of prolonged storage and pre-incubation thermal conditioning on the average rectal temperature of Plymouth Rock hybrid chickens measured hourly within 24 hours after hatching

Factor		Chick rectal temperature (^{0}C)
SD	14	40.64[b]
	21	41.16[a]
	SEM	0.260
	P-value	0.033
PTC	non-PTC	41.92[a]
	6hr-PTC	39.88[b]
	SEM	0.261
	P-value	0.013
PTC × SD	non-PTC × 14d	41.58[a]
	6hr-PTC × 14d	39.69[b]
	non-PTC × 21d	42.25[a]
	6hr-PTC × 21d	40.07[b]
	SEM	0.368
	P-value	0.045

Means of different superscripts ([a, b]) within the column differ significantly at $p < 0.05$. SD: Storage duration; PTC: 6-hour preincubation thermal conditioning, non-PTC: Eggs that did not receive any PTC (control); 6hr-PTC: Eggs that received 6-hour PTC, PTC × SD: Non-PTC × 14d: Eggs stored for 14 days and did not receive PTC (control), 6hr-PTC × 14d: Eggs stored for 14 days and received PTC for 6 hours, non-PTC ×21d: Eggs stored for 21 days and did not receive PTC (control), 6hr-PTC × 21d: Eggs stored for 21 days and received PTC for 6 hours. SEM: Pooled standard error of means. P-value: Probability value.

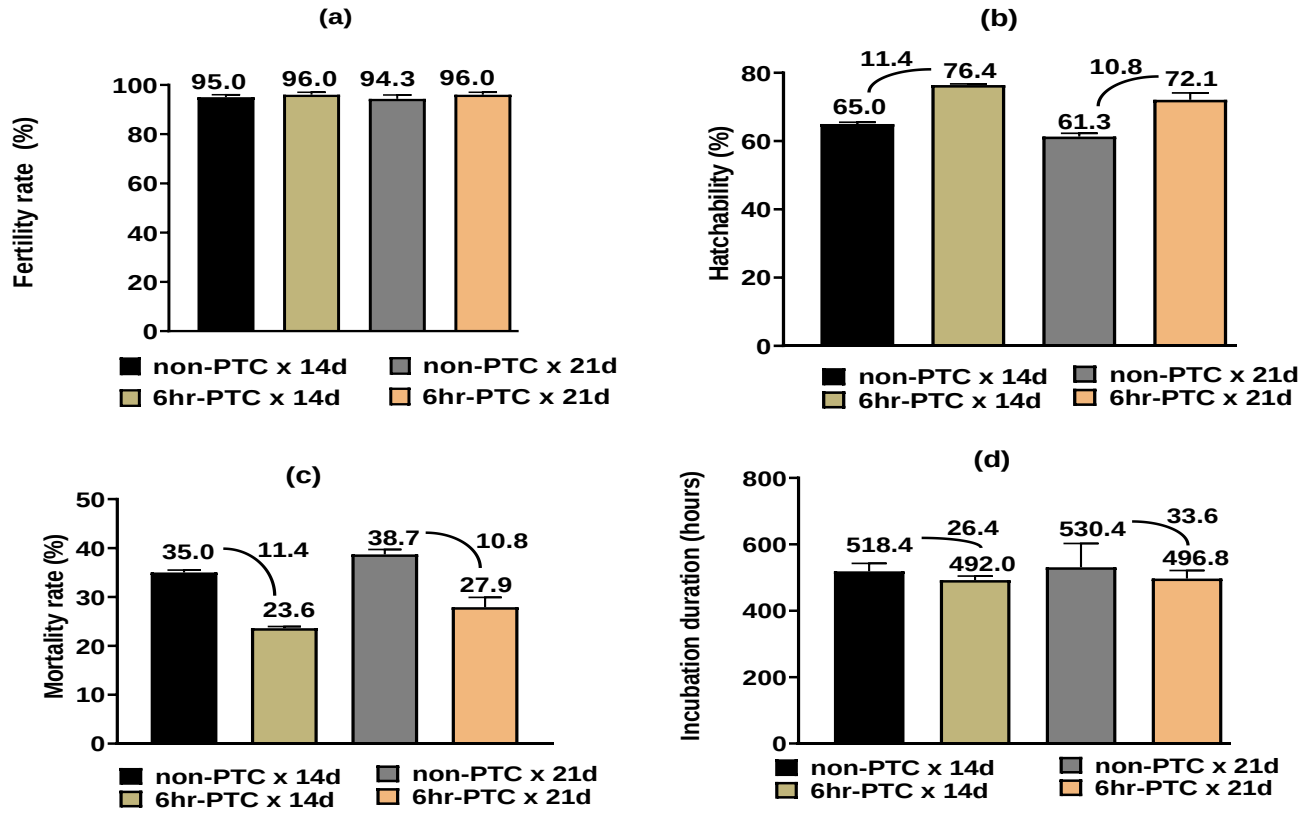

Figure 4. Effect of prolonged storage and pre-incubation thermal conditioning on fertility rate (a), hatchability rate (b), mortality rate (c), and incubation duration (d) in Plymouth Rock Hybrid chickens. Data bars are average values pooled from the two separate incubators. non-PTC × 14d: Eggs stored for 14 days and did not receive PTC (control), 6hr-PTC × 14d: Eggs stored for 14 days and received PTC for 6 hours, non-PTC × 21d: Eggs stored for 21 days and did not receive PTC (control), 6hr-PTC × 21d: Eggs stored for 21 days and received PTC for 6 hours.

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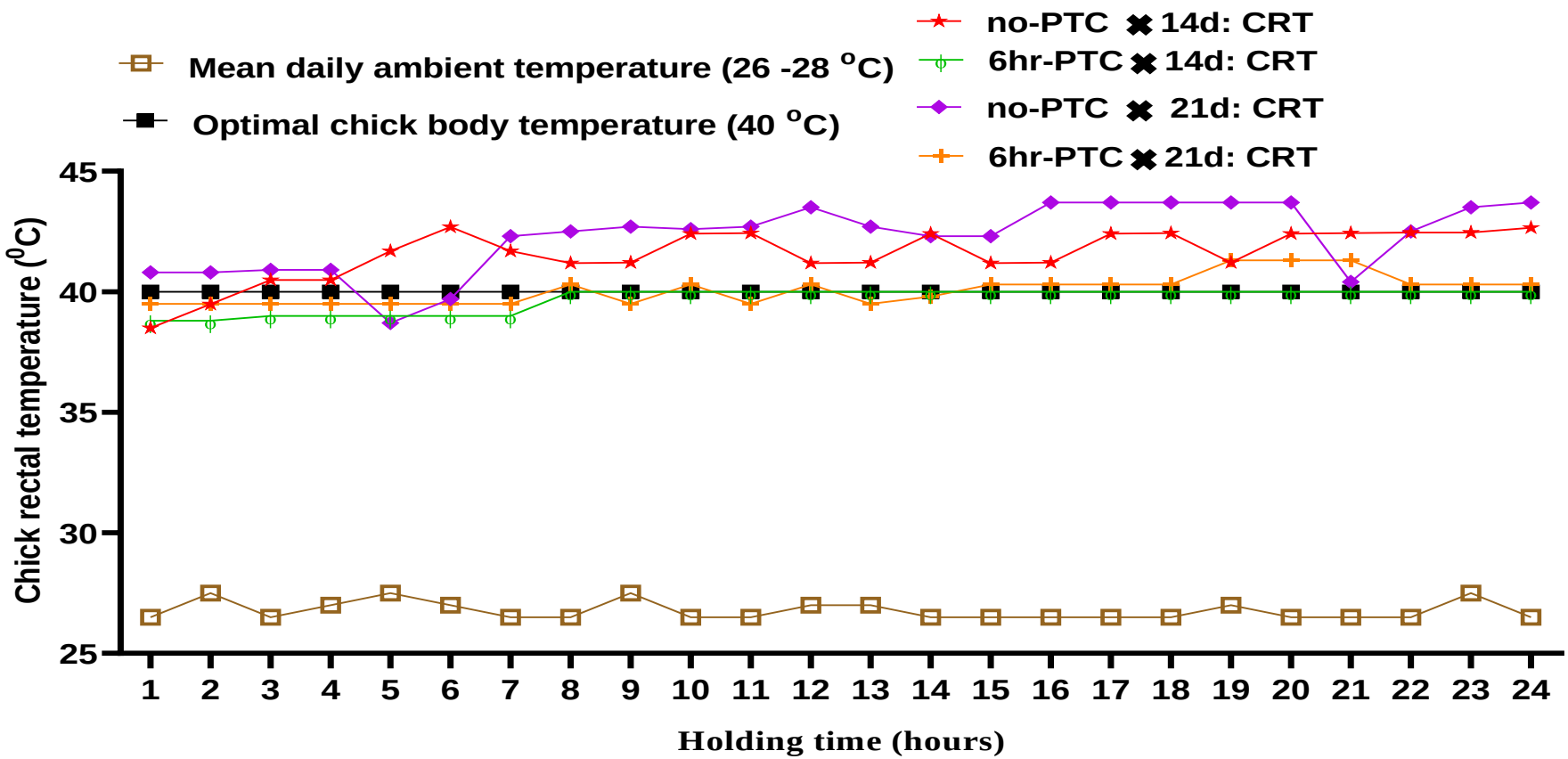

Figure 5. Effects of prolonged egg storage and pre-incubation thermal conditioning on patterns of rectal temperature of Plymouth Rock hybrid chickens measured within the first 24 hours post-hatching. CRT: Chick rectal temperature, non-PTC × 14d: Eggs stored for 14 days and did not receive PTC (control), 6hr-PTC × 14d: Eggs stored for 14 days and received PTC for 6 hours, non-PTC × 21d: Eggs stored for 21 days and did not receive PTC (control), 6hr-PTC × 21d: Eggs stored for 21 days and received PTC for 6 hours.

Table 7. Effects of prolonged storage and pre-incubation thermal conditioning on hematological profile in Plymouth Rock Hybrid chickens

	Erythrocytes						Platelets			
Factor	**RBC (10^{12}/L)**	**HGB (g/dL)**	**HCT/ PCV (%)**	**MCV (f/L)**	**MCH (pg)**	**MCHC (g/dL)**	**PLT (10^9/L)**	**MPV (fL)**	**PDW (%)**	**PCT (%)**
SD										
14	2.00	8.93^{a}	30.9^{a}	135^{a}	29.2	252^{a}	22.6^{a}	15.9^{a}	14.9	0.021
21	2.13	7.92^{b}	28.6^{b}	132^{b}	29.5	246^{b}	18.5^{b}	13.7^{b}	14.8	0.023
SEM	0.140	0.100	0.08	0.12	0.11	0.11	0.15	0.11	0.037	0.002
P-value	0.546	< 0.001	< 0.001	< 0.001	0.330	< 0.001	< 0.001	< 0.001	0.058	0.465
PTC										
non-PTC	2.21	6.42	29.1^{b}	132^{b}	29.3	248^{b}	19.8^{b}	14.2^{b}	14.5^{b}	0.016^{b}
6hr-PTC	1.93	8.03	30.3^{a}	135^{a}	29.3	251^{a}	21.2^{a}	15.4^{a}	15.2^{a}	0.027^{a}
SEM	0.140	0.100	0.08	0.12	0.11	0.11	0.15	0.11	0.037	0.003
P-value	0.181	0.973	< 0.001	< 0.001	0.841	< 0.001	< 0.001	< 0.001	<0.001	0.001
PTC × SD										
non-PTC × 14d	2.10	6.08^{bc}	28.9^{b}	130.0^{c}	28.9^{b}	246^{c}	17.8	13.2	14.6	0.015
6hr-PTC × 14d	2.37	8.67^{ab}	29.5^{b}	135.0^{a}	29.8^{a}	249^{b}	21.8	15.1	14.4	0.018
non-PTC × 21d	1.65	6.66^{b}	27.8^{c}	134.0^{b}	28.7^{b}	247^{c}	19.1	14.2	15.2	0.027
6hr-PTC × 21d	2.21	9.20^{a}	32.9^{a}	136.0^{a}	29.9^{a}	255^{a}	23.4	16.6	15.2	0.028
SEM	0.20	0.14	0.11	0.17	0.16	0.16	0.21	0.160	0.053	0.002
P-value	0.056	0.006	< 0.001	< 0.001	< 0.001	< 0.001	0.545	0.153	0.153	0.658

Means of different superscripts ($^{a, b, c}$) within a column differ significantly at $p < 0.05$. RBC (10^{12}/L): Red blood cell, HGB (g/dL): Haemoglobin, HCT/PCV: Haematocrit / Packed cell volume, MCV (f/L): Mean corpuscular volume, MCH (pg): Mean corpuscular haemoglobin, MCHC (g/dL): Mean corpuscular haemoglobin concentration, PLT (10^9/L): Platelet count, MPV: Mean platelet volume, PDW (%): Platelet distribution width, PCT (%): Plateletcrit. SD: Storage duration; PTC: 6-hour preincubation thermal conditioning, non-PTC: Eggs that did not receive any PTC (control); 6hr-PTC: Eggs that received 6-hour PTC, PTC × SD: non-PTC × 14d: Eggs stored for 14 days and did not receive PTC (control), 6hr-PTC × 14d: Eggs stored for 14 days and received PTC for 6 hours, non-PTC ×21d: Eggs stored for 21 days and did not receive PTC (control), 6hr-PTC × 21d: Eggs stored for 21 days and received PTC for 6 hours. SEM: Pooled standard error of means. P-value: Probability value.

Table 8. Effect of prolonged storage and pre-incubation thermal conditioning on serum metabolites in Plymouth Rock Hybrid Chickens

	Proteins			Carbohydrate	Lipids		Thyroid hormones	
Factor	**T-Pro (g/L)**	**GBL (g/L)**	**ALB (g/L)**	**Gluco (g/L)**	**T-Chol (g/L)**	**Trig (g/L)**	**T3 (pmol/L)**	**T4 (pmol/L)**
SD								
14	22.40	7.26^{a}	17.92^{a}	2.84	6.38	1.66	2.59^{b}	1.89^{b}
21	20.83	2.91^{b}	15.13^{b}	3.63	6.31	1.39	3.21^{a}	2.48^{a}
SEM	0.495	0.505	0.674	0.120	0.495	0.212	0.170	0.144
P-value	0.055	< 0.001	0.019	0.087	0.928	0.394	0.019	0.020
PTC								
non-PTC	18.93^{b}	4.33^{b}	14.60^{b}	3.84^{a}	6.67	1.81	3.95^{a}	3.03^{a}
6hr-PTC	24.29^{a}	5.84^{a}	18.45^{a}	2.63^{b}	6.02	1.25	1.86^{b}	1.34^{b}
SEM	0.495	0.505	0.674	0.120	0.495	0.212	0.170	0.144
P-value	< 0.001	0.048	0.004	0.018	0.384	0.099	<0.001	<0.001
PTC × SD								
non-PTC × 14d	18.83^{c}	5.95^{ab}	12.88^{c}	3.18^{ab}	6.70	1.30	3.60^{a}	3.62^{a}
6hr-PTC × 14d	25.96^{a}	8.58^{a}	17.38^{a}	2.49^{b}	6.05	2.02	1.58^{b}	1.34^{b}
non-PTC × 21d	19.03^{c}	2.71^{c}	16.32^{ab}	4.49^{a}	6.63	1.19	4.29^{a}	2.44^{a}
6hr-PTC × 21d	22.62^{b}	3.10^{bc}	19.52^{a}	2.77^{b}	5.99	1.59	2.13^{b}	1.35^{b}
SEM	0.701	0.715	0.953	0.170	0.264	0.300	0.241	0.204
P-value	0.035	0.036	0.014	0.024	0.701	0.608	0.045	0.020

Means of different superscripts ($^{a, b, c}$) within a column differ significantly at $p < 0.05$. T-Pro (g/L): Total protein, GBL (g/L): Globulin, ALB (g/L): Albumin, Gluco (g/L): Glucose, T-Cho (g/L): Total cholesterol, Trig (g/L): Triglyceride, T3 (pmol/L): Triiodothyronine, T4 (pmol/L): Thyroxine. SD: Storage duration; PTC: 6-hour preincubation thermal conditioning, non-PTC: Eggs that did not receive any PTC (control); 6hr-PTC: Eggs that received 6-hour PTC, PTC × SD: non-PTC × 14d: Eggs stored for 14 days and did not receive PTC (control), 6hr-PTC × 14d: Eggs stored for 14 days and received PTC for 6 hours, non-PTC ×21d: Eggs stored for 21 days and did not receive PTC (control), 6hr-PTC × 21d: Eggs stored for 21 days and received PTC for 6 hours. SEM: Pooled standard error of means. P-value: Probability value.

Table 9. Pairwise Pearson correlation analysis between incubation duration and key physiological parameters in Plymouth Rock Hybrid chickens

	Incubation duration (hours)	Chick rectal temperature (^{o}C)	HGB (g/dL)	HCT/ PCV (%)	MCV (f/L)	MCH (pg)	MCHC (g/dL)	Total protein (g/L)	Globulin (g/L)	Albumin (g/L)	Glucose (g/L)	T3 (pmol/L)
Chick rectal temperature (^{o}C)	0.854*											
HGB (g/dL)	-0.044	-0.788*										
HCT/ PCV (%)	-0.254	-0.498	0.855*									
MCV f/L	-0.000	-0.545	0.505	0.524								
MCH (pg)	0.055	-0.930*	0.729*	0.523	0.496							
MCHC (g/dL)	-0.185	-0.567	0.799*	0.950*	0.728*	0.600*						
Total protein (g/L)	-0.086	-0.819*	0.692*	0.394	0.673*	0.713*	0.482					
Globulin (g/L)	0.052	-0.607*	0.303	-0.161	-0.075	0.541*	-0.196	0.634*				
Albumin (g/L)	0.046	-0.700*	0.584*	0.623*	0.903*	0.723*	0.823*	0.623*	-0.012			
Glucose (g/L)	0.830*	0.093	-0.434	-0.450	0.010	-0.162	-0.304	-0.385	-0.264	0.036		
T3 (pmol/L)	0.683*	0.426	-0.621*	-0.560	-0.404	-0.457	-0.532*	-0.730*	-0.362	-0.358	0.886*	
T4 (pmol/L)	0.655*	0.173	-0.389	-0.562	-0.433	-0.247	-0.616	-0.427	0.11	-0.454	0.697*	0.794*

* Significant at $p < 0.05$. HGB (g/dL): Haemoglobin, HCT/PCV: Haematocrit/Packed cell volume, MCV (f/L): Mean corpuscular volume, MCH (pg): Mean corpuscular haemoglobin, MCHC (g/dL): Mean corpuscular haemoglobin concentration, T3 (pmol/L): Triiodothyronine, T4 (pmol/L): Thyroxine

Effect of prolonged storage and pre-incubation thermal conditioning on chick serum metabolites

Table 8 presents the effects of storage duration and PTC on chick serum metabolites. The findings show that storage duration significantly influenced several serum parameters, except for total protein (T-Pro), total cholesterol (T-Chol), glucose, and triglycerides (Trig, $p < 0.05$). Chicks hatched from eggs stored for 21 days had significantly lower globulin (GBL) and albumin (ALB) levels but higher triiodothyronine (T3) and thyroxine (T4) levels compared to chicks from eggs stored for 14 days ($p < 0.05$). However, PTC application significantly mitigated these effects, increasing total protein, globulin, and albumin levels while reducing glucose and triiodothyronine levels compared to the non-PTC group ($p < 0.05$). Specifically, eggs stored for 14 days and subjected to PTC resulted in chicks with significantly higher total protein (25.96 g/L), globulin (8.58 g/L), and albumin (17.38 g/L) levels, but lower glucose (2.49 g/L), triiodothyronine (1.58 pmol/L), and thyroxine (1.34 pmol/L) compared to eggs stored for 14 days without PTC, which had total protein (18.83 g/L), globulin (5.95 g/L), albumin (12.88 g/L), glucose (3.18 g/L), triiodothyronine (3.60 pmol/L), and thyroxine (3.62 pmol/L, $p < 0.05$). Similarly, eggs stored for 21 days and subjected to PTC produced chicks with significantly higher total protein (22.62 g/L), globulin (3.10 g/L), and albumin (19.52 g/L) levels but lower glucose (2.77 g/L), triiodothyronine (2.13 pmol/L), and thyroxine (1.35 pmol/L) compared to eggs stored for 21 days without PTC, which recorded total protein (19.03 g/L), globulin (2.71 g/L), albumin (16.32 g/L), glucose (4.49 g/L), triiodothyronine (4.29 pmol/L), and thyroxine (2.44 pmol/L, $p < 0.05$). No significant interaction effects were found for total cholesterol and triglycerides ($p > 0.05$). The lower plasma protein levels and elevated glucose concentrations in the non-PTC chicks suggest an increased metabolic rate as they may be adjusting to thermal stress (Tanizawa et al., 2014). Their heightened levels of thyroxine (T4) further suggest that the thyroid gland was more active, while the elevated triiodothyronine (T3) levels indicate intensified peripheral deiodination, both mechanisms aimed at coping with thermal challenges (Yahav et al., 2004). In contrast, PTC-treated chicks demonstrated lower levels of T3 and T4, indicating a reduced metabolic rate (Yahav and Hurwitz, 1996), likely as part of a key mechanism in thermotolerance acquisition, where heat production is suppressed by decreasing T3 concentrations (Yahav and McMurtry, 2001). These findings in conjunction with the improved haematological profiles and serum protein levels in the PTC-treated chicks suggest that PTC application not only improves metabolic regulation of chicks but also enhances their ability to withstand post-hatch thermal stress.

Pearson correlations between incubation duration and key physiological parameters

Table 9 provides crucial insights through the Pearson correlation analysis, shedding light on the physiological mechanisms behind the observed findings in the study. These correlations help explain the challenges faced by non-PTC chicks and how PTC application improved their physiological responses. A strong positive correlation between incubation duration and rectal temperature ($r = 0.854$, $p < 0.05$) supports the observation that non-PTC chicks, which underwent prolonged incubation, exhibited elevated rectal temperatures. This finding aligns with the idea that these chicks struggled with thermoregulation, as newly hatched chicks are dependent on their environment to maintain body temperature. The inability to dissipate heat effectively indicates that these chicks faced metabolic stress, further explaining the thermoregulatory challenges observed in non-PTC chicks. In contrast, the normal rectal temperatures observed in PTC-treated chicks can be attributed to the positive influence of PTC on their ability to manage thermal stress. The correlation suggests that PTC improved the chicks' thermotolerance by stimulating mechanisms within the sympathetic nervous system, which plays a critical role in temperature regulation post-hatch (Wekstein and Zolman, 1969). This perhaps enhanced thermoregulation and allowed PTC chicks to better cope with the environmental stressors they encountered after hatching. Furthermore, the negative correlations between elevated rectal temperatures and critical blood parameters, such as hemoglobin (HGB, $r = -0.788$, $p < 0.05$), mean corpuscular hemoglobin (MCH, $r = -0.930$, $p < 0.05$), and total protein ($r = -0.819$, $p < 0.05$), reinforce the earlier observation of dehydration and reduced oxygen-carrying capacity in non-PTC chicks. These correlations suggest that the increased rectal temperatures contributed to a breakdown in homeostasis, where dehydration likely impaired protein synthesis, leading to reduced plasma protein levels and poorer growth and development observed in these chicks (Xin and Rieger, 1995). Additionally, the positive correlation between incubation duration and glucose levels ($r = 0.830$, $p < 0.05$) helps explain why non-PTC chicks exhibited higher plasma glucose concentrations. This increase

reflects a stress-induced metabolic response, where the chicks needed to boost energy production to meet the demands of thermoregulation. This heightened glucose reliance underlines the increased metabolic strain experienced by these chicks in the absence of PTC. Finally, the significant correlations between triiodothyronine (T3, $r = 0.683$, $p < 0.05$) and glucose (T3: $r = 0.886$, T4: $r = 0.697$, $p < 0.05$) further explain the hormonal changes observed in non-PTC chicks. Elevated T3 and T4 levels are indicative of increased metabolic activity, as the chicks ramped up their energy production to compensate for thermal stress. These findings are consistent with previous studies showing that increased thyroid hormone activity is a physiological response to thermal challenges, enabling chicks to manage the energy demands of thermoregulation (Yahav and McMurtry, 2001).

CONCLUSION

The present study demonstrated that six-hour pre-incubation thermal conditioning (PTC) effectively mitigates the adverse impacts of prolonged egg storage in Plymouth rock hybrid chickens. Notably, the application preserved egg quality, enhanced embryonic development, restored hatchability, and improved post-hatch chick vitality and overall health, indicating its potential as a beneficial strategy in hatchery operations. However, the variability in PTC efficacy across the different storage durations necessitates further research to assess its broader applicability and economic feasibility in different poultry breeds, particularly in tropical climates.

DECLARATIONS

Availability of data and materials

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

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Authors' contributions

Prince Sasu conceptualized the study. Data collection was conducted by Prince Sasu, Felicia Emmanuella Ellison, Edna Mariam Ackah, Richard Koblah Agbehadzi, and Benjamin Adjei-Mensah. Data analysis and the initial manuscript draft were carried out by Prince Sasu and Benjamin Adjei-Mensah, with co-supervision provided by Cynthia Amaning Danquah, Kokou Tona, Jacob Alhassan Hamidu, and Were Pitala. All authors contributed to the editing and review of the manuscript and confirmed the last edition of the manuscript before submission to the journal.

Competing interests

The authors declare no conflicts of interest, either personal or professional, that could affect the interpretation of the findings presented in this manuscript.

Ethical considerations

All authors confirm adherence to ethical standards, including those concerning plagiarism, consent for publication, research misconduct, data fabrication, duplicate publication, and redundancy.

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Evaluation of Zootechnical Performance of Muscovy Ducks in South Benin

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ABSTRACT

Studies on the zootechnical performance of Muscovy ducks are scarce in Benin. The current study aimed to evaluate the performance of these ducks in a controlled environment for a better valorization of potentialities. Data were collected from 193 ducks for growth performance, 30 ducks for egg-laying performance, and 71 eggs for egg characteristics of Muscovy ducks in South Benin. The ducks were raised in controlled conditions. At hatching, male and female ducks had similar weights and body measurements. From week 2 to week 68, males had significantly higher weight than females. Males had higher initial specific growth than females (0.52 vs 0.63 per week), while females were older than males regarding age inflection point (33.10 vs 25.98 weeks). In addition, males had longer bodies, wider thoraces, and longer tarsus than females. Regarding the wingspan, the difference between males and females was observed from week 8, with higher values in males. Individual feed intake and feed conversion ratio increased as the ducks grew older. In the first week, the individual feed intake was 20.08 g per day, and the feed conversion ratio was 1.51. After 20 weeks of age, Muscovy ducks consumed 136 g daily with a high feed conversion ratio of 26. The age of the first egg of Muscovy duck was 6.17 months, and the average number of eggs laid per brooding was 15.37 eggs. The brooding duration was 35.63 days, with a hatching rate of 73.06%. The duckling's survival rate at hatching was 95.28%, of which 97.47% were weaned. The average weight of a duck egg was 63.56 g, and that of the shell was 8 g, while albumen and yolk amounted to 30.01 g and 23.86 g, respectively. Duck eggshell dominant color was white (60.5%), followed by dirty white (26.31%), and finally brown (13.64%). These results on the zootechnical performance of the Muscovy duck can be considered a reliable basis for this species' potential improvement in South Benin.

Keywords: Average daily gain, Benin, Feed conversion ratio, Muscovy duck, Weight, Zootechnical performance

INTRODUCTION

Muscovy duck (*Cairina moschata*, Linné, 1758) is a domestic species of the Anatidae family, derived from the Musk duck, native to South America (Teulier, 2010). It is from a large family of aquatic palmipeds with webbed feet (Anatidae) and is bred worldwide for egg and meat production. Their down, feathers and fatty liver are well-sold products (Guy et al., 2006). *Cairina moschata* is the most common species in Sub-Saharan Africa and is known for its great zootechnical performance and high resistance to various avian pathogens (Akpla, 2013; Houessionon et al., 2020). In Benin, its breeding is widespread in traditional poultry farming throughout the country, particularly in the South. Despite its importance in traditional poultry farming, Muscovy ducks are fewer in number and have a lower contribution to food security than other poultry species. Therefore, an update on this species' breeding has been carried out in the three agroecological zones of southern Benin to diagnose bottlenecks that limit this speculation development to set up an improvement strategy (Houessionon et al., 2020).

The update revealed that duck breeding modes differ between the three agroecological zones of southern Benin and within each of them, making it difficult to formulate recommendations. Duck farm typology has indicated three types of duck breeding in south Benin. Type 1 is characterized by a traditional system based on ducks' scavenging and the absence of health monitoring. Breeding type 2 includes a semi-improved family system, and breeding type 3 is characterized by the dominance of livestock buildings and rangelands where ducks are reared free range (Houessionon et al., 2019). The characteristics of these three breeding types are different, and integrated proposals have been formulated for each type (Houessionon et al., 2019). The implementation of proposals will then improve ducks' productivity but not their genetic potential. Given this, an evaluation of Muscovy duck's zootechnical performance is necessary to have a reliable basis for effective selection or crossbreeding program. The general objective of the study was to assess the zootechnical performance of Muscovy ducks in a controlled environment for a better estimation of their potential while minimizing the effect of environmental factors. In particular, the present study aimed to assess body weight performance and morphometric traits of Muscovy ducks according to sex, feed intake, and feed conversion ratio of this species, and finally, laying performance and egg characteristics of Muscovy duck.

MATERIAL AND METHODS

Ethical approval

Ducks in the current study were raised and treated according to the letter N° 23-2017/LBATV/DPSA/Se of 16 March 2017 of the Laboratory of Animal Biotechnology and Meat Technology of the University of Abomey-Calavi (Benin), approved by the Animal Welfare Committee of Benin.

Study area

The experiment was carried out at the poultry station of the Laboratory of Animal Biotechnology and Meat Technology of the University of Abomey-Calavi in South-Benin, precisely in the Township of Abomey-Calavi, district of Togba, area of Agori, at 6° 42' 6'' North longitude and 2° 32' 4'' East latitude. The Township of Abomey-Calavi is bounded to the North by the Township of Zè, to the South by the Atlantic Ocean, to the East by the Townships of Sô-Ava and Cotonou, to the West by the Townships of Tori-Bossito and Ouidah. It has an area of 539 km^2 and a population of more than 656,358 inhabitants in 2013 (INSAE, 2015). The climate is of subequatorial type with two rainy and two dry seasons. The major rain is from April to July, and the minor is from September to November. These seasons are separated by two dry seasons.

Breeding mode

The zootechnical characteristics study was carried out on Muscovy ducks (*Cairina moschata*). The breeding stocks were 2 males and 10 females bought from the three agroecological zones of southern Benin (depression, Ferralsols, and fishery), where consanguinity was not recorded, and other breeds were not used for crossbreeding. They were 5-7 months old, and the females had not yet started to lay eggs. After a month of quarantine, these reproducers were put together and reared in a coop with pens having access to a water pond. They were housed in a coop measuring 15 m² divided into three pens of 5 m² using wire netting in which 20 ducklings born during the same week were reared. The house roof was made of corrugated aluminum sheets. The floor was cemented, and the walls were 90 cm in height, topped with wire mesh. The inside of the pens was heated to 33°C by brooders made of jars filled with charcoal every evening until ducks aged three weeks. The litter was made of 3 cm wood chips. A footbath was installed on the floor at each lodge entrance for foot disinfection. Each lodge is extended by a courtyard of 300 m². Feeders, drinkers, and nest boxes were installed in the coop.

Matings were performed daily at random, and each duck laid, incubated, and hatched her eggs. At hatching, ducklings were given an identification number and were recorded in the database with their mother's name, parity number, and hatching date.

Three feeds were distributed during the animal breeding, including a starter feed, a growing feed, and a laying feed. For all the ducks, the starter feed was used for eight weeks and was followed by the growing feed, from week 8 to the laying onset at month 6. The laying feed was served to birds from 6 months onward. Feeds given to animals were bought in the commerce, and their nutritional values can be seen in Table 1.

The study set up a health and medical prophylaxis program for health monitoring. Sanitary prophylaxis consisted of hygiene rules and strict observance. The drinkers were washed daily, and the litter was renewed when altered. A footbath containing 5% cresyl disinfectant solution was placed at the coop entrance. As for medical prophylaxis, preventive treatments against infections and

coccidiosis were performed using Alfaceryl® (ALFASAN-Veto Service SA, Benin), erythromycin thiocyanate, oxytetracycline + vitamins A, D3, E, K, B1, B6, B2, B12, PP, C) and Amprolium® (LAPROVET-Veto Service SA, Benin) and Amprolium chloridrate in powder form. Ducks were vaccinated against Newcastle disease at 3 weeks with the second dose at two weeks later (CEVA® NEW L: A lentogenic strain of LaSota by oral route). The samples were dewormed every two months from the weaning against gastrointestinal parasitosis (Alfamisole®: levamisole) and also received a vitamin complex (Amin'Total®: vitamins, amino acids, and trace elements) by oral route. Oxytetracycline 50% (ALFASAN-Veto Service SA, Benin) was the most commonly used antibiotic for possible infectious diseases.

Table 1. Nutritional values of diets in Muscovy ducks

Items	Starter (1-8 weeks)	Grower (9-24 weeks)	Laying (>24 weeks)
Energy (kcal/kg)	2900	2800	2500
Crude protein (%)	21	19	18.5
Lysine (%)	1.1	1	0.9
Methionine (%)	1	0.44	0.44
Calcium (%)	1.08	1.01	3.5
Total phosphorus (%)	0.55	0.5	0.5
Crude ash (%)	7.37	7.12	13
Crude cellulose (%)	2.5	3.32	-
Sodium (%)	0.2	-	-
Crude fat (%)	5.54	5	4.5
Flavomycin (%)	0.007	0.007	0.005
Chloride (%)	0.19	-	-

Data collection and processing

Body weights, body length, tarsus length, thoracic perimeter, and duck wingspan were measured at hatching (P0), 2 weeks (P2), and 4 weeks (P4) of age and then once a month until 68 weeks of age. The ducks were weighed individually in the mornings before food service, with KERN brand balances of 1g, 5g, 10g, and 50g of precisions, respectively, for weights of 100g, 600g, 1000g, and 5000g. Average daily gain (ADG) was then calculated as t1 (0 to 12 weeks), t2 (12 to 24 weeks), t3 (24 to 36 weeks), t4 (36 to 48 weeks), and t5 (48 to 60 weeks). The food leftovers were recorded daily. The growth curve parameters were calculated using the Gompertz equation (Laird et al., 1965) according to the following formula: $W_t = W_0\, e^{L(1-\exp(-Kt))\,/\,K}$

Where W_t is the weight recorded at t age, W_0 denotes the estimated birth weight, L signifies the initial specific growth rate $(1/W^t) \times (dW_t/dt)$ when $t \rightarrow 0$), K is the maturity rate and TI, the age at inflection point corresponding to the period of the maximum growth. The following formula calculates the age inflection point.

$$TI = \left(\frac{1}{K}\right)\ln\left|\frac{L}{K}\right|$$

These growth curve parameters were estimated from the nonlinear regression using the NLIN procedure and the SAS Marquardt method taking into account the weight by age from hatching at the age of 68 weeks.

Feed intakes were obtained by the difference between the food quantities served and the leftovers of the day. The feed conversion ratio (FCR) was calculated by dividing an animal's feed intake by its average daily gain over a given period. The feed conversion ratio (FCR) was calculated for the first 20 weeks.

Concerning the laying performance, the age of the first egg, the number of eggs laid, the number of eggs brooded, the number of eggs hatched, the brooding duration, the number of alive ducklings at hatching, the number of weaned ducklings and the interval between two successive broods were recorded per duck for a total of 30 ducks. Annual egg production was determined by considering the total number of eggs laid by a female in one year from the first laid egg.

As for egg characteristics, the weight of the egg, shell, albumen, and yolk weight were recorded on 71 eggs. The egg variables were weighed using a KERN brand balance of 0.1 mg of precision with a capacity of 220 g. The eggshell color (white, brown, cream, tinted, or other) was also recorded.

Statistical analysis

The Statistical Analysis System software (SAS, 2013) was used to analyze data. A linear model with sex fixed-effect was adjusted to data on weight, average daily gain, and body measurements for analysis of variance. The F-test was used to determine the significance of the sex effect on each variable. The proc corr procedure was used to calculate correlations between the egg characteristics and those between the body measurements taken at 36 weeks of age for all ducks (all sexes combined) and by sex. The proc means procedure was used to calculate averages of individual feed intake and weekly feed conversion ratio as well as those of egg laying performance. Finally, the proc freq procedure was used to calculate the color class frequencies of eggshells. The means were compared using the student's t-test and the frequencies were compared paired by the Z-test. A significant level of $p < 0.05$ was used for all comparisons in both tests.

RESULTS

Body weight performance

At hatching, males and females had similar weights. From week 2 to week 68, males' weight was significantly higher ($p < 0.05$) than that of females (Figure 1). At the end of the experiment, the males' weight amounted to 3490 g, compared to 1900 g for females. The average daily gains for males were significantly higher than those of females ($p < 0.05$, Table 2). As for growth curve parameters, the maturity rate was similar for males and females (Table 2). However, males had higher initial specific growth than females, while females were older than males at the age inflection point.

Morphometric traits

At the hatching, body length, thoracic perimeter, and tarsus length of females and males were similar. From week 2 to week 68, males had a longer body, a wider thorax, and a longer tarsus than females ($p < 0.05$). Regarding the wingspan, the difference between males and females was observed from week 8 in favor of the males ($p < 0.05$). The growth curve of body length, the thoracic perimeter, the tarsus length, and the wingspan of females and males from hatching to week 68 are respectively shown in Figures 2-5.

Regardless of the sex, the correlations between body length, thoracic perimeter, tarsus length, live weight, and wingspan were positive and significant in all ducks ($0.699 \leq r \leq 0.944$, $p < 0.05$). Each variable was positively and significatively associated with the other ones. The correlations between the morphometric measurements of Muscovy ducks of both sexes are presented in Table 3. In females, the correlation between thoracic perimeter and live weight was positive and significant ($r = 0.415$, $p < 0.05$), but the correlations between the other body measurements were not significant. In males, wingspan had no significant correlation with other body measurements. The correlation between thoracic perimeter and body length also was insignificant. A low correlation was observed between body weight, body length, and tarsus length ($0.305 \leq r \leq 0.344$, $p < 0.05$) on one hand and between thoracic perimeter, tarsus length, and live weight ($0.355 \leq r \leq 0.432$, $p < 0.05$) on the other hand. In males, body length was significantly correlated with tarsus length. The correlations between the morphometric measurements of 36-week Muscovy ducks (female and male) reared in a station in southern Benin are presented in Table 4.

Feed intake and feed conversion ratio

The individual feed intake and the feed conversion ratio increased overall with age (Table 5). In the first week, individual feed intake was 20.08 g per day, and the feed conversion ratio was 1.51. After 20 weeks of age, the ducks consumed 136 g daily with a high feed conversion ratio of 26.

Laying performance

Muscovy duck's age at first egg was 6.17 months, and the average number of eggs laid per brood was 15.37. The brooding duration was 35.63 days, with a hatching rate of 73.06%. Ducklings' viability rate at hatching was 95.28%, of which 97.47% were weaned. The hatch-weaning mortality rate was 2.53%. The brood interval was 101 days, and the annual egg production was 47.54. The laying performance of Muscovy ducks reared in a station in South Benin is presented in Table 6.

Egg characteristics

The average egg weight of the duck was 63.56g and the shell weight was 8 g representing 12.59% of the egg weight. The albumen and the yolk weighed 30.01 and 23.86g or 47.22% and 37.54% of the egg weight, respectively. The egg characteristics of Muscovy ducks are presented in Table 7. Although there was an insignificant correlation between yolk weight and albumen, all other egg characteristics were highly correlated with each other ($p < 0.05$, Table 8). The dominant eggshell color was white (60.5%), followed by dirty white (26.31%), and brown (13.64%, Table 9).

Table 1. Nutritional values of diets in Muscovy ducks

Items	Starter (1-8 weeks)	Grower (9-24 weeks)	Laying (>24 weeks)
Energy (kcal/kg)	2900	2800	2500
Crude protein (%)	21	19	18.5
Lysine (%)	1.1	1	0.9
Methionine (%)	1	0.44	0.44
Calcium (%)	1.08	1.01	3.5
Total phosphorus (%)	0.55	0.5	0.5
Crude ash (%)	7.37	7.12	13
Crude cellulose (%)	2.5	3.32	-
Sodium (%)	0.2	-	-
Crude fat (%)	5.54	5	4.5
Flavomycin (%)	0.007	0.007	0.005
Chloride (%)	0.19	-	-

Table 2. The body weight of Muscovy ducks reared in South-Benin

Variable	Female			Male			ANOVA
	Number	Average	SE	Number	Average	SE	
ADGT1 (g/j)	102	15.15	0.37	79	24.67	0.42	***
ADGT2 (g/j)	102	14.17	0.38	79	22.17	0.42	***
ADGT3 (g/j)	93	19.61	0.28	72	38.22	0.30	***
ADGT4 (g/j)	95	20.56	0.40	79	37.90	0.46	***
ADGT5 (g/j)	83	20.27	0.46	71	38.17	0.63	***
K (1/week)	102	0.13	0.01	79	0.15	0.01	NS
L (1/ week)	102	0.52	0.04	79	0.63	0.05	*
Ti (week)	102	33.10	2.34	79	25.98	2.66	*

SE: Standard error; ADGT: Average daily gain of the term; K: Maturity rate, Ti: Age at an inflection point; L: Initial specific growth; NS: Not significant, */***: $p < 0.05$.

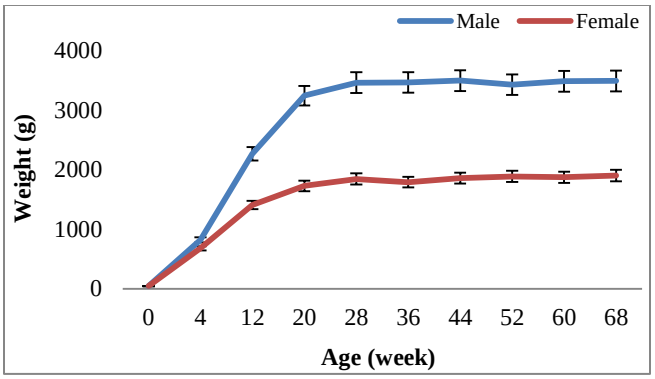

Figure 1. Body weight performance of Muscovy ducks according to sex

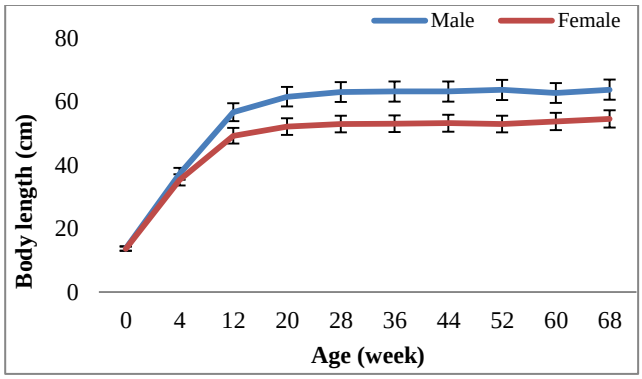

Figure 3. Body length of Muscovy ducks according to sex

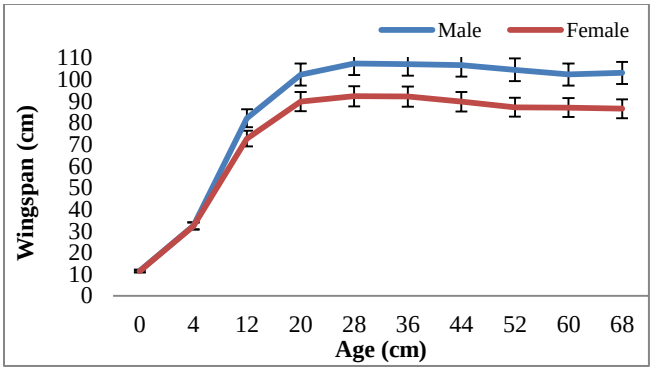

Figure 2. Wingspan of Muscovy ducks according to sex

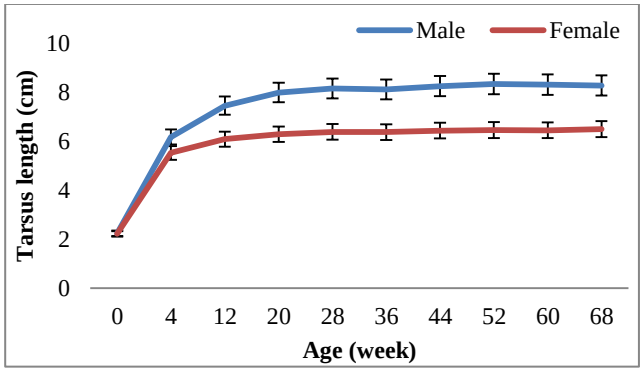

Figure 4. Tarsus length of Muscovy duck according to sex

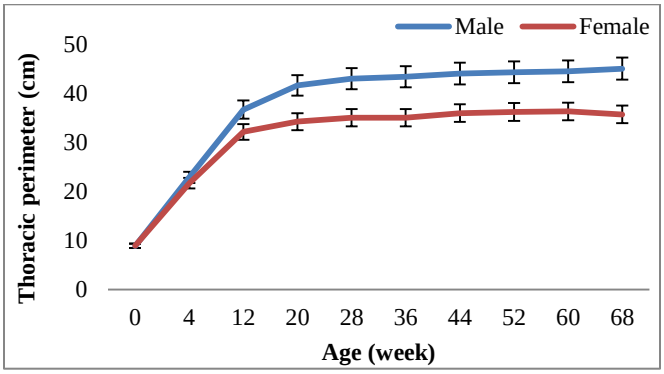

Figure 5. The thoracic perimeter of Muscovy ducks according to sex

Table 3. Correlations between the morphometric measurements of Muscovy ducks reared in South Benin

Variables	Body length (cm)	Tarsus length (cm)	Live weight (kg)	Thoracic perimeter (cm)
Wingspan (cm)	0.717***	0.705***	0.764***	0.699***
Body length (cm)		0.894***	0.895***	0.872***
Tarsus length (cm)			0.943***	0.918***
Live weight (kg)				0.944***

*** : $p < 0.05$

Table 4. Correlations between morphometric measurements of females (above the diagonal) and of males (below the diagonal) of Muscovy ducks reared in South Benin

Variables	Wingspan (cm)	Body length (cm)	Tarsus length (cm)	Live weight (cm)	Thoracic perimeter (cm)
Wingspan (cm)	1	0.183 NS	-0.094 NS	0.050 NS	-0.039 NS
Body length (cm)	0.01NS	1	-0.069 NS	-0.110 NS	0.133 NS
Tarsus length (cm)	-0.11 NS	0.449**	1	0.198 NS	0.131 NS
Live weight (cm)	0.20 NS	0.344*	0.30572*	1	0.415**
Thoracic perimeter (cm)	-0.13 NS	0.18 NS	0.355*	0.432*	1

NS : $p > 0.05$; * / ** : $p < 0.05$.

Table 5. Individual feed intake and feed conversion ratio of Muscovy ducks reared in South Benin

Age (week)	Number	Individual feed intake		Feed conversion ratio	
		Average (g)	Standard Deviation	Average (g)	Standard Deviation
1	56	20.08	10.36	1.51	0.78
2	56	49.81	15.71	3.74	1.18
3	56	63.14	13.38	1.88	0.40
4	56	79.14	17.53	2.36	0.52
5	56	92.29	18.80	3.31	0.67
6	53	100.30	20.17	3.60	0.72
7	49	103.96	21.28	3.73	0.76
8	47	103.93	19.30	3.73	0.69
9	41	104.28	24.49	10.89	2.56
10	39	104.00	23.47	10.86	2.45
11	28	107.60	34.54	11.23	3.61
12	28	110.92	50.17	11.58	5.24
13	26	110.29	39.28	12.06	2.36
14	21	108.30	34.89	15.31	2.10
15	21	104.58	33.93	15.69	2.04
16	16	111.92	17.73	16.73	1.07
17	14	109.49	24.54	20.89	4.68
18	14	100.77	27.54	19.23	5.26
19	11	127.88	23.71	24.40	4.53
20	7	136.31	26.10	26.01	4.98

Table 6. Laying performance of 24 to 26-month-old Muscovy ducks reared in South-Benin

Variables (N=30)	Average	Standard Deviation	Coefficient of variation (%)
Age at first egg (months)	6.17	0.38	6.15
Number of laid eggs	15.37	4.25	27.69
Number of brooded eggs	15.37	4.25	27.69
Brood duration (day)	35.63	2.50	7.01
Number of hatched eggs	11.23	4.01	35.74
Number of alive ducklings	10.70	4.15	38.82
Number of weaned ducklings	10.43	4.12	39.45
Annual eggs production	47.54	21.96	46.19
The interval between two broods (day)	101.11	49.12	48.58

Table 7. Egg characteristics of 7-month-old Muscovy ducks reared in South Benin

Variable (N=71)	Average	Standard deviation	Coefficient of variation (%)
Egg weight (g)	63.56	6.28	9.88
Shell weight (g)	8.09	0.69	8.54
Albumen weight (g)	30.01	3.34	11.14
Yolk weight (g)	23.86	4.38	18.35

Table 8. Correlations between variables of egg characteristics (egg weight, shell weight, and albumen weight) of Muscovy ducks reared in the South Benin

Variable	Shell weight (g)	Albumen weight (g)	Yolk weight (g)
Egg weight (g)	0.744^{***}	0.629^{***}	0.801^{***}
Shell weight (g)		0.415^{***}	0.641^{***}
Albumen weight (g)			0.152^{NS}

NS: $p > 0.05$; ***: $p < 0.05$.

Table 9. Eggshell color of Muscovy ducks reared in South Benin

Variables	Number	Frequency (%)	Confidence interval
White	40	60.5^{a}	15.15
Dirty white	17	26.31^{b}	20.93
Brown	9	13.64^{b}	22.42

Percentages followed by different superscript letters differ significantly at the threshold of 5%

DISCUSSION

Body weight performance and morphometric traits

At hatching, males and females had similar weights, but from week 2, males were heavier than females until week 68. These ducks grow gradually from hatching to adulthood, and males' live weight is almost double that of females. At the end of the experiment in the present study, males weighed 3490 g, compared to 1900 g for females. This difference must be from the genetic and hormonal origin and is observed in many species. Sex hormones improve conformation and growth potential depending on sex (Ismoyowati et al., 2017). In Nigeria, Oguntunji and Ayorinde (2014) found this influence of sex on the Muscovy duck's weight. In an extensive system, they report a live weight of 2640 g in males and 1600 g in females. According to Bati (2017), sexual dimorphism is a remarkable trait in Muscovy ducks. His study on the zootechnical performance of black, white-black, white, and gray varieties of Muscovy ducks in Congo indicated this difference in weight. Similar to the findings of the present study, Bati (2017) reported a significant difference in the second week of age, thereby becoming more important throughout birds' growth, indicating a faster growth rate in males. Yakubu et al. (2011) also reported similar results to the present study in Nigeria. Similar results are observed in previous studies on other poultry species such as chickens, guinea fowl, and geese. Indeed, Youssao et al. (2012) and Tougan et al. (2013) reported the influence of sex on the birds' weight in various studies on local poultry populations of *Gallus gallus* species. Likewise, Dahouda et al. (2008) and Uhlířová et al. (2018) also obtained similar results in guinea fowl and geese, respectively.

