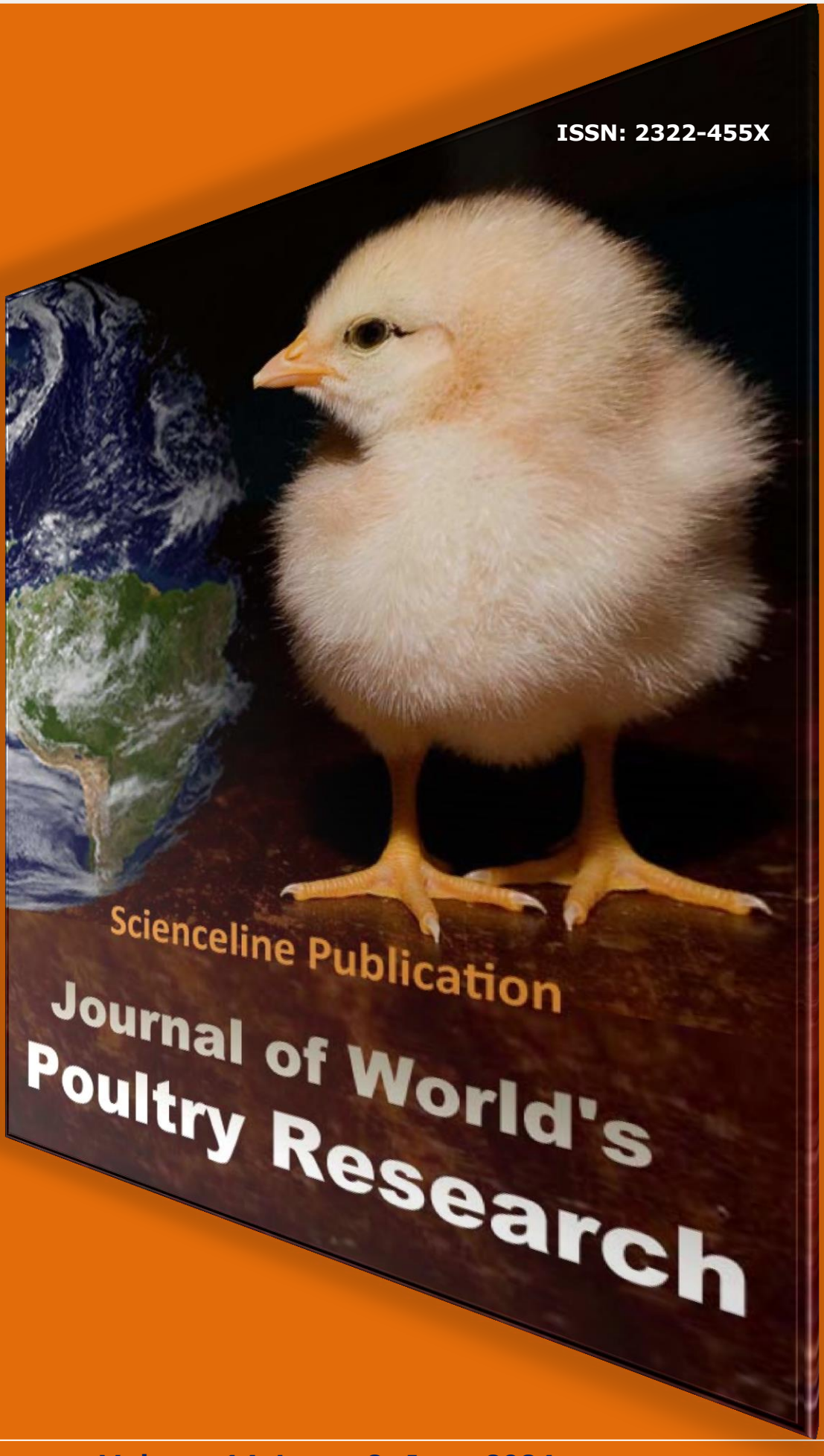
Journal of World's Poultry Research

BOOKLET



Volume 14, Issue 2, June 2024

JWPR
Journal of World's Poultry Research

2024, Scienceline Publication

J. World Poult. Res. 14 (2): 124-263, June 25, 2024

Advisory Board

TABLE OF CONTENT

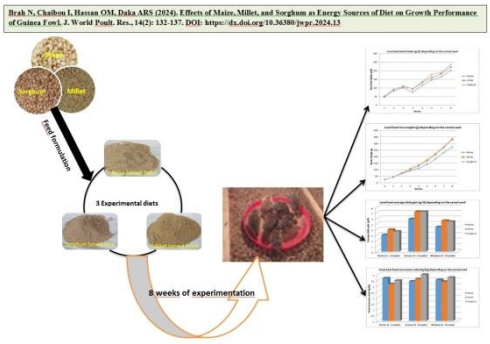
Volume 14 (2); June 25, 2024

Research Paper

Isolation and Molecular Identification of *Candida albicans* from the Oral cavity of Domestic Chickens using 28S rDNA in Diyala Governorate, Iraq

Kadhim MA, Abdulameer SJ, Al-Dulaimi OGJ, and Al-Azzawi AK.

J. World Poult. Res. 14(2): 124-131, 2024; pii: S2322455X2400012-14
DOI: https://dx.doi.org/10.36380/jwpr.2024.12



ABSTRACT: *Candida albicans* (*C. albicans*) is an opportunistic fungal pathogen that affects humans, animals, and birds. It is one of the most prevalent microbes found in clinical specimens. Candidiasis refers to endogenous fungal overgrowth under conditions of microbiota disruption or other host impairments. The study aimed to detect and isolate *C. albicans* based on morphological, cultural, and biochemical analysis. The present study examined 36 oral cavity samples from domestic chickens suspected of having candidiasis across various regions of Diyala Governorate in Iraq from September to November 2023. A total of twenty *C. albicans* were isolated from collected samples. PCR amplification of the 28S rDNA fungal gene, yielding the expected 260bp products, confirmed the identification of the yeasts. Using the Vitek 2 automated system, minimum inhibitory concentrations (MICs) for 6 common antifungal drugs were determined to test the antifungal susceptibility of a *Candida* clinical isolate. No resistance was found to Amphotericin B, Fluconazole, Flucytosine, Voriconazole, Caspofungin, or Micafungin in isolates from the oral cavity. In conclusion, the current study identified twenty *C. albicans* strains from chicken oral cavities and found them to be susceptible to all major classes of antifungals, indicating a lack of antifungal resistance in these isolates.
Keywords: *Candida albican*, demostic chicken, Vitek 2, 28S rDNA.