Females were older at the inflection point than males (33.10 vs. 25.98 weeks). This age indicates when animals have reached their maximum growth (Youssao et al., 2012), which is an ideal age as the cost/growth ratio is optimal, and it is advisable to take out fattening animals. Most often, the age at the inflection point corresponds to puberty. Generally, animals reach this point 2/3 of their adult weight. Growth in the majority of Muscovy ducks is relatively rapid in the beginning phase (from week 1 to week 12), which corresponds to the accelerated growth phase. A duckling born with a weight of 44.36 g multiplies its weight by 10 after 4 weeks. According to Teulier (2010), Muscovy ducklings have exponential growth during the first 4 weeks of their life. The results of the present study are in line with other poultry studies, indicating that local chickens of *Gallus gallus* species in free-range farming have good growth during the first 12

weeks of age (Youssao et al., 2009; Akouango et al., 2010). Muscovy ducks' weight changes gradually and stabilizes at the finishing period when adult weight is reached, which corresponds to the slow growth phase. In addition, weight stabilizes with age and does not increase following the normal bird growth curve. This stabilization is explained in females by the laying onset, which results from the use of the ingested feed for egg production to the detriment of muscle growth. In males, the age inflection point corresponds to puberty. This is the period when they start mating females, or they seek to demonstrate their dominance in the backyard. In the experiment, this period is characterized in males by physical and recurring confrontations and chases in the courtyard, causing bodily injuries. These physical efforts cause daily considerable energy loss that justifies body weight stabilization after the age inflection point. Djitie et al. (2014) made similar observations reporting that in the reproductive phase or beyond 24 weeks of age, weight growth is almost null and does not change significantly.

Body weight performance in the present study is better than those reported by Oguntunji and Ayorinde (2014) on adult male and female Muscovy ducks, and this difference in weight is related to the breeding system. On the other hand, in an intensive system in France, for example, Muscovy ducks are heavier than the males and females in the present study. Thus, the live weight of force-fed Muscovy ducks is 6393 g, and that of lean Muscovy ducks is 5418 g (Chartrin et al., 2006; Baeza et al., 2013). This difference in weight must be justified by genetic selection. No selection was performed on the Muscovy ducks in the present study. This explains the high coefficients of variation values of body weight performance of the species in the three agroecological zones of southern Benin, from which originate parents.

On the other hand, the European Muscovy duck has been selected for lean meat or fatty liver production. The results of Larzul et al. (2006) on the zootechnical performance of Muscovy ducks, Pekin ducks, and their crosses (Hinny and Mulard) also showed a significant difference in data on weight. In other words, Muscovy ducks (6520 g) were the heaviest and Pekin ducks the lightest (4095 g), and the two different genetic types of Hinny and Mulard were intermediate (5714 g and 5774 g, respectively).

Concerning morphometric traits, from week 2 to week 68, males had longer bodies, larger thoraxes, and longer tarsus than females. Likewise, Oguntunji and Ayorinde (2014) reported that the thoracic perimeter, body length, wing length, and whole thigh-drumstick total length of males (46.93 cm, 30.69 cm, 35.23 cm, and 17.18 cm, respectively) are higher than those of females (38.7 cm, 23.96 cm, 26.71 cm, and 14.55 cm, respectively).

Feed intake and feed conversion ratio

Individual feed intake and feed conversion ratio increased overall with age in this study. Body nutritional needs explain the increase in feed intake with age for birds' maintenance and growth. In the starting phase, animals consume little feed, with a higher average daily gain, which explains the low feed conversion ratio observed during this phase. When an animal becomes older, the average daily gain decreases, while feed intake increases with an increase in feed conversion ratio. The feed conversion ratio and growth rate averages recorded in this study are close to those obtained in Nigeria by Igwebuike and Anagor (2013). Besides, the increase in feed intake and feed conversion ratio with Muscovy ducks' age was also recorded in other studies. Makram et al. (2017) reported a feed intake of 1036.00 ±76.33 g for week 2 to week 4 and 2489.34 ± 77.06 g for week 8 to week 10 in Muscovy ducks. Concerning feed conversion ratio, they recorded 2.16 ± 0.10 and 2.98 ± 0.28, respectively, for these two periods. This same trend of feed intake and feed conversion ratio evolution with age was observed in other strains, such as Sudani and Pekin ducks (Makram et al., 2017; 2021).

Egg laying performance and egg characteristics of female duck

The Muscovy duck's age at the first egg was 6.17 months in the present study. This age confirms the observations of Retailleau and Blanchet (2003), who report that Muscovy ducks reach sexual maturity after 6 months of age. The average number of eggs laid per brood was 15.37 eggs, with an annual production of 47.54 eggs and an average weight of 63 g. These testify that Muscovy duck is a very good layer and prolific, compared to local chickens. This annual production is, however, below the results of Yakubu et al. (2011), who report that ducks can lay between 60 and 80 eggs each year with an average egg weight of around 72 g under better breeding conditions. The results of this study on laying performance confirm the results of Etuk et al. (2011), who observed that a duck is a good brooder and a good mother due to its reproductive performance. In Nigeria, Adeyeye (2009) recorded ducks' egg, shell, albumen, and yolk weights of 63.61g, 8.11g, 28.63g, and 24.08g, respectively. In addition, Banga-Mboko et al. (2007) study on the reproductive performance of Muscovy ducks in Congo revealed a brood size of 14.6

eggs and an average egg weight of 72 g. Widianingrum et al. (2020) also indicated close results.

CONCLUSION

The evaluation of the zootechnical performance of ducks in a controlled environment revealed that males had higher weights by aging and also had higher morphometric traits than females. The gap in sex performance increases with age. Regardless of sex, variables of live weight, body length, thoracic perimeter, tarsus length, and wingspan were significantly and positively correlated in all ducks. These correlations were more pronounced in males than in females. Individual feed intake and feed conversion ratio increased with age. The results of the present study can be considered as a reference for its potential improvement through subsequent studies.

DECLARATIONS

Authors' contributions

The experimental study was conceived and designed by Finagnon Josée Bernice Houessionon and Issaka Youssao Abdou Karim in consultation with Assouan Gabriel Bonou, Mahamadou Dahouda, and Guy Apollinaire Mensah. Tossou Jacques Dougnon and Issaka Youssao Abdou Karim supervised the experimental study, collection of data, and analysis. The manuscript was written and drafted by Finagnon Josée Bernice Houessionon. All authors read, reviewed, and approved the final manuscript for submission and publication.

Competing interests

The authors declare that they have no competing interests.

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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 the authors.

Availability of data and materials

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

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Effects of Dietary Supplementation of *Spirulina* on Health Status, Growth Performance, and Slaughter Traits in Quails

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ABSTRACT

The supplementation of sustainable alternative sources such as nutrient-rich algae, especially rich in proteins, in animal feed is a promising and innovative strategy to improve feed autonomy, especially in poultry diets. This study evaluated the effect of *Spirulina platensis* (SP) supplementation on growth performance and slaughter characteristics in Japanese quails (*Coturnix japonica*). A total of 180 unsexed, 2-day-old quail chicks with a mean body weight of 9 ± 1.42 g were randomly assigned to three dietary groups, each containing 60 quails. Each group was divided into 4 subgroups, with 15 quails in each (4 repetitions per group). Three groups were provided with commercial diets (starter, grower, and finisher) for five weeks. These diets were supplemented with *Spirulina* at concentrations of 0.5 g/kg (SP0.5), and 1 g/kg (SP1), while the control group (SP0) received no *Spirulina* supplementation. Growth performance was monitored, and at the end of the trial (35 d), 60 quails (20 per group) were slaughtered for carcass evaluation including hot and cold carcass weight and liver weight. Results showed that *Spirulina* supplementation at 1 g/kg (SP1) significantly increased feed intake and weight gain compared to the control and SP0.5 groups. Significant differences in growth performance and feed intake were observed between the *Spirulina*-supplemented groups (0.5 and 1 g/kg) and the control group. Carcass characteristics, including hot carcass yield and liver weight, were significantly higher in the SP0.5 and SP1 groups compared to the control group (SP0). In conclusion, supplementing quail diets with 0.5 and 1 g/kg *Spirulina* improved growth performance and carcass quality without negative effects on overall performance. This supplementation can be considered as a cost-effective diet ingredient for enhancing meat quality in quail production.

Keywords: Carcass trait, Growth, Japanese quail, Performance, *Spirulina platensis*

INTRODUCTION

In animal production, feed constitutes the largest share of production costs, representing a significant portion of overall expenditure (Poppi and McLennan, 2010; Karadağ et al. 2022). The rising cost and limited availability of feed are major challenges, particularly in countries reliant on imported raw materials (OCDE-FAO, 2023). This situation has led to higher prices for animal products, making them less affordable for low-income populations, thus contributing to food insecurity (FAO, 2021).

In Algeria, poultry farming is a key sector supported by the government, playing a crucial role in diversifying animal protein sources (Kaci, 2015). However, it faces challenges related to the availability of quality feed, breeding stock, and veterinary products (Mouhous et al., 2015). Feed alone accounts for approximately 70% of production costs, with soy being the primary and most expensive protein (Belaid-Gater et al., 2022). The limited availability and rising global prices of soy have hindered the profitability and sustainability of poultry farms (Kaci, 2015).

To address these challenges, there is growing interest in developing alternative, non-competitive, and sustainable protein sources to reduce dependency on soy and enhance the sustainability of poultry production (Holman et al., 2013; Draaisma et al., 2013; Malila et al., 2024).

Aquatic plants, such as *Spirulina platensis*, offer promising alternatives due to their nutrient-rich composition and sustainability (Caporgno et al., 2018). *Spirulina* is a blue-green alga known for its high protein content (50-70%) and rich composition of essential fatty acids, vitamins, minerals, and carotenoids (Belay, 1993; Becker et al., 2007; Costa et al., 2024). The *Spirulina platensis* is also a source of flavonoids, and has some biological activities including antioxidant, anti-inflammatory (Dianursanti et al., 2020), immuno-modulating (Jamil et al., 2015), antiviral, antibacterial, and antihepatopathic (Bitam and Aissaoui, 2020). Additionally, the naturally highly concentrated essential nutrients and numerous biochemical and physiological benefits of *Spirulina* (Hadeel et al, 2023) make it ideal as a natural feed additive in animal and poultry nutrition, since *Spirulina* is safe when added to different food products (Oliveira et al., 2010; Selim et al., 2018). Billah et al. (2022) reported that *Spirulina* has digestibility (up to 86%) due to the polysaccharide-rich composition of its cell walls, making it easily absorbed by the human and animal body. Several studies have reported that supplementing poultry diets with *Spirulina* improves growth performance, carcass quality, and overall animal health (Ismail et al., 2009; Doreau et al., 2010; Selim et al., 2018; Spínola et al., 2023).

In Algeria, although *Spirulina* has been studied for its antioxidant, anti-inflammatory, and immunological properties (Aouir et al., 2016; Lafri, 2017; Ahounou, 2018), limited research has focused on its use in poultry feed. Therefore, the present study aimed to evaluate the effects of dietary supplementation with dried *Spirulina platensis* on the growth performance and carcass characteristics of fattening quails.

MATERIALS AND METHODS

Ethical approval

This research project adheres to the ethical guidelines established by the Algerian Association of Experimental Animal Sciences (88-08/1988) and aligns with the European Union Guidelines (2010/63/EU) for animal welfare in research.

Feed, birds, and management

The *Spirulina platensis* (SP) used in this study was obtained in dried form from the Biological Farm ALKIRAM® in Biskra, Algeria, in 100g bags. The composition of *Spirulina*, according to the manufacturer, is as follows, 65% protein, 10% carbohydrates, 5% lipids, 7% fiber, and 8% minerals. *Spirulina* was incorporated into the diet as a supplement at two doses including 0.5 g/kg and 1 g/kg of feed.

The diets used in this study are commercially locally available products obtained from a feed factory located in Ain Bessam, Algeria. Three distinct dietaries were employed: starter (1 to 11 days), grower (12 to 24 days), and finisher (25 to 35 days). The composition of the commercial diets is provided in Table 1.

Table 1. The composition of the three types of diets used in the present study for Japanese quails

Ingredients (%)	Starter	Growth	Finisher
Maize	61	67	67
By-products of milling	5.33	2.86	12.2
Soybean meal	29.5	27.5	18
Common salt (NaCl)	0.6	0.9	1
Monocalcium Phosphate	1.34	0.7	0.8
DL-Methionine	0.03	0.04	-
Anti-stress	1	-	-
premix[1]	1	1	1
Chemical composition (% DM)			
DM*	86	87	88
CP**	20.58	19.71	19.02
Calcium	0.53	0.32	0.8
Metabolizable energy (ME), kcal/kg	2860	2850	2750

*DM: Dry matter, **CP: Crude protein. [1:] Mineral and vitamin composition (g/kg premix): Se, 0.025; Mg, 5; Mn, 7.5; Zn, 7.5; I, 0.12; Fe, 3.6; Cu, 2.25; Co, 0.04; Thiamin, 0.1; Riboflavin, 0.45; Calcium d-Pantothenate, 0.6; Pyridoxine, 0.15; Biotin, 0.0015; Nicotinic acid, 2; Choline chloride, 35; Folic acid, 0.4; Vitamin K3, 0.2; dl-α-tocopheryl acetate, 1.35; Biotin, 0.0015; Folic acid, 0.04; Cyanocobalamin, 0.0006; Vitamin A, 850000 IU; Vitamin D3, 170000 IU.

Design of the study

The experiment was conducted at the Pedagogical Animal House of the Department of Agronomic Sciences, University of Bouira (Algeria) from March to April 2023, with a duration of 5 weeks. In this study, 180 unsexed Japanese quails (*Coturnix japonica*), two days old and weighing an average of 9.18 ± 1.42 g, were selected for the study. The quails were randomly divided into three experimental groups of 60 quails each. Each group was further divided into four replicates with 15 quails per replicate. The quails were obtained from a local private farm. Each quail chick was individually identified and each subgroup was used in wire mesh cages (45 x 56 x 19 cm), designed for female breeders, which were adapted for the study. The quails were monitored from 2 to 35 days of age. The experimental groups were fed commercial diets,

tailored to the birds' age (starter, grower, and finisher phases), with *Spirulina platensis* supplementation at the following doses: 0 g/kg (control group, SP0), 0.5 g/kg (SP0.5 group), and 1 g/kg feed (SP1 group).

During the 5-week trial, quails were fed *ad libitum* with their respective diets. Weekly measurements of live weight and feed intake were recorded, and daily monitoring of mortality was conducted. Drinking water was provided *ad libitum* via nipple drinkers. The light inside the building was maintained at 24 hours for the first 5 days, then gradually reduced to 14 hours between days 6 and 35. The temperature was initially set at 40°C for the first three days, then decreased by 5°C each week (from 40 to 20°C). The humidity level recorded varied between 55% and 70%. At the end of the trial, 20 quails per group (five quails per replicate randomly selected) were humanely slaughtered under controlled conditions. The slaughter was performed without anesthesia, and the quails' main jugular veins were severed to ensure effective bleeding. The live weight and hot carcass weight were measured using a precision digital scale (0.01 g), and the carcasses were then chilled in a ventilated cold room at 4°C for 2 hours. After chilling, the cold carcass weight and liver weight were measured by the same digital scale.

Statistical analyses

All statistical analyses were conducted using R (version 3.5.1; R Foundation for Statistical Computing, Vienna, Austria) through RStudio (version 1.1.383, RStudio Inc., Boston, MA). The authors of the current study performed repeated-measures analysis of variance (ANOVA) for all measured variables. Diagnostic plots for assessing the normality of residuals and model effects were generated using the qqnorm function in R. If the model was not normally distributed, a logarithmic transformation of the independent variables was applied. Post-hoc comparisons were conducted using the lsmeans function from the lsmeans package. Results are presented as least square means (LSM) ± standard deviation (SD). A stacked line plot of the studied variables was created using Prism 6.07 (GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS

Growth parameters

The results in Figure 1 showed the effect of supplementing *Spirulina* on feed intake, body weight, and body weight gain over the first five weeks of measurement. There was no significant interaction between treatment and week, however, the week of measurement had a significant impact ($p < 0.05$).

As shown in Figure 1, during week 3, a significant effect of 0.5 g/kg *Spirulina* supplementation (SP0.5) on body weight was observed. Specifically, quails fed 0.5 g/kg *Spirulina* exhibited a higher body weight compared to the control group (130.22 ± 1.42 g vs. 122.83 ± 1.42 g, respectively). However, no significant differences were found between the SP1 (1 g/kg *Spirulina*) and control groups or between the SP1 and SP0.5 groups.

Quails in the SP1 group (1 g *Spirulina*/kg feed) demonstrated significantly higher body weight gains in both weeks 1 and 3 compared to the other groups. Specifically, weight gains of 3.49 ± 0.159 g/day and 6.78 ± 0.159 g/day were observed for SP1 in weeks 1 and 3, respectively.

Additionally, quails fed 0 g (control) and 0.5 g *Spirulina* exhibited higher feed intake in weeks 2 and 4 compared to those fed 1g Spirulina. For week 2, feed intake was 19.29 ± 0.295 g and 18.95 ± 0.295 g for SP0 and SP0.5, respectively, compared to 16.39 ± 0.295 g for SP1. Similarly, in week 4, feed intake was 30.71 ± 0.295 g in SP0 versus 28.92 ± 0.295 g in SP1. Over the entire treatment period, no significant treatment effect (group effect) was observed on body weight ($p > 0.05$). However, a repeated measure ANOVA revealed significant effects of *Spirulina* supplementation on feed intake and body weight gain (BWG) throughout the entire period. Indeed, in the growing phase, quails fed diets supplemented with 0.5 g and 1 g *Spirulina* showed significantly higher BWG compared to the control group ($p < 0.05$ for SP0.5 and SP1 respectively). Furthermore, quails supplemented with 0 g and 0.5 g *Spirulina* had higher feed intake during both the grower and finisher phases, compared to those receiving 1 g *Spirulina*/kg feed (Figure 2, $p < 0.05$).

Carcass traits

The effects of increasing levels of *Spirulina* supplementation (SP 0, SP 0.5, and SP 1) on carcass traits are presented in Table 2. The addition of *Spirulina* resulted in a quantitative increase in both hot and cold carcass weights, though the differences among the three groups were not statistically significant ($p > 0.05$). However, carcass yields in the SP0.5 and SP1 groups were significantly higher compared to the control group (SP0, $p < 0.05$). Regarding liver weight, a significant increase was observed in the groups supplemented with 0.5 g and 1 g of *Spirulina* camper in the control group. The SP1 group showed the most pronounced improvement compared with the SP0 control group ($p < 0.05$).

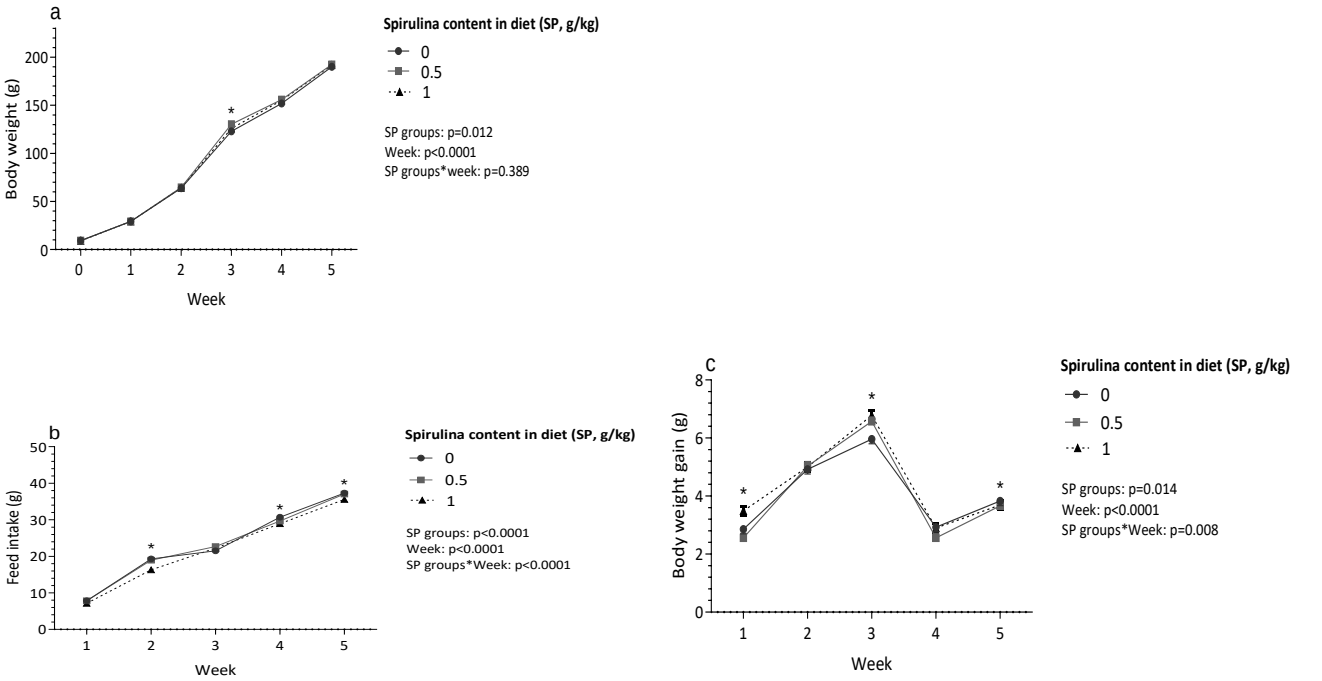

Figure 1. Effects of dietary supplementation of *Spirulina* on body weight (**a**), feed intake (**b**), and weight gain (**c**) during the five weeks of the study in quails. The effect of (p-value) groups, weeks, and the interaction of groups and weeks are indicated in the figures.

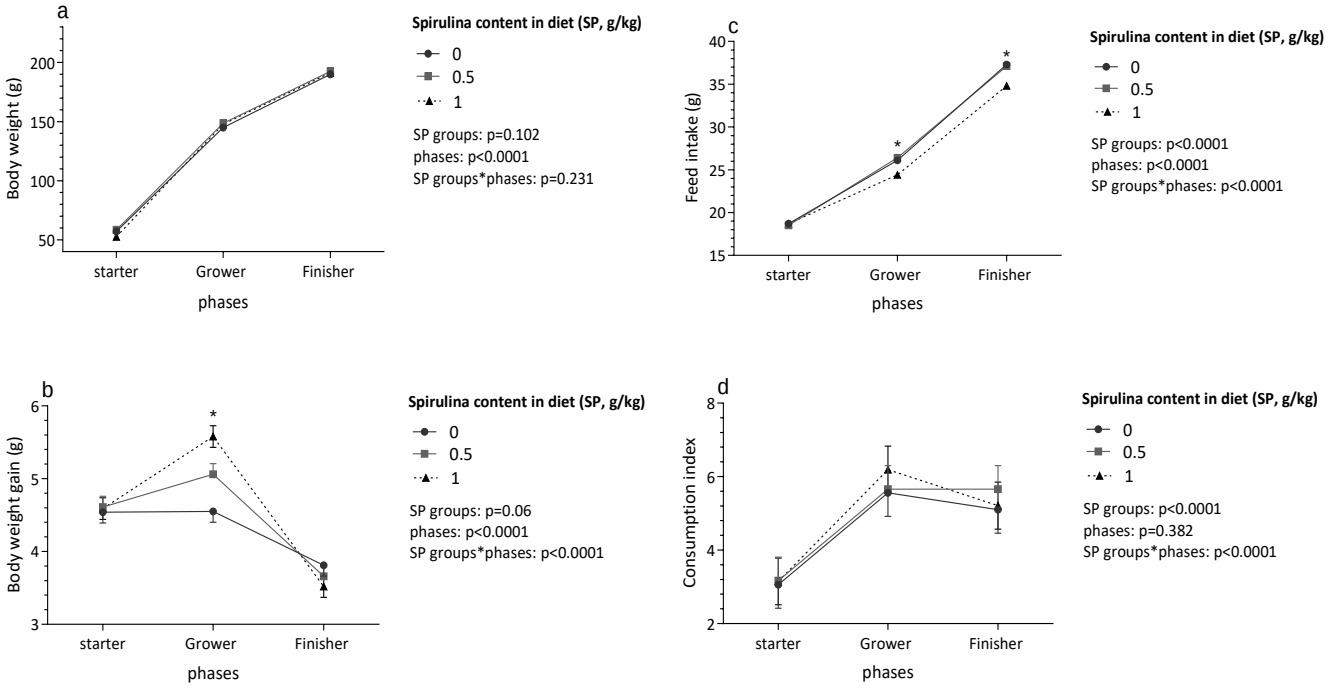

Figure 2. Body weight (**a**), body weight gain (**b**), feed intake (**c**), and consumption index (**d**) of quail throughout the Starter, growing, and finishing phases as affected by levels of *spirulina* supplementation. The effect of (p-value) groups, weeks, and the interaction of groups and weeks are indicated in the figures.

Table 2. Body weight and carcass traits of quails at 35 days of age fed different levels of *Spirulina*-supplemented

Parameters	**SP0**	**SP0.5**	**SP1**

Body weight (BW, g)	189.20 ±16.12	191.20± 9.53	190.67± 8.62
Hot carcass weight (g)	128.60 ± 13.16	133.19 ± 7.77	132.93 ± 6.95
Cold carcass weight (g)	124.71 ±17.11	127.80 ±8.77	126.24 ±9.08
Yield of hot carcass (%)	67.93 ±2.69^{a}	69.68 ± 2.71$_{b}$	69.73 ± 2.20^{b}
Yield of cold carcass (%)	65.71 ± 2.46^{a}	66.79± 1.65^{b}	66.14± 2.22^{b}
Liver weight (g)	3.99± 1.30^{a}	4.97 ± 0.83^{b}	4.95± 0.82^{b}

SP: Control without *Spirulina*, SP0.5: Group supplemented with 0.5g of *Spirulina*, SP1: Group supplemented with 1g of *Spirulina*. Different subscript letters (^{a,b,c}) within a row indicate significant differences between treatments ($p < 0.05$).

DISCUSSION

Ensuring food safety and sustainability requires the efficient use of natural resources. The incorporation of microalgae, such as *Spirulina*, into poultry diets can play a significant role in enhancing both production and health outcomes, as previously reported by Hajati et al. (2020) and Abdel-Wahab et al. (2023). In the present study, supplementation of quail diets with *Spirulina* at levels of 0.5 and 1 g/kg resulted in notable improvements in growth performance, carcass traits, and overall health, which is consistent with findings from several studies on the positive effects of *Spirulina platensis* on poultry.

Growth performance

In this study, quails fed *Spirulina* enriched diets exhibited similar live weights to the control group, corroborating the findings of Hajati and Zaghir (2019), AbdElzaher et al. (2023), and Alghamdi et al. (2024). However, the results of this trial showed superior growth performance compared to Gongnet et al. (2001), who reported a decline in the performance of quails supplemented with *Spirulina*. Notably, the group supplemented with 1 g *Spirulina* per kg of feed achieved the best weight gains. This aligns with studies by Kaoud (2015), Abouelezz (2017), and AbdElzaher et al. (2023), who observed improved growth parameters in quails supplemented with *Spirulina*. The positive effects of *Spirulina* supplementation are likely due to its high nutrient density and beneficial physiological properties (Bono et al, 2016; Selim et al, 2018; Hadeel et al., 2023).

Abdelwahab et al. (2020) and Alghamdi et al. (2024) reported a significant improvement in body weight and weight gain in chickens and quails-fed diets supplemented with *Spirulina*. More broadly, numerous studies have highlighted that the incorporation of microalgae into poultry diets contributes to increased body weight (Pratiwi, 2020; Mawed et al., 2020). This beneficial effect of *Spirulina* can be attributed to its high nutritional value and its potential role in positively modulating the gut microbiota (Ma et al., 2022). These improvements could also be attributed to the high digestibility of its proteins, its balanced amino acid profile, and its positive impact on intestinal health, particularly to its antimicrobial and prebiotic properties (Holman and Malau-Aduli, 2013). Similarly, Mariey et al. (2012) reported that incorporating *Spirulina* into diets has the potential to increase the population of *Lactobacilli* and enhance the efficiency of dietary vitamin absorption.

Similarly, Abdel-Wahab et al. (2023), demonstrated that *Spirulina* supplementation up to 4.5% improved weight gain in quails. These findings are consistent with Shanmugapriya et al. (2015), who showed that 1% *Spirulina* supplementation in broiler diets enhanced growth by stimulating the height of intestinal villi and increasing gut absorptive capacity. Furthermore, Alagawany et al. (2024) reported that *Dunaliella salina*, a microalga, improved weight gain and feed conversion in quails at supplementation levels of 0.25, 0.5, and 1 g/kg of feed.

Bellof et al. (2013) found that a 5% *Spirulina* supplementation in the diet of broiler chickens improved weight gain. Gongnet et al. (2001) reported that high levels (5%, 10%, and 15%) of *Spirulina* in broiler diets negatively affected performance. In contrast, the present study suggests that *Spirulina* supplementation at 0.5 and 1 g/kg positively influenced quail growth, likely due to the algae's rich nutrient profile, which supports metabolic processes and enhances growth performance (Park et al., 2018).

Throughout the study period, the feed intake and feed conversion ratio of quails fed a diet supplemented with 1 g of *Spirulina* were significantly higher than those of the control (SP0) and 0.5 g *Spirulina* (SP0.5) diets. These results contradict those of Ekýzoðlu et al (2020), who reported no significant effect of *Spirulina* supplementation on feed intake and feed conversion of growing quails. However, studies by Cheong (2015) and Sherif et al. (2022) have previously shown that supplementing diets with *Spirulina* at 1-2 g/kg improved feed efficiency during the quail's growth phase. Göçmen (2022) also found that lower concentrations of *Spirulina* (2.5%) improved feed

consumption, further supporting the beneficial role of *Spirulina* in enhancing feed utilization efficiency. In addition, Ibrahim et al. (2018) demonstrated improved feed conversion in quails when *Spirulina* was added to drinking water, highlighting its positive impact on feed utilization.

Bird mortality

During the study, mortality was observed only in the control and 0.5 g *Spirulina* groups (7 and 3 quails, respectively), while no mortality occurred in the group supplemented with 1g *Spirulina* per kg of feed. The mortalities might be attributed to the quality of the feed and the stress induced by environmental conditions. This is consistent with the findings of AbdElzaher et al. (2023) and Youssef et al. (2023), who reported reduced mortality due to the beneficial physiological effects of *Spirulina*, such as enhanced metabolism and improved intestinal health (Park et al., 2018). The lack of mortality in the 1g *Spirulina* group could reflect the improved overall health and immunity of poultry supplemented with this microalga (Lordan et al., 2011; Dewi et al., 2018). Billah et al. (2022), further support this hypothesis, proposing that the inclusion of *Spirulina* in poultry diets could potentially enhance disease resistance. Nevertheless, further studies are required to confirm the effect of *Spirulina* on mortality.

Carcass traits

While no significant differences were observed in the total carcass weight across the groups, certain carcass components, notably liver weight and carcass yield, were significantly increased in the groups receiving 0.5 g and 1 g of *Spirulina* supplementation. These results align with the observations of Alghamdi et al. (2024), who reported comparable enhancements in carcass yield following *Spirulina* inclusion in the diet. In contrast, Göçmen (2022) found no significant differences in overall carcass yield but reported improved breast meat quality, including color and tenderness, in quails supplemented with 2.5% *Spirulina*. Additionally, Shanmugapriya and Saravana Babu (2014) observed a reduction in abdominal fat in chickens fed *Spirulina*, which suggested that *Spirulina* supplementation may positively influence fat distribution and improve carcass quality.

CONCLUSION

In conclusion, the results of this study suggest that the supplementation of quail diets with *Spirulina platensis* at 0.5 and 1 g/kg can improve growth performance, feed efficiency, and certain carcass traits without adverse effects on health or mortality. These findings support the potential of *Spirulina* as a sustainable and beneficial dietary supplement for quails, contributing to enhanced production efficiency and meat quality. Additional research is essential to comprehensively evaluate the digestibility of *spirulina* and its influence on various physiological and immunological parameters, intestinal health, and product quality, including meat and eggs. Furthermore, assessing its economic efficiency is crucial for optimizing its use in poultry nutrition, ensuring both effectiveness and sustainability.

DECLARATIONS

Availability of data and materials

The data for this study are available on reasonable request from the corresponding author.

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Authors' contributions

Harouz-Cherifi Zakia conceived the idea, designed the experimental setup, and collected and interpreted the data. Harouz-Cherifi Zakia, Messad Sara, and Habbi-Cherifi Assia collaboratively contributed to organizing the data and drafting the initial version of the article. Abdelli Amine performed the statistical analysis and finalized the document. All authors reviewed and approved the final manuscript.

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Competing interests

The authors have declared that no competing interest exists.

Ethical considerations

Authors have ensured compliance with ethical standards, including plagiarism, data integrity, fabrication and/or falsification of data, duplicate publication and/or submission, and publication practices, in order to avoid violations.

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Effect of Cassava Silage Diet on Performance and Internal Organs of Male Ducks

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ABSTRACT

It would be beneficial to consider supplementary feeding of livestock as a means of increasing production, although this may be constrained by the residues produced. Silage is one of the forage feed conservation techniques that has also been the subject of interest in recent years concerning poultry feed. The present study aimed to gain insight into the potential impact of feeding cassava-based silage (CS) on the internal organs and performance of male ducks. The study was conducted using 200 male local ducks aged one day, which were then reared in cages for 10 weeks. The research design was based on a completely randomized design (CRD) with five treatments and four replications. The treatments were arranged based on the amount/percentage of silage used in the basal ration and were as follows S0 (silage ration 0% CS/control), S25 (silage ration 25% CS), S50 (silage ration 50% CS), S75 (silage ration 75% CS), and S100 (silage ration 100% CS). In further observations, several variables were considered, including body weight gain (BWG), ration consumption, ration conversion, abdominal fat percentage, spleen percentage, liver percentage, kidney percentage, heart percentage, gizzard percentage, pancreas percentage, thyroid percentage, serum thiocyanate levels, and mortality, as well as serum thiocyanate. The results indicated a notable decline in performance ($p < 0.05$) in BWG observations when CS was provided in amounts exceeding 25% and consumption exceeded 50%. Furthermore, there was a notable increase in the weight of internal organs, which appeared to coincide with an increase in the level of use of cassava-based silage in duck rations. Based on the results of the study the use of cassava-based silage could be considered as a potential alternative or replacement for up to 50% of basal rations, without necessarily resulting in significant changes in the performance and internal organs of livestock.

Keywords: Body weight, Complete ration, Cassava, Silage, Internal organ, Male duck.

INTRODUCTION

Domestic ducks represent a potential source of both eggs and meat in Indonesia. The domestic duck population in Indonesia is estimated to have reached 58.35 million heads, with meat production of 4,725.80 thousand tons and egg production of 6,322.55 million tons (Ditjennak, 2021). However, national duck egg and meat production is still relatively low compared to similar products from poultry farms. Some related factors are assumed to include the feed consumed by ducks whose development is relatively inadequate when compared to other competitors, such as broilers and layers. this can be seen from the last few years of research related to supplementation innovation is still very minimal and only focuses on the supply of protein in the ratio (Sjofjan et al., 2020; Suwignyo et al., 2021; Pramestya et al., 2021; Suwignyo et al., 2023; Mappanganro et al., 2024). Physiologically ducks have an advantage in terms of the ability to digest fiber better when compared to poultry production (El-katcha et al., 2021).

Based on these advantages, in-depth attention is needed to produce feed innovations that can increase duck productivity through the use of its physiological advantages. Furthermore, the process of extracting information for the production of quality feed is constrained by the high dependence on the use of soybean meal as a source of protein and corn as a source of energy. From an economic point of view, these are still very expensive because they are imported commodities. Therefore, efforts are needed to reduce import dependence by optimizing the production of feed. It would be beneficial to consider alternative sources of raw materials. Some potential feed ingredients that have been demonstrated to be effective in livestock diets are non-conventional agricultural by-products, such as cassava (tubers, leaves, and skins) and tapioca processing waste, which is locally known as onggok.

In the last two years, studies on the tuber, peel, or industrial waste (onggok) part of cassava, which is rich in carbohydrates, conducted as an energy source for poultry (Soeprijanto et al., 2022; Hasanudin et al., 2023). On the other hand, leaves are a source of protein, vitamins, minerals, and essential amino acids (Pereira et al., 2016; Li et al., 2019), which can be used by poultry in their daily activities. Furthermore, according to Hermanto and Fitriani (2019), the protein concentration in cassava leaves is between 20% and 36%. Calcium content is 1.10-1.14% and phosphorus content is 0.25-0.30% (Fasae and Yusuf, 2022). Given the considerable impact of this potential, the utilization of cassava as a raw material for animal feed has been widely implemented. However, this is still limited by the high cyanide content, which can adversely impact poultry productivity. Consequently, there is a clear necessity for a method that can effectively reduce the cyanide content. A promising technique for achieving this is through cassava-based silage technology, which involves the addition of rumen fluid enzymes and Leuconostoc mesenteroides bacteria (Hawashi et al., 2019; Khota et al., 2023; Mukhtar et al., 2023).

The combination of cassava and its waste through silage processing, with the addition of rumen fluid enzymes and leuconostoc mesenteroides bacteria, has been identified as the most effective strategy for optimizing the use of cassava. This approach is believed to reduce the content of toxins in feed through acid hydrolysis, which is then expected to support optimal duck productivity. Toxin compounds in the form of cyanide contained in cassava can reduce the productivity of poultry (Bakare et al., 2021). In addition, the present study was conducted to provide new insights and data for the poultry feed industry in developing feed processing methods and maximizing the use of local raw materials. Therefore, it is necessary to conduct a study on the utilization of cassava-based complete ration silage on the performance and internal organs of male ducks.

MATERIALS AND METHODS

Ethical approval

An investigation was conducted at the experimental station of the Department of Animal Science, Faculty of Agriculture, Sriwijaya University, South Sumatra, Indonesia. The animals were treated in accordance with the Animal Welfare Guidelines of the Indonesian Institute of Sciences. The experiment was granted approval by Sriwijaya University, reference number KPPHP-2023-2.

Experimental design and duck preparation

The research design was a completely randomized design (CRD) composed of five treatments and four replications. The treatments represented a combination of basal/control rations with the addition of cassava silage (CS) at a specified percentage and were designated as coded S (silage). The proportions used were as follows included S0 (0% CS/100% control ration), S25 (25% CS ration), S50 (50% CS ration), S75 (75% silage ration), and S100 (100% CS ration). As a source of observation/data collection, 200 one-day-old male ducks were used in this study and housed in 2 square meter cages for 10 weeks, with each cage containing 10 ducks. Moreover, the preparation of the DODs involves the provision of water dissolved in sugar at a concentration of 1-2% for the initial four hours, whereby the energy source serves to restore the condition of the DODs due to the effects of transport stress. Subsequently, the wing band was attached to one side of the duck's wing. Subsequently, the DODs were weighed and randomized based on their initial body weight. Immediately following this, the sugar water was replaced with drinking water, and the treatment ration was distributed. During the maintenance period, rations and drinking water were provided twice daily, in the morning and evening. The observations conducted as part of this study were carried out over 10 weeks, with the duck's body weight and consumption of treatment rations weighed weekly.

Diets and silage production process

The preparation of silage was conducted in accordance with the methodology proposed by Ogbuewu and Mbajiorgu (2023). Before the formulation of feed rations, the initial step was the preparation of cassava raw materials (CS). This process involved the cutting of each raw material, including leaves, skin, and tubers, into a uniform size of 1-2 cm. Following the cutting process, each ingredient was washed with running water and subsequently treated with rumen fluid enzyme at a dose of 1% (b/v) for each raw material. The

ingredients were then stirred until homogeneous and incubated in a 10 kg plastic bag with a thickness of 1.5 mm for 24 hours at room temperature in a closed room. Once the incubation period had elapsed, each ingredient was transferred to a 10 kg silo bag and the Leuconostoc mesenteroides inoculum was added at a dose of 1% (10-6 cells/ml) by spraying gradually. Subsequently, the silo was compacted to prevent the ingress of oxygen and was then incubated for 30 days in a closed room at room temperature. After this period, the silage was opened and dried in an oven for three days. The raw materials from the silage process that had been dried were then ground with a milling machine and used as a mixture of duck rations.

The second stage was the preparation of the ration, which began by analyzing the nutritional content of the raw materials that make up the ration through proximate analysis. The results of the nutrient composition analysis obtained were used as a reference in the preparation of the ration. The ration prepared in this study was based on the recommendations of NRC (1995) with protein content (16%) and metabolic energy (2,900 kcal/kg). The percentage values of ration composition along with the nutrient content of the experimental rations were presented in Tables 1 and 2.

Table 1. Experimental ration composition of male ducks

Raw materials	Control (%)	Treatment (%)
Corn	50.60	-
Fine bran	21.75	-
Coconut meal	3.65	-
Soybean meal	10.00	-
Leaves	-	35.00
Peels	-	23.00
Tubers	-	17.10
Onggok	-	10.20
Fish meal	10.00	10.00
Vegetable oil	3.31	3.31
Premix	0.69	0.69
DL-methionine		0.35
L-lysine		0.30

Table 2. The male duck ration ingredients used in the present study

Nutrients \ Treatment	S_0	S_{25}	S_{50}	S_{75}	S_{100}
Dry matter (%)[1]	88.34	88.50	88.09	88.21	87.53
Crude protein (%)[1]	19.91	17.24	17.30	18.50	18.25
Crude Fat (%)[1]	8.68	7.83	7.62	4.91	4.35
Crude Fiber (%)[1]	7.73	7.06	7.21	7.58	7.91
Ca (%)[2]	1.22	1.07	0.82	0.77	0.68
P (%)[2]	0.48	0.40	0.41	0.41	0.40
GE (Kcal/kg)[3]	4 091.55	4 085.92	4 054.93	4 008.45	4 019.72
HCN/Cyanide (ppm)[2]	0	15.69	21.77	25.07	27.80
Methionine (%)[2]	0.37	0.37	0.37	0.37	0.37
Lysine (%)[2]	0.99	0.89	0.92	0.94	0.85

S0: 100% control ration, S25: 25% CS silage ration, S50: 50% CS silage ration, S75: 75% CS silage ration and S100: 100% CS silage ration. [1]Analysis results of PAU laboratory of IPB,[2] Analysis results of Dairy Animal Nutrition Laboratory, Faculty of Animal Husbandry, IPB,[3] Analysis results of Feed Technology and Industry Laboratory, Faculty of Animal.

Observed variables

The measured variables in the present study were body weight gain, ration consumption, and ration conversion referring to the method used by Palupi et al. (2023). Moreover, the characteristics of the digestive organs, including the percentage of abdominal fat, the percentage of spleen, the percentage of liver, the percentage of kidney, the percentage of heart, the percentage of gizzard, and the percentage of pancreas, were determined using the method proposed by Huang et al. (2022). while thyroid levels and thiocyanate levels in serum based on the method used by Pettigrew and Fell (1972).

Feed consumption

The investigated parameters included consumption of ration (g/head/day), which was measured based on the difference between the ration given (g) and the rest of the ration given (g) during a specific period (days)

Body weight gain

body weight gain (g/head/day), which was measured by weighing the difference between body weight at the end of the study (g) and the initial body weight (g), then divided by the length of rearing time (days)

Feed conversion ratio

Feed conversion ratio (FCR), is measured based on the ratio between weight gain and ratio consumption.

Digestive organ characteristics

To calculate the percentage of abdominal fat, spleen, liver, kidney, heart, gizzard, and pancreas in poultry, it was first necessary to weigh each organ separately after the slaughtering process was complete. The weight of each organ should be recorded in grams using a precision scale. Additionally, the total body weight of the chicken prior to slaughter should be recorded as a reference point for calculating the percentage of each organ. Once the data on organ weight and total chicken weight have been obtained, the percentage can be calculated using the following formula:

Organ percentage = (organ weight/total chicken weight) × 100 (Formula 1)

To illustrate, if the total weight of the chicken was 2000 grams and the weight of the chicken liver was 50 grams, the liver percentage was calculated as (50/2000) × 100, which equates to 2.5%. This process was repeated for each additional organ, including abdominal fat, spleen, kidney, heart, gizzard, and pancreas. This allows for the calculation of the percentage of each organ relative to the total body weight of the chicken.

Thyroid and thiocyanate

The methodology employed comprises a series of steps for the determination of thiocyanate levels in biological fluids. The initial step involved the collection of a plasma or urine sample. To separate the plasma from the blood, the anticoagulant lithium heparin was employed. Subsequently, the sample was subjected to a deproteination process, whereby trichloroacetic acid was added. Subsequently, the test solution was treated with bromine water, which serves to oxidize thiocyanate into intermediate compounds, such as bromosyanide. The residual bromine was then neutralized with an arsenic oxide solution. Following this, the pyridine-p-phenylenediamine reagent was added, resulting in a pink color reaction. Measurements were then taken using a spectrophotometer at a wavelength of 520 nm. The concentration of thiocyanate in the sample was calculated based on the resulting absorbance of the test solution in comparison to the thiocyanate standard solution. The accuracy and specificity of this procedure have been tested through a series of recovery experiments, both for plasma and urine, in order to ensure that the method was effective in the precise detection of thiocyanate.

Statistical analysis

The data obtained will be processed to obtain an analysis of variance (ANNOVA) using Statistical Product and Service Solutions (SPSS) software version 20 (2018) according to the design used. If there were differences between treatments, the Duncan Multiple Range Test (DMRT) will be tested.

RESULTS AND DISCUSSION

Body weight gain

Cassava silage (CS) in the ration had a significant effect on reducing the value of body weight gain of ducks ($p < 0.05$). The decrease in BWG value in this study was strongly suspected to be due to the presence of cyanide content in all parts of cassava which further takes an important role in disrupting metabolism in the digestive system of ducks. The growth of ducks fed between 25% and 50% showed high weight gain. However, after feeding 75% silage, the weight gain achieved decreased. These results proved that ducks can be fed CS silage at the limit of 50% silage content without resulting in a decrease in body weight gain. Olayemi and Oso (2018) reported that the substitution of a mixture of cassava tubers and leaves with corn by up to 25% increased body weight gain but at the 50% substitution level decreased the body weight of ducks. Furthermore, Li et al (2019) stated that goose body weight gain decreased with increasing levels of cassava leaf feeding in the diet. Similarly, Sekarsari (2022) reported that the inclusion of cassava tubers in broiler rations can result in a reduction in body weight gain.

A reduction in the rate of body weight gain was observed in the 75% silage diet (S_{75}), which may be due to the presence of 25.07 ppm cyanide in the ration or 2.89 mg/kg/day cyanide consumption. The observed decrease in body weight gain (BWG) may have resulted from thyroid gland disorders and increased serum thiocyanate levels. This was evidenced by the increase in thyroid gland weight and thiocyanate levels with increasing levels of CS silage use. The increase in thyroid gland weight can be assumed to be due to increased activity in producing thiocyanate. The detoxification of cyanide in the body produces thiocyanate, and an increase in thiocyanate causes sulfur amino acids to be depleted (Njankouo et al., 2023). The function of thyroxine hormone plays the most important role in growth due to its contribution to metabolism and skeletal maturation. Thyroxine in performing its role in growth works together with growth

hormones such as somatotropin and somatropin. The thyroid gland produces thyroxine hormone using the basic ingredients of iodine. Iodine in thyroxine hormone was bound to the penol ring of tyrosine which was the active component. The cyanide acid contained in CS contains CN- which was a rival to the thyroid gland in transferring iodine. If the CN ingested by ducks was too high or exceeded the tolerance level, the thyroid gland's ability to produce thyroxine hormone was disrupted, which directly affects growth. Moreover, increased thiocyanate also inhibits the intra-thyroidal uptake of iodine, which causes an increase in thyroid-stimulating hormone (TSH) secretion and a decrease in the concentration of thyroxine which is necessary for growth (Muderawan et al., 2023).

Ration intake/consumption

The application of CS in the ration had a significant effect on reducing the value of ration consumption ($p < 0.05$). Average feed consumption during the study was lowest in the 100% CS treatment (S100) at 7227.18 g/head and the highest was achieved by ducks treated with 0% CS (S0) at 7789.30 g/head. These results indicated that the higher level of CS in the ration causes the duck's ration consumption to decrease linearly and has the same pattern of decreasing duck body weight presented in Table 3.

Table 3. Average body weight gain, feed consumption, and feed conversion of ducks with cassava silage diets

Variables \ Treatment	S_0	S_{25}	S_{50}	S_{75}	S_{100}	p-value
BWG (g/head)	1132.76±161.38^{ab}	1201.88±34.90^{a}	1080.58±84.38^{b}	1030.25±37.93^{bc}	977.52±30.36^{c}	0.01
Consumption (g/head)	7789.33±65.44^{a}	7709.51±70.21^{a}	7686.86±80.12^{a}	7472.45±161.68^{b}	7227.18±201.12^{c}	0.03
Convert	6.98 ± 0.98	6.42±0.22	7.15±0.59	7.26±0.38	7.40±0.42	0.66

S_0: 100% control ration, S_{25}: 25% CS silage ration, S_{50}: 50% CS silage ration, S_{75}: 75% CS silage ration and S_{100}: 100% CS silage ration. $^{a, b}$ different superscripts in the same row indicate significant differences ($p < 0.05$).

Table 4. Internal organ weight and thiocinates in serum of male ducks with cassava silage diets

Variables \ Treatment	S_0	S_{25}	S_{50}	S_{75}	S_{100}	p-value
Abdominal Fat (%)	1.23 ± 0.17^{a}	1.10 ± 0.06^{a}	1.12 ± 0.08^{a}	0.81 ± 0.04^{b}	0.76 ± 0.02^{b}	0.03
Spleen (%)	0.05 ± 0.02^{b}	0.06 ± 0.02^{b}	0.08 ± 0.02^{ab}	0.08 ± 0.01^{ab}	0.09 ± 0.01^{a}	0.03
Liver (%)	2.80 ± 0.39^{b}	2.91 ± 0.69^{b}	3.06 ± 0.34^{b}	3.30 ± 0.37^{ab}	3.81 ± 0.20^{a}	0.04
Kidney (%)	0.69 ± 0.22	0.71 ± 0.24	0.76 ± 0.11	0.90 ± 0.16	1.01 ± 0.12	0.058
Heart (%)	0.74 ± 0.11	0.70 ± 0.04	0.77 ± 0.34	0.77 ± 0.09	0.84 ± 0.12	0.08
Gizzard (%)	5.37 ± 0.94	5.46 ± 1.06	5.50 ± 0.53	5.83 ± 0.40	6.17 ± 1.21	0.1
Pancreas (%)	0.31 ± 0.87^{b}	0.34 ± 0.03^{b}	0.34 ± 0.03^{b}	0.37 ± 0.05^{ab}	0.43 ± 0.03^{a}	0.04
Thyroid (%)	0.08 ± 0.00^{c}	0.02 ± 0.01^{b}	0.02 ± 0.00^{b}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.04
Thiocyanate (µmol/l)	0.00 ± 0.00^{d}	23.57 ± 3.97^{c}	36.41 ± 4.94^{b}	42.92 ± 1.08^{a}	45.03 ± 1.57^{a}	< 0.01
Mortality (tail)	0	2	0	1	0	

S0: 100% control ration, S25: 25% CS silage ration, S50: 50% CS silage ration, S75: 75% CS silage ration and S100: 100% CS silage ration. ^{a,b} different superscripts in the same row indicate significant differences ($p < 0.05$).

The reduced feed consumption was thought to be because the feed composed of CS was more friable and turned into a paste when wet. In this study, leaves were the largest contributor to the CS ratio and are a large component of feed (Nova et al., 2021; Phoncharoen et al., 2022). These feed conditions indirectly cause the ducks to stop consuming because the capacity of the digestive tract has been reached, even though they still need additional energy. As a result, the ducks lack energy and other essential nutrients, which ultimately affect growth.

On the other hand, reduced feed consumption was also suspected to be due to the presence of cyanide in the CS diet. Generally, cyanide content correlates with bitter taste, although this is not absolute (Bakare et al., 2021). The bitter taste of cassava raw materials makes the ration less palatable, causing ducks to reduce the amount of feed consumption. Abouelezz et al (2022) found that duck

ration consumption decreased due to the presence of antinutritional substances such as cyanide, therefore limiting the use of cassava as animal feed (Widowati et al., 2022).

The cyanide content in the diet varied widely from 0-27.80 ppm, so it can be concluded that the higher the level of CS in the diet, the more cyanide was consumed by the ducks. Based on the assumption of the illustration obtained, the cyanide consumed by ducks every day in treatment S_{25} (25% CS silage) amounted to 1.73 mg/kg/day, S_{50} (50% CS silage) amounted to 2.39 mg/kg/day, S_{75} (75% CS silage) amounted to 2.67 mg/kg/day and S100 (100% CS silage) amounted to 2.87 mg/kg/day. However, the cyanide consumed by ducks daily during the study did not exceed the tolerance limit so the giving of CS up to 100% level did not cause mortality. As reported by Njankouo et al., (2023) who stated that the tolerance limit of broiler chickens to Hidrogen cyanide (HCN) administration was 2000 mg/kg. In another statement, Jayanegara et al., (2019) recommend that the HCN content in cassava should not be more than 10 mg HCN/kg cassava when given in poultry diets.

Feed conversion ratio

Analysis of variance results showed that providing CS in the diet had no significant effect on feed conversion. Bakare et al. (2021) reported that there was no difference in ration conversion with increasing levels of cassava tubers up to 20% in the ration in broilers. Moreover, It was reported that there was no difference in ration conversion when cassava peels were given up to 30% of local ducks (Ritonga and Munandar, 2018). But different from the results of research by Olayemi and Oso (2018) the substitution of a mixture of cassava tubers and leaves with corn up to 100% level gave significant results on the value of ration conversion. The average ratio conversion of this study ranged from 6.24-7.18. This conversion value was higher than the results of previous studies as reported. Ritonga and Munandar (2018), reported that the average ration conversion of local ducks fed with cassava peel flour was 4.13-4.32. The results of the study by Olayemi and Oso (2018) showed that the value of ration conversion in corn substitution and a mixture of cassava tubers and leaves ranged from 3.20-3.73. There were significant differences between these different ration conversions when the rations were made up of different types of feed (Depawole and Sudarma, 2020). This high rate of feed conversion was indicative of the ducks' uneconomic and inefficient use of feed, resulting in poor feed efficiency.

The lowest ration conversion was achieved in treatment S25 with 25% CS silage at 6.24, followed by treatment S0 (0% CS silage) at 6.78, S50 (50% CS silage) at 6.94, S75 (75% CS silage) at 7.06 and the worst was treatment S100 (100% CS silage) at 7.18. This result showed that the higher the use of cassava-based silage, the higher the feed conversion ratio, and that this was influenced by low feed intake which was not followed by adequate body weight gain. In addition, low feed intake reduces the amount of nutrients available to the body, which in turn affects growth.

Abdominal fat

The application of CS in the ratio had a significant effect on reducing the percentage of abdominal fat ($p < 0.05$). The highest percentage of abdominal fat was found in ducks fed 0% CS silage (S0) which amounted to 1.23% and the lowest in the treatment of 100% CS silage (S100) which amounted to 0.76%. These results indicated that the percentage of abdominal fat decreased linearly with increasing CS in the ratio. The results of the study by Widowati et al. (2022) showed that increasing the level of fermented cassava leaf flour in the ration reduced the percentage of abdominal fat in broilers. In this study, the decrease in abdominal fat percentage was also attributed to the low-fat content of the diet for relatively the same energy content of the diet. This can be seen in Table 1 low ration fat content will cause a low percentage of abdominal fat, otherwise high ration fat content will enhance a high percentage of abdominal fat. Widowati et al. (2022) stated that carcass fatness was influenced by the fat content in the ration. Furthermore, the presence of dietary cyanide, which increased with increasing CS silage in the diet, could be another reason for the decrease in abdominal fat percentage. Bakare et al. (2021) stated that the presence of antinutrients in the ration will inhibit the digestibility of protein, fat, and carbohydrates, which causes pathological changes in the intestines and liver tissue, thus affecting metabolism, inhibiting some enzymes, and binding nutrients which making it unavailable. El-Zayat et al. (2019) mentioned that the loss of body fat was affected by the inhibition of lipid synthesis in the liver and stomach tissues.

Spleen weight

The application of CS in the diet had a significant effect on increasing the percentage of spleen weight ($p < 0.05$). The lowest spleen weight percentage in the provision of 0% CS silage (S0) amounted to 0.05% and the highest in the treatment of 100% CS silage (S100)

amounted to 0.09% were obtained. Prasetya et al. (2015) reported that the percentage of spleen weight of male Balinese ducks was 0.12% of live weight. These results showed that the percentage of spleen continues to increase linearly along with the increase in the provision of CS silage in the ratio. The presence of toxic substances in the form of cyanide, which increases as CS silage is increasingly included in diets, influences the increase in the percentage of spleen weight. According to Ardiansyah et al. (2021), the size of the spleen can increase or decrease by effective factors such as diseases and antinutrition. In the case of rations containing toxic substances such as cyanide, the spleen will produce lymphocyte cells which will produce antibody substances. Lee et al. (2022) explained that the swelling that occurs in the spleen is a response to an infection that stimulates the lymphocyte cells in the lymphoid organs to produce antibodies.

Heart weight

The application of CS in the ration had a significant effect on increasing the percentage of liver weight ($p < 0.05$). The percentage of liver weight in this study ranged from 2.80-3.81%. This range was higher than that reported by Prasetya et al. (2015) in the percentage of duck liver weight was 2.62% of live weight. These results indicated that the percentage of liver weight increased linearly along with the increase in the provision of CS silage in the ration. The increasing percentage of liver weight in this study was due to an increase in cyanide content along with the increasing level of cassava raw material provision into the ration. Kadiri and Asagba (2019) reported that there was an increase in liver organs in chickens fed rations containing cyanide. According to Jayanegara et al. (2019), toxic compounds were subject to a process of detoxification in the liver. Excessive toxic compounds cannot be detoxified perfectly in the liver because the liver has a very complex function including bile secretion, and metabolic processes such as protein, fat, and carbohydrate metabolism, so its ability to neutralize toxins entering the body is very limited. Cyanide detoxification makes liver cells more active as a response to high thiocyanate concentrations. This active liver work was thought to allow adaptation of liver flexibility so that it will increase liver size. Cosmos et al. (2020) expressed that the detoxification process of cyanide poison in the liver was catalyzed by the enzyme rodanase which converts the thiosulfate-cyanide complex into thiocyanate which is then excreted from the body.

Kidney weight

The range of kidney weight percentage in the current study was 0.69%-1.01% of live weight. The average percentage of kidney weight was higher, as Kusmayadi et al. (2019) reported that the percentage of kidney weight of local ducks ranged from 0.51 to 0.86% of live weight. The feeding of CS silage, which increases in the ratio, leads to an increase in the average percentage of kidney weight, although it does not have a statistically significant effect. When toxic substances enter the body, the kidneys work harder to neutralize the toxins. According to Aqsa et al. (2016), one of the functions of the kidneys is to maintain the balance of blood composition by removing substances such as excess water, metabolic wastes, organic salts, and foreign substances dissolved in the blood. The kidneys were responsible for the maintenance of the integrity of the extracellular fluid volume, the process was the conservation of water and other substances, the material needed by the body will be returned to the body fluids, while the excess will be excreted in the urine. Furthermore, the kidneys remove nitrogen from protein metabolites, ions, and complex organic compounds, both endogenous and exogenous (Suzumoto et al., 2023).

The average percentage of heart weight obtained from this study ranged from 0.70-0.84%. This percentage was lower than that reported by Kokoszyński et al. (2019), which was the percentage of duck heart weight around 11-12.4% of live weight. The inclusion of more CS silage in the diet also increased the average percentage of heart weight, although the results of the analysis of variance were not significant. This shows that ducks fed with CS silage rations up to 100% produce the same percentage of heart weight as the control. The results of the study by Bakare et al. (2021) reported that giving cassava tuber waste did not affect duck heart weight. Aqsa et al. (2016) stated that the heart was a very sensitive organ to the poisons and anti-nutrients contained in the diet and that changes in the size of the heart were common in those affected by disease or poisoning. Rations with high cyanide content cause blockages in blood vessels so that the work of the heart muscle increases resulting in enlargement of the heart size from normal. Rosanti et al. (2021) indicated that enlargement of the heart was usually characterized by an increase in the size of the heart muscle, which could occur as the muscle adjusts to the excessive contractions of the heart.

Gizzard weight

The average percentage of gizzard weight obtained from this study ranged from 5.37 to 6.17%. The average

percentage had a higher value than the one reported by Kusmayadi et al (2019), which ranged from 2.28 to 3.03% of live weight. Providing increasing amounts of CS silage in the diet also increased the average percentage of gizzard weight, although the effect was not statistically significant. This showed that diets containing up to 100% CS silage (S100) produced the same percentage of gizzard weight as the control. Besides foreign bodies such as cyanide, crude fiber also interferes with the functioning of internal organs. In case the given ratio has a high crude fiber content, the work of the gizzard will be heavier and can increase the size and weight of the gizzard. The results of this study indicated that feed treatment affects the function and development of duck gizzard because the provision of CS silage up to 100% (S100) has a high crude fiber content (7.06-7.91%). Han et al. (2017) stated that increasing the percentage of crude fiber in duck rations can increase the percentage of gizzard weight to live weight. The increase in gizzard weight was due to its heavy function to digest feed containing high crude fiber (Kusmayadi et al., 2019). Jha and Mishra (2021) explained that the increased activity of the gizzard in grinding up incoming food can cause the muscles in the gizzard tissue to become thicker due to the contractions that occur so that the size of the gizzard also increases.

Pancreas weight

The application of CS in the ratio significantly influenced increasing the percentage of pancreas weight ($p < 0.05$). The lowest percentage of pancreas weight was treated with 0% CS silage (S0) at 0.31% and the highest treatment was 100% CS silage (S100) at 0.43%. The results of the study by Kusmayadi et al. (2019) showed that the percentage of pancreas weight of local ducks ranged from 0.21 to 0.45% of live weight. This result showed that the percentage of pancreas continues to increase linearly along with the increase in the provision of CS silage in the diet. Kadiri and Asagba (2019) reported that there was an increase in pancreatic organs in chickens fed rations containing cyanide. The increase in the percentage of the weight of the pancreas occurs as a form of adaptation effort by the organ to continue to produce digestive enzymes so that the digestive process can take place normally in the digestive tract. Furthermore, The increase in cyanide content in line with the increasing level of cassava raw materials into the ration causes disruption of the pancreas function in secreting digestive enzymes

Thyroid and thiocyanate weights in serum

Providing CS in the diet showed a significant effect on reducing the weight percentage of the thyroid gland and increasing the serum thiocyanate concentration. ($p < 0.05$). The lowest percentage of thyroid gland weight in the treatment of 0% CS silage (S0) amounted to 0.08% and the highest treatment of 100% CS silage (S100) amounted to 0.03%. Based on the pattern formed, it can be seen that an increase in the supply of CS silage in the diet leads to a linear increase in the decrease in thyroid gland weight and the increase in thiocyanate concentration. The increased thyroid gland weight and serum thiocyanate concentration are due to the presence of cyanide in the ration. The higher the CS silage causes the higher the cyanide content in the ration.

Mondal and Chandra (2019) suggested that the relationship between cyanide content in the ration and thiocyanate concentration in serum illustrated the function of the cyanide detoxification system in the body and the increase in thyroid gland weight. Thiocyanate levels in serum increase due to the provision of rations containing cyanide which accumulates continuously in cassava rations. According to Supapong et al. (2022), cyanide in the body will be converted into thiocyanate with the help of the function of rhodanese. Sulfur from dietary sulfur amino acids affects the ability of the animal's body to convert cyanide to thiocyanate. While Słupczyńska et al. (2023) have shown that there was a close relationship between the level and source of iodine in the animal's diet and thyroid activity, the presence of dietary compounds that may reduce iodine utilization, such as goitrogenic substances in some feed ingredients, should also be considered. Increased thyroid weight indicates increased activity of its epithelial cells to maintain the production of the thyroid hormones that ducks need to grow.

CONCLUSION

Based on the results of the study it can be concluded that giving 25% CS in the ratio gives the best results on the performance of male ducks and giving 100% CS can have a significant impact on the value of internal organs and thyroid and thiocinate levels in male ducks. The recommendation for giving cassava silage rations can be given a maximum of 50% with the lowest impact on performance compared to other treatments.

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Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Asep Sudarman, Komang Gede Wiryawan, and Djumali Mangunwijaya designed this research. Sofia Sandi contributed to the design and data analysis of this study and drafted the manuscript. Eli Sahara and Anggriawan Naidilah Tetra Pratama helped to improve the English version of the manuscript. All authors approved the final version of the manuscript

Availability of data and materials

The original contributions presented in the study are included in the article/supplementary material. For inquiries, please contact the corresponding author/s.

Ethical considerations

All authors have reviewed the manuscripts for ethical concerns, such as plagiarism, consent to publish, misconduct, data fabrication and falsification, double publishing and submission, and redundancy.

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Effects of Yogurt Supplementation on Feed Efficiency, Growth Performance, and Ileal Nutrient Digestibility in Broiler Chicken

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ABSTRACT

The use of probiotics, particularly fermented yogurt, in poultry diets has gained substantial interest due to their capacity to enhance growth performance, feed conversion efficiency, and nutrient absorption in broiler chickens. This study evaluated the effects of yogurt supplementation on broiler performance and nutrient utilization. Two hundred one-day-old Ross-308 male broiler chicks were randomly assigned to five dietary treatments using a completely randomized design. Each treatment group included five replicates with eight chicks per replicate. The dietary treatments consisted of a control diet (without yogurt), locally prepared yogurt (5 mL/L in drinking water), yogurt fermented with *Lactobacillus acidophilus* (LA, 5 mL/L), yogurt fermented with *Streptococcus thermophilus* (ST, 5 mL/L), and yogurt co-fermented with *L. acidophilus* and *S. thermophilus* (LA+ST, 5 mL/L). The performance and ileal digestibility of nutrients were measured. Results indicated that the average daily feed intake (ADFI) significantly decreased in the LA+ST group at 0-14 days, with an 11.7% reduction compared to the control. Broilers receiving yogurt demonstrated a higher average daily gain (ADG) at 0-14 days, with the LA+ST group showing an 8% improvement over the control. At 0-28 days, the LA+ST group maintained the highest ADG, 6.8% higher than the control. The feed conversion ratio (FCR) significantly improved with yogurt supplementation at 0-14 days. Compared to the control, FCR improved by 3.6%, 7.9%, 5.7%, and 15.7% in the Local, LA, ST, and LA+ST groups, respectively. Additionally, yogurt fermented with specific lactic acid bacteria (LAB) significantly enhanced the ileal digestibility of dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), ether extract (EE), and total ash (TA). These findings highlight the efficacy of yogurt fermented with *L. acidophilus* and *S. thermophilus* as a dietary supplement to enhance growth performance and nutrient utilization in broiler chickens.

Keywords: Broiler, Daily gain, Feed conversion ratio, Feed intake, Yogurt

INTRODUCTION

Chickens are unable to efficiently digest lactose present in yogurt due to the absence of lactase, the enzyme required for its digestion. However, when administered in limited quantities, yogurt may provide beneficial probiotics that support gut health, although excessive intake may result in gastrointestinal disturbances. The intricate interplay between diet, the gut microbiome, and host health is fundamental, as gut health plays a pivotal role in efficient nutrient absorption, optimal performance, and economic viability in broiler production systems (Ipçak, 2023; Perler et al., 2023). Tight junctions between the epithelium of the intestine are critical for maintaining gut barrier function, regulating nutrient assimilation, and safeguarding against pathogenic invasion. However, their disruption due to stress or disease can trigger inflammation and metabolic dysfunction (Casula et al., 2023; Chu et al., 2023). Emerging nutritional and microbiological interventions are increasingly recognized for their potential to enhance broiler productivity by modulating gut barrier function and

overall gut health (Ducatelle et al., 2023; Szabó et al., 2023).

Yogurt is a dairy product prepared by fermenting milk with probiotic bacterial cultures, commonly known as lactic acid bacteria (LAB). During the fermentation of milk, the exponential multiplication of bacteria causes the production of lactic acid sufficient enough to drop the pH of the milk to 4.4-4.8, resulting in the development of a distinctive color, a classic tangy taste, and a tart flavor (Aleman et al., 2023). The LAB count in yogurt may range from 90 to 500 billion colony forming units (CFU) per serving. The plenty of beneficial probiotics in yogurt can improve the health and performance of broiler chickens. Additionally, it supports digestive health by inhibiting pathogenic bacteria and maintaining a balance of beneficial bacteria. Furthermore, *Lactobacillus acidophilus* has been shown to reduce cholesterol absorption in the intestines thereby lowering blood cholesterol levels (Momin et al., 2023; Song et al., 2023). Above all, LAB can improve the digestibility of dietary protein and minerals such as Cu, Mn, Fe, Ca, and P in broiler chickens (Jin et al., 2000; Khayoon et al., 2024; Rodjan et al., 2018).

Previous studies have highlighted that the recommended levels of dietary yogurt improved ADG, ADFI, and FCR in broiler chickens (Mahmmod et al., 2014; Ghasemi-Sadabadi et al., 2019; Hossain and Momu, 2022). Yogurt further influenced carcass traits, meat yield, meat quality, intestinal length, and abdominal fat deposition in broiler chickens (Hossain and Momu, 2022). It is indicated that yogurt, being a probiotic-rich feed, may enhance gut health, improve nutrient absorption, and strengthen the immune response in broiler chickens. Understanding these effects can lead to optimized feed formulations, resulting in significant improvements in growth rates, feed conversion ratios, and overall health in broiler chickens (Mirsalami and Mirsalami, 2024). Therefore, this study aimed to investigate the effects of dietary yogurt supplementation on the performance and nutrient utilization of broiler chickens.

MATERIALS AND METHODS

Ethical approval

The experimental procedures involving animals were conducted in strict compliance with the Guide for the Care and Use of Laboratory Animals, with approval obtained from the relevant Bangladeshi regulatory authorities overseeing animal welfare (Memo No. CVASU/Dir (R&E) EC/2021/244) (6).

Study design

A total of 200 day-old Ross-308 male broiler chickens, with an average weight of 46.64 g, were randomly assigned to a completely randomized design into five dietary treatment groups designated as a diet without yogurt supplementation (control), a diet containing locally prepared (Local) yogurt (5 mL/L of drinking water), a diet containing *Lactobacillus acidophilus* (LA) fermented yogurt (5 mL/L of drinking water), a diet containing *Streptococcus thermophilus* (ST) fermented yogurt (5 mL/L of drinking water, and a diet containing *Lactobacillus acidophilus* and *Streptococcus thermophilus* (LA+ST) fermented yogurt (5 mL/L of drinking water). Each treatment had five replications, with eight broiler chickens per replicate. The experiment was conducted between May and June 2024 at the experimental poultry station of Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram-4225, Bangladesh. The broiler chickens used in the study were sourced from Nourish Agro Limited, Chattogram, Bangladesh.

Prior to the study, the shed underwent a thorough cleaning process using tap water and caustic soda. The floor, ceiling, corners, rearing cages, and brooding boxes were disinfected with a 1% (v/v) phenyl solution. After sanitization and disinfection, the building was left empty for 24 hours to ensure adequate drying. The shed entrances were securely sealed, and the shed was fumigated overnight using a gaseous fumigant comprising formalin and potassium permanganate ($KMnO_4$). Feeders and drinkers were cleaned and washed daily using a 0.3% (v/v) solution of Timsen® (San Vet, Brazil).

Each chick was examined for abnormalities and to confirm uniformity in size before starting the study. The shed was constructed with brick cement walls and included grooved metal wiring at the lower section. Each chick was allocated 0.17 square feet (sq. ft) of floor space in the brooding pen and 1 sq. ft in the cage. For the first three days, the broiler chickens were kept under light for 23 hours, with the lighting duration reduced by one hour each subsequent day until it reached 8 hours. The broiler chickens were raised for a period of 28 days for growth and performance trial and an additional three days for the digestibility trial. The temperature was maintained at 95°F during the first week, 90°F in the second week, 85°F in the third week, and 80°F in the fourth week and onward. The broiler chickens were vaccinated against Newcastle disease (RaniVax Plus Vet Initial, Incepta vaccine Ltd, Bangladesh) on days 5 and 17 and against Infectious Bursal Disease (GumboMed Vet, Incepta vaccine Ltd, Bangladesh) on days 12 and 22 via the ocular route.

Experimental diet and feeding trial

Three types of yogurts were prepared, and one type was purchased from the local market, all of which were tested for beneficial microorganisms before being supplemented with drinking water. The concentration of beneficial bacteria in the yogurt was 1.90×10^7 CFU for LA, 1.96×10^7 CFU for ST, and 1.98×10^7 CFU for the LA+ST-supplemented group. The broiler chickens were provided with mash-type feed (Formulated according to the breeder manual for Ross-308; Table 1). During the growth and performance trial (For the first 28 days), the broiler chickens had continuous access to feed and water.

Table 1. Starter (1-14 d) and finisher (15-28 d) diet for the experimental broiler chicken based on the broiler breeder manual (Ross-308)

Ingredients (kg)	Starter	Finisher
Maize	52.96	56.51
Rice polish	2.01	2.01
DDGS[1]	0.51	0.51
Fish oil	0.01	0.01
Soybean oil	1.81	3.01
Soybean meal	37.11	33.11
Protein concentrate	2.81	2.01
Limestone	1.11	1.21
Dicalcium phosphate	0.81	0.81
L-Lysine	0.165	0.135
DL-Methionine	0.21	0.19
Vitamin premix[2]	0.25	0.25
Feedzyme[3]	0.025	0.025
Common salt	0.21	0.21
Total	100.0	100.0
Calculated value		
Metabolizable energy (kcal/kg)	3001.1	3103.6
Crude protein (%)	23.06	21.13
Calcium (%)	0.96	0.95
Phosphorus (%)	0.70	0.68
Phosphorus$_{(avail)}$ (%)	0.43	0.41
Lysine (%)	1.50	1.34
Methionine (%)	0.56	0.52
Cystine-methionine	0.88	0.82
Tryptophan (%)	0.32	0.30
Crude fiber (%)	3.44	3.25
Ether extract (%)	4.52	5.61

[1]DDGS (Distiller's dried grain soluble): Dry matter (DM), 88.11%; Crude protein (CP), 30.69%; Ether extract (EE), 10.89%; Crude fiber (CF), 5.94%; Nitrogen free extracts (NFE), 44.56%; Total ash (TA), 7.92%; Calcium (Ca), 0.25%; P, 0.75%; Apparent gross energy (AGE), 3169.21 kcal/kg DM. [2]Per 2500 g contained: Beta-carotene (vitamin A) at 12,000,000 IU, cholecalciferol (vitamin D3) at 2,400,000 IU, alpha-tocopherol (vitamin E) at 23 g, menadione (vitamin K3) at 2 g, thiamine (vitamin B1) at 2.5 g, riboflavin (vitamin B2) at 5 g, pyridoxine (vitamin B6) at 4 g, vitamin B3 at 40 g, Ca-D-pantothenate at 12.5 g, vitamin B12 at 12 mg, folic acid at 800 mg, vitamin B7 at 100 mg, cobalt at 400 mg, copper at 10 g, iron at 60 g, iodine at 400 mg, manganese at 60 g, zinc at 50 g, selenium at 150 mg, DL-methionine at 100 g, L-lysine at 60 g, and calcium (Ca) at 679.6 g. [3]Per 100 g contained: cellulase at 20,000 IU, xylanase at 200,000 IU, protease at 20 IU, amylase at 40,000 IU, phytase at 20 IU, pectinase at 1,400 IU, invertase at 400 IU, hemi-cellulase at 500 IU, lipase at 20 IU, and α-galactosidase at 100 IU.

Ileal digestibility of nutrients

An additional 3 days (Days 28-30) beyond the actual growth and performance trial (Days 1-28) of the broiler chicken were considered for the digestibility trial. The earlier study thoroughly outlined the methodology (Hossain et al., 2023a). To summarize, titanium oxide (TiO_2) was administered to the diet of broiler chickens at a dosage of 5 g/kg for three days (Days 28-30) as a marker to evaluate digestibility (Short et al., 1996). On day 30 of rearing, three broiler chickens per group were ethically euthanized by severing the jugular veins and carotid arteries after a fasting period of three hours. To prevent contamination from the large intestine, ileal digesta was meticulously collected from the Meckel's diverticulum to the ileal-cecal-colon junction and preserved at −20°C (Hossain et al., 2023a). The collected digest was then freeze-dried and ground into a fine powder using a 0.25 mm mesh. Subsequently, the proximate composition of both the feed and ileal contents was analyzed (AOAC, 2019). The concentration of TiO_2 in both the experimental diet and ileal samples (3 replicates per treatment) was measured after the trial to calculate the digestibility of the nutrients using a UV-VIS spectrophotometer (UV 2600, Shimadzu, Japan). Following established procedures (Maynard, 2018), apparent ileal nutrient digestibility (AID) was calculated using specific formulae.

AID (%) = 100 − ([percentage of feed indicator/percentage of ileal indicator] × [percentage of ileal nutrient/percentage of feed nutrient] × 100) (Formula 1)

Apparent DM digestibility (%) = 100 − ([percentage of feed indicator/percentage of ileal indicator] × [percentage of ileal DM /percentage of feed DM] × 100) (Formula 2)

Apparent OM digestibility (%) = 100 − ([percentage of feed indicator/percentage of ileal indicator] × [percentage of ileal OM /percentage of feed OM] × 100) (Formula 3)

Apparent CP digestibility (%) = 100 − ([percentage of feed indicator/percentage of ileal indicator] × [percentage of ileal CP /percentage of feed CP] × 100) (Formula 4)

Apparent CF digestibility (%) = 100 − ([percentage of feed indicator/percentage of ileal indicator] × [percentage of ileal CF /percentage of feed CF] × 100) (Formula 5)

Apparent EE digestibility (%) = 100 − ([percentage of feed indicator/percentage of ileal indicator] × [percentage of ileal EE /percentage of feed EE] × 100) (Formula 6)

Apparent TA digestibility (%) = 100 − ([percentage of feed indicator/percentage of ileal indicator] × [percentage of ileal TA /percentage of feed TA] × 100) (Formula 7)

Performance parameter

Mortality among the chickens was monitored and recorded daily. Dead broiler chickens were excluded from the study, for accurate calculation of feed intake per broiler chicken, the cumulative feed intake of the dead broiler chickens was subtracted from the total feed intake of a particular replicate. Similarly, to calculate weight gain, the weight of dead chickens on the last weighing day was deducted from a replicate's total weight. On days 14 and 28, ADG and ADFI were calculated to determine FCR. The ADG was determined by deducting the initial body weight of the broiler chickens from their final weight. Feed intake was calculated by subtracting the remaining feed from the initial amount provided. The FCR was then obtained by dividing the total feed intake by weight gain.

Statistical analysis

The detailed statistical procedures have been described in previous studies (Hossain and Akter, 2022; Hossain et al., 2023a; 2023b). Performance and digestibility data from each pen were averaged before further analysis, with each pen treated as an independent experimental unit. Outliers and multicollinearity in the data were assessed using variance inflation factors and interquartile range tests. The normal distribution of the response variable was evaluated using a normal probability plot, and the Shapiro-Wilk test was employed to assess the equality of variances. The analysis of the data was conducted using a generalized linear model. To assess the suitability of the dataset for principal component analysis, the Kaiser-Meyer-Olkin test for sampling adequacy and Bartlett's test of sphericity were performed using the SAS 2022 platform. Orthogonal 'varimax' rotation (Kaiser off) was performed, leading to the identification of two primary components based on the top 'eigen' values in the 'scree plot. Duncan's New Multiple Range Test (DMRT) was utilized to compare means when significant effects were detected ($p < 0.05$). Statistical analyses were performed using SAS JMP Pro 16.2 2022 (SAS Institute, Cary, North Carolina, USA) and Stata 14.1 SE 2015 (Stata Corp LP, College Station, Texas, USA). The study employed the following additive model.

Y_{ijk}	$\mu + \alpha_i + \beta_j + \gamma_{k\ldots} + \varepsilon_{ijkn}$
Y_{ijk}	The effect observed for the 'n^{th}' repetition of the combination involving the 'i^{th}' level of factor 'α', the 'j^{th}' level of factor 'β', and the 'k^{th}' level of factor 'γ';
μ	The intercept of the regression model;
α_i	The effect of the 'i^{th}' level of factor 'α' on the observed value in Y_{ijk};
β_j	The effect of the 'j^{th}' level of factor 'β' on the observed value in $Y_{ijk;}$
γ_k	The effect of the 'k^{th}' level of the factor 'γ' on the value observed in $Y_{ijk;}$
ε_{ijk}	The random sampling error due to the 'i^{th}' level of the factor 'α', the 'j^{th}' level of the factor 'β', and the 'k^{th}' level of the factor 'γ' distributed as $\varepsilon i \sim NID(0,\sigma^2)$.

RESULTS

Average daily feed intake

The ADFI of the broiler chickens differed significantly ($p < 0.001$) among treatment groups during days 0-14 (Table 2). The lowest ADFI was recorded in the LA + ST- supplemented group which was 11.7% lower than the control group during this period. However, at days 15-28 and 0-28, no substantial differences in ADFI were observed between the control and treated groups ($p > 0.05$). At days 15-28, ADFI increased by 7.3%, 6%, 3.1%, and 5.4% in local, LA, ST, and LA + ST treated groups, respectively, compared to the control. Similarly, at days 0-28, ADFI was 5.2% higher in the local group, 3.8% in the LA group, and 2.2% in the ST-supplemented group compared to the control. An increased ADFI was associated with a concomitant increase in ADG at the expense of FCR (Figures 1-3). A strong positive correlation was observed between FW, ADG (Days 15-28), and ADG (0-28) while a strong negative correlation was noted between ADG (Days 15-28 and 0-28) and FCR (Days 15-28 and 0-28) (Figure 4). The FW, ADG (Days 0-28), and ADFI (Days 0-28) were identified as the principal eigenvectors determining major variations in broiler performance (Figures 5 and 6).

Average daily gain

The yogurt-supplemented groups exhibited a statistically significant improvement in ADG compared to the control group during days 0 -14 ($p = 0.031$; Table 2). Higher ADG was recorded in all the yogurt supplemented groups compared to the control. The ADG increased by 3.7%, 6.7%, 5.9%, and 4.3% in the Local, LA, ST, and LA+ST-supplemented groups, respectively, compared to the control ($p = 0.031$). Similarly, on days 15-28, the lowest ADG was recorded in the control group, while the highest ADG was recorded in the LA+ST-supplemented group, which was 8% higher than the control ($p = 0.484$). Accordingly, at days 0-28, the highest ADG was recorded

in the LA+ST-supplemented group, which was 6.8% higher than the control (p = 0.374).

Feed conversion ratio

The feed conversion ratio varied significantly among treatment groups at days 0-14 (p < 0.001) and 0-28 (p = 0.023). However, no significant differences were observed during days 15-28. During days 0-14, FCR improved by 3.6%, 7.9%, 5.7%, and 15.7% in local, LA, ST, and LA+ST yogurt-supplemented groups, respectively, compared to the control. Over the entire period (Days 0-28), the best FCR was observed in the LA+ST supplemented group, which was 6.6% better (p = 0.023) than the control group (Table 2).

Ileal digestibility

Overall, yogurt supplementation in the broiler diet significantly (p < 0.001) improved the ileal digestibility of DM, OM, CP, CF, EE, and TA except in the group supplemented with local yogurt, where digestibility of DM, CF, EE, and TA rather decreased than in the control group (Table 3). The highest digestibility of DM, OM, CP, and EE was recorded in the LA+ST supplemented group, with improvements of 23.82%, 29.51%, 20.27%, and 23.76%, respectively, compared to the control group (p < 0.001).

Table 2. Performance parameter of the broiler chicken provided with different types of yogurt supplements

Parameter	Dietary treatments[1]					SEM	p-value
	Control	Local	LA	ST	LA+ST		
IW[2] (g/b)	46.7	46.8	46.7	46.4	46.6	0.42	0.970
FW[3] (g/b)	1806.6	1834.0	1872.7	1853.6	1927.3	41.5	0.374
ADFI[4] (0-14 d, g/bird/d)	52.2^{a}	52.3^{a}	51.7^{a}	52.2^{a}	46.1^{b}	0.80	<0.001
ADG[5] (0-14 d, g/bird/d)	37.4^{b}	38.8^{ab}	39.9^{a}	39.6^{a}	39.0^{a}	0.53	0.031
FCR[6] (0-14 d)	1.40^{a}	1.35^{b}	1.29^{c}	1.32^{b}	1.18^{d}	0.02	<0.001
ADFI (15-28 d, g/bird/d)	118.4	127.0	125.4	122.1	124.8	3.16	0.373
WG (15-28 d, g/bird/d)	88.3	88.9	90.5	89.5	95.4	2.92	0.484
FCR (15-28 d)	1.34	1.43	1.39	1.36	1.31	0.03	0.133
ADFI (0-28 d, g/bird/d)	85.3	89.7	88.5	87.2	85.4	1.74	0.346
WG (0-28 d, g/bird/d)	62.9	63.8	65.2	64.5	67.2	1.48	0.374
FCR (0-28 d)	1.36^{b}	1.41^{a}	1.36^{b}	1.35^{b}	1.27^{c}	0.02	0.023

[1]Control: A diet without yogurt; Local: A diet containing locally prepared yogurt at 5 mL/L drinking water; LA: A diet containing *Lactobacillus acidophilus* fermented yogurt at 5 mL/L drinking water; ST: A diet containing *Streptococcus thermophilus* fermented yogurt at 5 mL/L drinking water; LA+ST: A diet containing *Lactobacillus acidophilus* and *Streptococcus thermophilus* fermented yogurt at 5 mL/L drinking water; [2]IW: Initial weight; [3]FW: Final weight; [4]ADFI: Average daily feed intake; [5]ADG: Average daily gain; [6]FCR: Feed conversion ratio; [abcd] Means bearing different superscripts in the same row differ significantly at p < 0.05.

Table 3. Nutrient digestibility in broiler chickens provided diets supplemented with various types of yogurts

Parameter (%)	Dietary treatments[1]					SEM	p-value
	Control	Local	LA	ST	LA+ST		
Dry matter	65.9^{d}	64.0^{e}	75.3^{c}	78.0^{b}	81.6^{a}	1.83	<0.001
Organic matter	61.0^{e}	64.9^{d}	71.1^{c}	75.7^{b}	79.0^{a}	1.77	<0.001
Crude protein	67.1^{e}	69.6^{d}	72.4^{c}	73.1^{b}	80.7^{a}	1.22	<0.001
Crude fiber	61.1^{d}	58.9^{e}	77.3^{b}	78.7^{a}	75.4^{c}	2.27	<0.001
Ether extract	60.6^{d}	57.8^{e}	69.6^{c}	71.5^{b}	75.0^{a}	1.76	<0.001
Total ash	76.0^{d}	73.7^{e}	77.8^{b}	81.2^{a}	77.5^{c}	0.66	<0.001

[1]Control: diet without yogurt; Local: A diet containing locally prepared yogurt at 5 mL/L drinking water; LA: A diet containing *Lactobacillus acidophilus* fermented yogurt at 5 mL/L drinking water; ST: A diet containing *Streptococcus thermophilus* fermented yogurt at 5 mL/L drinking water; LA+ST: A diet containing *Lactobacillus acidophilus* and *Streptococcus thermophilus* fermented yogurt at 5 mL/L drinking water; [abcde] Means bearing different superscripts in the same row differ significantly at p < 0.05.

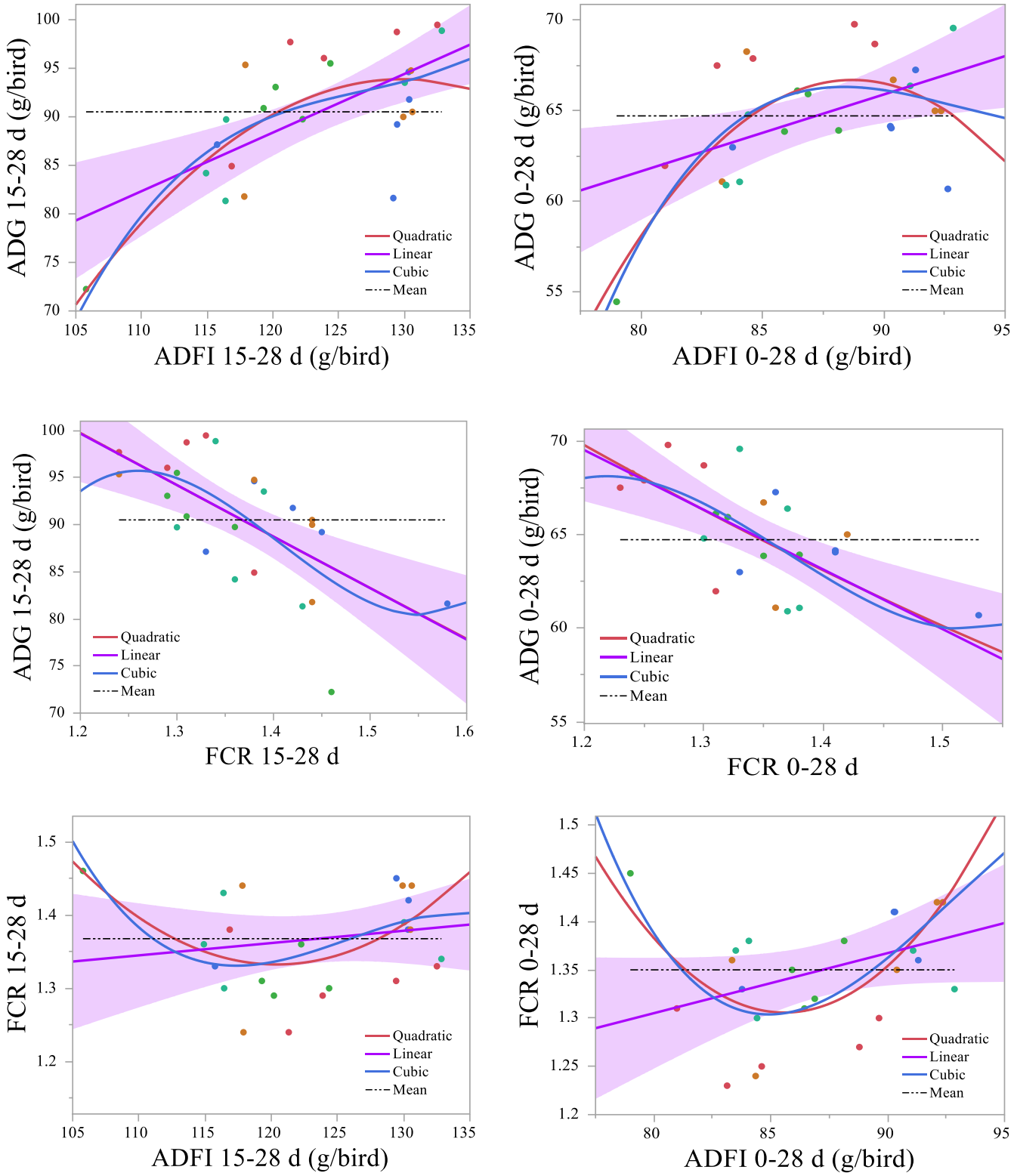

Figure 1. Bivariate distributions showing linear effects of diets supplemented with different types of yogurts on average daily feed intake, average daily gain, and feed conversion ratio in broiler chicken.

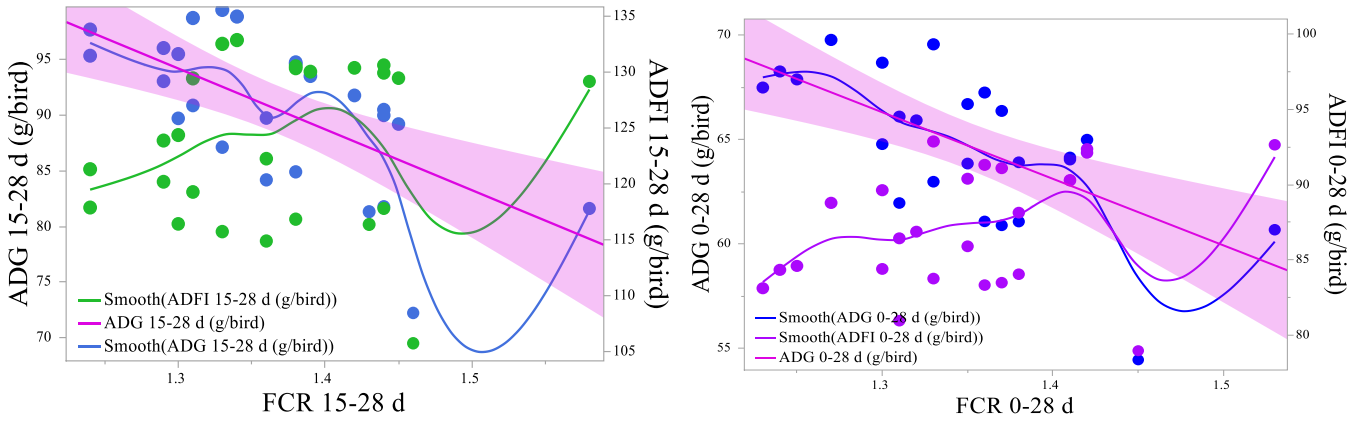

Figure 2. Locally weighted scatterplot smoothing showing effects of diets supplemented with different types of yogurts on feed conversion ratio, average daily feed intake, and average daily gain of the broiler chicken.

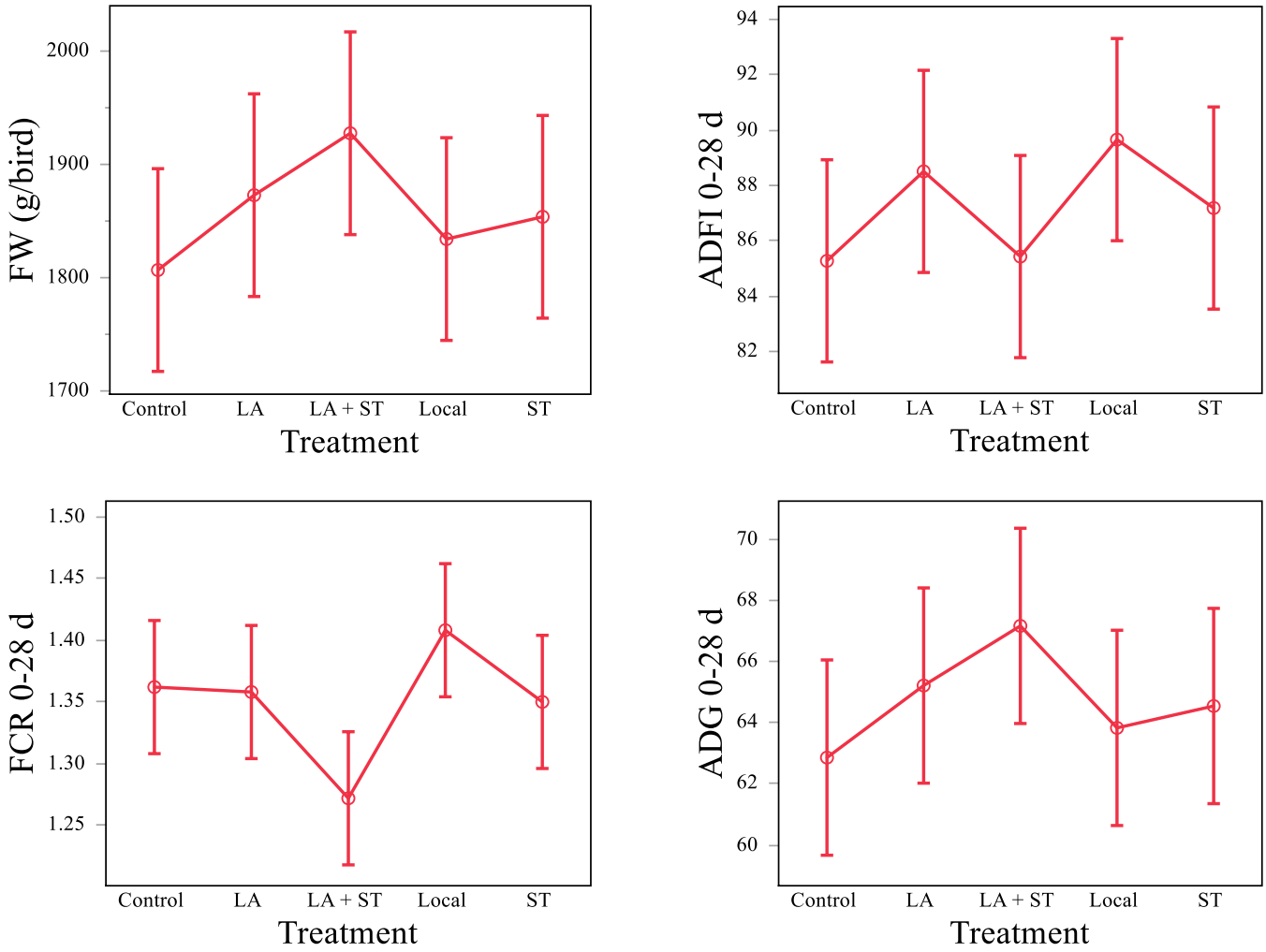

Figure 3. Least square means showing linear effects of different types of yogurts on final weight, average daily feed intake, average daily gain, and feed conversion ratio of the broiler chicken. Control: A diet without yogurt; Local: A diet containing locally prepared yogurt at 5 mL/L drinking water; LA: A diet containing *Lactobacillus acidophilus* fermented yogurt at 5 mL/L drinking water; ST: A diet containing *Streptococcus thermophilus* fermented yogurt at 5 mL/L drinking water; LA+ST: A diet containing *Lactobacillus acidophilus* and *Streptococcus thermophilus* fermented yogurt at 5 mL/L drinking water.

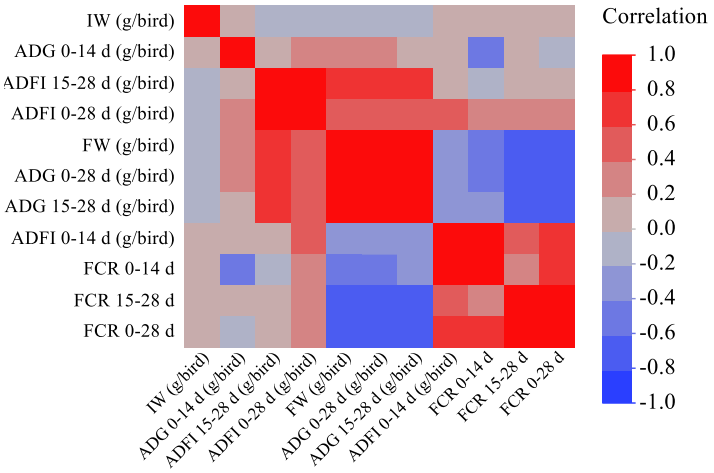

Figure 4. Heatmap showing clustered multiple correlation coefficient matrix of the performance parameter of the broiler chicken fed different yogurt supplemented diets

Eigenvectors

	Prin1	Prin2	Prin3	Prin4	Prin5
ADG 0-28 d (g/bird)	0.45798	0.07878	0.03576	0.35536	0.20695
FW (g/bird)	0.45796	0.07856	0.03535	0.35860	0.27601
ADG 15-28 d (g/bird)	0.45295	0.07299	0.16641	-0.48720	0.38306
ADFI 15-28 d (g/bird)	0.27262	0.48565	-0.20899	-0.57597	-0.27868
ADFI 0-28 d (g/bird)	0.17068	0.57588	0.03440	0.40841	-0.41651
FCR 0-14 d	-0.24075	0.29046	0.84750	-0.04929	-0.07736
FCR 15-28 d	-0.31448	0.38921	-0.45442	0.08089	0.11138
FCR 0-28 d	-0.33918	0.42302	-0.01267	0.01596	0.68171

Eigenvalues

Number	Eigenvalue	Percent	20 40 60 80	Cum Percent
1	4.6622	58.277		58.277
2	2.5815	32.269		90.546
3	0.7126	8.907		99.452
4	0.0399	0.499		99.951
5	0.0030	0.037		99.988
6	0.0008	0.010		99.999
7	0.0001	0.001		100.000
8	0.0000	0.000		100.000

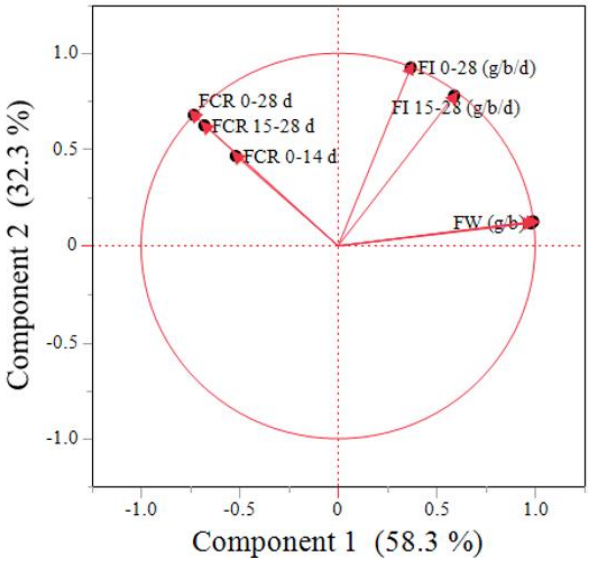

Figure 5. Principal component analysis with eigenvectors and values showing effects of different types of yogurts on the performance of the broiler chicken. Plotted on "x" as component 1 (58.3%) and "y" as component 2 (32.3%)

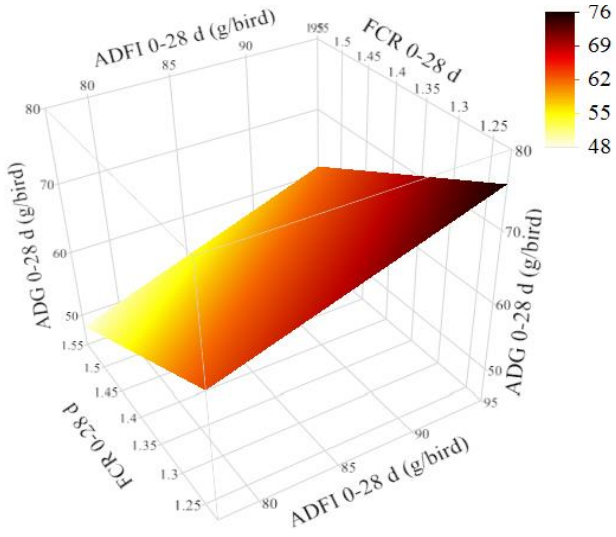

Figure 6. Response surface model with the central composite design showing desirable zone of average daily gain (ADG) optimized by linear combinations of the different values of average daily feed intake (ADFI) and feed conversion ratio (FCR) in broiler chickens provided with diets supplemented with different types of yogurts.