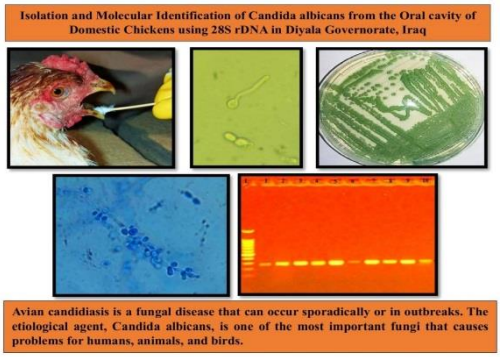
[Full text-PDF] [Crossref Metadata]

Research Paper

Effects of Maize, Millet, and Sorghum as Energy Sources of Diet on Growth Performance of Guinea Fowl

Brah N, Chaibou I, Hassan OM, Daka ARS.

J. World Poult. Res. 14(2): 132-137, 2024; pii: S2322455X2400013-14
DOI: https://dx.doi.org/10.36380/jwpr.2024.13



ABSTRACT: Guinea fowls are more valuable in the market in Niger than chickens; however, their ability to meet consumer demands is limited by low productivity. Therefore, suitable nutrition is an important factor for this productivity. The current study aimed to evaluate the growth performance of guinea fowl using maize, millet, and sorghum as energy sources in the diet. A total of 108 one-day-old unsexed local keets, with an average live weight of 25.5 ± 0.83 g were randomly distributed among three dietary treatments with four replicates per treatment. The keets were reared on the ground with litter at CERRA Maradi, Niger, for an 8-week experimental period, involving 36 keets per treatment and 9 keets per replication. The parameters monitored were feed intake (FI), live weight (LW), average daily gain (ADG), and feed conversion ratio (FCR). After 8 weeks of experimentation, the results indicated that the cereal used in the diet had no statistically significant effect on the keets' FI. Those fed millet-based diets had higher LW, compared to those fed maize and sorghum diets, respectively, however, this difference was not statistically significant. Millet also facilitated a higher ADG, compared to maize and sorghum, although the differences were statistically insignificant. The keets fed sorghum-based had higher FCR, compared to the FCR of the guinea fowls fed maize and millet diet. based on the growth performance assessed in this study, the recommended order for cereals in guinea fowl feed to ensure better growth is millet, followed by maize, and then sorghum.
Keywords: Cereals, Energy source, Growth performance, Keets

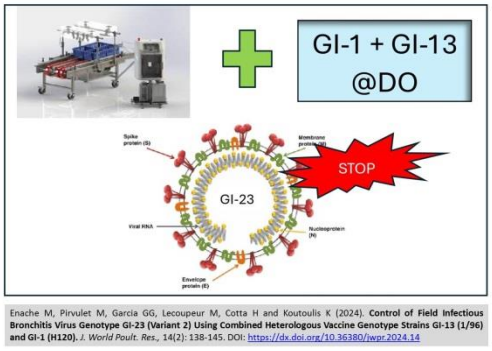
[Full text-PDF] [Crossref Metadata]

Research Paper

Control of Field Infectious Bronchitis Virus Genotype GI-23 (Variant 2) Using Combined Heterologous Vaccine Genotype Strains GI-13 (1/96) and GI-1 (H120)

Enache M, Pirvulet M, Garcia GG, Lecoupeur M, Cotta H and Koutoulis K.

J. World Poult. Res. 14(2): 138-145, 2024; pii: S2322455X2400014-14
DOI: https://dx.doi.org/10.36380/jwpr.2024.14



Enache M, Pirvulet M, Garcia GG, Lecoupeur M, Cotta H and Koutoulis K (2024). **Control of Field Infectious Bronchitis Virus Genotype GI-23 (Variant 2) Using Combined Heterologous Vaccine Genotype Strains GI-13 (1/96) and GI-1 (H120).** *J. World Poult. Res.*, 14(2): 138-145. DOI: https://dx.doi.org/10.36380/jwpr.2024.14

ABSTRACT: Infectious bronchitis (IB) is a disease with significant economic impacts both on the costs of control strategies and on productive losses. Various vaccination protocols are applied, depending on homologous or heterologous protection against IB and finding the optimal balance between costs and benefits as a choice by a responsible veterinarian. The current case study aimed to demonstrate the efficacy of a heterologous vaccine combination against field IBV GI-23 (Variant 2) infection when vaccination quality was properly monitored. Two groups, each consisting of six flocks, were examined before and after improving the quality of IB vaccine application in the hatchery. These groups were vaccinated with H-120 and 1/96 vaccine strains for heterologous protection. The study involved field visits, necropsies, serology via ELISA, and oropharyngeal sampling for RT-PCR follow-up activities. Moreover, performance parameters including average body weight, feed conversion ratio, and 7 days plus total mortality were analyzed at the end of the production cycle at 40-45 days of age. Results indicated that the group with enhanced vaccination quality in the hatchery exhibited a significant decrease in IBV titers and an absence of IBV GI-23 field infection. Additionally, there was an improvement in performance data in terms of average body weight, FCR and total mortality. Hatchery vaccination proved to be more controllable and practical compared to traditional on-farm vaccination, ensuring better control of the vaccination process and massive coverage of the farm population.
Keywords: Heterologous protection, Infectious bronchitis, Vaccine strain 1/96, Variant 2

[Full text-PDF] [Crossref Metadata]

Research Paper

Effects of Dried khat (*Catha edulis*) Leaves as a Natural Feed Additive on the White Leghorn Layers' Performance

Asfaw AG, Abebe MG, Senbeta EK, and Mulatu K.