DISCUSSION

Average daily feed intake

In the present study, the ADFI ranged from 46.1 to 52.3 g/bird/d at 0-14 days. Among the study groups, broiler chickens supplemented with LA+ST yogurt showed the lowest ADFI, whereas those provided with local yogurt showed the highest ADFI. Although significant differences were noticed between the yogurt and control groups at 0-14 days, no marked difference was found at 15-28 or over the entire 28-day period. These findings align with a previous study, which reported that supplementing broiler diets with yogurt at a concentration of 5 mL/L in drinking water did not significantly affect overall ADFI (Sultan et al., 2006). Likewise, Hossain and Momu (2022) reported that yogurt supplementation had a negligible effect on feed consumption in chickens. In contrast, earlier studies reported significantly higher feed intake in the broiler chickens supplemented with dried yogurt powder (1 kg/100 kg diet) compared to the probiotic-supplemented and control groups, which was consistent with the present study (Mamun et al., 2021; Hossain and Momu, 2022). Accordingly, another study found that dietary yogurt supplementation did not affect ADFI in broiler chickens during the first 10 days but had a substantial impact in the later stages (Ghasemi-Sadabadi et al., 2019). It implies that the influence of yogurt on feed intake may diminish over time, with noticeable differences in the early stages that do not persist. Hence, it may be concluded that the overall effects of yogurt on ADFI in broiler chickens are complex and vary depending on the study duration, the form and quantity of yogurt used, and the specific conditions under which the broilers were raised (Ghasemi-Sadabadi et al., 2019).

Average daily gain

In the current study, the ADG differed significantly among the dietary groups. The LA-supplemented yogurt group showed the highest gain at 0-14 days, with no notable difference in overall weight gain at 15-28 days. According to a former study, yogurt supplementation at 5 mL/L of drinking water significantly improved weight gain during both the starter and finisher phases in comparison to the control group (Sultan et al., 2006). The highest body weight gain at later stages in the LA+ST supplemented group was also aligned with an earlier study which reported better weight gain in broiler chicken supplemented with duo-strain probiotic (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*; Adriani et al., 2015). The highest ADG in the LA supplemented group at the initial stage obtained in the present study was also consistent with some other studies. For example, one study reported that supplementation with *Lactobacillus acidophilus* in broiler chickens infected with *Clostridium perfringens* improved ADG (Li et al., 2018). The observed improvement in performance may be ascribed to enhanced nutrient digestibility and absorption, maintenance of a stable intestinal microbiota, improved intestinal health, a stronger immune response, and reduced stress levels (Revolledo et al., 2006; Prado-Rebolledo et al., 2017; Li et al., 2018).

The inclusion of *L. acidophilus* in poultry feed prevents the growth of harmful bacteria and regulates intestinal flora via competitive exclusion (Forte et al., 2018). However, despite many similarities, these results contradict a previous study that found no effect of probiotic supplementation (*Lactobacillus spp.*) on ADG during phage feeding (Hosamani et al., 2006). The differences in weight gain could be attributed to factors such as sex, weather conditions, infectious diseases, and other variables (Aftahi et al., 2006). This study further revealed the highest body weight gain in the LA+ST-supplemented group at 15-28 days, indicating a synergistic effect of the multi-strain probiotic on broiler performance. The findings suggest that yogurt supplementation, particularly with *Lactobacillus acidophilus* and multi-strain probiotics (LA+ST), enhances early-stage ADG in broiler chickens. While initial improvements in weight gain are consistent with previous studies, long-term effects may vary due to environmental and health factors.

Feed conversion ratio

In the current study, notable differences in FCR were observed among the yogurt-supplemented groups compared to the control at 0-14 days. Among the yogurt supplemented groups, the LA + ST group showed the best FCR. Overall, FCR differed significantly, with the LA+ST group showing the highest feed efficiency. However, from days 15-28, no significant difference was found between the yogurt-supplemented group and the control group ($p = 0.133$). These results observed a better FCR in the LA + ST supplemented group compared to the control. Consistent with these findings, another study reported better FCR in the duo-strain probiotic supplementation in broiler chickens (Mirsalami and Mirsalami, 2024). Several previous studies also reported substantial impacts of yogurt supplementation on the FCR in broiler chicken (Sultan et al., 2006; Mansoub and Nezhady, 2011; Ghasemi-Sadabadi et al., 2019).

In contrast to the current study, a previous study reported that there was no statistically significant effect of yogurt supplementation on FCR in the broiler chicken (Mahmmod et al., 2014). Accordingly, no combined effect of *Streptococcus thermophilus* and *Lactobacillus sp.* on FCR was found, possibly due to insufficient bacterial count, as well as genotype and growth stage variations among experimental broiler chickens (Nafees and Pagthinathan, 2018). The present study indicated that yogurt supplementation, particularly with the LA+ST multi-strain probiotic, enhances FCR in broiler chickens, especially during the initial growth phase. However, discrepancies in findings across the studies may be due to variations in bacterial count, genetic factors, and growth stages (Mirsalami and Mirsalami, 2024).

Nutrient digestibility

Yogurt supplementation significantly improved the ileal digestibility of DM, OM, CP, CF, EE, and TA in the LA, ST, and LA + ST groups. Consistent with these results, a previous study reported enhanced ileal protein digestibility following dried yogurt powder supplementation (Abbas et al., 2020). The improved protein digestion within the gastrointestinal tract was likely due to increased lactic acid concentration, which enhances protease enzyme activity (Abbas et al., 2020). The reduction in pH induced by lactic acid bacteria boosts the enzyme activity, thereby facilitating feed breakdown, inhibiting the growth of pathogenic microorganisms, and promoting the proliferation of beneficial bacteria (Flint and Garner, 2009; Recoules et al., 2017). Another study demonstrated that a multi-strain probiotic containing lactic acid bacteria significantly enhanced the ileal digestibility of DM, CP, and gross energy compared to the control group (Kim et al., 2012). Similarly, another study reported that supplementation with lactic acid bacteria, specifically *Lactobacillus bulgaricus*, enhanced the digestion of nitrogen and fat, though it had no significant impact on fiber digestion. These observations suggested that lactic acid bacteria can improve the ability of broiler chickens to digest certain nutrients (Apata, 2008). On the contrary, it was indicated that the total tract apparent digestibility of DM, OM, CP, EE, and ash in broiler chickens remained unaffected despite the inclusion of yogurt acid whey powder at concentrations of 2.5%, 5%, and 10% of the diet (Paraskeuas et al., 2023). Similarly, it was reported that supplementation of dry whey powder and whey concentrate did not affect the digestibility of DM and CP, however, they significantly influenced the digestibility of minerals, particularly calcium, and phosphorus (Pineda-Quiroga et al., 2018).

CONCLUSION

Supplementation of yogurt in broiler diets improved their growth performance, feed intake, and nutrient utilization. Fermented yogurt containing *Lactobacillus acidophilus* and *Streptococcus thermophilus* (LA+ST) distinctively enhance feed efficiency, ileal nutrient digestibility, and growth performance of broiler chickens over 28 days. It may be concluded that probiotic-enriched yogurt was a viable dietary supplement to promote efficient growth and enhance nutrient utilization in broiler chickens. Future research should explore gut barrier functionality, intestinal histo-morphometry, bacterial translocation, and humoral immune responses in chickens fed yogurt supplemented diets.

DECLARATIONS

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Authors' contributions

Md. Emran Hossain conceptualized the study, managed the project, curated the data, conducted the generalized linear model, principal component analysis, and response surface modeling, interpreted the results, and finalized the initial draft. Minara Begum Munni, Umme Salma Amin, and Mahabub Alam prepared the yogurt culture, carried out the feeding trial, immunization, and ileal nutrient digestibility, and contributed to drafting the initial manuscript. Shilpi Islam, Nasima Akter, and Md. Ahasanul Hoque provided critical insights and oversaw the entire study. All authors reviewed and approved the final version of the manuscript.

Competing interests

The authors declare that there are no conflicts of interest.

Ethical considerations

The authors affirm that they have adhered to ethical research practices, avoiding plagiarism, misconduct, data fabrication or falsification, and duplicate submission or publication, and have provided their consent for this article's publication.

Availability of data and materials

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

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Broiler Farming in the Face of Accelerating Climate Change: Risks for Production and Food Security

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ABSTRACT

Climate change poses significant challenges to poultry farming, particularly when broiler farms rear chickens in suboptimal housing conditions. The objective of the present study was to examine the impact of climate change, expressed through the Temperature Humidity Index (THI), on quantitative (carcass yields, pectoral muscles, thighs and drumsticks, and abdominal fat rate) and qualitative production parameters (composition of muscles in dry matter, mineral matter, crude proteins, and fat). The study was conducted in two separate poultry buildings over 45 days in northern Algeria. A total of 300 one-day-old unsexed chicks were randomly allocated into three replicates of 50 broilers each per building. The conditions of temperature and relative humidity were strictly regulated in control group but it was unregulated, exposing birds to natural climate variations in the experimental group. The impact of climate change, represented by the Temperature Humidity Index (THI), on carcass yield, pectoralis major and minor (pectoral muscles), sartorius and gastrocnemius (thigh and drumstick muscles), as well as abdominal fat content were evaluated. The results revealed that the control group was exposed to THIs of 30.88, 20.45, and 19.19, while the experimental group was subjected to THIs of 33.07, 31.48, and 30.87 for the three growth phases. The increase in THI resulted in significant proportional deteriorations in the experimental group compared to the control group, for all the parameters under study, particularly at the end of breeding. There were reductions in yields of -6.12% for eviscerated carcasses, -8.16% for thighs and drumsticks, and -9.28% for pectoral muscles. Furthermore, the abdominal fat rate increased by +21.03%. The nutritional composition of pectoral muscles showed that chickens in the experimental group had +6.17% dry matter, +13.23% fat, -13.88% mineral matter, and -8.78% crude proteins. A similar trend was observed for thigh and drumstick muscles, with +6.10% dry matter, +14.39% fat, -12.28% mineral matter, and -12.50% crude proteins. The study highlighted the impact of climate change on poultry farming, which potentially affects production and threatens food security.

Keywords: Broiler chicken, Carcass, Climate change, Food security, Muscle, Nutritional quality, Yield

INTRODUCTION

The poultry sector, encompassing its two main branches of meat and egg production, plays a vital role in global human nutrition by providing animal-based proteins, notably meat and eggs. Poultry products, particularly meat and eggs, are widely consumed across diverse populations, especially in emerging economies where they serve as a critical dietary staple. According to data from FAOSTAT (2023), poultry meat accounts for approximately 33% of global meat production, underscoring the sector's critical contribution to protein intake for humans. With the global population projected to reach 9.9 billion by 2050 (PRB, 2020), including an additional one billion people in Africa alone (Thornton et al., 2009), demand for poultry products is expected to rise systematically. Projections indicate a 70% increase during this period (Searchinger et al., 2019). To meet this growing demand, fast-growing chickens, developed through over 70 years of genetic advancements, have significantly improved feed efficiency and production (Zuidhof et al., 2014; Tallentire et al., 2018). However, these advances have also accelerated metabolism, increasing metabolic heat production while leaving broiler chickens with underdeveloped

cardiovascular and respiratory systems. This combination renders them less thermotolerant and more susceptible to heat stress (Lu et al., 2007; Xu et al., 2018). Unlike mammals, broiler chickens lack sweat glands, relying instead on a thermoregulatory system that balances heat production and dissipation (Kumar et al., 2021). When this balance is disrupted, thermal stress ensues, leading to hyperthermia (Renaudeau et al., 2012; Rostagno, 2020). Hyperthermia is primarily characterized by an increased respiratory rate and significant loss of carbon dioxide to the environment.

Meanwhile, climate change, characterized by extreme deviations in climate patterns over extended periods (Ngaira, 2007), is expected to intensify further. Projections indicate that by 2050, climate patterns will include prolonged heatwaves, reduced rainfall, and overall atmospheric warming, particularly in equatorial, tropical, and Mediterranean regions (IPCC, 2018). These changes pose significant threats to agricultural and socio-economic development, with animal production systems being particularly vulnerable. Similarly, Surai and Fisinin (2016) reported that industrial poultry farming faces multiple stressors, including heat stress, adversely affecting production, reproduction performance, and overall poultry health. Attia et al. (2022) emphasized that emerging countries, particularly in Africa, are especially vulnerable to climate change due to inadequate infrastructure. As noted by Attia et al. (2022), poultry housing in these regions often fails to meet environmental standards, lacking proper insulation and climate control systems. Such conditions negatively impact the development and sustainability of poultry activities.

Findings indicate that high ambient temperatures induce heat stress, adversely affecting poultry farming, particularly broiler chickens (Lara and Rostagno, 2013). Thermal stress can be acute, characterized by a sudden temperature increase over a short period. Furthermore, it is classified as chronic when it persists over an extended period (Nawaz et al., 2021). Regardless of the type of stress, chain reactions are triggered in broiler chickens, which, in some instances, deteriorate carcass appearance and nutritional quality (Qu and Ajuwon, 2018; Zhao et al., 2019; Zhang et al., 2020), potentially compromising the availability of poultry products and, consequently, food security.

Given these considerations, the present study examines the impact of climate change, expressed through the Temperature Humidity Index (THI), on both quantitative and qualitative production parameters in Cobb 500 broiler chickens raised during the summer season.

MATERIALS AND METHODS

Ethical approval

The study was approved by the Faculty of Nature and Life Sciences and Earth Sciences at Djilali Bounaama University of Khemis Miliana. It was conducted over a period of forty-five days, from July 15 to August 30, 2023.

Methodological approach

The experimental study evaluated the impact of climate change, represented by the Temperature Humidity Index (THI), on carcass yield, pectoralis major and minor (pectoral muscles), sartorius and gastrocnemius (thigh and drumstick muscles), as well as abdominal fat content. Furthermore, the composition of significant nutrients was analyzed in the muscles used to measure yields and included the rate of dry matter, mineral matter, crude proteins, and fat. The study was conducted in two separate buildings during summer, lasting forty-five days between July 15th and August 30th, 2023. A total of 300 unsexed chicks from the Cobb 500 strain were received at one day of age (150 per building), and further divided into three random replicates, each with 50 broilers. The first building was modern, climate-controlled, and served as the control group (group C). The temperature was recorded as 32.95 ± 2.47, 21.14 ± 2.98, and 19.67 ± 1.81°C, and the relative humidity as 64.01 ± 2.56, 67.19 ± 4.83, and 69.44±4.75%, for the starter, grower, and finisher phases, respectively. In contrast, the second building was less equipped, had an uncontrolled environment, and was subject to natural climate variations, representing the experimental group (group E). The temperature in this building was 35.93 ± 1.77, 33.93 ± 2.63, and 33.44 ± 1.76°C, and the humidity was 57.21 ± 3.73, 59.57 ± 7.09, and 56.42 ± 2.62%, for the same breeding phases. Furthermore, parameters were analyzed during the starter, grower, and finisher phases at the fifteenth, thirtieth, and forty-fifth breeding days. Finally, broiler chickens were fed an identical commercial standard diet at each growth phase throughout the study. The composition and chemical analysis of these diets are reported in Table 1.

A standard lighting program was followed during the study. Lamps provided light, ensuring an approximate intensity of 5 watts/m². In addition, twenty-three hours of lighting were provided during the first week. From the 8th day of age onwards, a lighting duration of between eighteen and nineteen hours was maintained. For its part, vaccination procedures were standardized. Broilers were vaccinated against Newcastle and Gumboro diseases on the 8th and 18th days of age. On the day 23, vaccination

against coccidiosis was administered. Finally on day 29 coincided with a booster vaccination against Newcastle disease.

Table 1. Composition and chemical analysis of diet for the different growth phases

Composition (%)	Starter	Grower	Finisher
Maize	61	65	68
Soybean meal	27.5	24.3	21
Wheat bran	6	5.5	5.9
Calcium carbonate	2.7	2.7	2.7
Dicalcium phosphate	1.9	1.6	1.5
Mineral and vitamin premix	0.3	0.3	0.3
NaCl	0.3	0.3	0.3
L-lysine	0.1	0.1	0.1
DL-methionine	0.1	0.1	0.1
L-threonine	0.1	0.1	0.1
Chemical analysis			
Metabolizable energy (kcal/kg)	2900	2900	2950
Crude proteins (%)	21	19	17
Fat (%)	2.5	2.5	2.5
Crude fiber (%)	4	4	4
Lysine (%)	0.88	0.88	0.80
Methionine (%)	0.38	0.36	0.36
Calcium (%)	0.8	0.8	0.8
Phosphorus (%)	0.7	0.7	0.7

Methods for measuring ambient parameters

Ambient temperature and relative humidity were measured using Kimo KH 50 recording thermo-hygrometers manufactured by Testoon, France. These devices were placed at the center and both ends of the breeding buildings and recorded data every 30 minutes.

Method for measuring Temperature Humidity Index

The Temperature Humidity Index (THI) was calculated using the formula proposed by Marai et al. (2000).

$$THI = T- [(0.31 – 0.31xRH/100) (T-14.4)] \text{ (Formula 1)}$$

THI: Temperature Humidity Index, T: Temperature (°C), and RH: Relative Humidity (%).

Methods for measuring carcass yields

At 15, 30, and 45 days of age, 10 broiler chickens were selected based on an average live weight representative of each group. The live weights in the control group were 592.98 ± 39.00, 1827.13 ± 99.48, and 2926.64 ± 129.25g on days 15, 30, and 45, respectively. In the experimental group, the corresponding weights were 460.85 ± 31.01, 1521.92 ± 93.01, and 2165.90 ± 115.11g. The evaluated yields included eviscerated carcasses, thighs and drumsticks, pectoral muscles, and abdominal fat. To this end, after fasting for twelve hours, each chicken was slaughtered by bleeding the carotid artery and jugular vein. Following bleeding, the chickens were immersed in water at an average temperature of 60°C for two minutes to facilitate plucking, following the recommendations of Faria et al. (2010). Furthermore, the dissection of carcasses was performed according to the standard method described by Jensen (1984). After removing the head and legs, the carcasses were placed in dorsal decubitus on dissection trays. An abdominal opening was made using a scalpel blade, allowing the abdominal cavity to be lifted and folded forward. Abdominal fat, the digestive tract, and the giblets were removed. The eviscerated carcasses were weighed using Dawood brand electronic scales manufactured by Dongyang Zhibo Weighing Scales Factory, China. The yield of eviscerated carcasses was measured according to the following formula:

$$\text{Carcass yield (\%)} = \frac{\text{Eviscerated carcass weight (g)}}{\text{Slaughter weight (g)}} \times 100 \text{ (Formula 2)}$$

Isolation of the lower limbs of the carcasses was carried out by folding the pair of thighs until they were disarticulated from the pelvis. A horizontal incision was made to detach the pair of legs from the rest of the carcass. Additionally, the skin and fat covering the pectoral muscles were removed. An incision was made along the sternum and collarbones, allowing the pectoral muscles to be removed from the rib cage. The pair of legs, as well as the pectoral muscles, were weighed using the same scale. The yields of these cuts were calculated using the following formula:

$$\text{Cutting yield (\%)} = \frac{\text{Cutting weight (g)}}{\text{Slaughter weight (g)}} \times 100 \quad \text{(Formula 3)}$$

Abdominal fat was also weighed using the same scale and its proportion was calculated as follows:

$$\text{Fat proportion (\%)} = \frac{\text{Abdominal fat weight (g)}}{\text{Slaughter weight (g)}} \times 100 \text{ (Formula 4)}$$

Finally, after measuring the yields, samples of thigh and drumstick muscles and pectoral muscles were collected from each sample. They were stored at -18°C for subsequent determination of nutritional composition.

Methods for measuring the nutritional quality of muscles

Ten samples of thigh, drumstick, and pectoral muscles were thawed at room temperature. They were crushed and subjected to chemical analyses to determine their nutritional composition. Each analysis was carried out in triplicate for each sample. The analyses followed AOAC (2000) recommendations. Dry matter (DM%) was determined by drying a muscle sample in an oven manufactured by Memmert, Germany, for 24 hours at an average temperature of 105°C. Mineral matter (MM%) was determined by calcining a muscle sample in an incinerator manufactured by Nabertherm, Germany, for 1.5 hours at 200°C, followed by 2.5 hours at 500°C. The percentage of crude proteins (CP%) was measured after mineralizing a muscle sample using the Kjeldahl device manufactured by Buchi, Switzerland. The sample was mineralized with sulfuric acid in the presence of a catalyst; the organic nitrogen was transformed into ammoniacal nitrogen, and the ammonia was displaced by sodium hydroxide and measured after being absorbed in a boric acid solution. Finally, ethereal extract (EE%) was determined using Soxhlet extraction columns manufactured by Gerhardt, Germany. An organic solvent (diethyl ether) and anhydrous sodium sulfate catalyst were used to carry out this analysis.

Data analysis

The results of all measurements were expressed as means ± standard deviations, and calculations were carried out using Microsoft Excel software, version 2007. Data analysis was carried out using the same software, which performed a one-way analysis of variance (ANOVA 1) using Student's t-test. The significance level was set at $p < 0.05$, $p < 0.01$, and $p < 0.001$.

RESULTS

Ambient parameters

The ambient parameters revealed that the control group was reared under lower temperatures and higher humidity than those recorded in the experimental group, particularly during the grower and finisher phases (Table 2). Indeed, the ambient temperatures were established at 32.95 ± 2.47, 21.14±2.98, and 19.67 ± 1.81°C, respectively, for the three breeding phases in the control group. Furthermore, the temperatures were higher in the experimental group, where they were established, respectively, for the three growth phases at 35.93 ± 1.77, 33.93±2.63, and 33.44±1.76°C. The relative humidity was, on average, 64.01 ± 2.56, 67.19 ± 4.83, and 69.44 ± 4.75% during the three rearing phases in the control group. This atmospheric component was much lower in the experimental group, where the averages of 57.21 ± 3.73, 59.57 ± 7.09, and 56.42 ± 2.62% were noted.

Table 2. Ambient parameters during the study in Ain Defla, Algeria at summer 2023

	Temperature (°C)		Relative humidity (%)	
	Group C	Group E	Group C	Group E
Starter	32.95±2.47	35.93±1.77	64.01±2.56	57.21±3.73
Grower	21.14±2.98	33.93±2.63	67.19±4.83	59.57±7.09
Finisher	19.67±1.81	33.44±1.76	69.44±4.75	56.42±2.62

C: Control; E: Experimental.

Temperature humidity indexes

The temperature humidity indexes were higher in the experimental group than in the control group, regardless of the breeding phase (Table 3). Indeed, in the experimental group, these values were 33.07, 31.48, and 30.87 for the starter, grower, and finisher phases, respectively. On the other hand, they were established at 30.88, 20.45, and 19.17, respectively, for the same breeding phases in the control group.

Table 3. Temperature humidity indexes during the study in Ain Defla, Algeria at summer 2023

	Temperature humidity indexes	
	Group C	Group E
Starter	30.88	33.07
Grower	20.45	31.48
Finisher	19.17	30.87

C: Control; E: Experimental.

Carcass yields

The evolution of carcass yields revealed notable reductions in the experimental group compared to the control group (Table 4) at different sampling ages. Regarding the eviscerated carcass, a significant decrease was observed in the starter phase on the 15th day of age (-2.31%; $p < 0.01$). In the growth phase, the reduction was significantly minimal (-0.89%) on the 30th day. In contrast, a significant ($p < 0.001$) decrease was observed in the finisher phase on the 45th day, reaching -6.12%. Regarding the yields of thighs and drumsticks, during the starter phase, a non-significant decrease (-3.53%) was noted on the 15th day. In the grower and finisher phases, significant reductions were pointed out on the 30th day (-7.87%; $p < 0.01$) and the 45th day (-8.16%; $p < 0.001$). As for the pectoral muscles, the reduction in yield was significant (-9.39%; $p < 0.001$) from the first sampling in the starter

phase. However, this drop was less in the second sampling (-5.77%) and was not statistically significant. Furthermore, at the last sampling, a considerable decrease was observed (-9.28%; $p < 0.001$). Finally, the abdominal fat percentage revealed significant increases in broiler chickens of the experimental group compared to those of the control group, where increases of +10.81%, +12.15%; $p < 0.01$, and +21.03%; $p < 0.001$ were observed at the 15th, 30th, and 45th days of age.

Nutritional composition of the pectoral muscles

The overall trend of the results indicated that the pectoral muscles of broiler chickens in the experimental group exhibited higher dry matter and fat content, along with lower levels of mineral matter and crude proteins, compared to the control group (Table 5). Indeed, in the starter phase, dry matter showed a non-significant increase of +1.35% in terms of proportion, noted on the 15th day of age. Furthermore, in the grower and finisher phases, significant increases of +4.45% ($p < 0.05$), and +6.17% ($p < 0.01$) were observed on the 30th and 45th days. Regarding the mineral matter, in the starter and grower phases, significant decreases ($p < 0.05$) were observed on the 15th and 30th days, respectively at -10.27%, and -12.51%. The decrease was more marked in the finisher phase, reaching -13.88% on the 45th day. Similarly, crude protein levels followed a declining trend, with a significant decrease of 6.33% ($p < 0.01$) observed in the starter phase on the 15th day. The decrease was also substantial in the grower phase and stood at -7.18%; $p < 0.05$ on the 30th day, while in the finisher phase, a statistically significant decline of 8.78% ($p < 0.01$) was noted on the 45th day. Finally, the pectoral muscles contained more fat in the broiler chickens of the experimental group compared to those of the control group. Statistically significant increases ($p < 0.01$) were recorded in the starter and grower phases, reaching +6.35% and +10.48% on the 15th and 30th days of age, respectively. This increase was more significant in the finisher phase and stood at +13.23%; $p < 0.001$ on the 45th day.

Table 4. Carcass yields (%) during the study in Ain Defla, Algeria at summer 2023

	Eviscerated carcass	Thighs and drumsticks	Pectoral muscles	Abdominal fat
		D 15		
Group C	62.35±0.89^{a}	16.69±0.74^{a}	18.05±0.62^{a}	0.99±0.08^{a}
Group E	60.94±1,20^{b}	16.12±0,55^{a}	16.50±0.84^{b}	1.11±0.09^{b}
P-value	0.0081	> 0.05	0.00019	0.0043
		D 30		
Group C	65.53±1,07^{a}	18.78±0,86^{a}	19.26±1,26^{a}	1.59±0.14^{a}
Group E	64.95±0,77^{a}	17.41±0,69^{b}	18.21±1,09^{a}	1.81±0.18^{b}
P-value	>0.05	0.0010	> 0.05	0.0091
		D 45		
Group C	70.37±2.03^{a}	22.53±0.57^{a}	23.20±0.90^{a}	1.99±0.11^{a}
Group E	66.31±1.70^{b}	20.83±0.95^{b}	21.23±1.17^{b}	2.52±0.33^{b}
P-value	0.00013	0.00012	0.00052	0.00017

^{a,b} Means with different superscript letters in the same column represent significant differences at $p < 0.05$; C: Control; D: Day; E: Experimental.

Table 5. Nutritional quality of pectoral muscles during the study in Ain Defla, Algeria at summer 2023

	DM (%)	MM (%DM)	PB (%DM)	EE (%DM)
		D 15		
Group C	24.74±1.46^{a}	8.05±0,65^{a}	18.13±0.74^{a}	6.93±0.38^{a}
Group E	25.08±1.72^{a}	7.30±0.71^{b}	17.05±0.80^{b}	7.40±0.67^{b}
P-value	>0.05	0.024	0.0058	0.0091
		D 30		
Group C	26.85±1.27^{a}	7.73±0.56^{a}	19.41±1.21^{a}	9.05±0.78^{a}
Group E	28.10±0,71^{b}	6.87±0.86^{b}	18.11±1.13^{b}	10.11±0.66^{b}
P-value	0.014	0.016	0.022	0.0042
		D 45		
Group C	27.22±1.14^{a}	8.53±0.52^{a}	18.45±0.97^{a}	10.10±0.64^{a}
Group E	29.01±1.19^{b}	7.49±0.46^{b}	16.96±1.14^{b}	11.64±0.93^{b}
P-value	0.0029	0.00017	0.0055	0.00044

^{a,b} Means with different superscript letters in the same column represent significant differences at $p < 0.05$; C: Control; CP: Crude Proteins; D: Day; DM: Dry Matter; E: Experimental; EE: Ethereal Extract; MM: Mineral Matter.

Nutritional composition of thigh and drumstick muscles

The evolution of the nutritional composition of the thigh and drumstick muscles showed similarities with that of the pectoral muscles, where the broiler chickens in the experimental group contained more dry and fatty matter and less protein and minerals compared to those in the control group (Table 6). During the starter, grower, and finisher phases, dry matter content demonstrated statistically significant increases, recorded at +5.66% ($p < 0.05$) on the 15th day, +6.54% ($p < 0.01$) on the 30th day, and +6.10% ($p < 0.001$) on the 45th day of age. Mineral matter revealed a significant decrease (-7.77%; $p < 0.05$) in the starter phase on the 15th day. This was -9.84% in the grower phase, statistically insignificant on the 30th day. On the other hand, the decrease was significant in the finisher phase and revealed an amplitude of -12.28% ($p < 0.01$) on the 45th day. Crude protein levels exhibited notable decreases across all growth phases, with reductions of -11.69% ($p < 0.001$) on the 15th day, -8.54% ($p < 0.05$) on the 30th day, and -12.50% ($p < 0.001$) on the 45th day. The fat level was higher in broiler chickens in the experimental group than those in the control group. Non-significant increases were noted during the starter and grower phases, recorded at +10.05% and +12.87% on the 15th and 30th days of age, respectively. Furthermore, a significantly greater increase was observed in the finisher phase on the 45th day, when it reached +14.39%; $p < 0.001$.

Table 6. Nutritional quality of the thigh and drumstick muscles during the study in Ain Defla, Algeria at summer 2023

	DM (%)	MM (%DM)	PB (%DM)	EE (%DM)
		D15		
Group C	26.14±1.45^{a}	4.84±0.60^{a}	10.96±0.73^{a}	17.57±0.67^{a}
Group E	27.71±1.60^{b}	4.49±0.76^{b}	9.81±0.45^{b}	19.53±0.61^{a}
P-value	0.034	0.028	0.00053	>0.05
		D 30		
Group C	27.91±1.30^{a}	5.70±0.78^{a}	13.46±0.97^{a}	18.42±0.70^{a}
Group E	29.86±1.28^{b}	5.19±0.38^{a}	12.41±0.84^{b}	21.14±0.84^{a}
P-value	0.0033	> 0.05	0.017	> 0.05
		D45		
Group C	29.01±0.78^{a}	6.33±0,54^{a}	13.84±0.76^{a}	19.86±1.28^{a}
Group E	30.89±1.10^{b}	5.64±0.39^{b}	12.30±0.85^{b}	23.20±2.03^{b}
P-value	0.00033	0.0040	0.00048	0.00036

^{a,b} Means with different superscript letters in the same column represent significant differences at $p < 0.05$; C: Control; CP: Crude Proteins; D: Day; DM: Dry Matter; E: Experimental; EE: Ethereal Extract; MM: Mineral Matter.

DISCUSSION

Ambient conditions and temperature humidity indexes

This study aimed to investigate the potential impacts of climate change on broiler farming, as reflected through the Temperature Humidity Index (THI), and to assess its consequences on both quantitative and qualitative aspects of production. The experimental conditions exposed the broiler chickens in the experimental group to thermal stress. The breeding guide for the Cobb 500 (2008) recommends a reception temperature for broilers of 33°C. This temperature gradually lowers by 2 to 3°C every three days and eventually maintains between 18 and 20°C from the fourth week of age until marketing. In addition, the increase in temperature has also led to a drying of the atmosphere, as evidenced by the relative humidity values observed (less than 60% for the three breeding phases). In contrast, the same breeding guide suggests that the relative humidity values should be approximately 70%. Furthermore, the measurement of THI revealed that it exceeded 30 in the experimental group, regardless of the breeding phase considered. These findings confirm the presence of thermal stress, as classified by Duduyemi and Oseni (2012), who categorized THI into three levels, including values below 26 as the limit zone of comfort, values between 26 and 29 as the heat stress zone, and values exceeding 29 as the severe heat stress zone. Additionally, Kang et al. (2020) emphasized the applicability of the THI as a tool for assessing the effects of heat stress in poultry farming.

Carcass yields

The environmental conditions of the experiment, expressed through the Temperature Humidity Index, strongly impacted different yields. This was reflected in a reduction in carcass yield and major cuts, and an increase in the proportion of abdominal fat. Similar trends have been reported in previous studies, which have reported that heat stress situations induce a reduction in the yield of

eviscerated carcasses (Al-Sultan et al., 2019; Liu et al., 2019; Moustafa et al., 2021), in thighs and drumsticks (Shao et al., 2019; Moustafa et al., 2021), as well as in chest muscles (Zeferino et al., 2016; Cramer et al., 2018; Omran et al., 2020). In addition, an increase in the proportion of abdominal fat under heat stress conditions has been documented by Habibian et al. (2016), Zeferino et al. (2016), and Al-Sultan et al. (2019). To explain these findings, Zhang et al. (2012) suggested that reduced carcass yields could be associated with undesirable meat attributes, particularly in fast-growing poultry. Furthermore, Song et al. (2018) and Zaboli et al. (2019) suggested that the increase in abdominal fat could be linked to hormonal imbalances, particularly hypercorticosteronemia, which would slow down the protein synthesis process. Zhang et al. (2012) further postulated that excessive fat accumulation results from a decline in basal metabolism and physical activity, which would follow hypothyroidism. In addition, Lu et al. (2007) deduced that the impact of heat stress would be directly linked to ambient temperature rather than solely by a reduction in feed intake. Finally, Zhang et al. (2012) and Bayu et al. (2016) reported that fat accumulation leads to the degradation of carcass appearance and yields, which would have significant consequences on production and economic profits (Zhang et al., 2012).

Nutritional composition of muscles

The results demonstrated that broiler chickens in the experimental group experienced a deterioration in nutritional quality, which resulted in an increase in the dry and fat matter and a decrease in mineral matter and crude proteins, irrespective of the muscle type considered. These findings align with previous studies that reported similar deteriorations in carcass nutritional quality when rearing temperature exceeded the required standards. Indeed, under these stressful conditions, Attia and Hassan (2017) noted an increase in dry matter and a decrease in muscle mineral matter. For their part, Shao et al. (2019) observed a reduction in crude protein levels. Furthermore, Zhang et al. (2012) and De Antonio et al. (2017) noted an increase in the proportion of muscle fat. The deterioration in nutritional quality observed under thermal stress would result from the stimulation of the hypothalamic-pituitary-adrenal axis, which increased serum corticosterone concentration (Sapolsky et al., 2000). High levels of this hormone impact lipid metabolism by inhibiting the action of hormone-sensitive lipase, thus promoting hepatic lipogenesis (Vasilatos-Younken, 1995; Hausman et al., 2012), which leads to the storage of fats in adipocytes and the reduced release of free fatty acids into the bloodstream, causing fat accumulation in the abdomen, neck, and thighs (Cai et al., 2009; Wang et al., 2012 a; b). In addition, hypercorticosteronemia stimulated insulin production, a potent lipoprotein lipase activator, thus promoting muscle protein catabolism (Scanes, 2016). Collectively, the findings of this study suggested that thermal stress, driven by climate change and expressed through the Temperature Humidity Index, could profoundly affect protein and lipid metabolism, thereby deteriorating carcass yields and degrading nutritional quality.

CONCLUSION

Climate change, as reflected by the Temperature Humidity Index (THI), has had a direct impact on broiler farming, leading to declines in both production yields and carcass nutritional quality. Ultimately, this could negatively impact the entire realm of production and, consequently, the availability of products, the sustainability of the activity, and food security, given the role poultry products play in feeding populations. The present results underscored the importance of further research into the impact of heat stress in poultry farming in general and in broiler farming in particular. This would make it possible to conceptualize strategies to combat the harmful effects of heat stress, ensuring a balance between production efficiency and the long-term sustainability of the industry.

DECLARATIONS

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Availability of data and materials

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

Competing interests

The authors declare no conflicts of interest.

Authors' contributions

Abdelhak Karim Mouss played a key role in designing the study, conducting the experiment, collecting samples, measuring yields, performing laboratory

analyses, carrying out statistical analyses, drafting the manuscript, and making revisions. Dalila Hammouche participated in the study's design, followed the statistical analysis, supervised the analyses in the laboratory, and contributed to manuscript revisions. Rahla Meziane assisted in drafting the manuscript. All authors read and approved the final manuscript.

Ethical considerations

All authors were screened for ethical issues, including plagiarism, consent for publication, misconduct, fabrication of data, and duplicate publication or submission.

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Effects of Dietary Supplementation of *Chestnut tannin* on Growth Performance, Carcass Traits, and Meat Cholesterol in Ulu Chickens

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ABSTRACT

Tannin from chestnuts has garnered interest in poultry nutrition due to its potential impact on meat quality. The current study investigated the effects of *Chestnut tannins* (CT) which were derived from natural chestnut wood, on poultry health and meat characteristics. The primary objective was to determine the effects of CT supplementation in commercial feed on performance, carcass, and meat cholesterol in Ulu chickens. A total of 48 one-day-old Ulu chickens were divided randomly based on a completely randomized design into four treatment groups, with four replications each, and raised until 63 days of age. The treatments consisted of varying doses of CT (0.1%, 0.2%, and 0.3%) supplemented with a commercial diet. The parameters measured were performance, carcass traits, and meat cholesterol. The results showed that the supplementation of different levels of CT did not significantly alter performance, carcass traits, and meat cholesterol in ulu chickens. However, correlation and trend analysis indicated that the 0.3% CT treatment yielded the best growth performance, with a body weight gain of 934.85 g and a feed conversion ratio of 2.53, respectively. Conversely, the best treatment for reducing meat cholesterol was 0.2% of CT. It can be concluded that while CT supplementation did not influence the performance and carcass characteristics, it was effective in reducing meat cholesterol levels in Ulu chickens.

Keywords: Carcass, Cholesterol, Performance, *Chestnut tannins*, Ulu chicken

INTRODUCTION

Recently, the demand for indigenous chicken meat has grown alongside the increasing global population. Previous reports have highlighted that indigenous chicken meat is a valuable source of nutrients, including proteins, energy, and fat. Saidin (2010) reported differences in cholesterol content in broiler and indigenous chickens, noting that while broiler chicken meat contains approximately 100 mg of cholesterol per gram, indigenous chicken meat has a slightly higher cholesterol content of 116 mg/gram. Normally, cholesterol plays an important role in cell maintenance, but abnormal cholesterol levels in the blood can have adverse health effects. Mozaffarian et al., (2016) reported that one of the factors that may contribute to cardiovascular disease is the high level of low-density lipoprotein (LDL).

In the past, antibiotics were widely used to enhance chicken growth performance and health. However, that condition has been stopped since 2006 when the European Union banned antibiotics in feed diets due to concerns about antibiotic residues in poultry meat and the risk of bacterial resistance (Tian et al., 2021). This shift has heightened consumer awareness of meat quality and spurred interest in products with lower fat and cholesterol

content to meet health-conscious demands. Some efforts have been conducted to achieve these targets, particularly to ensure optimal chicken performance and manage subclinical diseases (Rafiq et al., 2022). Stamler et al. (1986) previously established a clear link between high concentrations of cholesterol and the risk of cardiovascular disease. Due to such challenges, a group of potential antibiotics for promoting growth can be plant-derived compounds such as organic acids, herbs, and essential oils. Among these, tannins stand out as phytochemicals with strong antimicrobial properties. These natural compounds have shown potential not only in promoting growth but also as viable substitutes for antibiotics in poultry (Farha et al., 2020).

The use of tannins as feed supplements has gained increasing attention as an alternative to antibiotics for promoting growth in poultry. Graziani et al. (2006) stated that hydrolyzable tannins may have the potential to substitute antibiotics. In addition, numerous studies have demonstrated the efficacy of natural chestnut extracts in reducing yolk cholesterol concentrations in quail eggs (Erwan et al., 2023). For instance, Buyse et al. (2021) revealed that meat quality, intestinal growth, and increased antioxidant status in broiler chickens could be stimulated by tannin supplementation.

Tannin from chestnuts has garnered interest in poultry nutrition due to its potential impact on meat quality. Tannins can reduce ABCA1 gene expression, which may minimize the risk of cardiovascular disease (Melo et al., 2023). This gene is related to the formation of high-density lipoprotein (HDL), thereby promoting blood pressure and reducing total cholesterol (Wang et al., 2008). Additionally, incorporating *Chestnut tannins* into poultry diets may enhance meat quality through their antioxidant properties, which can contribute to the improved colour, flavour, and overall nutritional value of chicken meat. Schiavone et al. (2008) reported that the supplementation of chestnut wood extract did not significantly affect apparent digestibility but exhibited quadratic or cubic effects on growth performance with increasing tannin levels in broiler chickens. Furthermore, Orzuna-Orzuna et al. (2021) reported that the inclusion of tannin in diets improved growth performance, carcass yield, and meat oxidative stability in sheep. Similarly, *Chestnut tannins* supplementation has been shown to reduce yolk cholesterol in Japanese quails (Erwan et al., 2023). Understanding the mechanisms through which tannins affect meat quality is important for optimizing poultry production and feed efficiency (Buyse et al., 2021). Tannins exhibit multiple beneficial functions, such as antioxidative properties, metal ion scavenging, and immune system stimulation (Fraga et al., 2010).

The present study aims to determine whether CT supplementation can reduce meat cholesterol levels in Ulu chickens. To advance experiments on CT, the effects of its supplementation on growth performance and carcass characteristics were also examined.

MATERIAL AND METHODS

Ethical approval

This study was approved by the Ethical Clearance Committee of the Faculty of Animal Sciences, Jambi University, Indonesia (Approval Number: 06UN21.7/ECC/2023).

Experimental design

A total of 48 one-day-old (DOC) Ulu chicks were randomly selected based on a completely randomized design with four treatments. Each treatment group contained three chickens per pen, and each treatment was replicated four times. These cages were maintained at a constant temperature of 30 ± 1°C and continuous lighting. All Ulu chickens were raised in groups from doc until 63 days of age at the Poultry Division Field Laboratory, Faculty of Agriculture and Animal Science, State Islamic University of Sultan Syarif Kasim Riau, Indonesia. Throughout the experiment, feed (Charoen Pokphand Ltd, Indonesia) and water were provided with *ad libitum* access. The treatments consisted of dietary supplementation with varying levels of CT, derived from chestnut wood extract, mixed into a commercial feed. The treatment groups were as follows 0% CT (control), 0.1% CT (low), 0.2% CT (medium), and 0.3% CT (high). The CT was a commercial feed supplement obtained from chestnut wood (Erwan et al., 2023). The chemical analysis of the commercial feed is presented in Table 1.

Table 1. The chemical analysis of commercial feed provided for Ulu chicken

Nutrient	Starter period[1]	Finisher period[2]
Crude protein (%)	23.50	19.00
Crude fiber (%)	1.88	6.00
Crude fat (%)	5.87	5.00
Ca (%)	0.29	0.80
P (%)	0.15	0.45
ME (Kcal/kg)	3,050	2,910

Ca: Calcium, P: Phosphor, ME: Metabolizable Energy, [1]CP511 and [2]CP512 Produce by PT. Charoen Pokphand, Indonesia.

Performance parameters measurement

Feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) were recorded weekly throughout the experimental period. The FCR was calculated by dividing the total feed intake by the BWG of chickens during the experiment.

Meat cholesterol measurement

Meat cholesterol concentrations were analyzed using an enzymatic color method with the following steps 1) One milliliter of cholesterol reagent kit was pipetted into a test tube, and 0.01 ml of the extracted sample was added, 2) The mixture was incubated for 20 minutes until the solution turned red, 3) A blank was prepared by pipetting 1 ml of the cholesterol reagent kit into a separate test tube. Blanks were prepared for each series of analyses, 4) The blank was placed into the spectrophotometer cell (Clinicon Autoanalyzer), and the reading was adjusted to zero at a wavelength of 500 nm before inserting the sample for measurement. Cholesterol levels were calculated by the numbers displayed on the spectrophotometer monitor. Carcass weight, breasts, whole thighs, wings, and abdominal fat were measured following the methodology described by Erwan et al. (2009).

Statistical analysis

All data were analyzed using a one-way analysis of variance (ANOVA), followed by the Tukey-Kramer test as a post-hoc test. Regression equations were fitted for data relating to the level of CT and performance, carcass characteristics, and meat cholesterol concentration. Significant differences were implied by $p < 0.05$. Results were presented as means ± standard error mean (S.E.M). All statistical analyses were performed using the commercially available package StatView (Version 5, SAS package, 1998). Prior to analysis, the Thompson rejection test was applied to all data to remove outliers ($p < 0.05$), with the remaining data used for analysis.

RESULTS

Effects of supplementing different levels of *Chestnut tannins* in commercial feed on performance in Ulu chickens

Table 2 presented the changes in growth performance after CT supplementation. There were no significant effects of CT supplementation on growth performance including FI, BWG, and FCR in Ulu chickens. However, FI and BWG exhibited a tendency to increase as the levels of CT in the diets increased. The correlation between CT and body weight is shown in Figure 1.

At a supplementation level of 0.3%, as shown in Figure 2, there was a significant negative correlation between CT levels and FCR ($p < 0.05$).

Effects of supplementing different levels of *Chestnut tannins* in commercial feed on carcass traits of Ulu chickens

Table 3 shows the effects of different levels of CT supplementation on carcass and carcass cutting. The findings indicated no significant effect on this parameter among all treatments ($p > 0.05$). The weight of the carcass, breasts, whole thighs, and wings were not altered by CT supplementation. However, as shown in Figures 3, and 4 a significant positive correlation was found between the levels of CT and carcass weight, breasts, and whole thighs ($p < 0.05$).

Effects of supplementing different levels of *Chestnut tannins* in commercial feed on abdominal fat and meat cholesterol of Ulu chicken

Figure 5 shows the effect of the supplementation of CT in commercial feed on the abdominal fat of Ulu chicken. While no statistically significant differences in abdominal fat were detected among the treatment groups, a decreasing trend was observed with increased CT supplementation. As seen in Figure 6, CT supplementation tended to reduce the cholesterol levels in Ulu chicken meat to low and medium levels compared to other groups ($P > 0.3$) though this reduction was not statistically significant ($P > 0.3$).

Table 2. The effect of supplementation of *Chestnut tannins* at different levels on the performance of Ulu chicken

Parameters	Treatments				P-value
	0	0.1	0.2	0.3	
Final body weight (g)	1,059±56.6	1,113±49.6	1,060±17.9	1,171±12.7	0.2
Average gain (g)	821±53.2	874±45.8	830±19.9	935±7.1	0.2
Feed intake (g)	2,305±76.8	2,233±9.4	2,234±51.9	2,362±29.4	0.2
FCR	2.84±0.2	2.58±0.1	2.73±0.1	2.53±0.0	0.3

FCR: Feed conversion ratio

Table 3. The effect of different supplementation levels of *Chestnut tannins* in commercial feed on carcass characteristics in Ulu chicken

Parameters	Treatments				P-value
	0	0.1	0.2	0.3	
Carcass (g)	570±22.9	625±55.9	618±24.8	692±30.6	0.2
Breasts (g)	160±9.4	169±10.4	174±4.4	185±7.0	0.2
Whole thighs (g)	212±14.9	222±9.6	209±6.9	245±2.1	0.1
Wings (g)	90±5.1	96±3.4	93±1.8	101±3.5	0.2

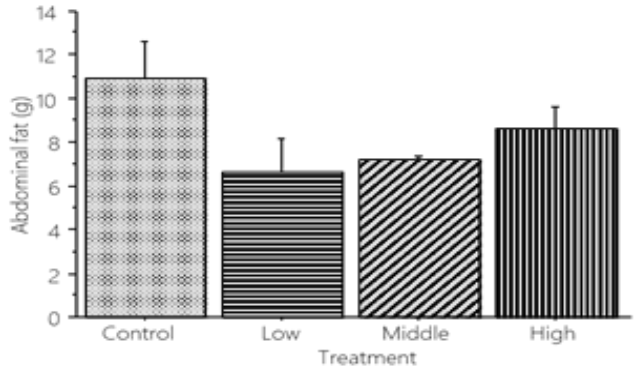


Figure 1. The correlation between *Chestnut tannins* and body weight

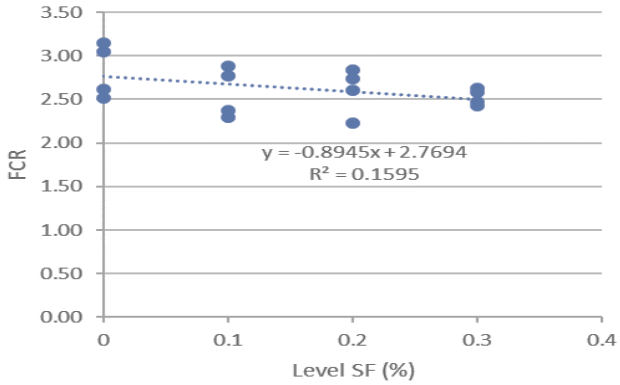


Figure 2 The correlation between *Chestnut tannins* and feed conversion ratio

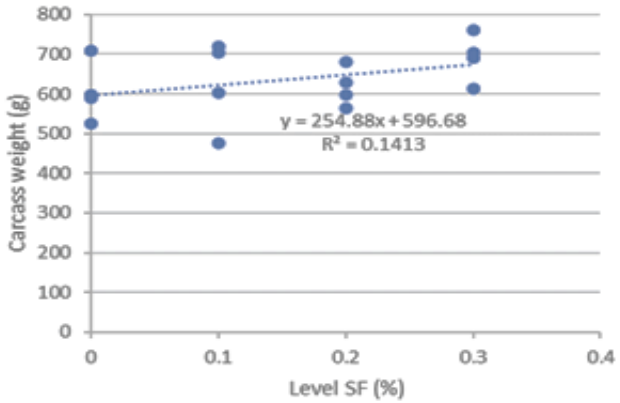
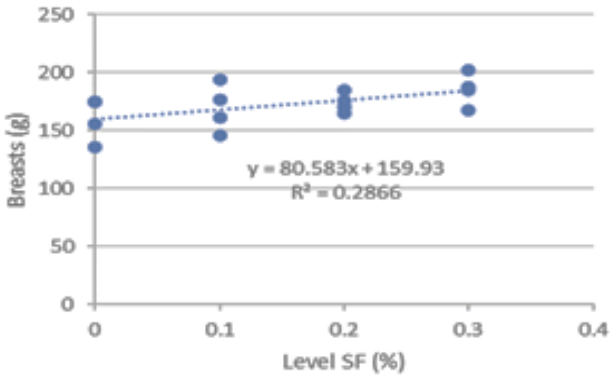


Figure 3 The correlation between *Chestnut tannins* and carcass weight



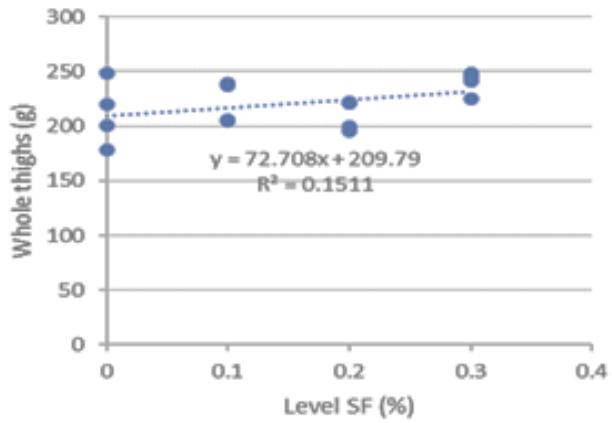


Figure 4 The correlation between *Chestnut tannins* and whole thighs

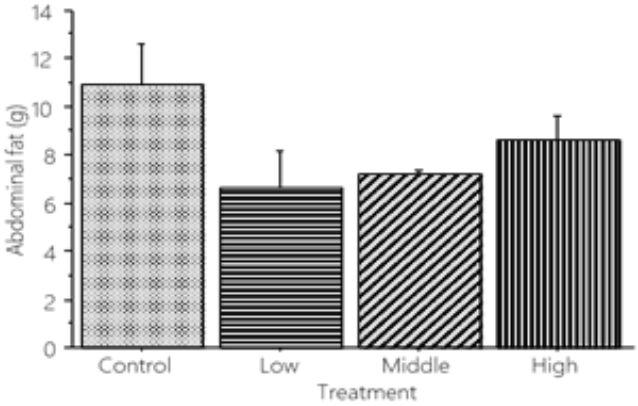


Figure 5. The effect of *Chestnut tannins* on abdominal fat

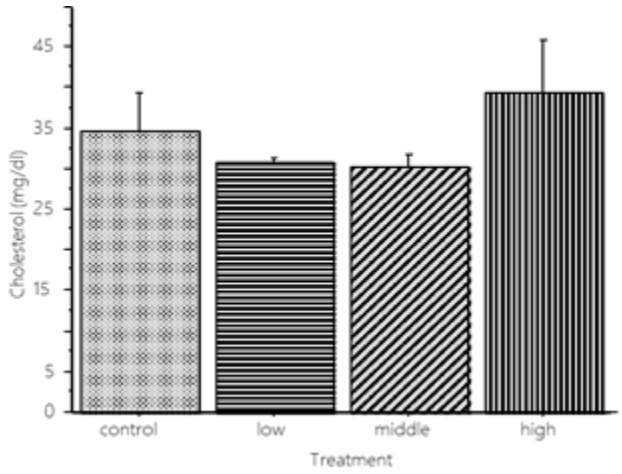


Figure 6. The effect between *Chestnut tannins* on meat cholesterol

DISCUSSION

The present study confirmed that supplementation of CT in commercial feed did not significantly impact the performance of Ulu chickens. However, a trend was observed where body weight at 63 days of age increased with higher levels of CT supplementation. These findings suggest that CT can support the growth of Ulu chickens. The findings were inconsistent with a previous report (Cengiz et al., 2017) which indicated that tannin supplementation from barley (2g/kg or 0.2% feed) decreased body weight, feed intake, and FCR while it can be considered as an alternative strategy to address foot dermatitis in broiler chickens.

Tannins in feed can impart a bitter, astringent taste, which may alter the poultry's perception of the feed. Chickens tend to avoid feed with a bitter or astringent taste, which can reduce their feed intake. The mechanism involved tannins binding to proteins in the chicken's saliva, which contains proline and hydrophobic groups, forming complex bonds that cause the feed to become astringent. Houshmand et al. (2015) noted that the primary adverse effects of tannins in monogastric animals were related to their protein-binding capacity, which reduces protein, starch, and overall digestibility. This ultimately leads to reduced palatability and decreased feed consumption in chickens. However, when administered in appropriate amounts, tannins have been reported to enhance feed intake in some studies.

In another study, hydrolyzed tannins were used as feed additives for broiler chickens. Tonda et al. (2018) evaluated the effects of hydrolyzed tannins at 0.5 g/kg of feed, both alone and in combination with *Bacillus coagulans*. Their study assessed the impact of hydrolyzed tannins on the performance and intestinal health of broiler chickens. Ethydrolyzed tannins could reduce intestinal lesion scores, the number of oocysts in feces, and the feed conversion ratio in broiler chickens challenged with *Eimeria* spp. Tannins from proanthocyanidin extract of grape seeds significantly reduced mortality and increase weight gain in broiler chickens after infection with *E. tenella* (Wang et al., 2008).

Investigation into the effects of tannins on carcass and meat quality in chickens revealed crucial findings. Perić et al. (2022) reported that the supplementation of *Chestnut tannin* extract in linseed oil-enriched diets improved intestinal morphology in broiler chickens. Additionally, the inclusion of sorghum with varying tannin levels in broiler diets resulted in inconsistent trends in performance and carcass characteristics, indicating that tannin levels may not be the sole factor affecting nutritional quality (Milton et al., 2023). Furthermore, substituting maize with low tannin sorghum in broiler diets was found to enhance the nutritional value of chicken meat, leading to lower cholesterol content and improved vitamin E levels in the thigh meat of chickens (Ochieng et al., 2020). However, a meta-analysis by Hidayat et al. (2021) cautioned that high dietary tannin levels might negatively affect amino acid digestibility, broiler performance, and lymphoid organs.

Chestnut tannins supplementation showed potential in reducing the cholesterol levels in Ulu chicken meat to a medium level. This result was in line with the findings of Starčević et al. (2015). Tannins, such as those from *Galla chinensis* and *quebracho* extracts, were significantly

effective in reducing total cholesterol concentrations in both the serum and liver of broiler chickens (Perin et al., 2019; Ren et al., 2023). Additionally, the inclusion of low-tannin sorghum in broiler diets has shown a hypocholesterolemic effect, leading to decreased cholesterol levels in the liver (Starčević et al., 2015). These findings suggested that dietary supplementation of tannins can effectively modulate cholesterol content in chicken meat, highlighting the potential of tannins as a natural approach to improve the nutritional quality of poultry products.

CONCLUSION

The optimal performance of Ulu chickens aged 63 days was observed with supplementation of 0.3% chestnut CT, resulting in a body weight gain of 934.85 g and an FCR of 2.53. The CT supplementation at 0.3% in commercial feed did not cause differences in the carcass characteristics, whereas supplementation levels of 0.1 and 0.2% tended to reduce cholesterol content in Ulu chicken meat. Further studies were recommended to determine the effect of CT supplementation on growth performance and plasma metabolites in other poultry species.

DECLARATIONS

Authors' contributions

Edi Erwan, Deni Fitra, Evi Irawati, and Afriadi conducted the experiment, performed statistical analyses, and prepared the draft manuscript. Vebera Maslami, Mozhdeh Emadi, and Edi Erwan further revised the manuscript. All authors have checked and approved the final version of the paper before submission.

Competing interests

The authors declare no conflicts of interest.

Availability of data and materials

All the data and materials are available upon request from the corresponding author.

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Ethical considerations

The authors have checked the ethical issues, including plagiarism consent to publish, misconduct, double publication and/or submission, and redundancy.

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Prevalence, Gross Pathology, and Histopathology of Marek's Disease in Backyard Chickens in Northeastern Tunisia

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ABSTRACT

Marek's disease (MD) is a common worldwide lymphomatous and neuropathic disease of chickens. Infection can cause significant losses in chicken production due to high mortality and morbidity. The present study aimed to determine the prevalence of MD in backyard flocks in the Grand-Tunis region of northeastern Tunisia and to analyze clinical cases over an eight-year and three-month period, from September 2012 to December 2020. A total of 798 cases were received for necropsy examination in the avian clinic of the National School of Veterinary Medicine of Sidi Thabet, Tunisia. Among these, chicks suspected of having MD underwent clinical observation, postmortem examination, and histopathological analysis. The results showed that 61 chickens (7.64%) were suspected to have MD. Clinical and postmortem examinations revealed different forms of MD including visceral (31 cases), mixed (20 cases), and nervous forms (10 cases). Postmortem examinations showed two types of lesions including hypertrophy and lymphomatous tumors. The highest frequencies of lesions were noted in the liver (74%), spleen (62%), sciatic nerves (48%), lungs (36%), and kidneys (31%). Hypertrophy predominated in the spleen (49%), sciatic nerves (48%), liver (28%), kidneys (25%), lungs (21%), proventriculus (18%), and gonads (17%). Conversely, lymphomatous tumors were more frequently observed in the liver (46%), heart (23%), lungs (15%), and spleen (13%). Histopathological investigations revealed pleomorphic infiltrations with lymphocytes and plasmocytes in visceral organs, sciatic nerves, and the skin. High histological scores were recorded in the liver, spleen, lungs, kidneys, and heart. The current study confirmed endemic MD in backyard chicken populations in Grand-Tunis région and confirmed that it can be a serious threat to poultry health in the study area.

Keywords: Backyard chickens, Clinical examination, Histopathology, Lymphoma, Marek's disease, Postmortem examination

INTRODUCTION

The traditional poultry sector plays a vital role in rural zones of Tunisia. It provides eggs and meat, as high-quality sources of animal proteins for both local market and household consumption. According to the latest official statistics from GIPAC (2010), this sector contributes an average of 7% to the national poultry production. Backyard poultry typically consists of small flocks of different poultry species reared under traditional conditions. Productivity in these flocks is often unsatisfactory because of serious health problems, malnutrition, and poor management conditions. In addition, potential threats to productivity, such as poor genetic potential due to lack of selection and predation, as well as infectious and non-infectious diseases, should be considered. Chickens raised in free-range systems are exposed to constant risks of infection by several pathogens, such as the Marek's disease virus (MDV).

Marek's disease (MD) is a worldwide viral and highly contagious neoplastic disease in poultry. The causative agent is Gallid-Herpesvirus 2 (GaHV-2), classified as a member of the family *Herpesviridae*, subfamily *alpha-herpervirinae,* and genus *Mardivirus*. Of the three

recognized three serotypes of MDV, only serotype 1 contains viral strains capable of inducing tumors (Morrow and Fehler, 2004).

Marek's disease is diagnosed in poultry-producing countries throughout the world. However, the incidence prior to the availability of vaccines was not uniform. Economic losses caused by MDV infections were especially high in intensive systems. The virus is transmitted through direct and/or indirect contact between chickens, most commonly via the airborne route (Abdul-Careem et al., 2009a). Fully infectious virus particles are replicated in the epithelial cells in the keratinizing layer of the feather (Abdul-Careem et al., 2009b). These cells serve as a source of environmental contamination. MDV associated with feathers and dander is infectious for at least several months at 20°C to 25°C and for years at 4°C (Calnek and Witter, 1997). Chickens can act as asymptomatic carriers and transmit the virus. The resilience of the virus and the ongoing shedding by infected birds make its prevalence readily understandable. To date, vertical transmission of MDV has not been confirmed. Similarly, transmission of the virus from breeder hens to progeny through external eggshell contamination has remained uncertain due to poor virus survival under temperature and humidity conditions of the incubation process (Pohjola et al., 2015; Mete et al., 2016).

The Marek's Disease Virus infections are characterized by T-cell lymphoma of peripheral nerves, viscera, skin, and eyes. Morbidity and mortality rates range from 5% to 30% when hypervirulent strains are incriminated. MD infection can occur from 3-4 weeks of age in mature chickens; however, clinical manifestations are often described at 12-30 weeks of age. The immunosuppressed condition due to MDV is a potential cause of vaccination failure against other contagious diseases, increasing the susceptibility of chickens to infection with other pathogens (Gimeno and Schat, 2018).

Symptoms of MD include depression, stunting, lethargy, characteristic unilateral paralysis of the legs, and mortality (Calnek and Witter, 1997; Nair, 2018). Detection of the virus, viral antigens, or nucleic acids in the absence of clinical disease does not confirm the occurrence of MD, resulting from the ubiquitous character of MDV and, subsequently, the presence of the virus in many poultry farms (Nair, 2018). Clinical signs of MD associated with lymphoma formation in multiple organs as well as enlarged peripheral nerves may suffice to make a tentative diagnosis (Nair, 2018). However, confirmation of the diagnosis can be performed by immunohistochemistry, histopathology, and polymerase chain reaction (PCR) (Calnek and Witter, 1997; Nair, 2018).

In Tunisia, MD has been documented in commercial poultry flocks, with clinical forms confirmed in broiler chickens and layer hens. Furthermore, the disease has been rarely reported in commercial meat-type turkeys and broiler breeders. However, there are few special reports on MD in backyard chickens (Kaboudi et al., 2019).

The control of MD infections is based on biosecurity measures and vaccination. Currently, vaccines provide effective prevention against MD. In Tunisia, different vaccine strains are available, including herpesvirus of turkey (HVT), Rispens, and SB-1. Vaccination is only provided for *Gallus gallus* breeders and layer hen flocks and is not routinely performed in commercial broiler chickens or turkey flocks.

The present study aimed to examine the prevalence of MD in free-range chickens received at the avian clinic of the National School of Veterinary Medicine of Sidi Thabet from various regions of Tunisia. Diagnosis was based on clinical signs, postmortem examination, and histopathological analyses of different tissue samples.

MATERIALS AND METHODS

Ethical approval

The experiment was approved by the Institution of Agricultural Research and Higher Education, National School of Veterinary Medicine of SidiThabet, University of Manouba, 2020 Sidi Thabet, Tunisia. It was conducted between September 2012 and December 2020.

Study area

The present study was conducted on backyard poultry flocks located in the "Grand-Tunis" region, comprising four governorates included Ariana (36°51′45″N, 10°11′44″E), Ben Arous (36°44′50″N, 10°20′0″E), Manouba (36°48′28″N, 10°6′4″E) and Tunis (36°48′23″N, 10°10′54″E; Figure 1). These governorates are divided into 48 districts, covering an area of 2.726 km^2 and a total population of 2.731.507 inhabitants. The agricultural surface and humid zone surface range from 24.3% (Tunis) to 78.3% (Manouba) and from 0.6% (Manouba) to 18.8% (Tunis), respectively. The mean annual rainfall and temperature range from 275 to 515 mm and 15.2 to 24.9°C, respectively. The average annual humidity is approximately 70%.

Animals

The current study was carried out between September 2012 and December 2020. A total of 798 chickens (dead and live), coming from 370 flocks and aged between 1 month and 3 years old, were admitted at the avian clinic of the National School of Veterinary Medicine of Sidi Thabet, Tunisia, for postmortem examination. The average weight of chickens ranged from 800 gr to 2.5 kg. Live chickens showed lethargy, anorexia, poor growing rate, respiratory distress, diarrhea, and leg paralysis. For each case, an individual data form was completed, and epidemiological information was recorded and analyzed to facilitate diagnosis. All chickens were obtained from flocks reared under traditional conditions. None of the flocks had a history of MD vaccination.

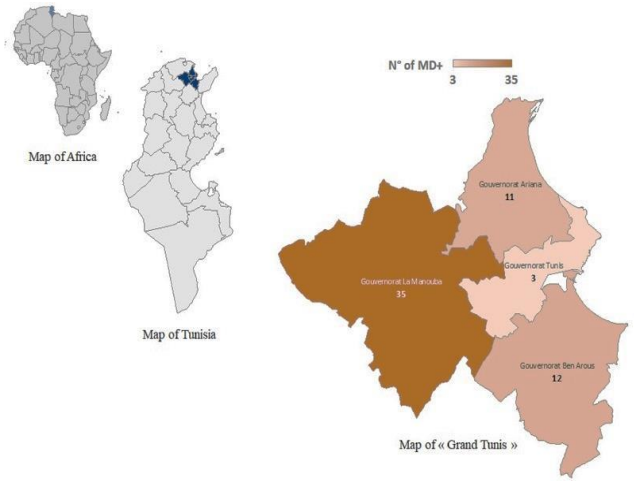

Figure 1. Tunisia and Grand-Tunis region showing the geographic location of sampled birds suspected of Marek's disease

Clinical and necropsy examinations

Live chickens were examined and their clinical signs were registered. Postmortem macroscopic examinations were conducted following standard protocols within 24 hours of death. After external examination, the general cavity was explored to remove and examine the heart, the liver, the digestive tract, lymphoid organs, the trachea, and the lung. The reproductive tract of adult hens was also removed. Finally, the kidneys, the locomotor systems, and the nervous systems were explored. All organs were closely dissected and examined for macroscopic changes relevant to MD (Schwartz and Bickford, 1986).

Tissues and organs suspected of being affected by MD were sampled for histopathological investigations. A total of 196 samples were fixed in 10% formalin solution and sent to the histology laboratory at the National School of Veterinary Medicine of Sidi Thabet, Tunisia, for microscopic examination.

Histopathology

Small tissue samples (0.5 × 0.5 cm) were collected from the liver (45 samples), kidneys (19 samples), proventriculus (12 samples), heart (14 samples), spleen (38 samples), lungs (22 samples), gonads (12 samples), pancreas (3 samples), skin (2 samples), and the lumbosacral plexus and sciatic nerves (29 samples). The samples were fixed in 10% neutral buffered formalin for 48 h. They were dehydrated in graded alcohol series, cleared in toluene, and then processed by the standard paraffin embedding technique. The slices were cut at 4 µm thick and mounted on microscope slides. They were stained with hematoxylin and eosin (HE) and finally examined under an optical microscope (Leitz, Germany) at 10x, 40x, and 100x magnifications for the detection of lesions. Histological scores designed for the present study were adopted from the methodology described by Mete et al. (2016). The severity of lesions was categorized as follows included few, mostly perivascular infiltration and/or scattered lymphocytic infiltrations (+), moderate numbers of lymphoid cells (++), and large multifocal to coalescing sheets of lymphocytes modifying the tissue architecture (+++).

Statistical analysis

Statistical analysis was performed using the SPSS software package (version 16.0, Chicago, SPSS Inc., 2007) for Windows. The Pearson chi-square test was used to evaluate the relationship between epidemiological criteria and the diagnosis of MD in the examined chickens at a threshold value of 5%.

RESULTS

Epidemiological and clinical signs

Out of 798 examined chickens, 61 (7.64%) were positive for MD. The highest MD prevalence was recorded in 2016, with 19.57% (18 cases out of 92), followed by 2015 with 13.16% (10 cases out of 76), and 2017 with 10% (9 cases out of 90). No cases of MD were diagnosed in the samples collected in 2014 (Table 1).

Marek's disease was concurrently diagnosed in all the selected governorates in Tunisia. The highest prevalence was observed in Manouba (35 cases/354; 9.89%),

followed by Ben Arous (12 cases/139; 8.63%). Conversely, the lowest prevalence rates were reported in Ariana (4.52%) and Tunis (4.84%). The disease was reported in young chickens (broilers) aged under 6 months (32 chickens; 5.18%) and adult chickens aged over 6 months (29 chickens; 16.11%; $p < 0.05$). The prevalence of MD infection was 8.84% (38 chickens) and 6.42% (23 chickens) in females and males, respectively ($p > 0.05$; Table 2). Clinical findings revealed that the visceral form of MD was predominant, with 31 cases (50.82%), followed by the mixed form with 20 cases (32.78%), and the nervous form with 10 cases (16.4%).

No specific clinical signs were noted in chickens with the visceral form (32 cases). The most commonly observed symptoms included prostration, respiratory distress, and diarrhea. In contrast, chickens with the nervous form predominantly showed leg paralysis (29 cases; Figure 2).

Necropsy findings

Lesions were observed in several viscera and tissues. The organs and tissues examined in the current study showed two types of lesions including hypertrophy (present in all samples except the heart, pancreas, and skin) and lymphomatous tumors (present in all samples except the nerves; Table 3).

The highest frequencies of lesions were noted in the liver (74%), spleen (62%), sciatic nerves (48%), lungs (36%), and kidneys (31%). Lesions were also observed, though less frequently, in the heart (23%), proventriculus (20%), and gonads (20%). Tumoral lesions in the pancreas (5%) and skin (3%) were rare.

Regarding the types of lesions, hypertrophy predominated in the spleen (49%), sciatic nerves (48%), liver (28%), kidneys (25%), lungs (21%), proventriculus (18%), and gonads (17%). In contrast, lymphomatous tumors were more frequently detected in the liver (46%), heart (23%), lungs (15%), and spleen (13%). No lymphomas were identified on the surface of the nerves.

Whereas organs with hypertrophy were pale and exhibited diffuse tumoral infiltration, viscera with nodular lymphomas displayed deformation and irregular surfaces (Figure 3).

Based on the findings, the mixed form was diagnosed in 20 chicks. The association between visceral and nervous lesions was the most common (17 cases). However, the nervous form associated with the cutaneous form was noted in one chicken. In addition, the visceral form associated with the cutaneous form was noted in another. Finally, the simultaneous evolution of the nervous, visceral, and cutaneous forms was observed in one chicken. No ocular, intestinal, mesenteric, or muscular tumoral lesions were identified in the present study.

Histopathological investigations

The histopathology of affected organs (196 samples) showed a marked cellular polymorphic lymphomatous infiltration. Tumoral lymphocytes and plasmocytes were arranged in multifocal or diffuse patterns. Pleomorphic neoplastic infiltration, characterized by cells of different sizes (small, medium, and large lymphocytes, as well as numerous lymphoblasts), was observed in different viscera and tissue samples (Figures 4 and 5). Histologic scores, based on lymphoproliferative changes, varied from mild (+) and moderate (++) to severe (+++). High histologic scores were most frequently observed in the liver, spleen, lungs, kidneys, and heart. The results regarding lesion severity are detailed in Table 4.

Figure 2. Backyard chickens suspected of Marek's disease with leg paralysis, Tunisia

Table 1. The distribution of Marek's Disease in backyard chickens of Grand-Tunis, North-Est of Tunisia during 2012-2020

Years	Total cases	N° negative MD	N° MD cases	Prevalence (%)
2012	84	83	1	1.19%
2013	95	91	4	4.21%
2014	88	88	0	0.00
2015	76	66	10	13.16%
2016	92	74	18	19.57%
2017	90	81	9	10.00%
2018	104	98	6	5.77%
2019	106	98	8	7.55%
2020	63	58	5	7.94%
Total	798	737	61	7.64%

Table 2. Prevalence of Marek's Disease in backyard chickens according to sample location, age, and sex of diseased chickens (Tunisia, 2012-2020)

		MD -	MD +	The total of examined animals	Prevalence (%)	Chi-square	p-value
Location	Ariana	232	11	243	4.52%	6.752	0.1
	Ben Arous	127	12	139	8.63%		
	Manouba	319	35	354	9.89%		
	Tunis	59	3	62	4.84%		
Age	Young	586	32	618	5.18%	23.602	0.001
	Adult	151	29	180	16.11%		
Sex	Female	402	38	440	8.84%	1.369	0.3
	Male	335	23	358	6.42%		

Table 3. Postmortem lesions types in different viscera and tissues of backyard chickens infected with Marek's Disease (Tunisia, 2012-2020)

Type of lesion	Liver	Spleen	Lung	Proventriculus	Kidney	Gonads	Heart	Skin	Pancreas	Nerve
Hypertrophy	17 (28%)	30 (49%)	13 (21%)	11 (18%)	15 (25%)	10 (17%)	0	0	0	29 (48%)
Lymphomatous tumors	28 (46%)	8 (13%)	9 (15%)	1 (2%)	4 (6%)	2 (3%)	14 (23%)	2 (3%)	3	0
Total (%)	45 (74%)	38 (62%)	22 (36%)	12 (20%)	19 (31%)	12 (20%)	14 (23%)	2 (3%)	3 (5%)	29 (48%)

Table 4. Histological score of lymphocytic infiltration in different organs and tissues (n = 61) of Marek's Disease in backyard chickens (Tunisia, 2012-2020)

Visceral organ/tissue	Mild (+)		Moderate (++)		Severe (+++)		Total	
	N°	%	N°	%	N°	%	N°	%
Liver	8	13%	17	28%	20	33%	45	74%
Spleen	4	7%	21	34%	13	21%	38	62%
Nerve	12	20%	10	16%	7	11%	29	48%
Lung	5	8%	6	10%	11	18%	22	36%
Kidney	4	7%	3	5%	12	20%	19	31%
Heart	4	7%	3	5%	7	11%	14	23%
Proventriculus	3	5%	5	8%	4	7%	12	20%
Gonad	2	3%	6	10%	4	7%	12	20%
Pancreas	0	0	2	3%	1	2%	3	5%
Skin	0	0	1	2%	1	2%	2	3%

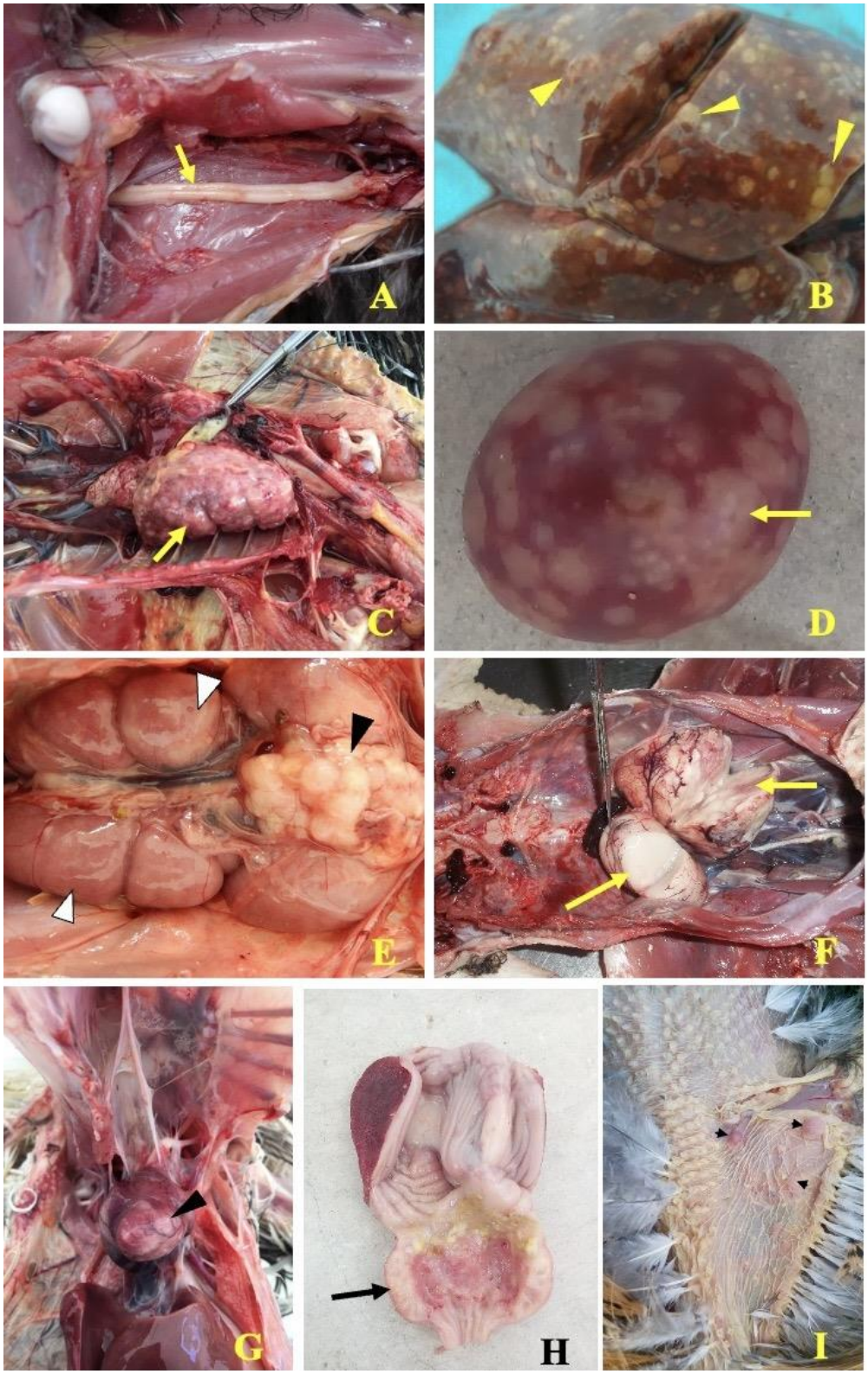

Figure 3. Gross pathology of suspected Marek's disease in backyard chickens in Tunisia during 2012-2020.
A: Sciatic nerve: hypertrophy (yellow arrow). **B:** Liver with numerous lymphomatous tumors (yellow head arrow). **C.** Lung: pale and hypertrophy caused by lymphomatous tumors (yellow arrow). **D:** Spleen: hypertrophy with lymphomatous tumors (yellow arrow). **E:** Kidneys and ovary: pale and hypertrophied (white head arrow). Ovaries appeared with lymphomatous tumors (blackhead arrow). **F:** Testis: dyssimetric and hypertrophied testicles (yellow arrows). **G:** Heart: lymphomatous tumors (blackhead arrow) **H:** Proventriculus: hypertrophied wall (black arrow). **I:** Skin: numerous lymphomatous tumors with varied sizes (blackhead arrow).

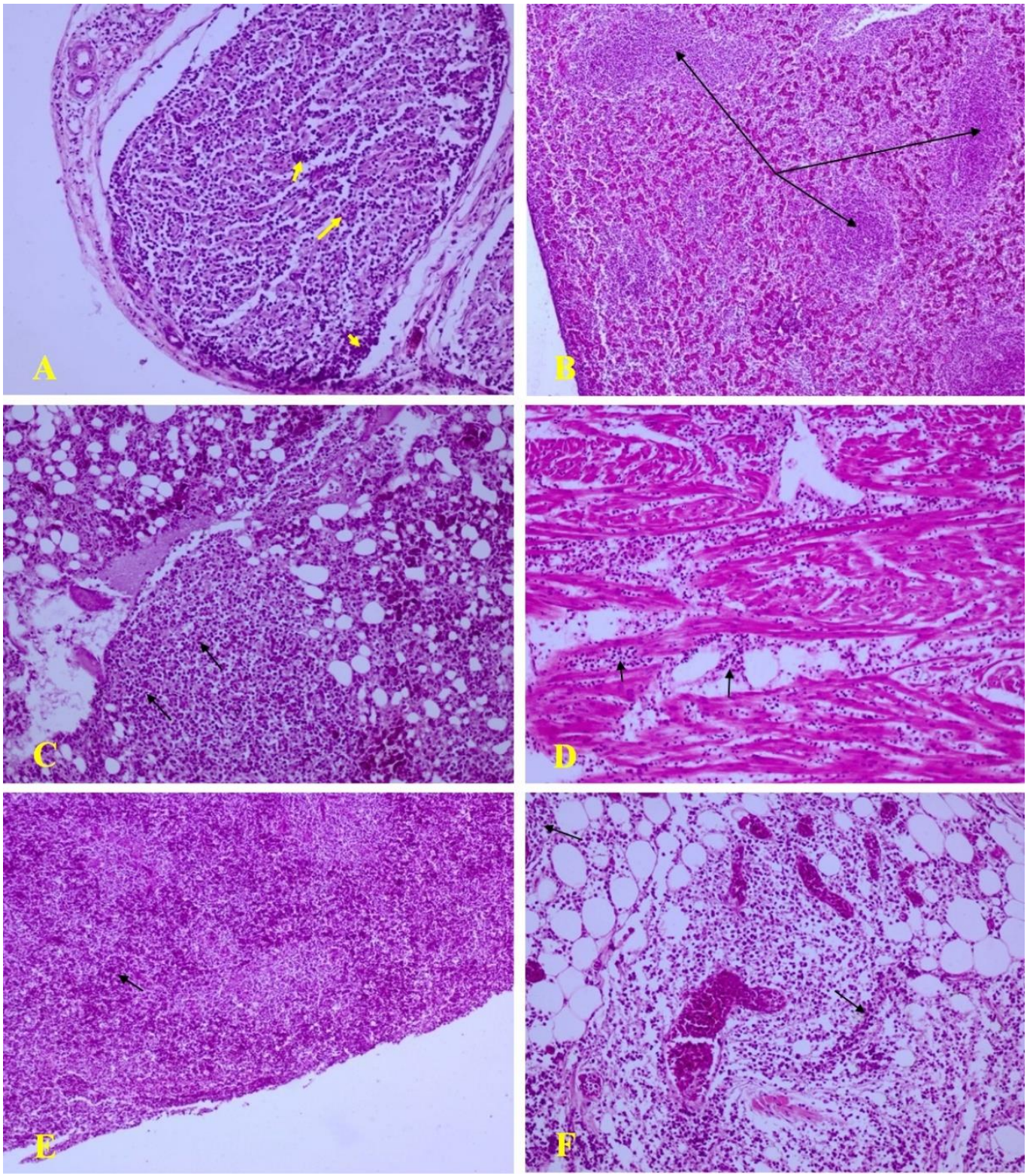

Figure 4. Microscopic lesions were consistent with Marek's disease in backyard chickens in Tunisia from 2012 to 2020.
A: Sciatic nerve: Pleomorphic lymphocytes and plasmocyte infiltrations (yellow arrows; Lesions type A; H&E x 200). **B:** Liver: pleomorphic lymphocytes and plasmocytes infirtations (black arrows; histologic score: +++; H&E x 100). **C:** Lung: pleomorphic lymphocytes and plasmocytes infirtations (black arrows; histologic score: +++; H&E x 200). **D:** Heart: Pleomorphic lymphocytes and plasmocytes infirtations (black arrows; Histologic score: ++; H&E x 200). **E:** Spleen: Pleomorphic lymphocytes and plasmocytes infirtations (black arrow; Histologic score: +++; H&E x 100). **F:** Skin: Pleomorphic lymphocytes and plasmocyte invitations (black arrows; Histologic score: ++; H&E x 200).

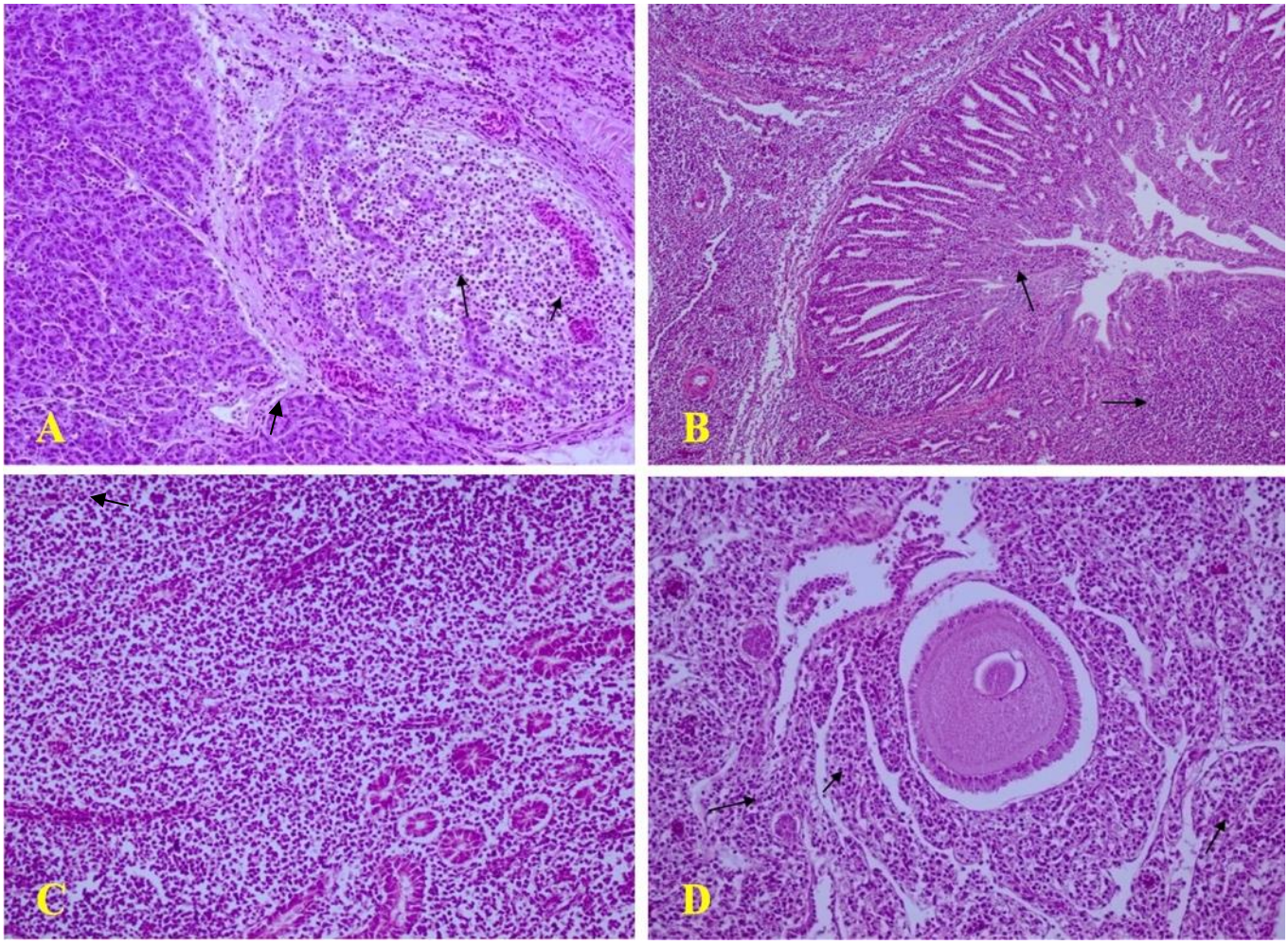

Figure 5. Microscopic lesions are consistent with Marek's disease in backyard chickens in Tunisia from 2012 to 2020. **A:** Pancreas: Pleomorphic lymphocytes and plasmocyte infiltration (black arrows; Histologic score: ++; H&E x 200). **B:** Proventriculus: Pleomorphic lymphocytes and plasmocyte infiltration (black arrows; Histologic score: ++; H&E x 40). **C:** Kidney: Pleomorphic lymphocytes and plasmocyte infiltrations (histologic score: +++; H&E x 200). **D:** Ovary: Pleomorphic lymphocytes and plasmocyte infiltration (black arrows; Histologic score: +++; HE x 200).

DISCUSSION

The positive cases of MD were diagnosed in the present study based on typical gross pathology and histopathological investigations, which remain critical for diagnosing field cases of MD (Pohjola et al., 2015; Wen et al., 2018; Brochu et al., 2019).

Out of the 798 necropsied free-range chickens, 7.64% were positive for MD. In the current study, the prevalence was lower than 13.96% reported recently by Azeem et al. (2023) in backyard chicken flocks in Pakistan. However, Sani et al. (2017) reported an even lower prevalence of 6.52% in Nigerian poultry, with Indigenous chickens showing a notably low rate of 0.43%.

Previous studies have reported MD as the most prevalent viral disease among backyard chickens in several regions. For instance, Crespo and Senties-Cue (2015) found a prevalence of 17.7% in chickens received at the Avian Health and Food Safety Laboratory in California from 2010 to 2014. Pohjola et al. (2015) reported a higher prevalence of 26.51% in Finland from 2000 to 2011, and Brochu et al. (2019) observed an 11% prevalence in submissions to the animal health laboratory at the Ontario Veterinary College, Canada, between 2015 and 2017. The variation in MD prevalence across studies can be attributed to differences in geographic location, sample sizes, biosecurity practices, chicken ecotypes, vaccination status, and the virulence of MDV strains.

Marek's Disease typically affects chickens older than 20 weeks. However, mortality due to MD lymphoma can be observed as early as 7-12 weeks (Calnek and Witter, 1997). In the present study, cases of MD were more

frequently diagnosed in adults above 24 weeks of age, a finding that contrasts with the results of Duguma et al. (2005),. who reported a higher incidence in Ethiopian local chickens aged 14- 20 weeks.

Gross pathology findings from this study showed lymphoma and/or diffuse tumoral infiltration in several visceral organs (liver, heart, spleen, kidney, gonads, and pancreas) of the chicks, as well as the involvement of sciatic nerves and skin. These observations are consistent with the findings reported in previous studies (Lobago and Woldemeskel, 2004; Haridy et al., 2019; Kaboudi et al., 2019; Birhan et al., 2023).

The predominance of the visceral form of MD (50.82%) in this study aligns with observations by Duguma et al. (2005). In general, lesions in visceral organs were observed in chickens with acute clinical signs of MD. Nair (2018) suggested that acute and /or visceral forms of the disease were correlated to the emergence of virulent viral strains of MDV. No tumor lesions were detected in the intestines, mesentery, and primary lymphoid organs, consistent with the findings of Sani et al. (2017), Ho et al. (2021), and Dwinna et al. (2023).

Paralytic symptoms, characteristic of the chronic form of MD, were associated with enlarged, pale, or grayish nerves that lacked cross-striations. These findings mirror those described by Crespo and Senties-Cue (2015). The mixed forms of MD described in this study (20 chickens) were in agreement with those reported by Duguma et al. (2005).

Multifocal nodular cutaneous lymphomas observed in this study resembled those described by Adedeji et al. (2019). Similar gross pathological lesions, particularly cutaneous and visceral forms, can be confused with avian leucosis and reticuloendotheliosis (Nair and Fadly, 2013). While skin lymphomas were common in MD cases, they were rare in avian leucosis, as compared to MD. In addition, visceral lymphomas in avian leukosis were soft, smooth, and glistening, with a grayish to creamy-white appearance (Nair and Fadly, 2013). Also comparably, the skin lesions in reticuloendotheliosis were lymphocytic infiltrates in and around feather follicles and the skin of the head and visceral lymphomas were nodular and firm. Bursal lymphomas, which were pathognomonic lesions in the diagnosis of avian leucosis and reticuloendotheliosis (Nair and Fadly, 2013), were not observed in this study.

The variation in the severity of symptoms, lesions, and mortality and morbidity rates observed in MD cases might be related to genetic resistance of chicks, age, immune status, infection pressure, and the virulence of MDV circulating strains (Nair et al., 2020). This was in agreement with the findings of Duguma et al. (2005) and Adedeji et al. (2019). Differential diagnosis between MD and lymphoid leukosis can be based on the morphology of lymphoid cells. In leucosis, the lymphocyte cells were homogeneous in shape with the constituent cells being lymphoblast, which were characterized by more cytoplasm and visible cell nuclei (Haridy et al., 2019).

The histopathological features described in different visceral organs were characteristic of MD (Calnek and Witter, 1997; Nair et al., 2020). In contrast, the predominant cells usually observed in avian lymphoid leucosis and reticuloendotheliosis are uniform, blast-like, pyroninophilic cells with B-cell markers (Nair and Fadly, 2013). The pleomorphic cells, neoplastic lymphocytes, and plasmocyte infiltrations observed in different organs and tissues in this study strongly suggest field infection with MD. The lesions in the present study were well-attached to those described in several previous reports (Lounas et al., 2021; Viet Thu et al., 2022; Azeem et al., 2023). Vieira-Pinto et al. (2003) noted that pleomorphic tumoral infiltrations revealed in nerves and viscera could serve as confirmatory evidence of MD. Pancreatic lesions, characterized by the proliferation of lymphocyte cells, were similar to those mentioned by Haridy et al. (2019). The massive proliferation of lymphocytes plays a significant role in the immune system's response in animals infected with MDV (Ali et al., 2014).

Overall, high histological lymphocytic infiltration scores were attributed to animals with gross tumors in the examined viscera. These findings are in agreement with the results of Mete et al.'s (2016) study. Severe histological lesions observed in this study might be explained by the circulation of extremely virulent MDV. Indeed, Lachheb et al. (2020) confirmed the circulation of very virulent MDV in Tunisian broiler flocks. In their study, MDV was detected directly from conserved tissue samples (liver, spleen, heart, and kidneys) using PCR, although no histological examination was performed.

CONCLUSION

The gross pathology and histological lesions observed in this study confirm that MDV has been circulating in traditional poultry flocks under study over the last decades (2012-2020). The application of rigorous biosecurity measures and regular vaccination against MD should be improved, particularly in nearby commercial poultry flocks located in the studied region, to reduce the risk of

contamination. Further molecular studies were also needed to characterize circulating strains in free-range chickens. In addition, a comparative molecular study on MDV isolated from industrial poultry flocks can be very useful to understanding the potential epidemiological role of backyard chickens in the spread and persistence of MD infection.

DECLARATIONS

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Competing interests

There are no conflicts of interest declared by the authors.

Ethical considerations

The article was written originally by authors from obtained data in current study. The content of the manuscript was checked for plagiarism before submission to the journal.

Authors contributions

KK performed the experimental protocol, interpreted statistical and post-mortem results, prepared figures and tables, and took the lead in drafting the manuscript. EM performed a necropsy examination, collected epidemiological information and samples, and drafted several sections of the manuscript. AA performed the histopathological analysis and prepared figures of histological sections. All authors read and approved the final manuscript.

Availability of data and materials

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

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Effects of Chitosan-Stearin on Quality of Chicken Egg Storage at Room Temperature

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ABSTRACT

Consumption of chicken eggs has perishable properties, the quality of eggs declines faster and the shelf life of eggs is considerably short at room temperature compared to cold temperatures. The present study aimed to evaluate the application of chitosan-stearin as a coating on the quality of chicken egg storage at room temperature. The present study used a Completely Randomized Design (CRD) 4 x 5 factorial pattern with three replications. Each replicate consisted of six fresh chicken eggs, resulting in 360 eggs. The groups included Without Coating (FD0), Virgin Coconut Oil (FD1), 1.5% Chitosan + 1% Stearin (FD2), and 3% Chitosan + 1% Stearin (FD3). The second effective variable in grouping was storage time 0 Days (ST0), 14 Days (ST14), 28 Days (ST28), 42 Days (ST42), and 56 Days (ST56). The current results indicated that the storage time and the formula dosage had a notable effect on haugh unit, yolk index, and albumen index, but no significant effect on the pH of the albumen. Formula dosage had no significant effect, but storage time had a significant effect on yolk pH and color, and weight loss. There was an interaction between formula dosage and storage time on haugh unit, albumen index, and yolk index, but there was no interaction on albumen pH, weight loss, yolk pH, and yolk color. The Chitosan-Stearin coating can maintain the quality of chicken eggs during storage for up to 56 days. The use of 3% Chitosan + 1% Stearin as a coating formula indicated the best results in maintaining the quality of chicken eggs during storage time at room temperature.

Keywords: Chicken egg, Chitosan, Coating, Room temperature, Stearin

INTRODUCTION

Purebred chicken eggs are widespread due to their high quality, easy availability, and relatively affordable prices (Abbas et al., 2024). Purebred chicken eggs are composed of 10% shell (eggshell/shell), 60% albumen, and 30% yolk (Almeida et al., 2021). The nutritional content of purebred chicken eggs is 12.9 g protein, 11.2 g fat, and 0.9 g carbohydrates per grain (BALITBANGTAN, 2021). Purebred chicken eggs have perishable properties, egg quality decreases faster at room temperature than at cold temperatures and the shelf life of the egg is typically only 10–14 days at room temperature before spoilage occurs (BSN, 2023). In addition, during the storage (room temperature) process, eggs undergo changes in their quality, namely increasing egg weight loss, albumen pH, and yolk pH and decreasing haugh unit values, albumen index, and yolk index (Sariyel et al., 2022).

In general, people handle chicken eggs using a refrigerator, although not everyone possesses this appliance. In addition, the Indonesian government program "free nutritious meal" utilizes eggs as a protein source (Desiani and Syafiq, 2025). Distribution to Eastern Indonesia requires careful consideration of the supply chain to ensure a consistent supply of quality eggs. An alternative for extending egg shelf life involves coating the eggshell to seal the pores that can control the transfer of moisture such as oxygen (O_2) and carbon dioxide (CO_2) (Rachtanapun et al., 2022). Different coating materials

have been studied, but the combination of Chitosan-Stearin has not been used (Fahmi et al., 2023).

Chitosan can be isolated from the shell of crab (*Portunus pelagicus*) which is one of the crustaceans. Crab shells have a lower mineral content than crab and shrimp shells at 22.93% and have a chitosan content of 20–30% (Nurhayati et al., 2022). Chitosan also has the properties and characteristics of biodegradable, antimicrobial, non-toxic, and a barrier to the escape of water vapor and gas in a product due to the polysaccharides of the strong chitosan layer (Picos-Corrales et al., 2023). Chitosan is the result of the deacetylation of chitin obtained by extraction (Nasution et al., 2020). Chitosan extraction is carried out at the stages of demineralization, deproteination, depigmentation, and deacetylation and chitosan can dissolve in acetic acid at a concentration of 1-2% (Mirwandhono et al., 2022). Due to the different sources and concentrations of materials employed in the extraction process, as well as the fact that chitosan is highly versatile, chitosan extraction can yield a diversity (Mirwandhono et al., 2024).

Stearin is a co-product of palm oil production, constitutes 20–30% of the oil, and has limited use as a coating material. In producing stearin through the stages of refining, bleaching, deodorizing, and cooling (Widowati et al., 2024). Stearin contains palmitic acid which acts as an antimicrobial (Sulaiman et al., 2022). Stearin is used as a coating that has a function to improve water vapor permeability, and flexibility, can cause a gloss effect, and maintain the structure and shape of the product during storage (Agusta et al., 2022).

Different studies on the manufacture and use of crustacean-based chitosan have been conducted from shrimp shells and crab shell waste in different applications, one of which was the coating effect (Ngasotter et al., 2023). However, no one has used a combination of chitosan from crab shell waste (*Portunus pelagicus*) and palm stearin applied to the fresh consumption of chicken eggs. Thus, the present study aimed to evaluate the effects of Chitosan-Stearin as a coating factor on the quality of chicken egg storage at room temperature.

MATERIALS AND METHODS

Materials

Materials used in the present study were crab shell chitin (*Portunus pelagicus*) degree of deacetylation (DD) 73.14% and palm stearin from the Palm Oil Research Center Bogor Unit (Indonesia), freshly harvested chicken eggs at 50–60 weeks of age (after peak production) with the Lohmann Brown strain and sorted with a weight of 60–65 g from Perseroan Terbatas (PT) Global Buwana Farm, Indonesia. Tools were haugh digital micrometer (PT. DIFOTEK, Indonesia), Roche yolk color fan (PT. DIFOTEK, Indonesia), pH meter (PT. DIFOTEK, Indonesia), hygrometer (PT. DIFOTEK, Indonesia), caliper, digital scale, glass table, filter paper, plastic, knife, basin, hotplate, stirrer, rubber binder, brush, egg yolk separator, and mica tray.