J. World Poult. Res. 14(2): 146-153, 2024; pii: S2322455X2400015-14
DOI: https://dx.doi.org/10.36380/jwpr.2024.15

Asfaw AG, Abebe MG, Senbeta EK, and Mulatu K (2024). Effects of Dried khat (*Catha edulis*) Leaves as a Natural Feed Additive on the White Leghorn Layers' Performance. *J. World Poult. Res.*, 14(2): 146-153. DOI: https://dx.doi.org/10.36380/jwpr.2024.15

ABSTRACT: Khat contains many bioactive compounds that are beneficial for chickens' health. Most of the chemical constituents found in Khat are biologically active and are used worldwide for the treatment of many diseases. However, there are few studies on the use of Khat in poultry as a nutrition, antioxidant, and antimicrobial activity. Therefore, the present study evaluated the effects of dried Khat leave (DKL) as a natural feed additive on the White Leghorn layer's performance, serum chemistry, and hematology. A total of 180 White Leghorn chickens, aged 168 days were randomly divided into four groups consisting of 45 chickens in three replications. Thirteen layers and two cocks were assigned to each replication and reared on a deep litter system. The diets of layers in T1, T2, T3, and T4 were supplemented by DKL at proportion of 0%, 0.2%, 0.4%, and 0.6%, respectively. The layers were evaluated for feed intake, body weight gain, egg production, feed conversion ratio, and some blood parameters, such as total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, albumin, globulin, red blood cells, hemoglobin, packed cells volume, white blood cells, and total protein. Feed intake in T1 (103.5 g) was significantly higher than T4 (98.5 g) and T3 (98.9 g). Layers in T1 significantly gained higher body weight than layers in other treatments. The use of DKL in layer chickens' diet did not affect egg production, egg mass, and feed conversion ratio. An increase in the level of DKL in the layers diet significantly reduced the serum high-density lipoprotein, cholesterol, and albumin. The present study showed that chickens that received a higher proportion of dried khat leaf (0.6%) significantly recorded lower levels of low-density lipoprotein and total cholesterol in their blood.
Keywords: Blood analysis, Dried Khat leaf, Layer, White Leghorn

[Full text-PDF] [Crossref Metadata]

Research Paper

Association of Different Body Sizes and Egg Quality Characteristics in White Leghorn Chicken Breed of South Africa

Tyasi TL, Sathekge LJ, and Hlokoe VR.

J. World Poult. Res. 14(2): 154-159, 2024; pii: S2322455X2400016-14
DOI: https://dx.doi.org/10.36380/jwpr.2024.16

Tyasi TL, Sathekge LJ, and Hlokoe VR (2024). **Association of Different Body Sizes and Egg Quality Characteristics in White Leghorn Chicken Breed of South Africa.** *J. World Poult. Res.*, 14(2): 154-159. DOI: https://dx.doi.org/10.36380/jwpr.2024.16

ABSTRACT: Egg quality could influence the price of the table and hatching eggs. However, the effects of hen's live body weight on egg characteristics are poorly understood. The present study evaluated the influence of body weight (BW) on egg characteristics, such as egg width (EWD), shell weight (SW), egg length (EL), albumen weight (AW), yolk weight (YW), shell surface index (SI), albumen ratio (AR), shell ratio (SR), and yolk ratio (YR) of White Leghorn chicken. A total of 100 White Leghorn chickens at 30 weeks of age were used in the current study of which 300 eggs were collected. Live body weight was classified into three groups namely, small (≤ 195g), medium (196-220g), and large (≥ 221g). The results revealed that the White Leghorn layer's live body weight was positively influenced by the eggshell index and egg length. Additionally, the results indicated that body weight did not affect EW, SW, EWD, SR, YW, AW, AR, and YR. In conclusion, the live body weight influences egg length and shell index in the White Leghorn chicken breed.
Keywords: Chicken, Egg length, Egg weight, Egg width, Shell index

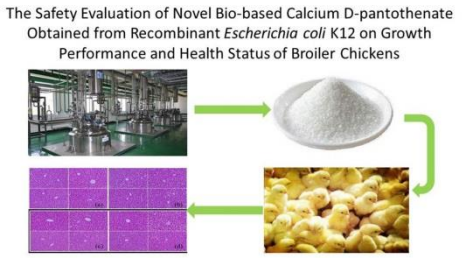
[Full text-PDF] [Crossref Metadata]

Research Paper

The Safety Evaluation of Novel Bio-based Calcium D-pantothenate Obtained from Recombinant Escherichia coli K12 on Growth Performance and Health Status of Broiler Chickens

Liu G, Xu X, Zhang L, Li C, Li M, and Zhang H.

J. World Poult. Res. 14(2): 160-171, 2024; pii: S2322455X2400017-14
DOI: https://dx.doi.org/10.36380/jwpr.2024.17



Liu G, Xu X, Zhang L, Li C, Li M, Zhang H (2024). *J. World Poult. Res.*, 14(2): 160-171. DOI: https://dx.doi.org/10.36380/jwpr.2024.17

ABSTRACT: The production of traditional synthesized calcium D-pantothenate (D-PA) is accompanied with chemical pollution, therefore, the eco-friendly bio-fermentation technology has received widespread attention. In order to verify the safety of a novel D-PA product produced by genetically engineered bacteria (*Escherichia coli* K12), the authors of the current study investigated the influence of adding D-PA to the diet on growth performance and health status of broiler chickens. A total of 192 day-old healthy Arbor Acres broiler chickens with similar weight (43.21 ± 0.12 g) were randomly divided into 4 treatments with 6 replicates and 8 broiler chickens in each replicate (male and female in half). The *Escherichia coli* K12 was genetically engineered for the production of D-PA. The control group was fed with the basal diet containing 20 mg/kg synthesized D-PA (CT group). The treatments were supplemented with 20 (TCaP1 group, recommended dose group), 100 (TCaP5 group, 5-fold-dose group), and 200 (TCaP10 group, 10-fold-dose group) mg/kg bio-based D-PA product, respectively. The experiment lasted for 42 days and the growth performance and health status of broiler chickens were determined. The results indicated that the addition of 5- and 10-fold doses of bio-based D-PA could increase the average daily weight gain during 22-42 days of age and decrease the feed conversion rate during 22-42 and 1-42 days of age of broilers. There were some differences in white blood cell count, intermediate cell absolute value (MID) count, absolute granulocyte count, absolute lymphocyte count, granulocyte percentage, mean corpuscular volume, red blood cell distribution width-standard deviation, mean platelet volume and serum phosphorus and total bilirubin in different groups, compared with the CT group. Histological observations of the liver, spleen, pancreas, and small intestines showed that the tissue structures of various organs of the broiler chickens fed with the bio-based D-PA were clear, and no abnormal changes such as inflammatory cell infiltration and fibrous tissue hyperplasia were observed in all groups. In summary, dietary supplementation of bio-based D-PA was safe within the 10-fold- dose (200mg/kg) to broiler chickens during 1-42 days.
Keywords: Biological safety evaluation, Broiler chicken, Calcium D-pantothenate, Growth performance, Vitamin B_5