Method

Egg quality data were analyzed using a factorial completely randomized design (CRD) with two factors and three replications were used in the present study. Each replicate consisted of six fresh eggs, resulting in 360 eggs. The grouping included (Fahmi et al., 2023) Without Coating (FD0), Virgin Coconut Oil (FD1), 1.5% Chitosan + 1% Stearin (FD2), and 3% Chitosan + 1% Stearin (FD3). The second effective factor in grouping was the storage time which included 0 Days (ST0), 14 Days (ST14), 28 Days (ST28), 42 Days (ST42), and 56 Days (ST56).

Deacetylation of chitin

To produce the DD findings applied to the coating of chicken eggs, 50% NaOH was used to deacetylate chitin into chitosan (Yunilas et al., 2023). 50% NaOH was added to crab shell (*Portunus pelagicus*) chitin (DD 73.14%) from the Palm Oil Research Center Bogor Unit, Indonesia in a 1:10 (w/v) ratio, mixed, and heated for 6 hours at 100 ^{o}C. filtered, cleaned to a pH of neutral, and then dried for 24 hours at 60 ^{o}C in an oven. Chitosan was the name given to the resultant particles.

Coating formula

The preparation formula of the chitosan as a coating from crab shells was carried out using 2% acetic acid at 40 ^{o}C while palm stearin was melted at 60 ^{o}C (Fahmi et al., 2023). Then the chitosan solution of the crab shell was mixed with the palm stearin solution and 2% tween 80 (Sigma-Aldrich, France) was added according to the variation of the ratio (Hanani et al., 2012). Then the solution was stirred for four minutes with a magnetic stirrer.

Coating application

Fresh-consumption chicken eggs were cleaned by dry cleaning using a sponge. The coating on eggs was done by dipping technique (Revanda and Puspitarini, 2024),

namely by dipping the egg sample into the coating solution for 15 minutes, then lifted and placed on a mica tray and aerated until the coating solution stuck to fresh chicken eggs, then stored at an average room temperature of 28 ^{o}C and an average humidity of 56%. Each chicken egg required one mL of coating formula solution that has been designed by the authors.

Variables

The variables including weight loss (Hintono, 1997), haugh unit (BSN, 2023), albumen index (BSN, 2023), yolk index (BSN, 2023), albumen pH (Eke et al., 2013), yolk pH (Eke et al., 2013), and yolk color (Thohari et al., 2022) were measured.

Data analysis

Data analysis was carried out using SAS software and data were analyzed using variance analysis (Mattjik and Sumertajaya, 2013). If the obtained results were confirmed to be real or highly credible, they were further analyzed using the Duncan Multiple Range Test (DMRT) with a significant level ($p < 0.05$).

RESULTS

Chitosan characteristics

Properties of chitosan, such as the color, texture, odor, solubility, and level of deacetylation were presented in Table 1. According to the current investigation, the chitosan derived from crab shells was odorless, powder-shaped, white-light brown, and soluble in 2% acetic acid.

Table 1. Characterization of chitosan obtained from crab shells

Parameters	Results
Odor	Odorless
Color	White-light brown
Texture	Powder
Solubility in 2% acetic acid	Soluble
Degree of deacetylation (%)	79.52

Quality of coated chicken egg

Results of this research with the parameters of weight loss, haugh unit, albumen index, yolk index, albumen pH, yolk pH, and yolk color were illustrated in Table 2. Weight loss of chicken eggs in the present study ranged from 0.00 to 6.20%. The percentage of weight loss in FD1, FD2, and FD3 increased compared to FD0. The results indicated that the formula dosage had no significant effect ($p > 0.05$) while the storage time had a significant effect ($p < 0.05$) on weight loss and there was no interaction between the formula dose and storage time ($p > 0.05$).

Haugh unit of chicken eggs in the present study ranged from 13.34 to 91.61%. The percentage of haugh units of eggs in FD1, FD2, and FD3 decreased compared to FD0. The results indicated that the formula dosage and storage time had a very significant effect ($p < 0.05$) on the haugh unit of chicken eggs and there was an interaction between formula dosage and storage time ($p < 0.05$).

The albumin index of chicken eggs in the present study ranged from 0.02 to 0.10%. The percentage of albumen index in FD1, FD2, and FD3 decreased compared to FD0. The results of the variance analysis indicated that the formula dosage and storage time had a significant effect on the albumen index of chicken eggs ($p < 0.05$) and there was an interaction between formula dosage and storage time ($p < 0.05$).

The yolk index of chicken eggs in the study ranged from 0.25 to 0.40%. The percentage of yolk index in FD1, FD2, and FD3 decreased compared to FD0. Results of the variance analysis indicated that the formula dosage and storage time had a significant effect ($p < 0.05$) on the yolk index of chicken eggs and there was an interaction between the formula dosage and storage time ($p < 0.05$).

The albumen pH of chicken eggs ranged from 7.97 to 8.17. The percentage of albumen pH in the present study in FD1, FD2, and FD3 did not experience a certain increase or decrease but seemed to fluctuate compared to FD0. Results of the analysis of variance showed that the formula dosage and storage time had no significant effect ($p > 0.05$) on the pH of chicken eggs albumen and there was no interaction between the dose and length of storage ($p > 0.05$). The yolk pH of chicken eggs in the current study ranged from 6.25 to 7.11. The percentage of yolk pH in FD1, FD2, and FD3 fluctuated compared to FD0. The results showed that the formula dosage had no significant effect ($p > 0.05$) while the storage time had a very significant effect ($p < 0.05$) on the pH of egg yolks and there was no interaction between the formula dosage and storage time ($p > 0.05$). The yolk color of chicken eggs in the current study ranged from 6.11 to 7.89. The percentage of yolk color in FD1, FD2, and FD3 fluctuated compared to FD0. The results showed that the formula dosage had no significant effect ($p > 0.05$) while the storage time had a significant effect ($p < 0.05$) on the yolk color of eggs and there was no interaction between the formula dosage and storage time ($p > 0.05$). Yolk color in the study showed that egg handling with coating can prevent high yolk color loss (Figure 1).

Table 2. Quality of coated chicken egg storage at 28 ^{o}C and an average humidity of 56%

Items	Weight loss (%)	Haugh unit (%)	Albumen index (%)	Yolk index (%)	Albumen pH	Yolk pH	Yolk color
Storage Time (ST)							
ST0	0.00^{A}	91.58	0.09	0.40	8.04^{NS}	6.27^{A}	7.78^{E}
ST14	2.05^{B}	70.14	0.07	0.38	8.08^{NS}	6.85^{B}	7.44^{D}
ST28	3.55^{C}	60.94	0.06	0.34	8.10^{NS}	6.97^{CD}	7.27^{C}
ST42	4.20^{CD}	55.13	0.05	0.32	8.12^{NS}	7.04^{CD}	6.72^{B}
ST56	5.11^{DE}	47.44	0.03	0.30	8.13^{NS}	7.08^{DE}	6.27^{A}
P-value	8.40**	1.46**	3.81**	1.55**	0.62^{NS}	1.06**	4.23**
Formula Dosage (FD)							
FD0	3.49^{NS}	42.84	0.05	0.32	8.07^{NS}	6.81^{NS}	6.97^{NS}
FD1	2.88^{NS}	73.93	0.06	0.35	8.10^{NS}	6.87^{NS}	7.17^{NS}
FD2	3.07^{NS}	69.18	0.06	0.34	8.08^{NS}	6.82^{NS}	7.04^{NS}
FD3	2.49^{NS}	74.24	0.07	0.37	8.12^{NS}	6.87^{NS}	7.20^{NS}
P-value	0.14^{NS}	1.82**	1.41**	1.01**	0.82^{NS}	0.34^{NS}	0.09^{NS}
Interaction (ST x FD)							
ST0FD0	0.00^{NS}	91.58^{A}	0.10^{A}	0.40^{A}	7.97^{NS}	6.25^{NS}	7.89^{NS}
ST0FD1	0.00^{NS}	91.61^{CD}	0.09^{C}	0.39^{C}	8.07^{NS}	6.26^{NS}	7.78^{NS}
ST0FD2	0.00^{NS}	91.54^{B}	0.10^{B}	0.40^{B}	8.04^{NS}	6.29^{NS}	7.67^{NS}
ST0FD3	0.00^{NS}	91.60^{CD}	0.10^{D}	0.40^{D}	8.08^{NS}	6.28^{NS}	7.78^{NS}
ST14FD0	2.34^{NS}	49.51^{A}	0.07^{A}	0.36^{A}	8.05^{NS}	6.83^{NS}	7.22^{NS}
ST14FD1	2.05^{NS}	79.52^{CD}	0.07^{C}	0.38^{C}	8.08^{NS}	6.88^{NS}	7.55^{NS}
ST14FD2	2.11^{NS}	71.80^{B}	0.07^{B}	0.38^{B}	8.08^{NS}	6.83^{NS}	7.44^{NS}
ST14FD3	1.69^{NS}	79.73^{CD}	0.08^{D}	0.39^{D}	8.10^{NS}	6.88^{NS}	7.55^{NS}
ST28FD0	4.10^{NS}	37.09^{A}	0.04^{A}	0.32^{A}	8.11^{NS}	6.91^{NS}	7.11^{NS}
ST28FD1	3.45^{NS}	70.35^{CD}	0.06^{C}	0.35^{C}	8.11^{NS}	7.06^{NS}	7.44^{NS}
ST28FD2	3.62^{NS}	65.86^{B}	0.06^{B}	0.33^{B}	8.08^{NS}	6.89^{NS}	7.22^{NS}
ST28FD3	3.03^{NS}	70.45^{CD}	0.07^{D}	0.37^{D}	8.11^{NS}	7.02^{NS}	7.33^{NS}
ST42FD0	4.80^{NS}	22.67^{A}	0.03^{A}	0.29^{A}	8.10^{NS}	7.01^{NS}	6.55^{NS}
ST42FD1	4.10^{NS}	67.95^{CD}	0.06^{C}	0.33^{C}	8.13^{NS}	7.06^{NS}	6.78^{NS}
ST42FD2	4.14^{NS}	61.76^{B}	0.04^{B}	0.30^{B}	8.10^{NS}	7.02^{NS}	6.66^{NS}
ST42FD3	3.77^{NS}	68.17^{CD}	0.06^{D}	0.36^{D}	8.14^{NS}	7.06^{NS}	6.88^{NS}
ST56FD0	6.20^{NS}	13.34^{A}	0.02^{A}	0.25^{A}	8.12^{NS}	7.05^{NS}	6.11^{NS}
ST56FD1	4.81^{NS}	60.22^{CD}	0.05^{C}	0.33^{C}	8.13^{NS}	7.11^{NS}	6.33^{NS}
ST56FD2	5.47^{NS}	54.93^{B}	0.03^{B}	0.30^{B}	8.10^{NS}	7.07^{NS}	6.22^{NS}
ST56FD3	3.97^{NS}	61.27^{CD}	0.05^{D}	0.34^{D}	8.17^{NS}	7.10^{NS}	6.44^{NS}
P-value	0.98^{NS}	1.72**	4.15**	2.49**	0.99^{NS}	0.99^{NS}	0.96^{NS}

$^{A-E}$ and ** Means values in a column with different superscript letters indicate a significant difference ($p < 0.01$). ^{NS} Means value in a column with similar superscript letters indicates a non-significant difference ($p > 0.05$).

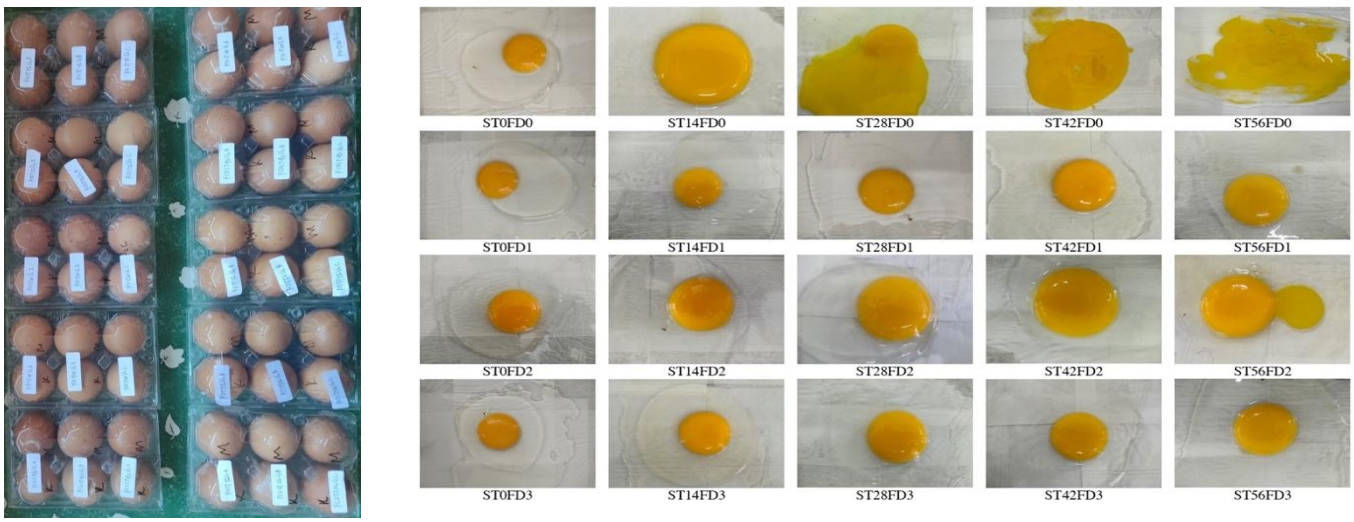

Figure 1. Chicken egg yolk shape during storage without coating (FD0), virgin coconut oil (FD1), 1.5% Chitosan + 1% Stearin (FD2), And 3% Chitosan + 1% Stearin (FD3). 0 days (ST0), 14 days (ST14), 28 days (ST28), 42 days (ST42), and 56 days (ST56)

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DISCUSSION

According to Alimi et al. (2023), the amount of chitosan produced depends significantly on the chitin source and how well the deacetylation step goes. Furthermore, by determining the acetyl group that was removed from the amide, the degree of deacetylation (DD) described the effectiveness of the deacetylation process of chitin into chitosan. Due to the extraction method, the resulting DD of this study was 79.52% (Table 1). Kahar et al. (2022), studied to obtain a DD of 79.35. According to Narudin et al. (2022), the extraction procedure or method resulted in both high and low DD.

In the present study from 0 to 56 days, samples without coating (FD0) experienced a high weight loss of 6.20%. Samples coated with virgin coconut oil (FD1) showed a weight loss of 4.81%. Samples coated with chitosan 1.5% + 1% stearin (FD2) had a weight loss of 5.47%, and samples coated with chitosan 3% + 1% stearin (FD3) experienced a weight loss of 3.97. In another current study conducted by Revanda and Puspitarini (2024), using virgin coconut oil for 45 days resulted in lower weight loss compared to the present study for 42 days. Virgin coconut oil-coated eggs for up to 42 days resulted in a weight loss of 4.10%, FD2 resulted in a weight loss of 4.14%, and FD3 resulted in a weight loss of 3.77%. Revanda and Puspitarini (2024), indicated that using virgin coconut oil with a storage period of 45 days resulted in a weight loss of 1.77%. According to Sheng et al. (2020) using chitosan with different origins, dosage, and DD for 42 days resulted in higher weight loss when compared to the present study for 42 days. Sheng et al. (2020), conducted that using crab-origin chitosan DD 90% at a dose of 2% + slightly acidic electrolyzed water and storage time for 42 days resulted in a weight loss of 5.35%.

Weight loss of eggs in the current study illustrated that the handling of eggs carried out by coating can prevent the occurrence of water vapor and coating treatment using chitosan, particularly FD2 and FD3, can prevent the transfer of water and gas to prevent the evaporation process which causes the weight loss of eggs to avoid experiencing a high decrease in eggs during storage. Because the high evaporation process happens because of the difference in water pressure inside and outside so that water vapor can come out, the low weight loss of eggs in the current investigation was caused by the low water pressure within and outside. In addition, eggs without coating experience high water pressure compared to eggs with coating. According to Rostamabadi et al. (2024), coating applications can prevent the occurrence of water vapor therefore preserving the high quality of the food products that have been coated. Aranaz et al. (2021) stated that the use of chitosan as a coating on a product such as food could act as a protective outer surface so, it would prevent the evaporation process. Luo et al. (2020) reported that during the storage of eggs, the internal water pressure exceeds the external pressure resulting in the water vapor release.

In the present study from 0 to 56 days, FD0 experienced a high decrease in haugh units of 13.34% compared to FD1 of 60.22%, FD2 of 54.93%, and FD3 of 61.27%. Sugiyono et al. (2022) studied virgin coconut oil for 42 days, resulting in a lower haugh unit of eggs than the present study. The FD1 up to 42 days resulted in haugh unit of 67.95%, FD2 up to 28 days resulted in haugh unit of 65.86%, and FD3 up to 28 days resulted in haugh unit of 70.45%. Sugiyono et al. (2022), conducted that using virgin coconut oil with a storage period of 42 days resulting in a haugh unit of 36.57%. According to a study by Zirabi et al. (2024), chitosan with different origins, dosage, and DD for 28 days resulted in a comparable egg haugh unit when compared to the present study for 28 days. Zirabi et al. (2024), indicated that using polyvinyl alcohol 5% + chitosan (DD 85%) with the concentrated use of 2% + montmorillonite 4% + garlic extract 4% with storage for 28 days resulted in haugh units of 70.00%.

As demonstrated in FD2 and FD3 treatments, handling eggs with coating can avoid a significant drop in haugh units of eggs, according to the current findings. Additionally, there was a correlation between the haugh unit and albumen height and weight. The haugh unit value increased with the albumen value. Conversely, the haugh unit value decreased as the albumen value decreased. In addition, small egg haugh units occur due to a change in the increase of water to the yolk. According to Gogo et al. (2021), eggs cannot have high quality if longer storage is carried out because eggs are easily damaged and the haugh unit of eggs can decrease with the length of storage. Ningtiyas et al. (2024), stated that the egg's haugh unit consistently correlated with egg weight and albumen height. The haugh unit value of eggs was influenced by the height of the albumen, with higher albumen values resulting in higher haugh unit value. Jaelani et al. (2021) reported that the haugh unit value of eggs can decrease during the storage period due to an increase in water to the yolk, and the length of egg storage carried out without coating can provide an opportunity to increase water to the yolk.

In the present study from 0 days to 56 days FD0 experienced a high decrease in albumen index of 0.02% compared to FD1 of 0.05%, FD2 of 0.03%, and FD3 of 0.05%. Another current study using virgin coconut oil for 21 days resulted in a higher albumen index compared to the present study for 28 days. The FD1 for up to 28 days resulted in an albumen index of 0.06%, FD2 for up to 28 days resulted in an albumen index of 0.06%, and FD3 for up to 28 days resulted in an albumen index of 0.07%. Irmawaty et al. (2022), conducted research using virgin coconut oil with a storage period of 21 days resulting in an albumen index of 0.07%. Likewise, the findings of Sapitri et al. (2020), chitosan with different origins, dosage, and DD for 28 days resulted in a lower albumen index when compared to the present study for 28 days. Sapitri et al. (2020) found that using sago starch + 2% chitosan with storage for 28 days resulted in an albumen index of 0.04%.

The albumen index in the present study indicated that preserving eggs with coating can prevent a high decrease in albumen index as shown in the FD2 and FD3 treatments. As the storage duration of eggs increased, the albumen index value decreased. During storage, egg white is the part that was quickly damaged due to the release of water vapor from the ovomucin nets which functioned as a structure builder in albumen. In addition, the water content in albumen was high, therefore the damage in albumen occurred faster. Uncoated albumen during storage was more susceptible to damage compared to coated albumen. By observing the dilution of the albumen, as the albumen became more diluted, the albumin index value decreased. According to Adriaensen et al. (2022), the albumen index decreased with the length of storage, so the albumen index encountered a small value. Jalili-Firoozinezhad et al. (2020) stated that the low or high albumen index value was caused by the evaporation of water from the ovomucin mesh, and albumen is a vitally important indicator because it is easily damaged, so it needs to be considered during storage. Amezua-Arranz et al. (2024) reported that during egg storage, attention must be paid to the quality of the albumen because the water content in the albumen is more than in other parts (yolk) and the albumen that seems to be damaged can be observed from the watery albumen (albumen that has a high-water content).

In the present study from 0 days to 56 days FD0 experienced a high yolk index decrease of 0.25% compared to FD1 of 0.33%, FD2 of 0.30%, and FD3 of 0.34%. In a study by Rahmawati et al. (2014) virgin coconut oil used for 30 days resulted in a lower yolk index compared to the present study for 28 days. The FD1 for up to 28 days produced a yolk index of 0.35%, FD2 for up to 28 days produced a yolk index of 0.33%, and FD3 for up to 28 days produced a yolk index of 0.37%. Rahmawati et al. (2014), indicated that using virgin coconut oil with a storage period of 30 days resulted in a yolk index of 0.30%. In addition, a study by Shurmasti et al. (2023) used chitosan with different origins, dosages, and DD for 28 days resulting in a lower yolk index when compared to the present study for 28 days. Shurmasti et al. (2023), conducted a study using chitosan from shrimp shells with a dose of 4%, DD 85%, and storage for 28 days combined with 5% polyvinyl alcohol resulted in a yolk index of 0.35%.

The yolk index in the present study illustrated that preparing eggs with coating can prevent a high decrease in the yolk index as shown in the FD2 and FD3 treatments. The decreasing yolk index value was caused by the yolk vitelline membrane, which was not strong due to the migration of water from the egg white that entered the yolk by diffusion, resulting in yolk enlargement. In addition, long storage of eggs can cause a mushy effect on the yolk which indicates that the yolk index has been damaged, so the yolk index obtained can be small (Mudawaroch et al., 2020). According to Biesiada-Drzazga et al. (2022), the lengthy storage period is the reason for the low yolk index during storage, and excessive storage duration can also result in a low yolk index. Kunz et al. (2023), stated that during egg storage the yolk vitelline membrane can experience the migration of water from the egg white which can enter the yolk, therefore the yolk appears larger. Mudawaroch et al. (2020), reported that during storage the egg yolk causes a mushy effect which indicates that the yolk index obtained can be small so that the quality of the yolk index decreases.

In the current study from 0 days to 56 days FD0 experienced an increase in albumen pH of 8.12 compared to FD1 of 8.13, FD2 of 8.10, and FD3 of 8.17. According to Senevirathne et al. (2022), virgin coconut oil used for 28 days resulted in a lower albumen pH compared to the present study for 28 days. The FD1 for up to 28 days resulted in an albumen pH of 8.11, FD2 for up to 28 days resulted in an albumen pH of 8.08, and FD3 for up to 28 days resulted in an albumen pH of 8.11. Senevirathne et al. (2022), conducted a study using virgin coconut oil with a storage period of 28 days resulting in an albumen pH of 7.17. Likewise, other current research using chitosan with different origins, dosages, and DD for 28 days resulted in higher albumen pH when compared to this study for 28 days. Kilinc et al. (2023), found that using 1.5% chitosan

+ 1.5% *Aloe vera* gel and storage for 28 days resulted in an albumen pH of 10.33.

Albumen pH in the current study indicated that egg preparation with coating can prevent a high increase in albumen pH as seen in the FD2 and FD3 treatments. Carbon dioxide vaporation in the present study was low so the buffer system mechanism was still pleasant. The increase in an albumen pH in the current study was not too high, but changes were observed in the gel structure, therefore the surface of the albumen expanded due to dilution that occurred in the albumen due to CO_2 (carbon dioxide) evaporation and the pH would increase. In addition, CO_2 lost through the pores of the eggshell causes the concentration of bicarbonate ions in the albumen to decrease and damage the buffer system, therefore the pH of the albumen increases. According to Hanifa et al. (2023), the pH of albumen does not increase rapidly because there is no high CO_2 evaporation, but when CO_2 evaporation is significantly high, the pH level of the albumen can swiftly rise. Kar et al. (2023), stated that the low pH value of albumen is due to insignificant changes in the gel structure of albumen. Anggita et al. (2023), reported that the increase in albumen pH due to the pores of the eggshell releasing CO_2 usually occurs in eggs that are not coated. Additionally, an increase in albumen pH can occur if the eggs are stored for too long at room temperature without coating. However, if coating is done, the pH of the albumen can remain stable and not increase.

In the present study from 0 days to 56 days FD0 experienced an increase in yolk pH of 7.05 compared to FD1 of 7.11, FD2 of 7.07, and FD3 of 7.10. Other studies that developed at this time using virgin coconut oil for 35 days resulted in lower yolk pH compared to this study for 42 days. Likewise, other current studies using different doses of chitosan and combinations with a storage time of 28 days resulted in comparable yolk pH when compared to the current study for 28 days. The FD1 for up to 42 days resulted in a yolk pH of 7.06, FD2 for up to 28 days resulted in a yolk pH of 6.89, and FD3 for up to 28 days resulted in a yolk pH of 7.02. Saputri (2011), found that using virgin coconut oil with a storage period of 35 days resulted in a yolk pH of 6.36. Awwaly et al. (2024), illustrated that using 1% chitosan + 1% casein + garlic essential oil and storage for 28 days resulted in a yolk pH of 6.83.

pH of yolk in the present study indicated that preparing eggs with coating can prevent a high increase in yolk pH as shown in the FD2 and FD3 treatments. The present study used room temperature, therefore high temperature at the time of the study (egg storage) contributed to a larger loss of CO_2. In addition, the high pH value of the yolk indicated that there is evaporation of water and CO_2 gas contained within the egg. According to Kim et al. (2024), the long storage of eggs would cause the pH of the yolk to increase because the pores of the eggshell can open as long as the storage lasts. Oliveira et al. (2020), indicated that egg storage carried out at room temperature would provide an opportunity for CO_2 to disappear so quickly that the pH value of the yolk would increase and storage of eggs could be carried out at room temperature with a coating treatment that would be able to control evaporation. Wibowo et al. (2023), reported evaporation of the egg's contents including water and CO_2 gas will also increase contained in the egg and if the egg is coated the evaporation that occurs will be resolved and the pH value of the egg yolk will also not increase.

In the present study from 0 days to 56 days FD0 decreased the yolk color by 6.11 compared to FD1 by 6.33, FD2) by 6.22, and FD3 by 6.44. Another current study using virgin coconut oil for 40 days resulted in lower yolk color compared to the present study for 42 days. In addition, other current studies using different doses of chitosan and combinations with a storage time of 14 days resulted in comparable yolk color when compared to this study for 14 days. The FD1 coating using virgin coconut oil for up to 42 days produced a yolk color of 6.78, FD2 using 1.5% chitosan + 1% stearin for up to 14 days produced a yolk color of 7.44 and FD3 using 3% chitosan + 1% stearin for up to 14 days produced a yolk color of 7.55. Todja et al. (2019), indicated that using virgin coconut oil with a storage period of 40 days resulted in a yolk color of 3.00. Thohari et al. (2022), conducted a study using 1% chitosan + 4% casein + 1% TiO_2 and storage for 14 days resulted in a yolk color of 7-9.

Yolk color in the present study did not decrease rapidly because the migration of H_2O from albumen to yolk was not significant. Different yolk color values are caused by the high productivity of chickens and low xanthophyll pigment content in the diet. The difference in yolk color in the present study is attributed to the different metabolic rates of the chickens, resulting in varying abilities to absorb xanthophyll pigments. Virgin coconut oil caused a distinctive aroma of coconut oil while treatment FD3 caused a distinctive aroma of chicken eggs, thus a reduction would be observed in the level of consumer preference. According to Li et al. (2022), the rapidly decreasing yolk color is due to the migration of H_2O from albumen to the yolk, so the resulting yolk color can also be small and pale. Yunitasari et al. (2023), stated that low yolk color values can occur due to high chicken

productivity factors and low xantophyl pigment content in the diet, so it needs to be considered so that the yolk color value does not decrease. Dansou et al. (2023) reported that each chicken has a different metabolic condition, so the ability to absorb xantophyl pigments in chickens is not similar.

CONCLUSION

The chitosan-stearin coating can maintain the quality of chicken eggs during storage for up to 56 days. Using a coating formula with a treatment level of 3% chitosan + 1% stearin indicated the best results in maintaining the quality of eggs during storage at room temperature.

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Authors' contributions

Trioso Purnawarman, Muheri Indra Aja Nasution, Mochammad Sriduresta Soenarno, and Siswanto contributed to designing the study, analyzing the study, and preparing the manuscript. Trioso Purnawarman, Muheri Indra Aja Nasution, Mochammad Sriduresta Soenarno, Siswanto, Yunilas, Uswatun Hasanah, and Sri Wahyuni analyzed the samples in the laboratory and contributed to the drafting and critical checking of the manuscript. All authors confirmed the final draft of the manuscript before submission to the journal.

Competing interests

The authors declared no competing interests in the publication of the present study.

Ethical considerations

All the authors had checked and confirmed the article through ethical issues such as plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy before the submission.

Availability of data and materials

All the data and materials are available on request from the corresponding author.

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Effects of Olive Leaf Extract on Growth Performance and Immunobiochemical Parameters in Turkey Poults

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ABSTRACT

Olive leaf extract (OLE) is known to have numerous bioactivities attributed to its high phenolic compound content. This study aimed to investigate the impact of OLE and Ceftriaxone on *Escherichia coli (E. coli)* in turkey poults. A total of 150 cloacal swabs were taken from turkey poults for isolation and identification of *E. coli*. Fifty-one-day-old turkey poults were divided into five equal groups. The first group served as the control, and the second group orally received 400 mg/kg body weight OLE daily for 35 days. The third, fourth, and fifth groups were infected with a culture suspension of *E. coli* O78 (0.3 ml, 3×10^7 organism/ml) via the nasal route. The third group was infected untreated. The fourth group was treated with 50 mg/Kg body weight of Ceftriaxone for 5 consecutive days. The fifth group received 400 mg/kg body weight of OLE from day to day 35 of age. Bacteriological examination revealed positive swabs in 18.18%, 46.67%, and 53.33% of healthy, diseased, and recently deceased poults, respectively. Serological identification of *E. coli* isolates included O157 (2), O78 (2), and O11 (1). Poults of the third group showed typical clinical signs, gross pathological changes such as congestion in various organs, and a 30% mortality rate. Additionally, significant reductions in body weight, weight gain, catalase (CAT), and superoxide dismutase (SOD) were observed, alongside anemia, hypoproteinemia, and hypoalbuminemia. Conversely, significant increases were noted in the phagocytic index, killing percentage, total globulin, immunoglobulins, and the albumin/globulin ratio. Furthermore, significant increases were observed in FCR, leukocytic counts, lysosome, tumor necrosis factor α (TNF-α), interleukin-10, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), uric acid, creatinine, and malondialdehyde (MDA) levels. Poults in the fourth and fifth groups showed fewer clinical signs, lower lesion scores, and reduced mortality rates. Additionally, there was a decrease in *E. coli* re-isolation, modulation of altered parameters, and improvement in pathological lesions compared to the infected, untreated poults. Both OLE and Ceftriaxone were found to modulate the haematological, biochemical, and immunological parameters, as well as mitigate performance changes and pathological lesions induced by *E. coli* infection in turkey poults.

Keywords: Blood parameter, Ceftriaxone, *E. coli*, Olive leaves extract, Performance, Turkey poult

INTRODUCTION

Turkey is an important poultry species in Egypt, ranking second only to chickens in global meat production. However, turkeys are susceptible to various diseases, including *Escherichia coli* (*E. coli*), which poses a substantial threat to the turkey industry and results in considerable economic losses (Guabiraba and Schouler, 2015). Although *E. coli* is a commensal bacterium in the alimentary tract of healthy birds, it is also recognized as one of the main bacterial pathogens affecting turkeys (Rosario

et al., 2004). *E. coli* is a rod-shaped, Gram-negative, non-spore-forming bacterium causing high mortality and economic losses in the poultry industry. *E. coli* secretes lipopolysaccharide molecules, which cause acute inflammatory reactions and induce hematobiochemical and pathological alterations (Mohamed et al., 2022). Lipopolysaccharides existing in the bacterial cell wall cause cell and tissue injury as well as multiple organ dysfunction (Van Amersfoort et al., 2003). Colibacillosis, instigated by *E. coli*, is one of the leading causes of morbidity and mortality in poultry worldwide, acting either as a primary or secondary pathogen (Lutful Kabir, 2010).

In veterinary medicine, cephalosporins are commonly used because of their broad-spectrum activity and safety profile. Among these, Ceftriaxone is widely used in clinical practice due to its excellent antibacterial efficacy (Ghandour et al., 2023). Ceftriaxone, a broad-spectrum antibiotic belonging to the cephalosporin class, exhibits strong activity against both Gram-positive and Gram-negative bacteria by inhibiting bacterial cell wall synthesis (Prescott, 2013). However, the extensive use of antibiotics in the treatment of bacterial infections in humans and animals in recent decades has increased the percentage of antibiotic-resistant bacterial species in various environments. This trend has posed significant challenges for the selective treatment of bacterial infections (Fazeli-Nasab et al., 2021). Consequently, there is a growing need to explore alternative antibacterial agents derived from traditional medicine.

Olive leaves, which are rich in oleuropein phenols and flavonoids, are utilized in Mediterranean traditional medicine (Sedef and Sibel, 2009). Olive leaf extract (OLE) possesses antioxidant, anti-inflammatory, and antimicrobial properties (Anusha and Mohamed, 2013). OLE contains several potentially bioactive compounds classified as secoiridoids (e.g., oleuropein), flavonoids including flavones (e.g., apigenin and luteolin), flavonols (e.g., rutin and quercetin, and catechin), and simple phenols (e.g., tyrosol, hydroxytyrosol, vanillin, vanillic acid, gallic acid, caffeic acid, and verbascoside) (Sedef and Sibel, 2009). Phenolic compounds extracted from olive leaves might be beneficial to broilers through their antimicrobial activity against intestinal pathogenic bacteria (Sarica and Ürkmez, 2016). Methanol solvent olive extract is highly effective against *E. coli*. Due to the increasing resistance of bacteria to chemical antibiotics, antibacterial compounds from olives and other plants are used in the treatment of bacterial infections (Fazeli-Nasab et al., 2021). Therefore, this study aimed to investigate the comparative effects of olive leaf extract and Ceftriaxone on growth performance, immune response, and biochemical markers in turkey poults infected with *E. coli.*

MATERIAL AND METHODS

Ethical approval

This animal protocol was approved by the Agriculture Research Center Institutional Animal Care and Use Committee (ARC-IACUC) under protocol number ARC-AHRI-79-23, Egypt.

Bacteriological examination and serological identification of isolated *E. coli*

A total of 150 cloacal swabs were aseptically taken from 150 poults (110 healthy, 20 diseased, and 20 freshly dead). The swabs were inoculated into the nutrient broth and incubated at 37°C for 12 hours, followed by subculturing on MacConkey agar and nutrient agar plates for 24 hours at 37°C. Bacterial colonies were identified using standard microbiological methods (Mahon et al., 2018). Isolated *E. coli* were serotyped using slide agglutination test against polyvalent and mono-valent standard serum obtained from Denka Seiken Co., Ltd., Tokyo (Boop et al., 1999).

Antibiotic sensitivity test for isolated *Escherichia coli* (*In vitro*)

Using screening and confirmatory assays recommended by CLSI (CLSI, 2020), isolated *E. coli* O78 was examined for OLE by disc diffusion in comparison to several antibiotics.

Drugs

Ceftriaxone (Ceftriaxone^{R}), supplied by Pharco Company, Egypt, was used for intramuscular or intravenous injection in strengths equivalent to 250 mg, 500 mg, and 1000 mg of Ceftriaxone sodium.

Preparation of watery olive leaf extract

Fresh olive leaves were collected, washed with water, dried, and ground into a fine powder. A total of 7 g of the powder was mixed with 200 ml of boiling distilled water, left to steep, and then filtered. The filtrate was dried in an incubator at 35-40°C (Sylvia et al., 2003).

Experimental diet

The diets used throughout the 35-day experimental period and the physical composition of feedstuff are detailed in Tables 1 and 2.

Table 1. Formulation of experimental diet used for turkey poults during the experiment and the calculated chemical analysis

Ingredient		Calculated chemical analysis	
Ground yellow corn	50.7 kg	Crude protein (%)	26.1103
Soybean meal	32.4 kg	Ether extract (%)	3.687
Fish meal	5.9 kg	Crud fiber (%)	3.602
Corn gluten 60%	6.3 kg	Ca (%)	1.307
Soybean oil	1.1 kg	available Phosphorus (%)	0.549
Lysine Hcl78%	0.1 kg	Metabolic energy (Kcal /Kg)	2916.15
DL- methionine 98%	0.2 kg		
Calcium dibasic phosphate	1.4 kg		
Calcium carbonate	1.7 kg		
Premix	0.1kg		
Toxinil	0.1 kg		
Total	100		

Crude protein percentage and ether extract percentage were chemically analyzed, source: AOAC (1990), and calculated according to the feed composition, source: NRC (1994).

Table 2. Physical composition of feedstuff used in the formulation of diets during the experimental period.

Nutrient (% as fed basis) / Ingredient	Crude protein (%)	Ether extract (%)	Crude fiber (%)	Ca (%)	Available Ph (%)	Metabolic energy (Kcal /Kg)
Ground Yellow corn	7.9	3.5	2.2	0.05	0.1	3350
Soybean meal	44	1.2	7.3	0.35	0.27	2230
Fish meal	65	5	1	0.3.73	2.43	2580
Corn gluten 60%	60	2.4	1.3	0..07	0.14	3720
Soybean oil	0.0	00	00	0.0	0.0	8800
Calcium dibasic phosphate	00	00	00	21.3	18.5	00
Calcium carbonate	00	00	00	38	00	00
Lysine Hcl78%	118	0.0	0.0	0.0	0.0	4600
DL- methionine	58	0.0	0.0	0.0	0.0	3600

Ca: Calcium Ph: Phosphorus Kcal/kg: Kilo calories/ kilogram

Turkey poults and experimental design

Fifty one-day-old turkey poults, confirmed to be free from bacterial infection, were divided into five equal groups: GP1 included healthy poults (negative control); GP2 had healthy poults receiving 400 mg/kg body weight of aqueous olive leaf extract orally for 35 successive days (Atef and Fawziah, 2019; Erener et al., 2020). At day 30 of age, poult sin groups GP3, GP4, and GP5 were inoculated with 0.3 ml of a culture suspension of isolated *E. coli* O78 (3×10^7 organism/ml) via the nasal route (Nakamura et al., 1992). Poults in GP3 were infected with *E. coli* (positive control), and poults in GP4 were infected with *E. coli* and treated with 50 mg/Kg body weight of Ceftriaxone for 5 successive days (Pardeep et al., 2011). Moreover, poults in GP 5 received aqueous OLE for 35 days and were infected with *E. coli* at 30 days of age. Five poults from each group were individually weighed on the first day and day 36 to determine the weight gain and feed conversion ratio (FCR). Mortality rates, clinical signs, and post-mortem lesions were recorded. At 36 days of age, five poults from each group were sacrificed. Samples from the ileum, caecum contents, and fecal matter were collected in sterile bags, incubated on nutrient broth at 37°C for 24 hours, and then subcultured on nutrient agar (Woldehiwet et al., 1990). Isolated bacteria were identified using standard methods (Adams and Moss, 1999) and counted according to AOAC (1990).

Three blood samples were collected from each group at 36 days of age. The first blood sample was collected in Ethylenediaminetetraacetic acid anticoagulant (EDTA) tubes for hematological examination (Feldman et al., 2000). The second blood sample was taken in heparinized tubes for the estimation of phagocytic percentage and killing percentage (Wilkinson, 1976; Lee and Bacon, 1982). The third blood sample was collected without anticoagulant to obtain serum for the measurement of total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), uric acid, and creatinine using a 7150 Automatic Blood Chemistry Analyzer (Ciba–Corning Diagnostic Crop). Additionally, serum levels of superoxide dismutase (SOD) (Nishikimi et al., 1972), catalase (CAT)

(Sinha, 1972), malondialdehyde (MDA) (Nielsen et al., 1997), immunoglobulin (IgA, IgG, and IgM) (Erhard et al., 1992), lysozyme activity (Schltz, 1987), interleukin-10 (IL-10), and tumor necrosis factor α (TNF-α) were measured using ELISA kits (WKEA MED Supplies) according to the manufacturer's instructions, using purified IL-2 and TNF-α antibodies, respectively.

Histopathological examination

Liver, kidney, and intestinal samples were taken from groups infected with *E. coli* (3, 4, and 5) at 36 days of age. Tissue samples were preserved and fixed in 10% neutral buffered formalin and then processed using the routine paraffin embedding technique employing alcohol and dehydrated in graded ethanol (70-100%), xylol, and melted paraffin wax at 60˚C, sectioned at the 4-5 micron thickness, and stained with H&E technique for routine examination (Suvarna et al., 2013).

Statistical analysis

Data was analyzed using one-way analysis of variance (ANOVA) with the SPSS program (version 16). Duncan's Multiple Range Test was used for post-hoc comparisons (Tamhane and Dunlop, 2000). Statistical significance was set at $p < 0.05$.

RESULTS

Of 110 healthy poults, 20 positive swabs (18.18%) were divided into 8 single isolates and 12 mixed isolates. Among 20 diseased poults, 6 positive swabs (30%) were detected, including 5 single isolates and 3 mixed isolates. Of 20 recently dead poults, 8 positive swabs (40%) were distributed as 3 single isolates and 5 mixed isolates (Figure 1 and Table 3). Serological identification of the isolated *E. coli* strains revealed 2 O157, 2 O78, and 1 O11 serotypes (Table 4). The antibiotic sensitivity test demonstrated that *E. coli* was more sensitive to Ceftriaxone compared to other antibiotics used in the study (Table 5).

Healthy poults receiving OLE (GP2) exhibited a significant increase ($p < 0.05$) in body weight gain compared to the normal control group (GP1; Figure 2). Hematobiochemical analysis revealed nonsignificant changes in ($p < 0.05$) in red blood cells (RBCs), hemoglobin (Hb), packed cell volume (PCV), heterophils, lymphocytes, eosinophils, basophils, monocytes, phagocytic percentage, phagocytic index, killing percentage, total globulin, white blood cells (WBCs), immunoglobulins (IgG, IGA, IgM), lysosome activity, interleukin-10 (IL-10), tumor necrosis factor-α (TNF-α), albumin/globulin (A/G) ratio, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), uric acid, and creatinine levels. Additionally, significant increases in serum total protein and albumin and improvement in FCR were observed in GP2 compared to the control group (Tables 6- 9; Figures 2 and 3).

Poults suffering from colibacillosis (GP3) showed clinical signs which included diarrhea, depression, dropping wings, listlessness, respiratory signs, frothy exudate in eyes, and loss of weight, with a mortality rate of 30%. Postmortem examination revealed congestion of the intestine, liver, and kidneys, along with hepatitis, degenerative changes hisopathologically, and a significant decrease ($p < 0.05$) in the body weight gain in poults infected with *E. coli* (GP3; Figure 2). Hematobiochemical findings revealed significant decreases ($p < 0.05$) in RBCs, Hb, PCV, serum total protein, albumin, total globulin, A/G ratio, CAT, SOD and significant increases ($p < 0.05$) in FCR, WBCs, heterophils, phagocytic percentage, phagocytic index, killing percentage, IgG, IgA, IgM, lysosome, TNF-α, IL-10, AST, ALT, ALP, uric acid, creatinine, and MDA. *E. coli* was reisolated from all infected poults in comparison to control poults (Tables 7, 8, 9, and 10; Figure 3).

Infected poults treated with OLE or Ceftriaxone (GP4 and GP5) showed mild clinical signs, lesion scores, reduction of mortality rate to 10 percent, reduction of *E. coli* re-isolation (Figure 1), a significant increase ($p < 0.05$) in body weight gain, RBCs, HB, PCV, phagocytic percentage, phagocytic index, killing percentage, IgG, IGA, IgM, CAT, SOD, total protein, albumin, total globulin, A/G ratio, as well as non-significant changes ($p < 0.05$) in WBCs, heterophil, lymphocyte, basophil, eosinophil, monocyte, coupled with improvement in FCR, AST, ALT, ALP, uric, creatinine, lysosome, TNF-α, IL-10, and MDA ($p < 0.05$) in comparison to the infected poults.

Histopathological findings

The liver of turkey poults infected with *E. coli* (GP3) at the end of the experiment exhibited focal necrotic areas (Figure 4A), hydropic degeneration, and dilated sinusoids (Figure 4B). The kidneys showed severe peritubular hemorrhage (Figure 4C) and the deposition of intra-tubular eosinophilic substance with some degenerative changes (Figure 4D). Intestinal samples showed focal destruction of the intestinal muscularis mucosa (Figure 4E). In contrast, the liver of turkey poults infected with *E. coli* and treated with Ceftriaxone (GP4) at the end of the

experiment showed mild focal lymphocytic cell infiltration in hepatic parenchyma (Figure 5A). Kidneys exhibited mild focal tubular cloudy swelling (Figure 5B), while the intestine showed a mild increase in goblet cells (Mucinous degeneration; Figure 5C). Poults that received OLE and were subsequently infected with *E. coli* (GP5) demonstrated mild focal hemorrhage in the liver (Figure 6A). Kidneys showed hydropic degeneration of some renal epithelial cells (Figure 6B), and the intestine displayed long, fused intestinal villi (Figure 6C).

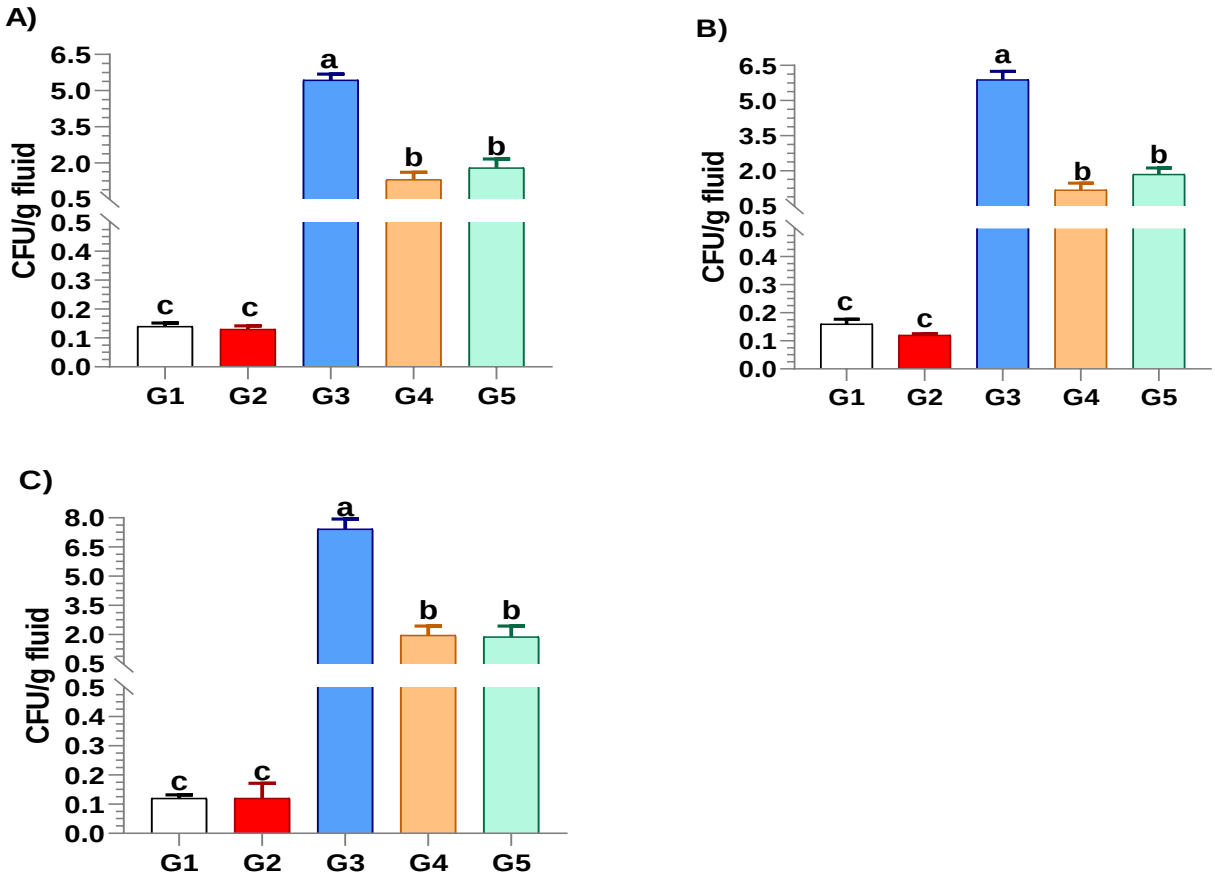

Figure 1. Effect of olive leaf extract and ceftriaxone on re-isolation and counts of *Escherichia coli* (CFU/g fluid) in Ileum (A), Caecum (B), and fecal matter (C) of pouts experimentally infected with *E. coli*. GP(1): Negative control GP(2): Treated with Olive leaf Extract GP(3): Infected with *E. coli* (positive control). GP(4): Infected with *E. coli* and treated with Ceftriaxone GP(5): Infected with *E. coli* and treated with OLE.