[Full text-PDF] [Crossref Metadata]

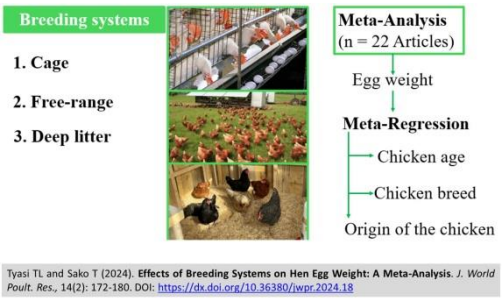
Review

Effects of Breeding Systems on Hen Egg Weight: A Meta-Analysis

Tyasi TL and Sako T.

J. World Poult. Res. 14(2): 172-180, 2024; pii: S2322455X2400018-14

DOI: https://dx.doi.org/10.36380/jwpr.2024.18



Tyasi TL and Sako T (2024). **Effects of Breeding Systems on Hen Egg Weight: A Meta-Analysis**. *J. World Poult. Res.*, 14(2): 172-180. DOI: https://dx.doi.org/10.36380/jwpr.2024.18

ABSTRACT: In the egg production industry, egg weight is a critical parameter influencing economic viability. The objective of the present study was to determine the effect of cage, free-range, and deep litter breeding systems on hen egg weight using meta-analysis. Articles were searched using Google Scholar, PubMed, ScienceDirect, and Web of Science yielding 175 articles of which 22 articles were included in the present study. Methodological quality was assessed using Joanna Briggs Institute guidelines. A model was used to determine the effect of breeding systems on average hen egg weight. Meta-regression analysis was used to examine the effect of the following moderators, publication year, region, chicken age, and breed. The Cochran's Q test and I^2 statistic were performed for h heterogeneity across used studies. According to the obtained results, there was no significant difference between cage and free-range on average hen egg weight (standardized mean difference (SMD) = 0.08, I^2 = 89%, 95%CI 0.19-0.34). The free-range breeding system had heavier hen egg weight than deep litter (SMD = 0.54, I^2 = 88%, 95%CI 0.01-0.08). The findings also revealed that deep litter and free range had no significant difference in average hen egg weight (SMD = -0.05, I^2 = 87%, 95%CI -0.28-0.17). Meta-regression findings showed that the origin of the used articles, the age of the chickens, and the chicken breed were observed as the main reasons for heterogeneity. This meta-analysis revealed that a free-range breeding system increased the average hen egg weight.
Keywords: Breeding system, Cage, Deep litter, Free-range, Meta-analysis

[Full text-PDF] [Crossref Metadata]

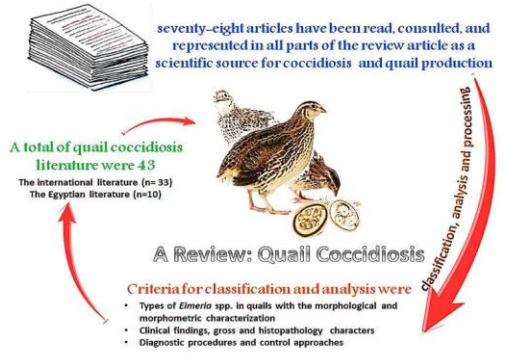
Review

Landmarks in Quail Coccidiosis Research with Special Scrutiny to the Available Egyptian Literature: A Review

ElBakrey RM, Helal SS, ElKholy AA, and Eid AAM.

J. World Poult. Res. 14(2): 181-195, 2024; pii: S2322455X2400019-14

DOI: https://dx.doi.org/10.36380/jwpr.2024.19



ElBakrey RM, Helal SS, ElKholy AA, and Eid AAM (2024). **Landmarks in Quail Coccidiosis Research with Special Scrutiny to the Available Egyptian Literature: A Review**. *J. World Poult. Res.*, 14(2): 181-195. DOI: https://dx.doi.org/10.36380/jwpr.2024.19

ABSTRACT: Quails are an important alternative to chicken production for protein sources, offering many advantages over other poultry species. However, raising quail faces certain challenges, such as a shortage of specified hatcheries and the lack of local markets for quail eggs and meat, particularly in Egypt. In addition, there is less interest in quail's medication and vaccine production. A significant disease affecting the health and productivity of quails is coccidiosis, which is associated with poor feed conversion ratio, lower growth performance, heightened mortality, and high cost of vaccination and treatment. Attention to quail coccidiosis and its clinical forms, diagnosis, morphological characterization, control, and prevention is very critical for improving quail meat and egg production. This review compiles scientific data on quail coccidiosis, with a focus on literature from Egypt, for classification, data analysis, and processing.

Keywords: Anticoccidial, Coccidiosis, Eimeria, Egyptian, Morphology characterization, Quail

[Full text-PDF] [Crossref Metadata]

Research Paper

Enterobacteriaceae Antibiotic Resistance Identification in Slender-billed Gull Species Migrating to Libya

Mansour A, Sharif E, Hamhoom A, Eldaghayes I, Etayeb Kh, Dayhum A and Kammon A.

J. World Poult. Res. 14(2): 196-203, 2024; pii: S2322455X2400020-14
DOI: https://dx.doi.org/10.36380/jwpr.2024.20

ABSTRACT: The Libyan coast has various types of wetlands that are passed by many migratory birds throughout the year, however, studies on bacterial isolation from these birds in Lybia are scarce. The present study aimed to isolate enteropathogenic bacterial species from the seagulls that migrated to the Libyan coast as well as identification of the antibiotics that are resistant to these bacteria. A total of 50 fresh fecal samples were collected from slender-billed gulls in January 2023 at Farwa Island near the city of Zuwara, in Western Libya. Bacteria were isolated by conventional culturing method, identified using the Enterosystem 18R, and antibiotic susceptibility tests were conducted on the isolated bacteria. The results revealed the isolation of 46 bacteria, but only 32 of them were identified using biochemical tests. These identified bacteria belong to six species of Enterobacteriacae, namely *Citrobacter* (*C.*) *freundii*, *Pantoea* (*P.*) *agglomerans, Escherichia (E.) coli*, *Enterobacter (En.) cloacae*, *Serratia liquifaciens*, and *Proteus mirabilis*, with percentages of 53.125%, 31.25%, 6.25%, 3.125%, 3.125%, and 3.125%, respectively. All isolated bacteria were 100% sensitive to gentamicin (10 µg) and ciprofloxacin (5 µg). The highest resistance result was observed against the antibiotic cefoxitin (30 µg), with both *C. freundii* (5 samples) and *P. agglomerans* (4 samples). Resistance was observed in 5 samples of *C. freundii* and 4 samples of *P. agglomerans* out of 11 samples. Resistance to antibiotics, such as azithromycin (15 µg), ceftriaxone (30 µg), and ampicillin (10 µg), was also noted in a few isolates. The results indicated that *C. freundii* was the most antibiotic-resistant bacterial species isolated in this study. The highest multiple antibiotic resistance index was demonstrated by bacteria *C. freundii*, *P. agglomerans*, and *En. cloacae*, with a value of 0.33 for each of them. In conclusion, slender-billed gulls carry multi-drug-resistant bacteria. The study recommends the implementation of a national program to survey antibiotic-resistant bacteria, determine their prevalence, and assess the presence of antibiotic residues in animal-derived food. Furthermore, the present study advises expanding scientific studies on risk analysis, and antibiotic alternatives in migratory birds.
Keywords: *Citrobacter freundii*, Enterobacteriaceae, Multiple antibiotic resistance, Prevalence, Slender-billed gull

[Full text-PDF] [Crossref Metadata]

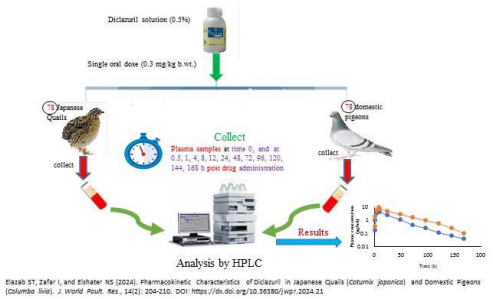
Research Paper

Pharmacokinetic Characteristics of Diclazuril in Japanese Quails (*Coturnix japonica*) and Domestic Pigeons (*Columba livia*)

Elazab ST, Zafar I, and Elshater NS.

J. World Poult. Res. 14(2): 204-210, 2024; pii: S2322455X2400021-14

DOI: https://dx.doi.org/10.36380/jwpr.2024.21



ABSTRACT: Coccidiosis, caused by the protozoan *Eimeria*, is a significant disease in poultry farms worldwide, leading to substantial economic losses. Triazines, benzene-aceto-nitrile derivatives, are widely employed in the field of veterinary healthcare to combat the hazardous impacts of protozoan parasite infestation comprising coccidiosis. The current research was designed to investigate the pharmacokinetic profile of diclazuril, a member of triazines, in Japanese quails (*Coturnix japonica*) and domestic pigeons (*Columba livia*) following single oral administration at 0.3 mg/kg body weight. 78 Quails (male: female, 1:1, 7 weeks old) and 78 pigeons (male: female, 1:1, 4 weeks old) were randomly divided into 13 groups for each species (n=6 birds/ group). Plasma samples were obtained at various time intervals (at time 0 [preceding diclazuril administration], and 0.5, 1, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours after diclazuril administration) to determine its concentration utilizing high-performance liquid chromatography (HPLC). The non-compartmental approach was applied to assess the pharmacokinetic parameters via the aid of WinNonlin 8.3 software. In quails and pigeons, the peak plasma concentrations were 5.35 and 9.14 µg/mL attained at 8 hours, respectively. Additionally, the elimination half-lives ($T_{1/2\lambda z}$) were 30.74 and 26.48 hours, and the area under the plasma concentration-time curve from time zero to the last sample ($AUC_{0\text{-last}}$) values were 155.67 and 343.57 µg h/mL, respectively. The mean residence time was 30.71 hours in quails and 39.68 hours in pigeons. Diclazuril exhibited favorable pharmacokinetic characteristics after oral administration at a dose of 0.3 mg/kg in quails and pigeons. However, to adjust the dosage

regimen for curing coccidiosis, a future study is warranted to determine the clinical efficacy against coccidia infection. Moreover, further investigation is needed to evaluate the tissue residues and calculate the withdrawal time of diclazuril in quails and pigeons.
Keywords: Diclazuril, High-performance liquid chromatography, Japanese quail, Pharmacokinetic, Pigeon

[Full text-PDF] [Crossref Metadata]

Research Paper

Evaluation of Salmonella Enteritidis Isolated from Layer Hens and Murine Fecal Pellets in Poultry Farms of Libya

Benlashehr I, Elmasry K, Kammon A, and Asheg A.