Table 3. Prevalence of bacterial isolates from cloacae swabs obtained from turkey poults

	Healthy (total examined 110)				**Diseased (total examined 20)**				**Freshly dead (total examined 20)**			
+ veswab	**20 isolate (18.18%)**				**7 isolate (35%)**				**8 isolate (40%)**			
Type of isolate	Single 8(40%)		Mixed 12 (60)		Single 4 (28.8)		Mixed 3 (71.4)		Single 3 (37.5%)		Mixed 5 (62.5%)	
Isolate	*E. coli*	2	*E. coli* + *Staph Aureus*	5	*E. coli*	2	*Strept spp* + *E. coli*	1	*E. coli*	1	*Sal. spp*+ *E. coli*	1
	Sal. spp.	2	*E. coli*+ *Proteus*	3	*Strept.sp*	1	*Sal. sp*+ *E. coli*	1	*Staph aureus*	1	*E. coli*+ *Staph Aureus*	3
	Strep spp	4	*Sal. spp* +*E. coli*	4	*Sal spp*	1	*sal* .+ *E. coli*	1	*Sal.spp*	1	*Sal. spp*+*E.coli*	1

Sal. Spp.: Salmonella species Staph: Staphylococcus

Table 4. Serological identification of *Escherichia coli* isolated from turkey poults

Serotype	O157	O78	O11
Healthy (2)	1	1	-
Diseased (2)	1	1	-
Dead (1)	-	-	1

Table 5. Susceptibility of *Escherichia coli* to olive leaf extract and some antimicrobial agents

	Olive	Ceftriaxone	Erythromycin	Gentamycin	Florfenicol
Mark and potency	00	CRO (30mg)	Define under the table (30mg)	Gm (10mg)	FF (30mg)
Inhibition zone	17	24	21	15	21
sensitive	++	++++	+++	++	++++

CRO: Ceftriaxone; Gm: Gentamycin; FF: Florfenicol

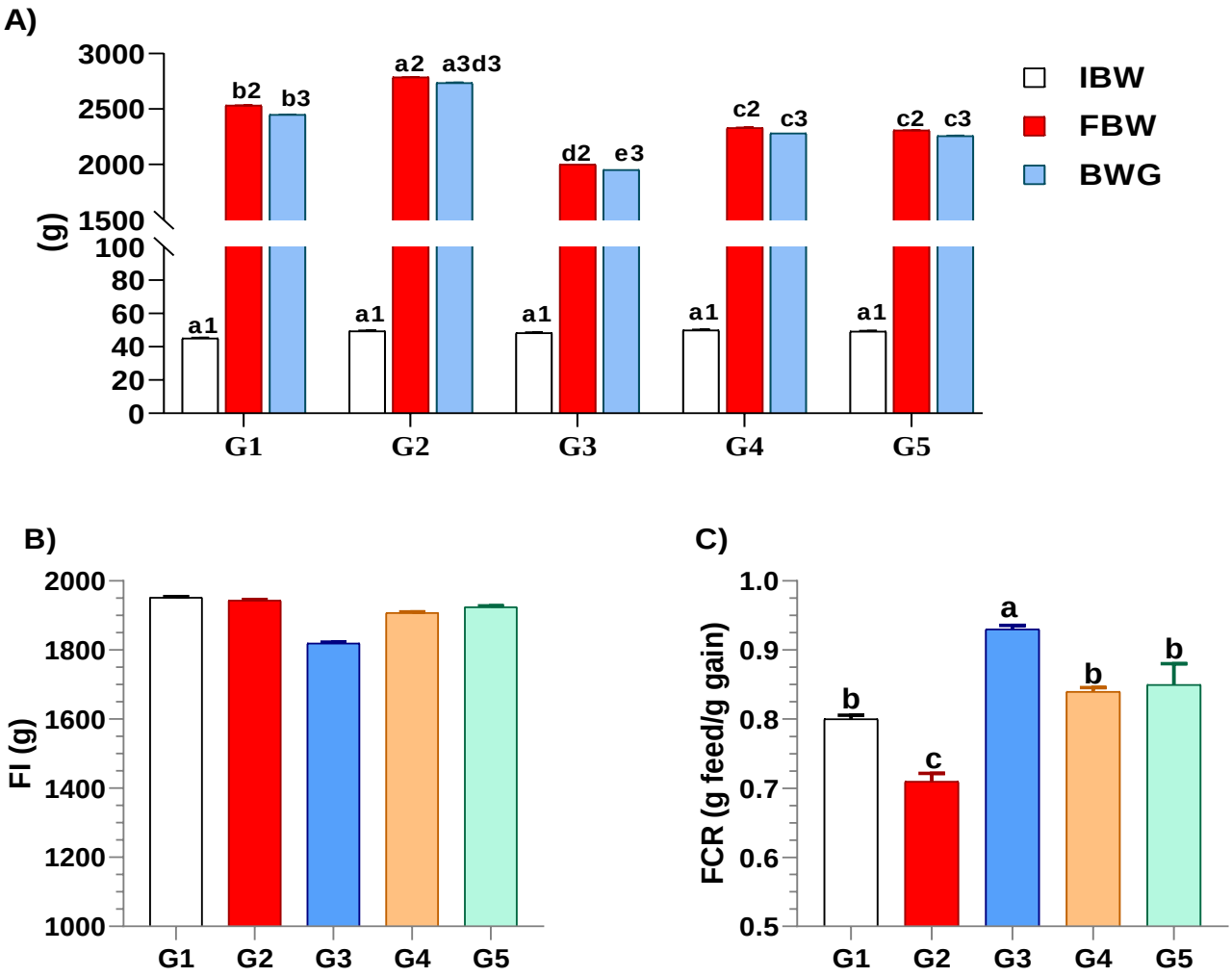

Figure 2. Effect of olive leaf extract and Ceftriaxone on body performance. **A:** Initial body weight, IBW; Final body weight, FBW; Body weight gain (BWG) and feed utilization. **B:** Feed intake, FI; Feed conversion ratio (FCR) in poults experimentally infected with *Escherichia coli*. GP(1): Negative control GP(2): Treated with olive leaf extract GP(3): Infected with *E. coli* (positive control) GP(4): Infected with *E. coli* and treated with Ceftriaxone GP(5): Infected with *E. coli* and treated with OLE.

Table 6. Effect of olive leaves extract and ceftriaxone on hematology of poults experimentally infected with *Escherichia coli* at 36 days of age

Group	Erythrogram			Leukogram					
	RBCs (10^6/cu mm)	HB (g/dl)	PCV (%)	WBCs $X10^3$/µl	Differential leukocytic count (X103/µl)				
					Heterophil	Lymphocyte	Eosinophil	Basophil	Monocyte
Gp (1)	6.06 ± 0.31[a]	12.32 ± 0.17[a]	29.21± 0.28[a]	11.16 ± 0.38[b]	3.31 ± 0.13[b]	4.39 ± 0.32[a]	1.19 ± 0.13[a]	1.21 ± 0.15[a]	1.06 ± 0.12[a]
Gp (2)	6.01 ± 0.22[a]	12.67 ± 0.13[a]	29.01 ± 0.19[a]	11.44 ± 0.16[b]	3.20 ± 0.11[b]	4.48 ± 0.69[a]	1.38 ± 0.18[a]	1.27 ± 0.17[a]	1.11 ± 0.10[a]
Gp (3)	4.44 ± 0.09[b]	8.23 ± 0.27[b]	17.32 ± 0.12[b]	12.02 ± 0.33[a]	4.26 ± 0.29[a]	4.48 ± 0.88[a]	1.15 ± 0.07[a]	1.18 ± 0.06[a]	1.03 ± 0.09[a]
Gp (4)	5.79 ± 0.27[a]	11.89 ± 0.29[a]	28.88 ± 0.46[a]	11.82 ± 0.16[a]	4.28 ± 0.42[a]	4.49 ± 0.47[a]	1.16 ± 0.10[a]	1.19 ± 0.11[a]	1.04 ± 0.07[a]
Gp (5)	5.96 ± 0.19[a]	11.89 ± 0.07[a]	28.68 ± 0.51[a]	12.06 ± 0.05[a]	4.89 ± 0.51[a]	3.98 ± 0.59[a]	1.08 ± 0.11[a]	1.13 ± 0.10[a]	1.01 ± 0.09[a]

Different superscripts (a, b, and c) within the same row indicate significant differences at $p < 0.05$. RBCs: Red blood cells, HB: Hemoglobin, PCV: Packed Cell Volume, WBCs: White blood cells. GP(1): Negative Control GP(2): Treated with olive leaf extract GP(3): Infected with *E coli*(positive control). GP(4): Infected with *E. coli* and treated with Ceftriaxone GP(5): Infected with *E. coli* and treated with OLE

Table 7. Effect of olive leaf extract and ceftriaxone on immunoglobulin of poults experimentally infected with *Escherichia coli*

Parameter	Immuonoglobuline (gm/100ml)		
Groups	**IgG**	**IgM**	**IgA**
Gp(1)	3.97 ± 0.89[c]	5.70 ± 0.57[c]	8.21 ± 0.91[b]
Gp(2)	4.12 ± 0.95[c]	6.66 ± 0.89[c]	8.77 ± 0.96[b]
Gp(3)	6.98 ± 0.89[b]	7.74 ± 0.23[b]	9.89 ± 0.78[b]
Gp(4)	6.91 ± 0.57[b]	6.87 ± 0.53[c]	9.81 ± 0.71[b]
Gp(5)	8.22 ± 0.78[a]	9.21 ± 0.62[a]	11.41 ± 0.87[a]

Different superscripts (a, b, and c) within the same row indicate significant differences at $p < 0.05$. IgA: Immunoglobulin A IgM: Immunoglobulin M IgG: Immunoglobulin G. GP(1): Negative control GP(2): Treated with olive leaf extract GP(3): Infected with *E coli* (positive control) GP(4): Infected with *E. coli* and treated with Ceftriaxone GP(5): Infected with *E. coli* and treated with OLE.

Table 8. Effect of olive leave extract and ceftriaxone on CAT, SOD, MDA, IL-10, and TNF-α, in poults experimentally infected with *Escherichia coli*

Parameter	Antioxidant enzymes		MDA	IL-10	TNF-α
Group	**CAT(U/mL)**	**SOD(U/mL)**	**(ug/ml)**	**pg/ml**	**pg/mL**
Gp(1)	9.37 ± 0.89[b]	25.55 ± 1.58[b]	9.58 ± 0.82[b]	0.89 ± 0.21[b]	0.99 ± 0.18[b]
Gp(2)	15.09 ± 0.63[a]	32.82 ± 1.21[a]	8.06 ± 0.94[c]	0.80 ± 0.18[b]	0.94 ± 0.15[b]
Gp(3)	7.68 ± 0.45[c]	23.65 ± 1.37[c]	10.94 ± 0.73[a]	0.94 ± 0.15[a]	2.84 ± 0.21[a]
Gp(4)	9.48 ± 0.66[b]	25.12 ± 1.83[b]	9.12 ± 0.98[c]	0.89 ± 0.19[a]	1.03 ± 0.16[b]
Gp(5)	14.21 ± 0.75[a]	30.17 ± 1.53[a]	9.73 ± 0.78[b]	0.91 ± 0.13[a]	1.02 ± 0.53[b]

Different superscripts (a, b, and c) within the same row indicate significant differences at $p < 0.05$. CAT: Catalase SOD: Soper Oxide Dismutase MDA: Malonaldhyde IL-10: Interleukin 10 TNF-α: Tumer Necrosis Factor-α. GP(1): Negative Control GP(2): Treated with olive leaf Extract GP(3): Infected with *E coli* (positive control) GP(4): Infected with *E coli* and treated with Ceftriaxone GP(5): Infected with *E. coli* and treated with OLE.

Table 9. Effect of olive leaf extract and ceftriaxone on liver enzymes and kidney function of poults experimentally infected with *Escherichia coli*

Parameters \ Groups	GP 1	GP 2	GP 3	GP 4	GP 5
Total protein	4.72 ± 0.41^{b}	5.93 ± 0.33^{a}	2.86 ± 0.30^{c}	4.01 ± 0.35^{b}	3.98 ± 0.34^{b}
Albumin	2.08 ± 0.21^{c}	3.00 ± 0.19^{a}	1.59 ± 0.17^{d}	2.10 ± 0.21^{b}	2.06 ± 0.19^{b}
Total globulin	2.64 ± 0.15^{a}	2.93 ± 0.19^{a}	1.37 ± 0.17^{c}	1.91 ± 0.21^{b}	1.88 ± 0.19^{b}
AG ratio	1.35 ± 0.19^{a}	1.33 ± 0.11^{a}	1.07 ± 0.16^{b}	1.36 ± 0.12^{a}	1.36 ± 0.08^{a}
AST	78.48 ± 0.98^{c}	76.39 ± 0.78^{c}	81.42 ± 0.82^{a}	79.75 ± 0.69^{b}	79.83 ± 0.91^{b}
ALT	37.32 ± 0.89^{c}	36.83 ± 0.54^{c}	40.61 ± 0.55^{a}	38.32 ± 0.71^{b}	38.41 ± 0.63^{b}
ALP	89.14 ± 0.79^{b}	87.87 ± 0.89^{b}	92.61 ± 0.77^{a}	90.33 ± 0.78^{b}	90.40 ± 0.55^{b}
Uric acid	5.41 ± 0.43^{b}	5.02 ± 0.43^{b}	8.12 ± 0.55^{a}	6.60 ± 0.75^{b}	7.66 ± 0.49^{ab}
Creatinine	1.15 ± 0.16^{b}	1.10 ± 0.13^{b}	1.89 ± 0.31^{a}	1.42 ± 0.27^{a}	1.50 ± 0.46^{a}

Different superscripts (a, b, and c) within the same row indicate significant differences at $p < 0.05$. AG ratio: Albumin/Globulin Ratio. AST: Aspartate amino transferase ALT: Alanin amino transferase. ALP: Alkaline phosphatase. GP 1: Negative control, GP 2: Treated with olive leaf extract, GP 3: Infected with *E. coli* (positive control), GP 4: Infected with *E. coli* and treated with Ceftriaxone, GP 5: Infected with *E. coli* and treated with OLE.

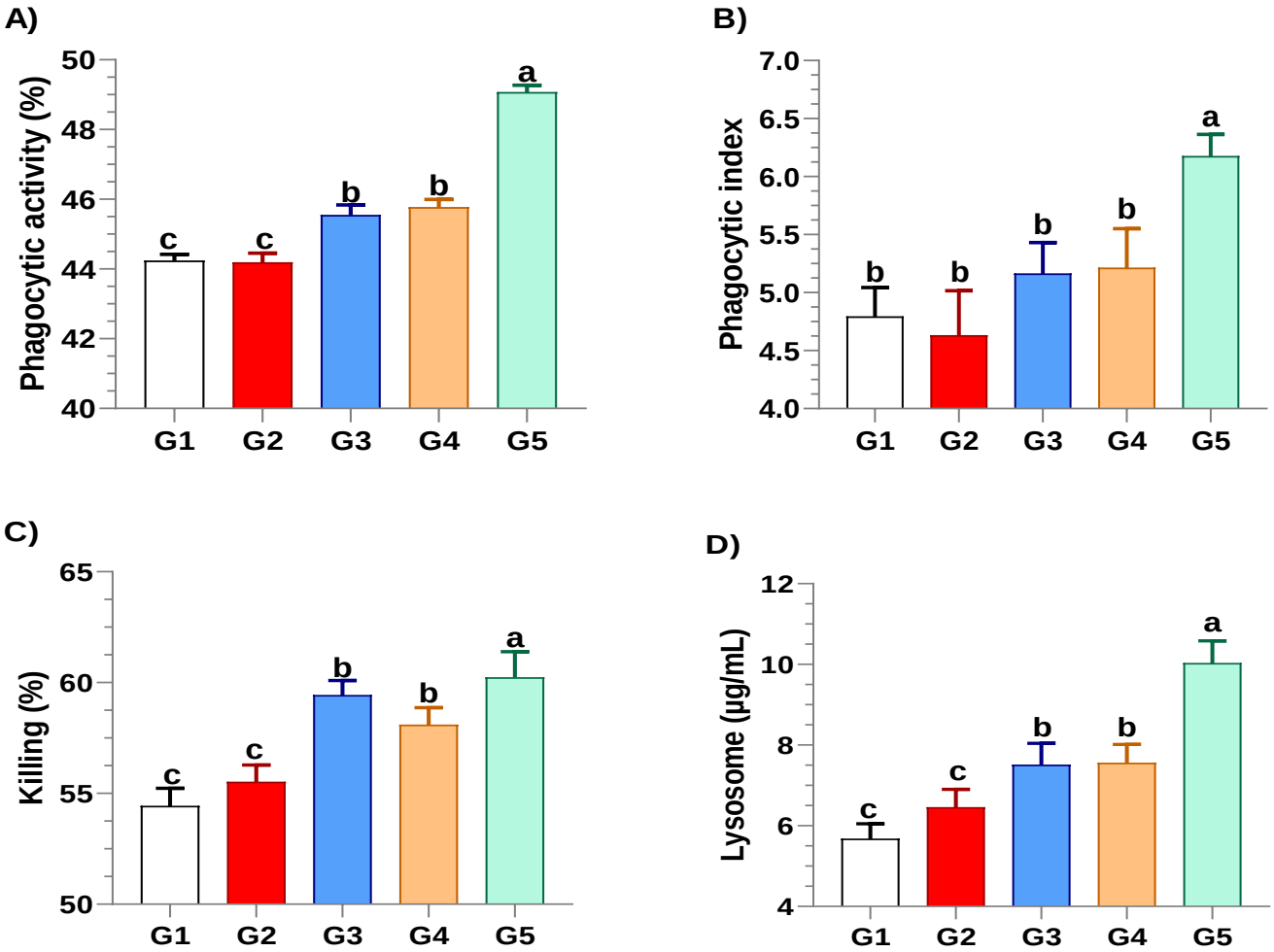

Figure 3. Effect of olive leaf extract and Ceftriaxone on phagocytic activity (A), phagocytic index (B), killing rate (C), and lysosome (D) in poults experimentally infected with *Escherichia coli*. GP 1: Negative control GP 2: Treated with olive leaf extract GP 3: Infected with *E. coli* (positive control) GP 4: Infected with *E. coli* and treated with Ceftriaxone GP 5: Infected with *E. coli* and treated with OLE.

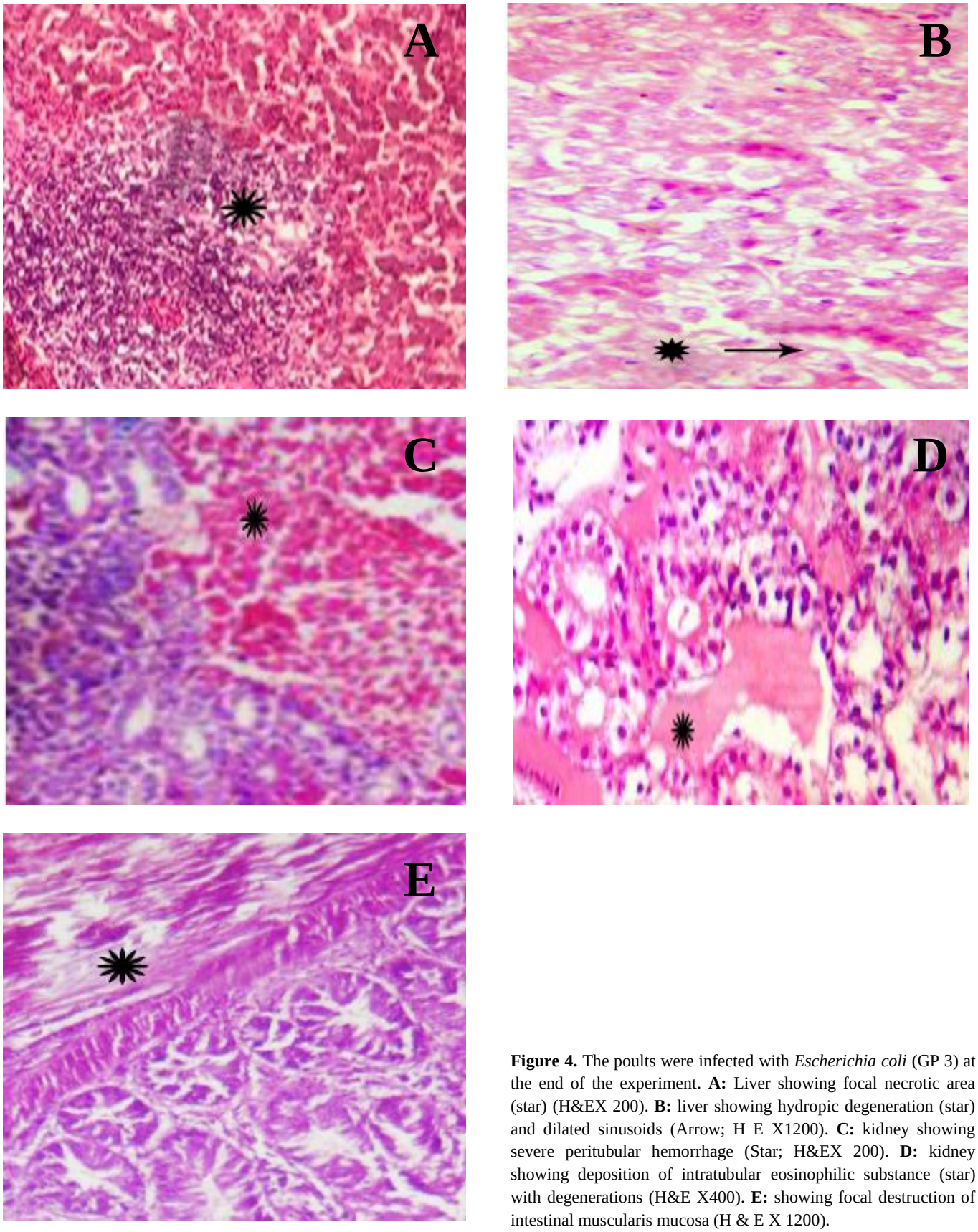

Figure 4. The poults were infected with *Escherichia coli* (GP 3) at the end of the experiment. **A:** Liver showing focal necrotic area (star) (H&EX 200). **B:** liver showing hydropic degeneration (star) and dilated sinusoids (Arrow; H E X1200). **C:** kidney showing severe peritubular hemorrhage (Star; H&EX 200). **D:** kidney showing deposition of intratubular eosinophilic substance (star) with degenerations (H&E X400). **E:** showing focal destruction of intestinal muscularis mucosa (H & E X 1200).

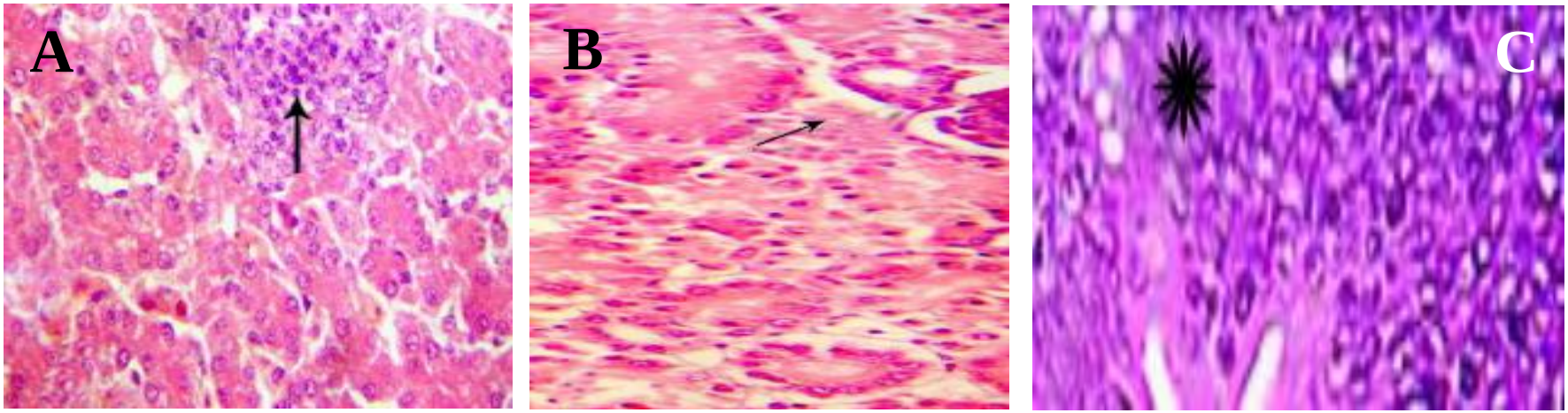

Figure 5. The poults infected with *Escherichia coli* and treated with ceftriaxone (GP 4) at the end of the experiment. **A:** liver showing focal mild lymphocytic cells infiltrated the hepatic parenchyma (Arrow; H&E, X1200). **B:** kidney showing mild focal tubular cloudy swelling (Arrow; H&E, X1200). **C:** The intestine shows a mild increase in goblet cells (Star; Mucinous degeneration ; H&E, X1200).

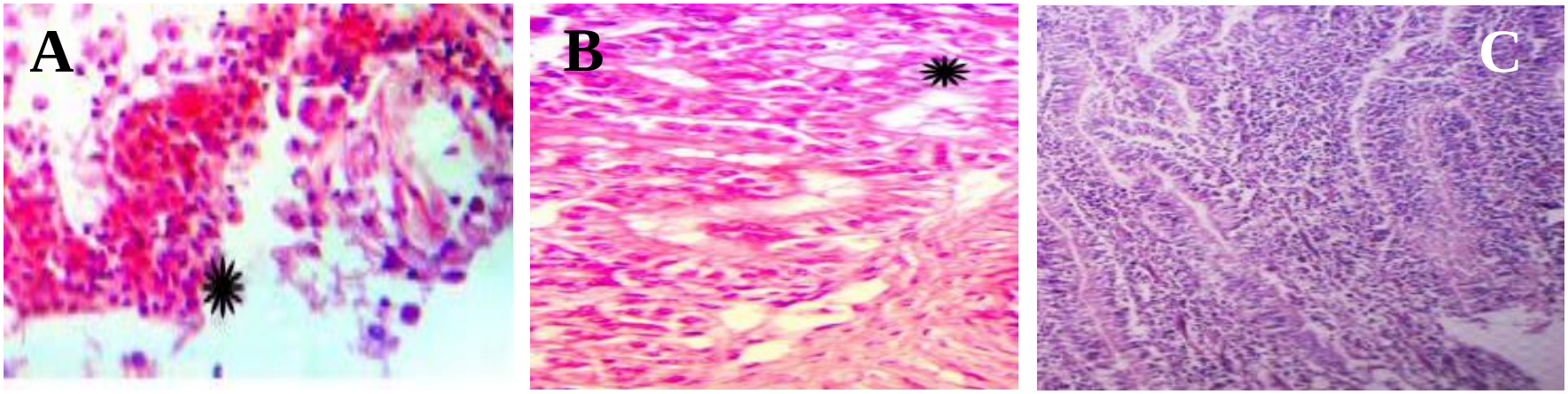

Figure 6. The poults receiving olive leaf extract and subsequently infected with *Escherichia coli* GP (5) at the end of the experiment. **A:** Liver showing mild focal hemorrhage (star) (H& E X1200). **B:** kidney showing hydropic degeneration of some renal epithelium (star), (H&E X300). **C:** Intestine showing long fused intestinal villi (H&E X300).

DISCUSSION

Avian colibacillosis, caused by *E. coli* (either as a primary or secondary infection), is one of the main causes of morbidity and mortality in poultry (Barnes et al., 2008). Moreover, there is a higher tendency to utilize medicinal herbs to alleviate diseases due to their reduced risk of side effects (Fazeli-Nasab et al., 2021). Certain herbs and medicinal plants, such as olives, have demonstrated beneficial effects on various physiological systems owing to their antioxidant properties (Cotelle et al., 1996). For instance, the disc diffusion test revealed that OLE induced an inhibitory zone of approximately 17 mm against isolated *E. coli*, with Ceftriaxone being the most effective antibiotic among those tested. Similarly, Gökmen et al. (2014) reported that OLE induced an 18 mm inhibitory zone against *E. coli*. Furthermore, *E. coli* has been shown to exhibit high sensitivity to ceftriaxone (Ghandour et al., 2023).

In the present study, poults infected with *E. coli* showed clinical signs including ruffled feathers, dropping wings, inappetence, depression, conjunctivitis, sneezing with frothy exudates in their eyes, diarrhea, and a mortality rate of 30%. These symptoms may be attributed to the effect of *E. coli* endotoxins on various organs. These results are partially consistent with those of El-Tahawy et al. (2022) in broiler chickens and align with Reham et al. (2021), who reported diarrhea, loss of appetite, mouth breathing, sneezing, ruffled feathers, weight loss, and a 30% mortality rate in chickens infected with *E.coli.*

The results of this study revealed a decrease in body weight gain and an increase in FCR as a result of inappetence, intestinal damage, poor digestion, and diarrhea. This finding was supported by El-Tahawy et al. (2022), who observed that experimental *E. coli* infection in broilers induced weakness, loss of appetite, depression, cough, and watery diarrhea in addition to a recorded mortality rate of 30% as well as a reduction in growth performance. The obtained results align with those of Stordeur and Mainil (2002), who stated that colibacillosis in broiler chickens induced low performance and weight gain.

Based on the current study, the postmortem examination of poults revealed congestion of the internal organs and hepatitis, likely caused by *E. coli* endotoxins. This finding is similar to that noted by Reham et al. (2021) and Ghandour et al. (2023), who observed the same postmortem lesions in chickens with *E.coli* infection.

In the current study, treatment of *E. coli*-infected poults with either OLE or Ceftriaxone resulted in improved clinical signs, reduced mortality, enhanced weight gain, and a decrease in re-isolation of *E. coli.* This improvement is likely due to the antimicrobial properties of OLE and ceftriaxone. These observations are consistent with the findings of Markin et al. (2003), who reported that OLE exhibited antibacterial effects against *E. coli*, reduced clinical signs, improved weight gain, and reduced re-isolation of *E. coli*. Similarly, Ghandour et al. (2023) demonstrated that Ceftriaxone was effective against *E. coli*, reducing clinical signs, eliminating mortality, improving weight gain, and reducing *E. coli* re-isolation in broiler chickens.

The obtained data revealed that healthy poults receiving OLE showed insignificant changes in RBCS, Hb, PCV%, and WBCs, as well as an insignificant increase in heterophils, lymphocytes, eosinophils, basophils, monocytes, phagocytic index, and killing percentage at the end of the experiment compared to healthy control poults. Similarly, olive leaf powder was found to induce elevation in RBCS, Hb, PCV %, and WBCs in El-Damarawy et al.'s (2013) study. The obtained results align with those of Ahmed et al. (2017), who stated that olive oil increased RBCS, Hb, PCV%, WBCs, lymphocytes, and neutrophils in rats and chickens.

In this study, the poults infected with *E. coli* showed a reduction of RBCs, Hb, and PCV and an increase in WBCs, heterophils, phagocytic index, and killing percentage, which may be due to *E. coli* endotoxins. These results corroborate with those of Allam et al. (2016), who stated that broilers infected with *E. coli* showed a reduction in RBCS, Hb, PCV, lymphocytes, monocytes, eosinophils, and basophils, as well as a significant increase in WBCs and heterophils. This finding aligns with El-Tahawy et al. (2022), who stated that *E-coli* induced a reduction in RBCS, Hb, and PCV in broilers. Moreover, Mithin et al. (2022) mentioned that broilers infected with *E. coli* showed an increase in WBCs and heterophils.

Poults infected with *E-coli* and treated with either OLE or Ceftriaxone showed a significant increase in RBCs, HB, PCV, phagocytic index, and killing percentage, coupled with a significant reduction in WBCs and heterophils in comparison with infected untreated poults. This may be due to the antibacterial effects of OLE against *E. coli*, leading to the improvement of the examined hematological and immunological parameters. The same observation was recorded by El-Kholany et al. (2022), who stated that OLE was effective against *E. coli* and improved the hematological parameters. Phenolic compounds extracted from olive leaves were found effective due to their antimicrobial activity in broilers

(Sarica and Ürkmez, 2016). Ceftriaxone was powerful in the treatment of colibacilosis and improved RBCS, Hb, PCV, WBCs, and phagocytosis (Mithin et al., 2022). These findings partially agree with Ghandour et al. (2023), who reported that broilers infected with *E. coli* and treated with Ceftriaxone showed an elevation in RBCS, Hb, and PCV together with a reduction in WBCs when compared to infected untreated broilers.

In the present study, poults suffering from colibacillosis showed a significant decrease in CAT and SOD, besides a significant increase in IgG, IgA, IgM, lysosome, IL-10, TNF-α, and MDA levels compared to healthy control poults. According to Coleman (2001), this might result from severe inflammation and oxidative stress caused by Colibacilosis. *Escherichia coli* infection induced inflammation and oxidative stress, causing damage to the internal organs and leading to an increase in the lysosome, MDA, IL-10 a, and TNF-α as well as a decrease in CAT and SOD (Huda et al., 2020; Mohamed et al., 2022).

Poults suffering from colibacillosis and treated with OLE or ceftriaxone showed a significant increase in IgG, IgA, IgM, CAT, and SOD as well as a significant decrease in the lysosome, IL-10, TNF-α, and MDA levels compared to infected untreated poults. This may be due to the antioxidant properties of OLE and the antibacterial effects of both Ceftriaxone and OLE. In the same line, Markin et al. (2003) reported that OLE possesses antibacterial effects against *E. coli* and enhances antioxidant enzyme levels. Additionally, OLE has been shown to improve antioxidant enzymes, immunoglobulins (IgG, IgA, IgM), and MDA levels, demonstrating its efficacy against various bacterial infections (Lee and Lee, 2010). In previous studies, adding olive leaves to turkey diets led to increased serum concentrations of polyphenols such as oleuropein, hydroxytyrosol, tocopherol, carotene, sitosterol, and triglycerides, which are responsible for antioxidant activity and protection from blood lipid oxidation (Moudache et al., 2016; Lins et al., 2018). Furthermore, treatment of colibacillosis with cefepime has been shown to improve TNF-α and IL-10 (Coleman, 2001). These findings are consistent with Huda et al. (2020), who reported that broilers infected with *E. coli* and treated by cephalosporin (cephradine) showed improvements in CAT, SOD, and MDA levels.

In this study, poults infected with *E. coli* displayed a significant reduction in the total protein, albumin, total globulin, and A/G ratio, together with a significant elevation in AST, ALT, ALP, uric acid, and creatinine. These changes may be attributed to inappetence, diarrhea, inflammation of the intestine, and histopathological damage to liver and kidney tissues. The significant elevation in the hepatic enzymes in infected poults is likely due to degenerative changes and necrosis of the hepatic tissues, leading to increased hepatic permeability and the subsequent release of excessive liver enzymes into the serum. These findings are consistent with those of Manimaran et al. (2003), who reported that *E. coli* infection in broilers induced a significant reduction in the protein profile due to the malabsorption of amino acids from the inflamed intestine. Turkeys infected with *E. coli* showed an increase in serum liver enzymes, uric acid, and creatinine (Huff et al., 2008). In addition, infection with *E. coli* induced a reduction in protein profiles in broiler chickens and poults (Reham et al., 2021; Mohamed et al., 2022).

Poults infected with *E. coli* and treated by either OLE or Ceftriaxone showed a significant increase in total protein, albumin, and total globulin as well as a reduction in AST, ALT, ALP, uric acid, and creatinine in comparison with infected untreated poults. This improvement may be due to the antibacterial effect of both OLE and Ceftriaxone. The present study supports the results of Osman and Tantawy (2017) and Ghandour et al. (2023), who stated that olive leaf had hepatoprotective effects and improved the protein profile and liver enzymes. Olive leaf extract demonstrated antibacterial effects against *E. coli* due to the presence of phenolic compounds and the improvement of liver and kidney function (Takó et al., 2020).

Compared with the normal control group, the histopathology of the liver of *E. coli* infected turkey poults (GP3) showed the congestion of hepatic blood vessels and hepatic sinusoids, and the hydropic degeneration of hepatic cells, Their kidneys showed severe peritubular hemorrhage, pale eosinophilic substance, degenerative changes, and focal destruction of intestinal muscularis mucosa. These changes may be due to endotoxins present in *E. coli*. This suggestion was reinforced by Huff et al. (2008), who reported congestion, coagulative necrosis in the renal and hepatic parenchyma, degenerative changes in the hepatocytes and epithelium lining of renal tubules, and leukocytic infiltrations in *E. coli* infected broilers and turkeys. Reham et al. (2021) reported that the liver, kidneys, and intestines of broiler chickens suffering from colibacillosis revealed severe pathological abnormalities, inflammation, congestion, and degenerative changes, along with the sloughing of epithelium.

In this study, poults receiving OLE and then infected

with *E. coli* (GP5) showed mild focal hemorrhages in the liver and mild hydropic renal degeneration in the kidneys. They also demonstrated long fused intestinal villi in the intestine. This improvement may be due to the antimicrobial and antioxidant effects of OLE, as discussed by Ahmed et al. (2017). OLE enhances the mitochondrial membrane to prevent the disintegration of liver cells, eliminates liver blood vessels and sinusoids, and prevents the secretion of more liver enzymes, as reported by Vahidi-Eyrisofla et al. (2019). Such beneficial effects of OLE in improving intestinal absorption and nutritional digestibility are similar to those reported by Ahmed et al. (2017), who stated the reduction in the proliferation of pathogenic microorganisms and the production of toxins. Olive leaves possess anti-inflammatory and gastroprotective properties that improve intestinal epithelial cells (Mahmoud et al., 2021). The results of the current study are similar to those found by Papadopoulos et al. (2023), who reported that the gross lesions and histological structure of liver incorporation of OLE had a beneficial effect.

The livers of turkey poults in GP4 showed mild lymphocytic cells infiltrated in the hepatic parenchyma. Moreover, cloudy swelling and mucinous degeneration of the intestine were observed in their kidneys. Our results are supported by El-Tahawy et al. (2022) in their study on broiler chickens infected with *E. coli* and treated with another cephalosporine (cefquinome), showing improvement in the pathological lesions in the liver and kidneys. Similar improvements in the pathological lesions were reported by Ghandour et al. (2023), who stated that broiler chickens infected with *E. coli* and treated with Ceftriaxone demonstrated mild lesions in the liver, kidneys, and intestine.

CONCLUSION

The use of the aqueous extract of olive leaves (OLE) as a prophylactic measure and Ceftriaxone as a treatment for experimental *E. coli* infection not only improves body weight gain and FCR but also alleviates the deleterious effects induced on hematological and immunological parameters. Additionally, both OLE and Ceftriaxone modulate the pathological lesions induced by *E. coli* in turkey poults. Further research is recommended to evaluate the effects of OLE on different turkey breeds, the use of varying doses, its effects on other parameters, and its effects on meat quality.

DECLARATIONS

Authors' contributions

All authors contributed to the study conception and design, material preparation, data collection and analysis, and funding. The experimental diet and growth performance were performed by Ghada M. El Khedr. The hematological and biochemical investigations were performed by Doaa I.A. Mostafa, Marwa M. Sarhan, and Sara A. Abd El Wahab. The microbiological investigation was performed by Shaimaa A. Abd El-kader. The histopathological studies and histomorphometric measurements were performed by Heba A. Ewis, and Mohammed Kassem participated in the design of the study, writing, and revision of the manuscript, and in the approval of its final draft. The first draft of the manuscript was written by all authors who commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Availability of data and materials

The data and materials are available upon reasonable request from the authors.

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Competing interests

The authors declare that there are no conflicts of interest.

Ethical considerations

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

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Impacts of Zinc, Selenium, and Vitamin E Supplementation on Growth Performance, Hematological and Biochemical Parameters of Blood in Broiler Chickens

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ABSTRACT

Metabolism, lipid synthesis, and reducing oxidative stress contribute to broiler chickens' growth and immunity. The current study examined how zinc, vitamin E, and selenium impact broiler growth, carcass characteristics, hematological and serum biochemical parameters, and profitability. There were 300-day-old straight-run chicks (Indian River) raised in a deep litter system until 28 days old. On day 7, the chicks were randomly divided into four groups of 75 chicks, each group replicated into 3 replications. Supplementation of zinc, selenium, and vitamin E through water was conducted from day 7 to day 28. This experiment was performed during the lifespan of chickens from 0 to 28 days of age. The treatment groups were control (drinking water with no supplementation), Zn (drinking water with 4 ml/L zinc), Se+Vit E (drinking water with 0.25 ml/L E-Sel), and Zn+Se+Vit E (drinking water with both 4 ml/L zinc and 0.25 ml/L E-Sel). The results indicated significant changes in growth and feed conversion ratio among Zn, Se, and Vit-E supplemented groups. Among the supplemented groups, the Zn+Seleium+VitaminE group exhibited higher growth performance, lower cholesterol, and lower production costs. The findings showed no significant changes in dressing characteristics and feed consumption among groups. The combined group of Zn, Se, and Vit-E had a lower abdominal fat content than other supplemented groups. Supplemented with Zn, Se, and Vit-E groups had lower cholesterol and LDL levels than the control group. Serum differential leukocyte count (eosinophils, lymphocytes, neutrophils, and monocytes) and liver and kidney function tests (ALT, AST, creatinine) showed no significant variations between the groups. Antioxidants increased profitability, with the Zn+Se+Vit E group having a higher profit per kg broiler and cost-benefit ratio. Broiler growth performance, dressing characteristics, biochemicals, and hematological indicators are associated with supplementation Zn, Se, and Vit- E. The addition of Zn (4 ml/L) and Se and Vit E solution (E-Sel) (0.25 ml/L) to drinking water could enhance broiler growth performance and reduce cholesterol and high-density lipoprotein (HDL) concentration.

Keywords: Broiler, Selenium, Vitamin E, Zinc, Performance

INTRODUCTION

Despite its limited area, Bangladesh has a rapidly expanding population and generates 9.22 million metric tons of meat per year (DLS, 2024), with the broiler sector making a significant contribution. The nation has a variety of broiler farms, ranging from small to large, although many confront suboptimal environmental circumstances detrimental to chick health. The rapid growth of broiler chickens, often associated with compromised immune systems, exacerbates their health management challenges (Oke et al., 2024). Antioxidants are chemical substances that are crucial contributors to animal survival, health, productivity, and reproductive success. Several minerals and vitamins function as antioxidants, such as zinc, selenium, and vitamin E (Surai and Fisinin, 2016).

Zinc (Zn) is identified as essential for the production of the antioxidant superoxide dismutase enzyme that helps to break down potentially harmful oxygen molecules in cells (Long et al., 2020). Zinc is an important biological activity and is required for skeletal and improved immunological responses. The antioxidant capabilities of zinc might be attributed to its role as the catalytic core of superoxide dismutase, an essential enzyme in maintaining cellular redox balance and preventing oxidative damage

that can impair immune function (Saleh et al., 2018). Zinc could be incorporated into broiler diets in the form of either organic Zn (zinc protein, zinc amino acid, or zinc picolinate) or inorganic Zn (zinc chloride, zinc sulfate, or zinc oxide). Inorganic zinc sulfate has a positive effect on broiler growth performance, serum biochemistry, and antioxidant function when compared with other zinc sources (Xie et al., 2024). $ZnSO_4$, a highly bioavailable form of zinc, is often incorporated into chicken diets to satisfy their growth-related Zn requirement (Leeson and Caston, 2008). Xie et al. (2024) demonstrated that zinc methionine and $ZnSO_4$ are equally efficacious in enhancing growth and zinc levels in day-old broiler chickens. Selenium (Se) is considered to be an essential trace mineral for both animals and humans. Selenium has the potential to enhance growth, immunity, reproductive performance, and resistance to sickness (Ghazi Harsini et al., 2012; Habibian et al., 2014). Selenium improves feed utilization by acting on the biosynthesis of amino acids, lipids, and carbohydrates (Stapleton, 2000). Selenium is a highly effective antioxidant in nature, which has a vital health-boosting effect. When selenium is paired with vitamin E, it could enhance immunity and reduce the risk of cancer (Lü and Jiang, 2005; Huang et al., 2012). Vitamin E (Vit-E) is crucial for growth and physiological and immunological function (Gao et al., 2010). Vitamin E supplementation is frequently incorporated into chicken feed as DL-α-tocopherol acetate; it has equivalent amounts of eight stereoisomers. Studies indicated that D-α-tocopherol, in contrast to synthetic alternatives, shows superior retention in blood and tissues (Cheng et al., 2017), alleviates lipopolysaccharide-induced inflammatory reactions (Kaiser et al., 2012), and improves the quality of chicken meat's quality (Gao et al., 2010; Rey et al., 2015). Vitamin E enhances the resistance of the body against free radical damage during metabolic and inflammatory processes (Sheikh et al., 2020). This function inhibits the lipid peroxidation of unsaturated fats inside the cell, therefore safeguarding the cell from the harmful effects of free radicals (Khan, 2011). Vitamin E plus selenium are important food compounds that not only have high antioxidant properties but can also affect different biological processes of the body (Alyari Gavaher et al., 2022). Selenium and vitamin E may interact synergistically to affect biological functions, including immunology and antioxidant activity (Spears and Weiss, 2008). This interaction is especially evident in neutrophil activity, lymphocyte proliferation, and cell-mediated immunity in livestock. Ribeiro et al. (2021) demonstrated similar findings regarding the impact of selenium, along with vitamin E and zinc, on the growth performance of broiler chickens. The current study is intended to assess the implications of using several micronutrients (zinc, vitamin E, and selenium) as antioxidants on broiler growth performance, dressing characteristics, and hematological and serum biochemical parameters.

MATERIALS AND METHODS

Ethical approval

All protocols and ethical use of experimental animals were approved by the Ethical Standard of Research Committee, Bangladesh Agricultural University, Mymensingh-2202 (No. BAURES/ESRC/AH/73).

Experimental layout

In this experiment, 300-day-old Indian River straight-run broiler chicks were reared. The study was conducted at a poultry farm under the Department of Poultry Science, Bangladesh Agricultural University, Mymensingh (Bangladesh). The chicks were assigned to 4 dietary treatments in 3 replications with 25 chickens per replication for 28 days, following a completely randomized design. The treatment groups were control (drinking water with no supplementation), Zn (drinking water with 4 ml/L zinc), Se+Vit-E (drinking water with 0.25 ml/L E-Sel), and Zn+Se+Vit-E (drinking water with both 4 ml/L zinc and 0.25 ml/L E-Sel).

These solutions were employed according to the manufacturer's suggestions. Inorganic zinc was used as Zesup-Vet solution, and Vitamin E and selenium solutions were provided as E-Sel® solution, which includes liquid alpha-tocopherol acetate and sodium selenite as vitamin and mineral supplements (water-soluble nutrients), both manufactured by Square Pharmaceuticals PLC, Bangladesh.

Chickens' management

The trial was performed in a semi-monitored open-sided building. The starter diet (ME 2950 Kcal and Crude protein 22%) was fed for the first 10 days, followed by the grower diet (ME 3050 Kcal and Crude protein 20%) for the broilers until they reached 4 weeks of age. This experiment was performed during the lifespan of chickens from 0 to 28 days of age. Chickens were fed twice daily, ensuring feeders were never empty and fed *ad-libitum*. Treated and non-treated water was administered twice daily. Weekly feed refusals were examined. The brooding temperature started at 34°C and decreased by 2°C weekly until the fourth week. The lighting was constant during the

brooding period, followed by 23 hours of illumination and 1 hour of darkness. All chickenchickens were vaccinated against infectious bursal disease by Nobilis Gumboro 228E (I/O 1 drop at days 11 and 24) and Newcastle disease by Nobilis ND clone 30 (I/O 1 drop at days 4 and 24). Fencing and additional biosecurity measures, such as rat traps and wild chicken protector nets, secured the entire research site from rats and wild animals.

Data collection and record-keeping

After the first day of the experiment, chicks were weighed from each replication once a week up to the end of the experiment. The initial body weight was subtracted from the end body weight to get the average body weight increase for each replicate group under the four treatments. Weights were recorded in the morning before feeding. Every week's feed consumption for the chickens in each replication of all treatment groups was determined by subtracting the quantity of excess feed from the total amount provided. One broiler with equivalent body weight was pulled out from each pen after the study in order to record the parameters and quality of the meat yield. They were killed following the Halal technique (Riaz et al., 2021), which involves cutting the neck and holding the chicken until the bleeding is completely stopped. Skin, feathers, viscera, giblets, legs, and heads were removed from the carcass. Meat yield data, including weights of live, dressed, liver, gizzard, abdominal fat, spleen, thighs, drumsticks, and breast, were recorded by replicating and converted into percentages of live weight before statistical analysis.

Hematological and serum biochemical parameters

To collect 4 or 5 ml of blood from each replication, a series of sterile blood collection tubes having the anticoagulant EDTA at a ratio of 1:10 was utilized. After slaughtering, blood samples were taken from the jugular vein of the broiler chicken. After two hours, the separated blood serum was extracted to an Eppendorf tube and centrifuged for ten minutes at 4000 rpm. The serum was separated using a Microfuge 20R centrifuge (Beckman Coulter Inc., USA). The serum was then put into a different Eppendorf tube and preserved at -20°C until it was time to be assessed. Serum cholesterol, triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), liver enzyme aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were estimated using a commercial kit (Monlab S.L., Spain) with a standard method using the double-beam UV-visible spectrophotometer LAMBDA 365 (Perkin Elmer, Inc., USA). The creatinine test is based on a modified picrate reaction and uses a cromatest solution (Linear Chemicals S.L.U, Spain). For differential lymphocyte count, a blood smear was prepared with Wright's stain, and then methanol fixing was done. In terms of the total number of leucocytes, the percentage of differential leucocyte count (DLC) was represented.

Cost-benefit analysis

The cost-benefit ratio indicates the investment's net benefits and capital cost over time. The profitability index measures the present value of benefits per dollar invested. For the cost-benefit analyses (in US dollars) of the experiment, different parameters were computed, as described by Alabi and Aruna (2005), where Total Revenue (TR) referred to the total income generated from chicken sales, and Net Profit (NP) was determined by subtracting the total cost of production from total revenue. The Cost-Benefit Ratio (CBR) was determined using the TR ÷ TCP method, indicating the experiment's financial feasibility.

Statistical analysis

SAS (2009) general linear models performed a fully randomized variance analysis of broiler chickens' body weight, body weight gain, feed intake, feed conversion ratio (FCR), dressing parameters, and hematological and serum biochemical parameters. Duncan's Multiple Range Test was carried out to assess mean value variation. The significance and higher significant levels were chosen at $p < 0.05$ and $p < 0.01$, respectively.

RESULTS

Growth performances of broiler chicken

Table 1 presents higher significant variances in the body weight at 28^{th} days of age in the supplemented groups ($p < 0.01$). Higher significant variations were noticed in the body weight gain during 15-28 days and a total 0-28 days of age among the treated groups ($p < 0.01$). In supplemented groups, the Zn + Se + Vit-E group represented the highest body weight and weight gain, followed by the Se + Vit-E and control groups. No significant feed consumption changes were seen in treated and non-treated throughout the periods of 0-14 days, 15-28 days, and total 0-28 days of age ($p > 0.05$). Figure 1 indicates a significant variation ($p < 0.01$) of FCR in growing (15-28 days) and total period (0-28 days) among the treated groups. The group supplemented with Zn + Se + Vit-E had an improved FCR value than other treated groups for the period of 0-28 days and 15-28 days of age.

In considering the entire time frame, the Zn + Se + Vit-E group revealed lower FCR in comparison to the other groups.

Dressing parameters

Table 2 shows that no substantial variations were noticed in dressing parameters (dressing %, spleen, liver, abdomen fat, gizzard weight), breast meats, thigh meats and bone, and drumstick meats and bone among the groups ($p > 0.05$). The Zn and Zn + Se + Vit-E treated groups reported a numerically low level of abdominal fat ($p > 0.05$).

Hematological and serum biochemical parameters

Table 3 illustrates that significant variations were found in LDL and cholesterol levels across the dietary groups ($p < 0.05$). The groups supplied with antioxidants showed low concentrations of LDL and cholesterol, while both Zn and Zn + Se + Vit-E groups had relatively similarly low values to the Se + Vit-E supplemented group. However, no significant differences were seen in the levels of creatinine, TG, HDL, ALT, and AST in the treated groups ($p > 0.05$). According to the results, Zn, Se, and Vit-E had no influence on liver and kidney function. The serum differential leukocyte count (DLC) results are listed in Table 4, which suggested no significant variations ($p > 0.05$) among the supplemented groups. All treated groups had a numerical increase in eosinophil and lymphocyte concentrations compared to the control.

Cost-benefit analysis of broiler chicken

Figure 2 shows that the control group possessed the higher production cost per kilogram of chicken, while the Zn + Se + Vit-E group was the lowest. The research estimated the profit per kilogram of chicken to be $0.33 for the control, $0.34 for the Zn, $0.34 for the Se + Vit-E, and $0.35 for the Zn + Se + Vit-E group. As a result, the cost-benefit ratio was greater for the antioxidant-fed chickens compared to the control. Nevertheless, there was no economic variation in the Zn and Se + Vit-E groups.