J. World Poult. Res. 14(2): 211-218, 2024; pii: S2322455X2400022-14

DOI: https://dx.doi.org/10.36380/jwpr.2024.22

ABSTRACT: The rodents play a significant role in the transmission of *Salmonella* between farms and regions. The present study aimed to compare the virulence of *Salmonella enteritidis* isolated from fecal samples of laying hens and murine within the same poultry house but different regions in Libya using Vivo-quantitative measurement of invasiveness (chicken intestinal loop model). A total of 540 cloacal swabs from laying hens (Hy-line brown chickens) aged 36 weeks and 200 batches of murine fecal pellets were collected from the same poultry house at Gaser Bin Gisher and Furnag regions in Libya. The samples were passed on pre-enrichment broth (Buffered Peptone Water) and enrichment broths (Rappaport Vassiliadis, Selenite broth, and tetrathionate), then the samples were cultured onto Xylose Lysine Deoxycholate agar, brilliant green agar, *Salmonella* Shigella agar, and Hektoen enteric agar. Single colonies were selected and stained by gram stain and tested biochemically using analytical profile index (API) 20 tests. *Salmonella enteritidis* was isolated from all the collected samples. The invasion of *Salmonella enteritidis* isolated from laying hens and murine feces was significantly higher in the anterior inoculation position compared to the posterior position of jejunum in both regions. The account of *Salmonella enteritidis* isolated from laying feces of hens and murine at Gaser Bengasher region was significantly higher than that isolated from the AlFurnge region. In the present study, the rodents act only as mechanical transmitters without affecting *Salmonella* invasiveness capacity. Furthermore, the invasion of *Salmonella enteritidis* depends on the inoculation position in the jejunum. Moreover, the invasiveness variation of *Salmonella enteritidis* isolated from the Gaser Bengasher and AlFurnge regions could be attributed to the presence of different *Salmonella* strains in the studied area. *Salmonella enteritidis* isolated from poultry and murine in the current study was sensitive to gentamicin, ciprofloxacin, and enrofloxacin and resistant to doxycycline, chloramphenicol, sulfafurazol, and ampicillin.
Keywords: Invasiveness, Layer chicken, Murine infestation, *Salmonella enteritidis*

[Full text-PDF] [Crossref Metadata]

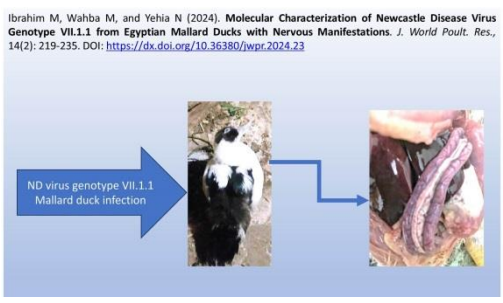
Research Paper

Molecular Characterization of Newcastle Disease Virus Genotype VII.1.1 from Egyptian Mallard Ducks with Nervous Manifestations

Ibrahim M, Wahba M, and Yehia N.

J. World Poult. Res. 14(2): 219-235, 2024; pii: S2322455X2400023-14

DOI: https://dx.doi.org/10.36380/jwpr.2024.23



ABSTRACT: In Egypt, Newcastle disease virus (NDV) strains of genotype VII are known to be mild in domestic waterfowl and considered reservoirs. This is the first report for the detection of NDV GVII.1.1 from ducks showing severe clinical signs with high mortalities and nervous manifestations, additionally, isolation of NDV and molecular characterization for full HN and F genes were performed. In the current study, 16 backyard mallard duck flocks showing severe nervous signs with high mortalities were investigated by real-time RT-PCR using primers specific for the Fusion gene of NDV and matrix gene for avian influenza virus (AIV). Fourteen duck flocks tested positive for AIV and only two flocks tested positive for NDV infection. NDV was isolated from the trachea and brain of the same duck from each flock then full HN and F genes were sequenced. The phylogenetic analysis of the F and HN genes indicated that these strains were clustered with NDV genotype VII 1.1. The F gene had a specific mutation that cluster them in a new branch with with A11T in the

signal peptide, N30S, T324A, and 480K in the hydrophobic heptad repeat (HRc) compared to the Lasota strain. The duck strains of NDV isolated from the brain had N294K in the hydrophobic heptad repeat-b (HRb) of F protein compared to the strains isolated from the trachea of the same duck, which may have a role in crossing the blood-brain barrier. The HN protein had a specific mutation that clustered them in a new branch with mutations of A4V, R15K in the cytoplasmic region, A28T in the transmembrane domain, and S76L in the HRa. In addition, HN protein had A50T, S54R T232N, P392S, and T443V, and multiple mutations were detected in the neutralizing epitopes specific to strains in the present study (N120G, K284R, S521T) that can alter virus antigenicity. The current study indicated the continuous evolution of NDV strains from genotype VII circulating in Egypt with increasing pathogenicity in ducks. The present findings demonstrated the urgent need for the vaccination of ducks and geese with killed NDV vaccines to reduce economic losses due to virus infection and prevent transmission to chickens helping in ND control in Egypt.
Keywords: F gene, Genotype VII 1.1, Mallard duck, Newcastle disease virus, Protein

[Full text-PDF] [Crossref Metadata]

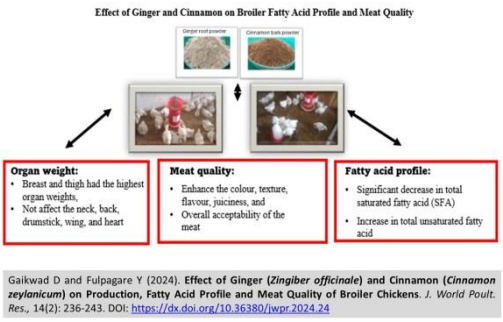
Research Paper

Effect of Ginger (*Zingiber officinale*) and Cinnamon (*Cinnamon zeylanicum*) on Production, Fatty Acid Profile and Meat Quality of Broiler Chickens

Gaikwad D and Fulpagare Y.

J. World Poult. Res. 14(2): 236-243, 2024; pii: S2322455X2400024-14

DOI: https://dx.doi.org/10.36380/jwpr.2024.24



ABSTRACT: Phytogenic feed additives play an important role in broilers' nutrition, contributing to the improvement of the performance and quality of meat. The study aimed to evaluate the effect of Ginger (*Zingiber officinale*) and Cinnamon (*Cinnamon zeylanicum*) on broiler chicken production, fatty acid profile, and meat quality. In the present study, 140-day-old Vencob-400 broiler chicks were divided into 7 groups, including the control group (with no additives, T0), and T1 to T6 groups receiving varying concentrations of cinnamon and ginger. Accordingly, the chickens' diet in T1 was supplemented with 1.0% cinnamon, T2 with 2.0% cinnamon, T3 with 3.0% cinnamon, T4 with 1.0% ginger, T5 with 2.0% ginger, and T6 with 3.0% ginger, all calculated based on dry matter. The carcass traits, weight of immune organs, organoleptic tests, and fatty acid profile of meat (breast and thigh) were recorded after the age of 42 days. The findings indicated that the breast and thigh had the highest organ weights in group T4 compared to other groups, however, the neck, back, drumstick, wing, and heart were not affected. The inclusion of 2% cinnamon (T2) and 1% ginger (T4) in the diet, significantly enhanced the color, texture, flavor, juiciness, and overall acceptability of the meat, compared to the diet of the control group. Adding a supplement of 2% cinnamon or 1% ginger powder to the diet of broiler chickens significantly decreased the percentage of total saturated fatty acid and increased the total unsaturated fatty acid (breast and thigh). The improvement in fatty acid composition is beneficial for the quality of the broiler meat. Based on these findings, it is recommended to supplement the diet of the broiler with either 2% cinnamon or 1% ginger powder to improve the carcass parameters and quality of the meat.
Keywords: Broiler meat, Characteristic, Cinnamon, Ginger, Quality

[Full text-PDF] [Crossref Metadata]

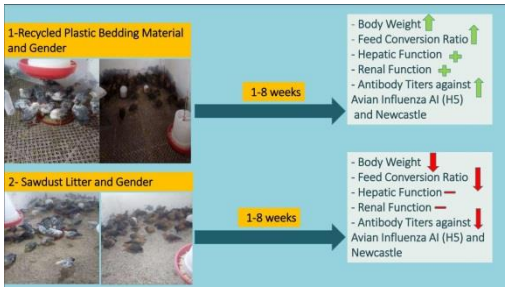
Research Paper

Efficiency of Recycled Plastic Bedding Material and Gender in Improvement of Productive Traits, Physiological, and Immunological Parameters of Hybrid Broiler Chickens

EL-Masry MA, Hassan MS, Arafa AA, El-Afifi TM, Bealish AM, Ouda MM, Fathey IA, Fahmy HA and Abd El-Atty HK.

J. World Poult. Res. 14(2): 244-254, 2024; pii: S2322455X2400025-14

DOI: https://dx.doi.org/10.36380/jwpr.2024.25



ABSTRACT: Litter management is important for poultry housing husbandry and affects chicken performance. The present study evaluated the effect of bedding material and gender on the productive, physiological, and immunological performance of a new hybrid chicken (WINZY Line 105) under cold stress for 56 days of age. A total of 540

one-day-old broiler chicks were divided into two groups. The groups, including 270 males (M) and 270 females (F) were further divided into two sub-groups, including sawdust litter (SL) and plastic slatted floor (PSF), three replicates, and 45 chickens each. Broiler chickens were raised during the winter with an average temperature of 10oC for 56 days. Productive, physiological, and immunological performance parameters were measured. The obtained results indicated that M reared on PSF (M x PSF) recorded the highest values in body weight (BW), body weight gain (BWG), carcass characteristics, and the best values in feed conversion compared with other interaction groups during all experimental periods. In addition, F reared on PSF had higher BW, carcass, and thigh percentages, low feed intake (FI), and best feed conversion compared to females reared on SL. The M reared on PSF had the highest hepatic enzymes except AST which was higher in F reared on PSF than other treatments. However, renal function biomarkers (Creatine, Uric acid, Urea) were higher in both M and F that were reared on SL than those reared on PSF. Moreover, there was a significant interaction detected for antibody titters against avian influenza (H5) and Newcastle disease at 21 days of age suggesting that the highest values observed for M reared on PSF (M x PSF) compared with other interaction groups during the experimental period, and it was higher in F that reared on PSF than F reared on SL at 21 days of age. It can be concluded that plastic slatted floors could be an alternative to substitute wood shavings to raise broiler chickens since it was efficient from the perspective of environmental conditions and production rates.
Keywords: Bedding material, Environmental adaptation, Hybrid chicken, Immunological parameter, Productive trait, Plastic slatted floor, Performance

[Full text-PDF] [Crossref Metadata]

Research Paper

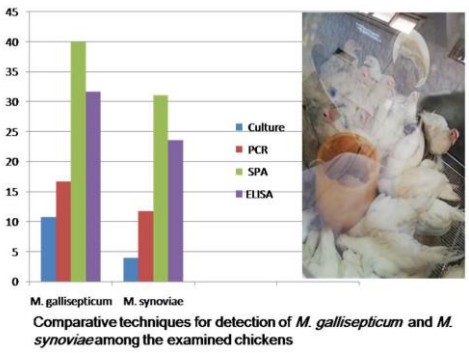
Comparative Study of Various Diagnostic Methods for Detection of Mycoplasma gallisepticum and Mycoplasma synoviae in Egyptian Chicken Flocks

Emam M, Hashem YM, Ismael E, El Hariri M, and El-Jakee J.

J. World Poult. Res. 14(2): 255-263, 2024; pii: S2322455X2400026-14

DOI: https://dx.doi.org/10.36380/jwpr.2024.26

ABSTRACT: The significance of *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) in the poultry industry underscores the critical importance of diagnosing avian mycoplasmosis within the field of veterinary medicine. The present study aimed to compare various diagnostic methods for detecting MG and MS in Egyptian Chicken Flocks. A total of 360 samples were collected from breeder, layer, and broiler chickens from four governorates in Egypt. Conventional isolation methods and polymerase chain reaction (PCR) were used for the direct detection of MG and MS, while serum plate agglutination test (SPA) and Enzyme-linked immunosorbent assay (ELISA) were used for detecting antibodies against MG and MS. The highest detection rate of MG was found in commercial layers, followed by breeders, and broilers. Regarding MS, the highest detection rate was found in breeders, followed by commercial layers, and broilers. By comparing the used diagnostic methods, MG and MS were determined by the SPA test (40% and 31.1% respectively), ELISA test (31.7% and 23.6%), PCR (16.7% and 11.7%), and by the conventional culture method (10.8% and 3.9%). It could be concluded that the serological methods and PCR gave better sensitivity than culture methods and can be used in the diagnosis of avian mycoplasmosis.