Table 1. Effects of zinc, selenium, and Vit-E on body weight, body weight gain, and feed intake of broiler chickens

Parameters	Control	Zn	Se+Vit-E	Zn+Se+Vit-E	P Value	LS
Body weight (g/chicken)						
14 days	510.08±2.57	506.72±7.44	507.81±3.47	507.64±5.25	0.964	NS
28 days	1577.53^{c}±2.13	1649.96^{b}±9.51	1636.21^{b}±0.15	1672.00^{a}±7.75	0.0001	**
Body weight gain (g/chicken)						
Period (0-14) days	467.50±2.57	464.27±7.44	466.21±3.47	465.06±5.25	0.970	NS
Period (15-28) days	1067.45^{c}±1.53	1143^{ab}±15.50	1128.40^{b}±3.36	1164.94^{a}±6.57	0.0003	**
Total Period (0-28) days	1543.95^{c}±2.13	1607.5^{b}±9.51	1594.6^{b}±0.15	1630.00^{a}±7.75	0.0001	**
Feed intake (g/chicken)						
Period (0-14) days	561.69±1.42	546.71±6.77	560.12±1.63	557.13±4.58	0.125	NS
Period (15-28) days	1810.55±20.75	1828.40±10.15	1833.96±5.28	1806.31±8.96	0.395	NS
Total Period (0-28) days	2372.25±19.39	2375.11±9.39	2394.08±4.36	2363.44±4.42	0.333	NS

^{abc} means different superscript letters in the same row show significant differences; Data presented as mean ± standard error; **: $p < 0.01$; LS: Level of Significance; NS: Non-significant

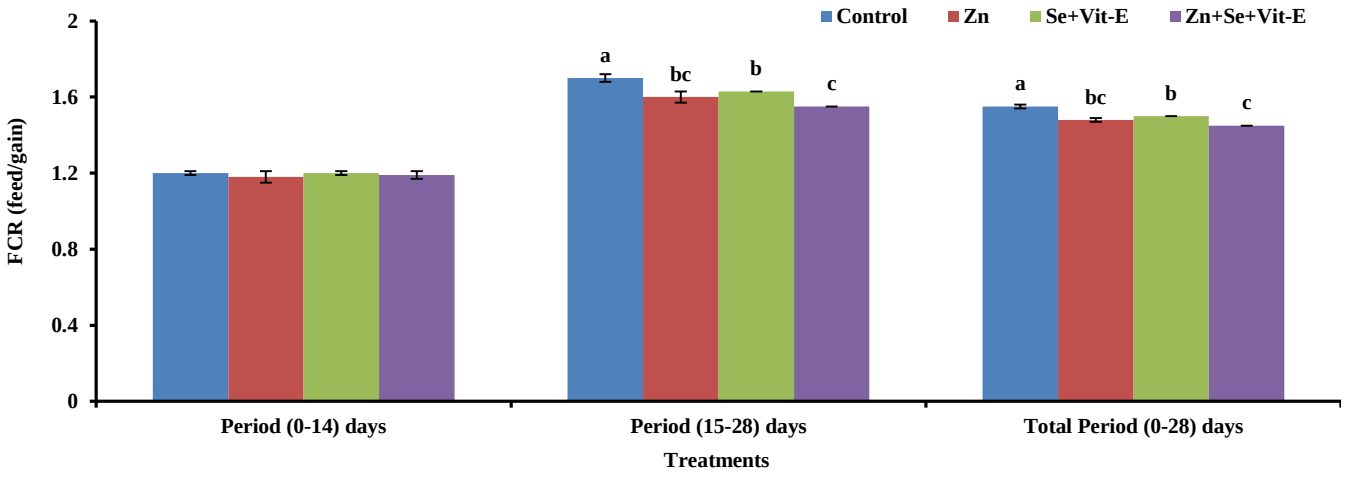

Figure 1. Effects of zinc, selenium, and Vit-E on feed conversion ratio (feed/gain) of broiler chickens. Values are expressed as means ± SD. Bars within a time class not sharing a common letter are significantly different ($p < 0.05$).

Table 2. Effects of zinc, selenium, and Vit-E on dressing parameters of broiler chickens (relation percentage to body weight)

Parameters	Control	Zn	Se+Vit-E	Zn+Se+Vit-E	P Value	LS
Dressing yield	73.06±1.76	70.64±1.45	72.40±0.10	72.31±0.99	0.583	NS
Spleen	0.18±0.03	0.21±0.05	0.19±0.00	0.14±0.03	0.623	NS
Liver	2.84±0.16	2.94±0.07	2.76±0.22	3.29±0.39	0.465	NS
Abdominal fat	0.98±0.06	0.88±0.08	1.08±0.24	0.84±0.04	0.603	NS
Gizzard	1.20±0.05	1.43±0.26	1.35±0.01	1.41±0.16	0.727	NS
Breast meat	11.81±0.26	10.83±0.34	10.71±0.81	10.19±0.22	0.191	NS
Thigh meat	5.06±0.58	5.14±0.34	5.36±0.28	4.99±0.33	0.925	NS
Drumstick meat	3.58±0.28	3.88±0.23	3.29±0.08	3.69±0.23	0.352	NS
Thigh bone	0.87±0.17	0.98±0.08	0.72±0.05	0.98±0.06	0.300	NS
Drumstick bone	1.24±0.08	1.40±0.18	0.99±0.07	1.03±0.32	0.440	NS

Data presented as mean ± standard error; LS: Level of Significance; NS: Non-significant

Table 3. Effects of zinc, selenium, and Vit-E on blood biochemical parameters of broiler chickens (mg/dl)

Parameters	Control	Zn	Se+Vit-E	Zn+Se+Vit-E	P Value	LS
Cholesterol	174.62^{a}±3.67	148.39^{c}±3.36	164.51^{ab}±3.69	152.69^{bc}±6.86	0.014	**
TG	88.33±7.26	91.87±8.34	77.25±9.11	92.60±0.91	0.450	**NS**
HDL	43.70±1.45	37.69±0.69	41.64±1.11	39.11±2.23	0.081	**NS**
LDL	113.25^{a}±3.17	92.33^{b}±4.41	107.22^{ab}±4.88	95.05^{b}±5.30	0.032	*
ALT	27.48±4.43	32.27±3.41	27.59±5.76	27.17±6.67	0.882	**NS**
AST	214.37±3.18	225.32±4.63	229.77±6.86	214.80±1.66	0.101	**NS**
Creatinine	0.61±0.06	0.96±0.15	1.02±0.18	0.95±0.15	0.245	**NS**

^{abc} means having not similar superscripts in the same row differed significantly; Data presented as mean ± standard error; *: $p < 0.05$; **: $p < 0.01$; LS: Level of Significance; NS: Non-significant

Table 4. Effects of zinc, selenium, and Vit-E on hematological (%) parameters of broiler chickens

Parameters	Control	Zinc	Se+Vit-E	Zn+Se+Vit-E	P Value	LS
Neutrophils	31.33±1.45	28.67±2.19	30.33±0.67	31.00±1.73	0.665	NS
Eosinophils	1.33±0.88	1.67±0.67	1.67±0.88	2.00±0.58	0.941	NS
Lymphocytes	64.67±0.88	68.67±1.86	66.00±1.00	65.00±1.52	0.236	NS
Monocytes	2.67±0.33	1.00±0.58	2.00±0.57	2.00±0.58	0.244	NS

Data presented as mean ± standard error; LS: Level of Significance; NS: Non-significant

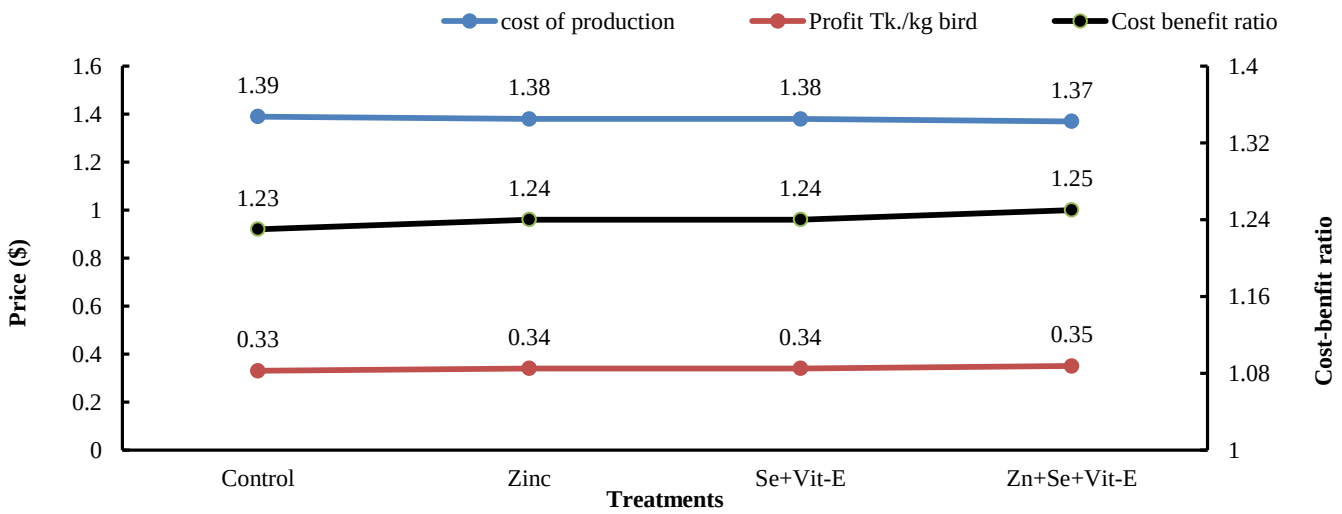

Figure 2. Effects of zinc, selenium, and Vit-E on the cost-benefit ratio of broiler chickens. 1 USD = 119.67 BDT

DISCUSSION

Growth performances of broiler chickens

Human health widely uses zinc, selenium, and vitamin E as medicinal products. The addition of Zn, Se + Vit-E, and Zn + Se+Vit-E to drinking water positively impacted the growth performances of broiler chickens. Zn (80 mg/L) added to drinking water significantly enhanced growth performance (Mohammed et al., 2023). The results of the present study aligned with the findings of Khalifa et al. (2021), who discovered that nutritional supplementation of chickens with Vit-E (100 mg/kg) and Se (0.3 mg/kg) significantly enhanced growth compared to the control. However, Tayeb and Qader (2012) indicated that supplementation of Vit-E and Se (0.45 mg Se +150 mg Vit-E/kg feed) failed to cause significant changes in the growth performance of broiler chicken. Zinc increases glucose utilization and insulin metabolism, which affects weight growth. The Se and Vit-E influence the growth hormone receptor (GHR) and insulin-like growth factor 1 (IGF1), which promote growth performance in broiler chickens (Khalifa et al., 2021). This study revealed no significant variations in feed intake across the dietary groups. The present study corresponded with Tayeb and Qader (2012) and Albuquerque et al. (2017), who demonstrated that no significant changes were in feed consumption across all treatments (Vit-E and Se) and controls. Zhang et al. (2018) stated that Zn did not influence feed intake. This study contradicted the findings of Vit-E and Se by Laganá et al. (2007) and Zn and Vit-E by Hosseini-Mansoub et al. (2010), where supplemented broiler chickens consumed decreased feed intake. Additionally, El-Sebai (2000) stated that supplementation with Se and Vit-E significantly improved feed intake. The current study demonstrated that supplementation of Zn, Se, and Vit-E exhibited improved feed efficiency compared to the control group. The combination of Zn, Se, and Vit-E achieved the maximum feed conversion ratio, followed by the groups receiving Zn, Se + Vit-E, compared to the control group. Hosseini-Mansoub et al. (2010) reported the optimal feed conversion ratio when broilers supplemented with zinc and Vit-E (100 and 50 mg/kg) relative to the control. Ao et al. (2009) discovered that $ZnSO_4$ (31 mg/kg) supplementation affected FCR. The present study agreed with the findings of Laganá et al. (2007), who showed that supplemented chickens with Zn who found that adding Zn (50, 75, and 100 mg/kg) caused a significant improvement in serum cholesterol in broiler breeders; this increase may be attributed to the role of steroid hormones, though the mechanism was not entirely clear. Similarly, Aljumaily and Aljumaily (2021) provided chickens with Se and Vitamin E (0.25 mg and 300 mg/kg), (40 ppm/kg), and Se (0.3 ppm/kg) had a much-improved feed conversion ratio. Ghazi Harsini et al. (2012) reported that adding Vit-E (1 mg/kg) and Se (0.5 mg/kg) enhanced the feed efficiency of chickens in turning feed into meat. Zinc, a cofactor in over 240 enzymes, metabolizes foods including carbohydrates and proteins, boosting growth and reproduction (Chand et al., 2014). The Zn supplementation improved enzyme activity and efficiency of feed by increasing the digestibility of nutrients in broiler chickens (Kucuk et al., 2003). Selenium and Vit-E reduce oxidative stress that impairs growth and feed efficiency. Tayeb and Qader (2012) observed no major changes in the feed conversion ratio among all treatments (Vit-E, 100mg/kg and Se 0.3mg/kg) and controls, while Zhang et al. (2018) mentioned that Zn did not influence feed efficiency.

Dressing parameters

The present study did not observe any significant changes in dressing characteristics among the supplemented groups. Lu et al. (2014) found no significant variations in chicken breast meat, liver, and heart weight in Vit-E-supplemented groups. Perić et al. (2009) found no noteworthy impacts in broiler dressing parameters for the selenium-supplemented group, while Aljumaily and Aljumaily (2021) noticed Se and Vit-E had no consequence on dressing parameters in broiler chickens. Attia et al. (2016) and Yusof et al. (2023) observed no impacts of Zn (0.05 mg/kg and 40 mg/kg consecutively) on dressing percentage in broiler chickens. Moreover, the chickens fed Zn (20 mg/kg) demonstrated a decrease in abdominal fat (Kucuk et al., 2003). In addition, Vit-E had an important role in fat reduction in broiler chickens (Zhang et al., 2021).

Hematological and serum biochemical parameters

The study indicated that the supplemented groups had significantly lower cholesterol and LDL levels than the control group. This study aligned with the findings of Kucuk et al. (2003), Aksu and Ozsoy (2010), and Moustafa et al. (2021) in broiler chickens and Babazadeh et al. (2022) in rats. Mangayarkarasi et al. (2015) found that Zn, Vit-E, and Se (80 mg, 0.25 mg, and 50 mg/kg) administration reduced serum cholesterol and LDL concentrations in broiler chickens. However, the present study contradicted the study of Al-Daraji and Amen (2011), who found that adding Zn (50, 75, and 100 mg/kg)

caused a significant improvement in serum cholesterol in broiler breeders and this increase may be attributed to the role of steroid hormones but mechanism was not quietly clearly, as well as Aljumaily and Aljumaily (2021), who gave chickens Se and Vit-E (0.25 mg and 300 mg/kg). Antioxidant supplements (Se and Vit-E) did not affect TG or HDL. According to Habibian et al. (2014), there were no significant changes in HDL and cholesterol, however, LDL concentrations were higher when chickens' diets supplemented Se and Vit-E. A diet supplemented with Se, Zn, and Vit-E may reduce malondialdehyde production in the liver by increasing glutathione peroxidase enzyme, reducing oxidative damage, and lowering serum cholesterol and LDL levels (Yanardag et al., 2007). The results demonstrated no significant changes in AST, ALT, and creatinine among the supplemented groups. Yusof et al. (2023) found no effect of dietary Zn (40 mg/kg on basal diet) on AST and ALT activity. According to Arslan et al. (2001), Vit-E and Se had no impacts on plasma AST or ALT levels. Conversely, Gul et al. (2022) observed that Vit-E and Se elevated AST, ALT, and creatinine levels in comparison to the control group. The Vit-E and Se decrease oxidative damage to muscle tissues, hence reducing muscle degradation and the consequent synthesis of creatinine (Ryan et al., 2010). Mashkoor et al. (2013) and Aljumaily and Aljumaily (2021) reported that supplementing broiler diets with Se and Vit-E increased lymphocytes. It also supported the findings of Sridhar et al. (2015), who found that supplementing with Zn raised the concentration of lymphocytes. This study deviated from the conclusions of Da Silva et al. (2009), who claimed that Vit-E did not impact broiler hematological parameters and had no noticeable impact on lymphocyte depletion analysis. The present study confirmed the findings of Chand et al. (2014), who reported that although heterophils and eosinophils did not alter, lymphocytes in the treated groups significantly increased. Selenium, a vital component of the antioxidant enzyme glutathione peroxidase (GPx), plays a crucial role in maintaining cellular redox balance and thereby protecting lymphocytes. In response to intestinal infections, Zn stimulates the generation of circulating lymphocytes and antibodies (Lamberti et al., 2013). However, Vit-E and Se treatment had no significant effects on any hematological parameters, according to a study by Tras et al. (2000).

Cost-benefit analysis of broiler

The present study has shown that supplementing with Zn, Se, and Vit-E was the most cost-effective way to improve productivity. The antioxidant-supplemented groups were able to increase their total profit due to increased body weight gain by increasing nutrient metabolism and reducing oxidative stress. Salami et al. (2015) indicated that a combination of dietary antioxidants (Vit-E, Vit-C, Se) might offer significant financial benefits in chickens. Jayanthi et al. (2018) and Abou–Ashour et al. (2022) reported that selenium supplementation increased body weight and reduced production cost in chickens.

CONCLUSION

Diets supplemented with antioxidants, including Zn (4 ml/L), Se, and Vit-E solution (0.25 ml/L), exhibited improved growth and feed efficiency in broilers, leading to greater profit relative to the other treatment groups. Moreover, antioxidant treatments led to a significant decrease in cholesterol and LDL concentrations. Future studies should be conducted to estimate better dosages of these micronutrients in broiler chicken production.

DECLARATIONS

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Authors' contributions

Jakir Hossain contributed to the research, data collection, data analysis, and manuscript preparation. Md. Elias Hossain and Musabbir Ahammed supervised and revised the manuscript. The final version of the article was reviewed and approved by all the authors, who also looked over the information submitted in this publication.

Availability of data and materials

This article incorporates all research data, with additio nal material attainable upon reasonable request from the corresponding author.

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Ethical considerations

The manuscript underwent scrutiny for ethical issues, including plagiarism, permission to publish, misconduct, data fabrication and falsification, double publishing, and redundancy by all authors.

Competing interests

All authors have declared that they have no conflicts of interest.

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Association Between Genetic Polymorphisms of Growth Hormone Gene and Egg Production Traits in Chickens: A Systematic Review

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ABSTRACT

Chicken performance traits are affected by the chicken growth hormone (cGH) gene due to its essential part in metabolism and growth, and genetic polymorphisms may be useful as a genetic marker for growth traits. However, no comprehensive review provides information on the cGH polymorphisms and their correlation with egg production traits. The study systematically reviewed the single nucleotide polymorphisms (SNPs) of the growth hormone gene and their association with the chicken's egg production traits. Four databases, Google Scholar, ScienceDirect, PubMed, and Web of Science, were used to search the literature where the keywords 'growth hormone, single nucleotide polymorphisms, genetic variations, genetic effects, egg production traits, and chickens were the keywords during the literature search. The outcomes revealed that four articles published in 2013, 2014, 2015, and 2018 were included. The results indicated that four SNPs (T185G, G662A, T3094C, and C3199T) were identified, with allelic frequencies ranging from 0.020 to 0.964 and genotypic frequencies ranging from 0.007 to 0.930. The findings indicated that some of the articles used more than one breed. The present reveiw revealed that egg number was found to be significantly associated with discovered genotypes six times, while body weight at first egg and egg weight at first egg were found to be significantly related to discovered genotypes four times. However, additional research is required to validate the identified SNPs. Furthermore, identified SNPs could serve as possible molecular markers to genetically improve egg production in chickens.

Keywords: Average egg weight, Body weight, Egg number, Egg weight, Genotype

INTRODUCTION

The poultry industry has a noticeable position as one of the main sources of animal protein for human consumption. Poultry is crucial in genetic research due to breeding practicality, quite short generation intervals, and phenotypic variations. The enhancement of economic traits in chickens has progressively gained attention, and the documentation and use of quantitative trait loci (QTLs) offer the possibility for genetic enhancement in selection programs with no slaughtering (Kazemi et al., 2018). The chicken growth hormone (cGH) gene is one of the highly vital genes that affect chicken performance traits due to its significant role in growth and metabolism, and it has four exons and five introns with an overall length of 4098 bp (Makhsous et al., 2013). Single nucleotide polymorphisms (SNPs) studies help in the identification of gene variants and their possible relationships with the phenotypic expression of useful traits and are a significant aspect of the development of breeding systems that make use of marker-assisted selection (MAS) of traits that influence the economic value of the animal in that breeding program (Kulibaba, 2015). SNPs and their relations with egg production traits has been conducted in different chicken breeds around the world such as Fars Native and

Mazandaran Native fowls in Iran (Makhsous et al., 2013; Kazemi et al., 2018), Recessive White and Qingyuan partridge chicken breeds in China (Su et al., 2014) and Poltavskaya Glinistaya Chickens in Ukraine (Kulibaba, 2015). Based on the authors' knowledge, the literature on SNPs of chicken growth hormone gene and their relationships with egg production traits has not yet been systematically investigated. To address the documented knowledge gap, the study conducted a systematic review of the literature on the impact of single nucleotide polymorphisms in the chicken growth hormone gene on egg production traits. The current study aimed to guide chicken breeders and researchers in identifying potential genetic markers that can be used in the selection of chickens for the improvement of egg production traits during breeding.

MATERIALS AND METHODS

Eligibility criteria

The articles with the word "chicken", "egg production traits", and "single nucleotide polymorphisms" were used for the literature search.

Literature search

The authors searched the research articles using the following databases, including Google Scholar, ScienceDirect, PubMed, and Web of Science up to August 15, 2023, where the keywords used were growth hormone (GH), single nucleotide polymorphisms, genetic variations, genetic effects, egg production traits, and chickens.

Inclusion criteria

The eligibility of all the acquired articles was set to the extent where the following words were fulfilled by the studies: The chickens' GH gene was under investigation, included the polymorphisms of the GH gene, and chickens were included as the animal of interest.

Exclusion criteria

The criteria for exclusion involved articles with duplicated records, not published in the English language, other poultry species, such as ducks, and lacking the association of chicken GH polymorphisms to egg production traits.

Data extraction

The authors extracted the articles from databases independently. The information retrieved from the articles included the name of the first author, the chicken breed, the year of publication, population size, the country, egg production traits, and genotyping procedure.

RESULTS

Literature reveiw

A total of 96 articles were retrieved for the systematic review from the following databases, including Google Scholar (n = 37), Web of Science (n = 27), ScienceDirect (n = 16), and PubMed (n = 16), as displayed in Figure 1. The duplicates (n = 21) found in the search databases were eliminated, and the remaining articles were evaluated for inclusion and exclusion criteria. The remaining articles (n = 75) were screened for their title, and sixty-eight (n = 68) were excluded. The screening for the abstract was done, and three (n = 3) were excluded. The study systematically reviewed all the articles searched and excluded articles not eligible for the study, and a total of four articles were included in the systematic review.

Characteristics of included articles

About four studies of the 96 articles were reserved for inclusion in the literature review (Table 1). The included articles all investigated growth hormone (GH) gene single nucleotide polymorphisms (SNPs) and their correlation with egg production traits in chickens. Out of 4 included articles, 3 (75%) of them (Makhsous et al., 2013; Kulibaba, 2015; Kazemi et al., 2018) used PCR-RFLP as a genotyping method, except 1 article (25%) used PCR-LDR genotyping method (Su et al., 2014). All the articles included in the present study used different chicken breeds, however, all used native chicken breeds.

Targeted chicken growth gene genomic regions

The findings displayed that all the reviewed articles targeted the intron 1 region (Table 2). The results showed that one article (Kulibaba, 2015) out of the four articles reviewed targeted intron 1 and intron 4 regions, while the other articles reviewed targeted intron 1 only. Two articles (Makhsous et al., 2013; Su et al., 2014) out of four articles did not identify the regions targeted.

Identified single nucleotide polymorphisms

Table 2 shows the SNPs identified and their positions in the articles involved. The outcomes displayed that one article (Su et al., 2014) out of four involved articles discovered the SNPs and their positions, and 4 similar SNPs (T185G, G662A, T3094C, C3199T) were identified. The findings also revealed that the 4 SNPs were discovered from two different chicken breeds (Qingyuan partridge and Recessive White chickens).

Allelic and genotypic frequencies

Table 2 displays allelic and genotypic frequencies discovered from the included articles. The findings indicated that all four reviewed articles showed the allelic frequencies, while three (Makhsous et al., 2013; Kulibaba, 2015; Kazemi et al., 2018) of the included articles showed the genotypic frequencies. The findings indicated that the allelic frequency ranged from 0.020 to 0.964, while the genotypic frequency ranged from 0.007 to 0.930.

Identified SNPs' genotypes and their relationship with the egg production traits

Identified genotypes and their correlation with egg production traits of included articles are presented in Table 3. Five chicken breeds (Recessive White, Qingyuan partridge, Mazandaran native, Poltavskaya Glinistaya, and Fars Native) were used to study the relationship between identified SNPs' genotypes and egg production traits. The article studied in Recessive White and Qingyuan partridge chicken breeds found that the genotypes of SNPs T185G and T3094C were associated with BFE, EWFE, and EN (Su et al., 2014). The article investigated the Poltavskaya Glinistaya chicken breed and found that the genotypes were associated with EN and WE (Kulibaba, 2015), while the article examined the Fars Native chicken breed and found that identified genotypes were associated with EN and ELR (Makhsous et al., 2013). These results found that EN was significantly related to genotypes six times, while BFE and EWFE were found to be significantly related to genotypes four times.

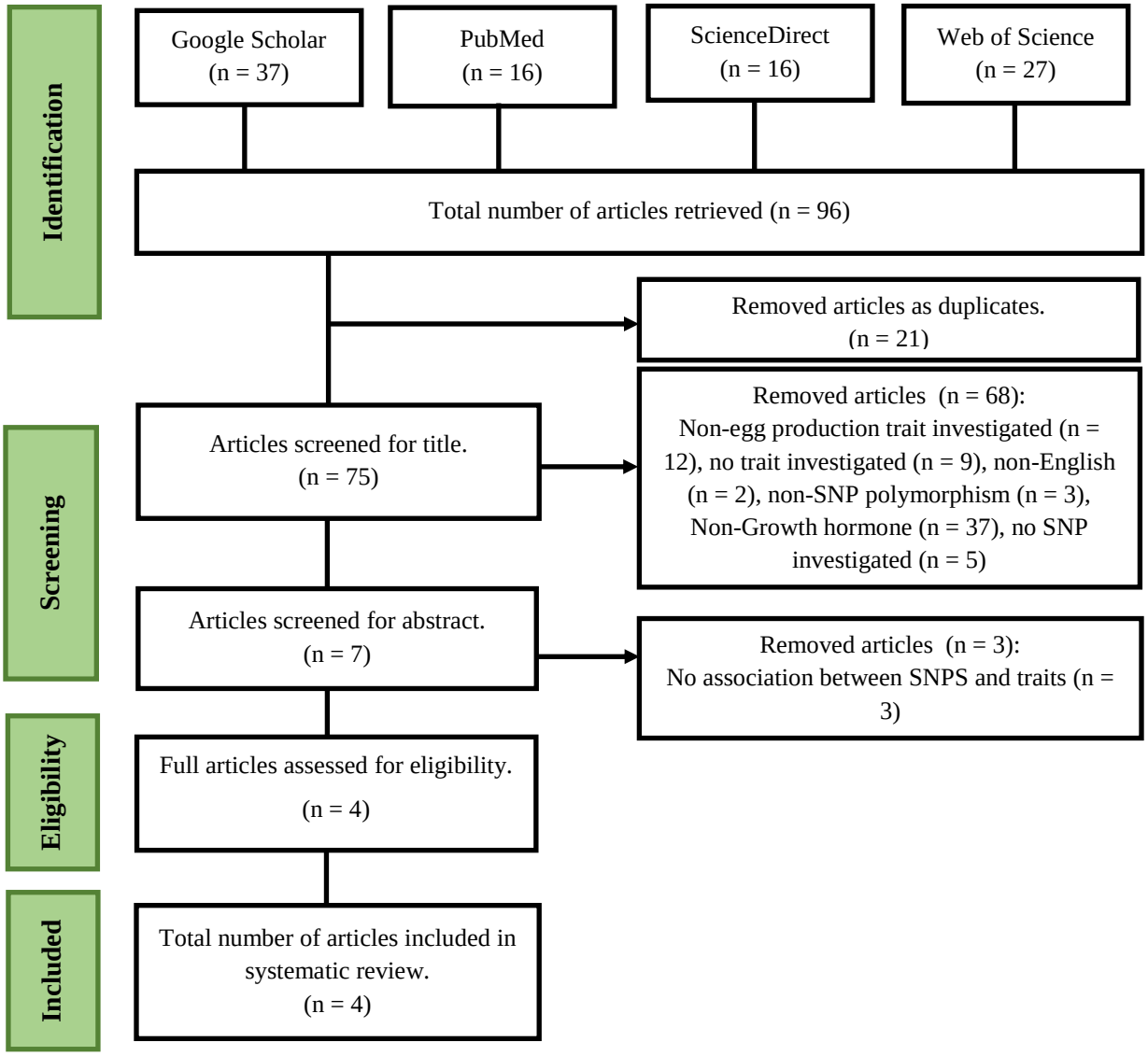

Figure 1. The process of article selection in the present study

Table 1. Characterization of the articles included for the present systematic review

Author	Year	Country	Breed	N	Egg production traits	Genotyping method
Kazemi et al.	2018	Iran	Mazandaran native Fowls	380	EN, LI, EW, AEW, PH	PCR-RFLP
Kulibaba	2015	Ukraine	Poltavskaya Glinistaya chickens	98	EN, EW	PCR-RFLP
Makhsous et al.	2013	Iran	Fars Native Chickens	142	EW, AFE, ELR	PCR-RFLP
Su et al.	2014	China	Recessive White chickens and Qingyuan partridge chickens	136; 187	EN, BWFE, EWFE, AFE	PCR-LDR

N: Sample size, EN: Egg number, LI: Laying intensity, EW: Egg weight, AEW: Average egg weight, PF: Percentage fertility, PH: Percentage hatchability, AFE: Age at first egg, ELR: Egg laying rate, BWFE: Body weight at first egg, EWFE: Egg weight at first egg, PCR-LDR: Polymerase chain reaction-ligase detection reaction, PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism.

Table 2. Identified single nucleotide polymorphisms and their positions in the included articles

Breed	SNP	Region	Allelic frequencies	Genotypic frequencies	Author
Recessive White chickens	T185G	Not identified	T (0.812), G (0.188)	Not identified	Su et al. (2014)
Recessive White chickens	G662A	Not identified	G (0.435), A (0.565)	Not identified	Su et al. (2014)
Recessive White chickens	T3094C	Not identified	T (0.200), C (0.800)	Not identified	Su et al. (2014)
Recessive White chickens	C3199T	Not identified	C (0.713), T (0.287)	Not identified	Su et al. (2014)
Qingyuan Partridge chickens	T185G	Not identified	T (0.951), G (0.049)	Not identified	Su et al. (2014)
Qingyuan Partridge chickens	G662A	Not identified	G (0.287), A (0.713)	Not identified	Su et al. (2014)
Qingyuan Partridge chickens	T3094C	Not identified	T (0.122), C (0.878)	Not identified	Su et al. (2014)
Qingyuan Partridge chickens	C3199T	Not identified	C (0.831), T (0.169)	Not identified	Su et al. (2014)
Mazandaran Native fowls	Not identified	Intron 1	A (0.305), B (0.098), C (0.597)	AA (0.10), BB (0.01), CC (0.36), AB (0.07), AC (0.34), BC (0.12)	Kazemi et al. (2018)
Poltavskaya Glinistaya chickens	Not identified	Intron 1	A (0.908), B (0.020), C (0.072)	AA (0.820), AB (0.040), AC (0.140)	Kulibaba (2015)
Poltavskaya Glinistaya chickens	Not identified	Intron 4	A (0.036), B (0.964)	AB (0.070), BB (0.930)	Kulibaba (2015)
Fars Native Chickens	Not identified	Not identified	A (0.599), B (0.102), C (0.299)	AA (0.338), AB (0.113), AC (0.409), BB (0.007), BC (0.070), CC (0.063)	Makhsous et al. (2013)

Table 3. Identified single nucleotide polymorphisms and their association with egg production traits

Author	Breed	SNP	Egg production traits	Genotypes			Sig
Su et al. (2014)	Recessive white chickens	T185G	AFE	TT	TG	GG	ns
		T185G	BFE	TT	TG	GG	*
		T185G	EWFE	TT	TG	GG	*
		T185G	EN	TT	TG	GG	*
		T3094C	AFE	TT	TC	CC	*
		T3094C	BFE	TT	TC	CC	*
		T3094C	EWFE	TT	TC	CC	*
		T3094C	EN	TT	TC	CC	*
Su et al. (2014)	Qingyuan partridge chickens	T185G	AFE	TT	TG	GG	ns
		T185G	BFE	TT	TG	GG	*
		T185G	EWFE	TT	TG	GG	*
		T185G	EN	TT	TG	GG	*
		T3094C	AFE	TT	TC	CC	*
		T3094C	BFE	TT	TC	CC	*
		T3094C	EWFE	TT	TC	CC	*
		T3094C	EN	TT	TC	CC	*
Kazemi et al. (2018)	Mazandaran native fowls	Not identified	BFE	AA	BB	CC	ns
			EW	AA	BB	CC	ns
			EN	AA	BB	CC	ns
			LI	AA	BB	CC	ns
			PH	AA	BB	CC	ns
			AEW	AA	BB	CC	ns
Kulibaba (2015)	Poltavskaya Glinistaya chickens	Not identified	EW	AA	BB	AB	*
			EN	AA	BB	AB	*
Makhsous et al. (2013)	Fars native chickens	Not identified	EN	AA	BB	CC	*
			EW	AA	BB	CC	ns
			ELR	AA	BB	CC	*

EN: Egg number, LI: Laying intensity, EW: Egg weight, AEW: Average egg weight, PF: Percentage fertility, PH: Percentage hatchability, AFE: Age at first egg, ELR: Egg laying rate, BFE: Body weight at first egg, EWFE: Egg weight at first egg, Sign: Significant, *: Significant at $p < 0.05$, ns: Non-significant.

DISCUSSION

Egg production traits are economically important in poultry production, and they can be affected by the genetic makeup of the birds (Su et al., 2014). Enhancing the economic traits in chickens has progressively gained attention, and the documentation and exploitation of QTLs offer a possibility for genetic enhancement in selection programs without slaughtering (Kazemi et al., 2018). The findings indicated that out of the 4 articles included in the study, only 1 article identified four similar SNPs (T185G, G662A, T3094C, C3199T) in the two chicken breeds, namely, Recessive White chickens and Qingyuan partridge (Su et al., 2014). The findings further displayed that out of the four SNPs identified, only two SNPs showed significant association with the egg production traits. Su et al. (2014) indicated the T185G SNP and discovered that it is associated with three egg production traits: Egg weight at first egg, body weight at first egg, and egg number, however, there was no significant association with age at first egg laying. In addition, T3094C SNP was associated with body weight at first egg laying, age at first laying, egg weight at first laying, and egg number in Recessive White chickens and Qingyuan partridge chickens. Kazemi et al. (2018) reported that the SNP found in the study had no significant association with all the studies' egg production traits, namely egg weight, body weight at first egg, laying intensity, egg number, percentage hatchability, and average egg weight in Mazandaran native fowls. Kulibaba (2015) found that the SNP linked significantly with egg weight and egg number in Poltavskaya Glinistaya chickens, while Makhsous et al. (2013) reported that the SNP was found to correlate with egg number and egg laying rate, but no significant association with egg weight in Fars Native Chickens. The present systematic review confirms the influence of the chicken growth hormone gene on egg production traits. Kazemi et al. (2018) targeted the intron 1 genomic region during the study, while Kulibaba (2015) targeted intron 1 and 4 genomic regions. According to Su et al. (2014), the chicken growth hormone gene intron is highly polymorphic. According to the knowledge of the authors, this systematic review is one

of the first studies on the relationship of the growth hormone gene SNPs with egg production traits. Thus, the current study does not compare with other systematic review findings.

CONCLUSION

Two SNPs associated with egg production traits can be used as genetic markers to improve egg weight at first laying, body weight at first laying egg, and egg number. According to the present systematic review, there is insufficient information on the identified SNPs of the growth hormone gene and their relationship with egg production traits. The growth hormone gene influences the egg production traits, and the T185G and T3094C SNPs may be employed as possible genetic markers for improving the egg production traits in chickens during breeding.

DECLARATIONS

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Availability of data and materials

All data are presented in this article but the prepared data are available upon reasonable request from authors.

Ethical considerations

The ethical issues considered by all authors while this review results were checked for plagiarism, informed consent, misconduct, and data manipulation.

Authors' contributions

The conceptualization of the study was done by Victoria Rankotsane Hlokoe and Thobela Louis Tyasi. Victoria Rankotsane Hlokoe was responsible for the methodology and original draft preparation, and Victoria Rankotsane Hlokoe and Thobela Louis Tyasi were responsible for reviewing and editing the manuscript. The manuscript's final edition has been read and agreed upon by all authors.

Competing interests

The authors state that there is no conflict of interest.

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Roles of Environment, Nutrition, and Genetics in Woody Breast Condition in Chickens

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ABSTRACT

Woody breast (WB) condition is a muscle disease in broiler chickens that makes breast meat hard and rubbery, and it has a negative impact on the texture and appearance of fast-growing broiler chicken breast muscle. This condition is safe for consumers, but the meat generally goes under extra meat processing, such as making pet foods, because of lower consumer acceptance, which is an additional cost for the industry. The exact etiology of myopathy is unknown. Although there is no promising solution for the issue, several strategies, such as nutrition, have been introduced to reduce the WB rate. The present study reviewed the strategies that improved WB conditions, including genetics, nutritional, and environmental factors such as temperature and air quality.

Keywords: Broiler chicken, Environment, Genetic, Mitochondria, Nutrition, Woody breast

INTRODUCTION

Woody breast (WB) negatively impacts the texture and appearance of chicken breast meat. The fillets show noticeable stiffness and distinct histological composition (Sun et al., 2022). Although the meat has no health risks to humans, it significantly costs the poultry industry (Caldas-Cueva and Owens, 2020), as the meats are mostly used for byproducts that require further processing, and the final products are cheaper than the breast muscle.

Studies linked the problem to the disruption of ATP production (Wang et al., 2023) and mitochondria function (Shakeri et al., 2024). However, it has been indicated that more factors are related to the severity of WB, such as genetics, nutrition, and environment (Caldas-Cueva and Owens, 2020). Several approaches have been used to improve the WB, but so far, none of the strategies mentioned above have offered a completely promising solution. Therefore, the present review aimed to offer a list of strategies that reduced WB severity in broiler chickens.

ENVIRONMENT

Improving environmental factors such as the housing condition and environmental temperature can significantly impact the development of WB in chickens, potentially, they can be related to oxygen deprivation in the breast muscles, which is considered a key contributor to this muscle condition (Caldas-Cueva and Owens, 2020).

Temperature

Short/long-term high environmental (2-3h for short-term and 8-12h for long-term) temperatures worsen the severity of WB (Al-Abdullatif et al., 2024). High environmental temperature exacerbates muscle oxygen deprivation and contributes to WB development. Under heat stress, a chicken's body generates an excessive amount of reactive oxygen species (ROS) (Shakeri et al., 2020), leading to higher damage to muscles by increasing fibrous connective tissue that contributes to WB (Shakeri et al., 2023).

Furthermore, heat stress disrupts normal muscle growth by reducing the absorption of essential nutrients such as minerals and vitamins, contributing to the development of WB. Studies indicated that additives such as minerals (Cauble et al., 2020; Kuttappan et al., 2021) and vitamins (Kuttappan et al., 2021; Meyer and Bobeck, 2023) or a combination of both could improve WB condition by reducing oxidative stress in breast muscle tissues. A combination of vitamin E and selenium improves enzyme activity while reducing oxidative stress, leading to improved chicken performance under heat stress (Shakeri et al., 2020). The optimum environmental temperature for adult broiler chickens is between 18-22 °C (Shakeri et al., 2020). Therefore, to reduce the impacts of heat stress on WB, proper solutions should be considered during warm seasons to cope with the problem, such as reducing the number of birds per area, increasing housing ventilation, and supplementing diets with additives such as antioxidants and betaine.

Humidity

Optimum relative humidity (50–70% after brooding and 60–80% during brooding, RH) plays a major role in the health and productivity of broiler chickens. Excessive RH may compromise gut health and the immune system (Chigwada et al., 2022), leading to higher oxidative damage to tissues (Mishra and Jha, 2019). A combination of RH and heat creates a stressful condition that elevates the production of ROS, leading to potential damage to tissues and affecting their growth performance and meat quality, including WB (Oke et al., 2024).

Stocking Density

Higher stocking density increases heat stress incidents during the summer or in tropical areas, which can negatively impact broiler chickens' health and performance (Shakeri et al., 2015). High stocking density leads to higher oxidative damage to tissues (Shakerı et al., 2015) by altering immunity and gut health (Chigwada et al., 2022). Previous works indicated that reducing the number of birds per area could reduce oxidative stress in broiler chickens, resulting in a reduction in WB (Pekel et al., 2020; Son et al., 2022). The optimum space required for a bird is 0.1m^2 (v) (Pettit-Riley et., 2002).

Ventilation

Poor ventilation can lead to reduced oxygen levels in the chicken house, further contributing to hypoxia in the breast muscles. WB links to a lack of sufficient oxygen supply to the breast muscle (Shakeri et al., 2024), causing a condition known as hypoxia, which leads to oxidative stress and muscle damage. Considering proper ventilation systems for industrial buildings could improve the air quality of broiler chickens. Ventilation is essential to provide healthy environmental conditions. Unfortunately, failing to maintain adequate house temperatures will force broilers to consume excess feed to maintain body temperature rather than spend on growth performance. The feed used for maintenance cannot be used for growth, and this will have a detrimental effect on the feed conversion ratio and flock performance (Tabler, 2014).

Nutrition

This strategy is the cheapest and most practical method to cope with WB issues. Diet composition, including the different levels of protein, energy, and certain nutrients, can influence muscle development and the likelihood of WB.

Minerals

Minerals such as selenium reduce the incidence of WB in broiler chickens (Kuttappan et al., 2021). Trace minerals such as selenium are bio-available and reduce oxidative stress in tissues (Shakeri et al., 2020). A study showed that trace minerals had a 44% reduction in severe WB (Kuttappan et al., 2016) compared to other groups in their study. The reason behind the benefits might be related to the antioxidant properties of the minerals (Horváth and Babinszky, 2018). The addition of minerals to a diet has been shown to maintain the antioxidant defense and lead to healthy longevity (Tan et al., 2018). Antioxidants are essential in reducing ROS in tissues and protecting them against oxidative damage. Additionally, mitochondria-targeted antioxidants have great potential against damage by eliminating excessive ROS (Oyewole and BirchMachin, 2015).

Vitamins

Vitamins B complex, C, E, and D play a major role in healthy muscle function and blood circulation, which can be important for preventing muscle damage that contributes to WB. Among all vitamins, vitamin C seems to be the most effective one against WB (Meyer and Bobeck, 2023). Supplementing diets with vitamin C has been evaluated to significantly reduce WB (Cemin et al., 2018). Vitamin C is a potent antioxidant that can help regulate the production of ROS and reduce oxidative stress damage. It can also protect the cardiovascular system by improving mitochondrial function and maintaining antioxidant levels (Zheng et al., 2024).

Amino Acids

There is a strong link between imbalances in amino acid availability and the development of breast muscle, which is associated with increased oxidative stress (Trithavisup et al., 2024). Amino acid oxidation helps the body to release energy. However, a higher protein intake may increase the oxidation of amino acids, which can lead to oxidative stress if the antioxidant defense is disrupted. Reducing the overall level of amino acids, particularly by lowering the ratio of arginine to lysine, can help mitigate the occurrence of WB (Meyer and Bobeck, 2023) by potentially preventing the loss of muscle mass and function.

GENETICS MODIFICATIONS

Fast-growing breeds, such as Ross and Cobb, and heavier broiler chickens have a higher incidence of WB (Caldas-Cueva and Owens, 2020). There are several studies indicating that high breast meat yield broiler chickens showed a higher incidence of WB compared to not genetically modified birds (Mazzoni et al., 2015; Petracci et al., 2013). Studies also indicated that WB can be detected at early ages, as early as 3 weeks, suggesting the problem could be related to genetics (Che et al., 2022). The genetic modifications may have side effects such as altering mitochondrial function by affecting different proteins (Shoop et al., 2023), potentially leading to disruptions in energy production within the cell and leading to various alterations, including WB. Previous studies associated WB with mitochondria function and energy production (Shakeri et al., 2023; 2024).

CONCLUSION

Several factors, including environment and genetics, impact the severity of WB, while appropriate supplementation of vitamin C, minerals, or amino acids could improve the condition by removing ROS and improving mitochondria function. However, the additives alone cannot fully eliminate the problem. Therefore, the current review suggests that environmental and genetic factors potentially are better strategies to cope with the WB.

DECLARATIONS

Availability of data and materials

All provided data in the text is available upon reasonable request.

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Authors' contributions

Majid Shakeri contributed to the preparation of the manuscript. Majid Shakeri checked and approved the final version of the manuscript for publication in the present journal.

Competing interests

The author has declared that no competing interest exists.

Ethical considerations

The author has checked the ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy.

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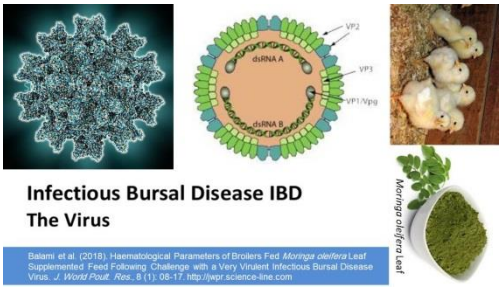

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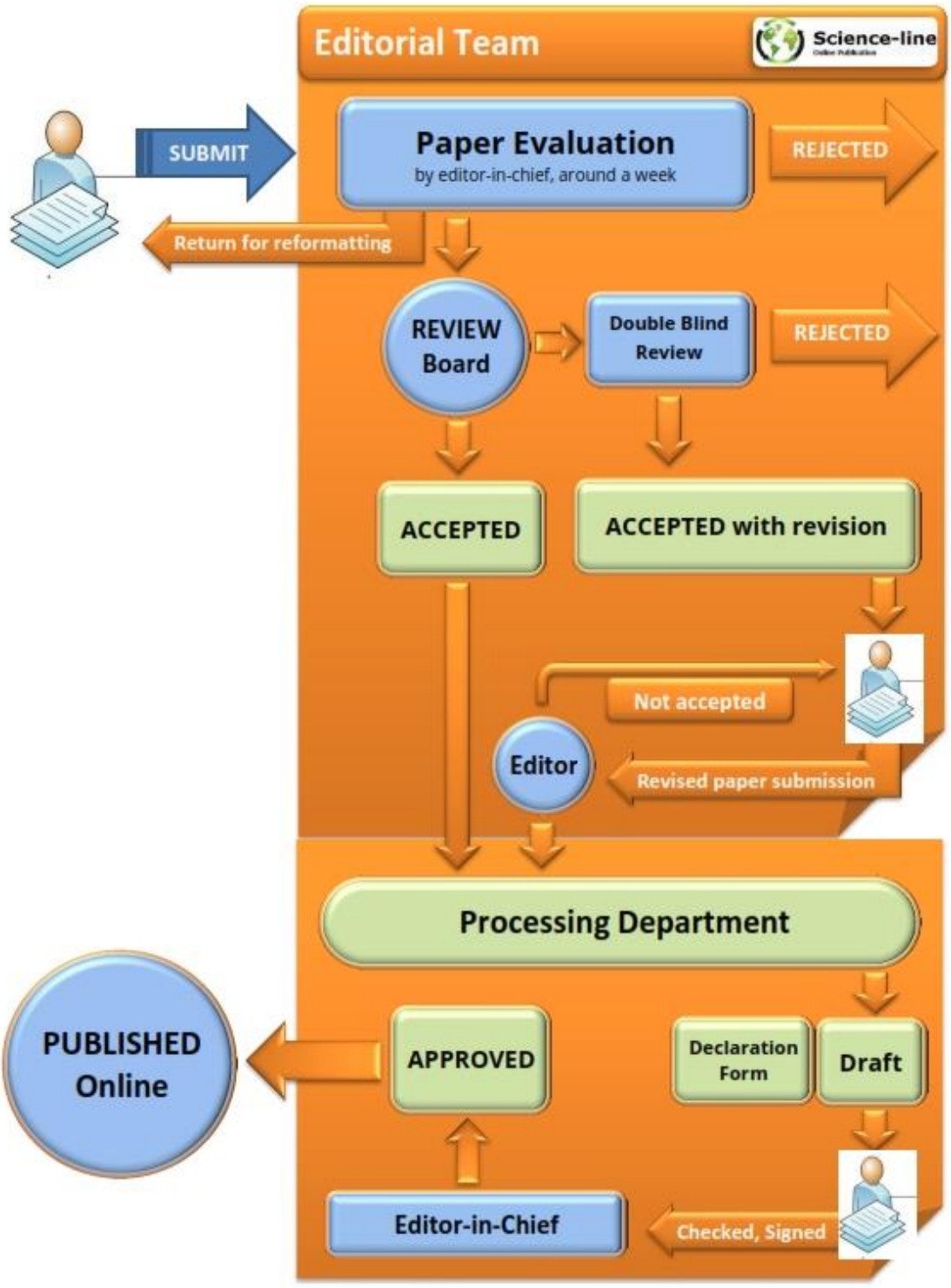

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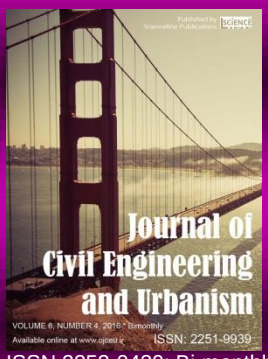

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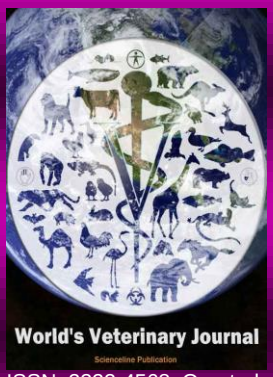

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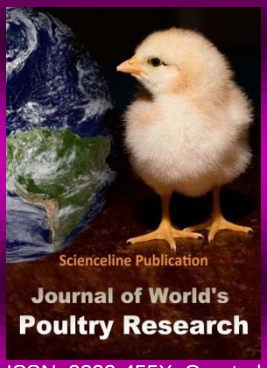

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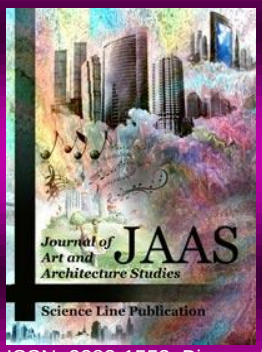

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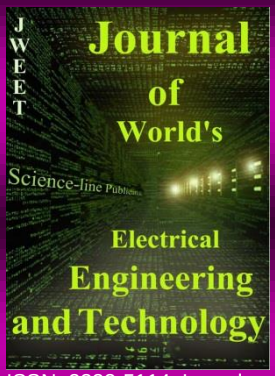

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