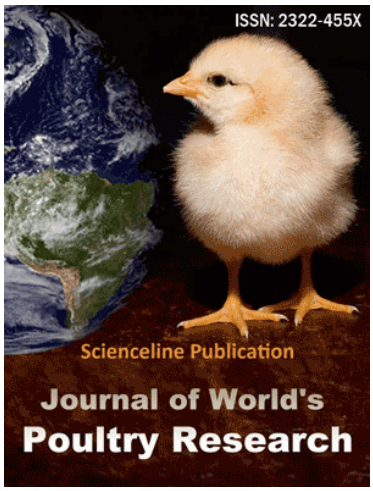
Comparative techniques for detection of *M. gallisepticum* and *M. synoviae* among the examined chickens

Keywords: Chicken, *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, Sensitivity

[Full text-PDF] [Crossref Metadata] [Scopus] [Export from ePrints]

Previous issue | Next issue | Archive

Journal of World's Poultry Research



ISSN: 2322-455X

Frequency: Quarterly

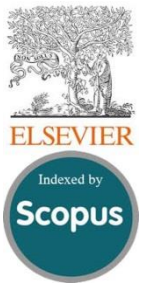
Current Issue: 2024, Vol: 14, No: 2 (June 25)

Publisher: SCIENCELINE

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

www.jwpr.science-line.com

» Indexed/covered by SCOPUS (CiteScore=1.3), NLM Catalog, AGRIS, NAAS (Score: 4.79), CIARDRING, Ulrich's™, PUBDB, TIB, WorldCat, EZB, Google Scholar...full index information



» Open access full-text articles is available beginning with Volume 1, Issue 1.

» Digital Archiving: Journal Repository (ePrints)

» Full texts and XML articles are available in Crossref and AGRIS.

» This journal is in compliance with Budapest Open Access Initiative and International Committee of Medical Journal Editors' Recommendations.

ICMJE INTERNATIONAL COMMITTEE of MEDICAL JOURNAL EDITORS

BOAI 10

» High visibility of articles over the internet.

» This journal encourage the academic institutions in low-income countries to publish high quality scientific results, free of charges... view Review/Decisions/Processing/Policy

Scienceline Publication, Ltd.
Editorial Office:
Ömer Nasuhi Bilmen Road, Dönmez Apart., G Block, No:1/6, Yakutiye, Erzurum/25100, Turkey
Homepage: www.science-line.com
Email: administrator@science-line.com
Phone: +90 538 7708824 (Turkey)

JWPR
Journal of World's Poultry Research
2024, Scienceline Publication
J. World Poult. Res. 14(2): 124-131, 2024
Research Paper
DOI: https://dx.doi.org/10.36380/jwpr.2024.12
PII: S2322455X2400012-14
ISSN: 2322-455X

Isolation and Molecular Identification of *Candida albicans* from the Oral cavity of Domestic Chickens using 28S rDNA in Diyala Governorate, Iraq

Mahmood Ahmed Kadhim, Sarah Jasim Abdulameer, Osama Ghazi Jalil Al-Dulaimi , and Amer Khazaal Al-Azzawi*

Department of Microbiology, College of Veterinary Medicine, University of Diyala, Baquba, Diyala, Iraq

*Corresponding author's E-mail: amer_alazawy@yahoo.com

Received: March 20, 2024, Revised: April 24, 2024, Accepted: May 18, 2024, Published: June 25, 2024

ABSTRACT

Candida albicans (*C. albicans*) is an opportunistic fungal pathogen that affects humans, animals, and birds. It is one of the most prevalent microbes found in clinical specimens. Candidiasis refers to endogenous fungal overgrowth under conditions of microbiota disruption or other host impairments. The study aimed to detect and isolate *C. albicans* based on morphological, cultural, and biochemical analysis. The present study examined 36 oral cavity samples from domestic chickens suspected of having candidiasis across various regions of Diyala Governorate in Iraq from September to November 2023. A total of twenty *C. albicans* were isolated from collected samples. PCR amplification of the 28S rDNA fungal gene, yielding the expected 260bp products, confirmed the identification of the yeasts. Using the Vitek 2 automated system, minimum inhibitory concentrations (MICs) for 6 common antifungal drugs were determined to test the antifungal susceptibility of a *Candida* clinical isolate. No resistance was found to Amphotericin B, Fluconazole, Flucytosine, Voriconazole, Caspofungin, or Micafungin in isolates from the oral cavity. In conclusion, the current study identified twenty *C. albicans* strains from chicken oral cavities and found them to be susceptible to all major classes of antifungals, indicating a lack of antifungal resistance in these isolates

Keywords: *Candida albican*, demostic chicken, Vitek 2, 28S rDNA.

INTRODUCTION

Candida albicans (*C. albicans*) is a common fungal pathogen and opportunistic yeast that is present in the digestive, reproductive, and urinary tracts of birds (Osorio et al., 2007; Vieira and Coutinho, 2009). While over 200 *Candida species* exist, *C. albicans* is the primary causative agent of candidiasis (Dhama et al., 2013; Mugale et al., 2015). In birds, *Candida* infections can occur as either primary or secondary infections (Vieira and Coutinho, 2009). Under immunosuppressive conditions brought on by factors like viral infections, steroid administration, prolonged antibiotic therapy, and subclinical malnutrition, invasive infections are more likely to develop (Razmyar et al., 2014). *C. albicans* grows as oval budding yeast cells measuring 3.5-6.0 × 6.0-10.0 µm in size. When *C. albicans* is cultured on agar plates or in animal tissues, round colonies will appear (Samour and Naldo, 2002).

Sequence analysis of ribosomal DNA (rDNA) is a common molecular method for fungal identification. Previous studies have used PCR with primers targeting the 28S rRNA gene, which contains conserved sequences shared among all fungal pathogens, to effectively detect fungi in clinical samples (Anand et al., 2001; Ninet et al., 2003; Zunaina et al., 2008; Nayak et al., 2011). These prior investigations found that PCR amplification of DNA fragments less than 300 bp in length, such as the 250-280 bp 28S rDNA amplicons, results in greater detection sensitivity compared to larger fragments (Evertsson et al., 2000; Gade et al., 2017).

Antifungal susceptibility testing plays a vital role in resistance detection, epidemiological research, and comparing the *in vitro* efficacy of novel and existing antifungal medications (Cejudo et al., 2010). The Vitek 2 system by bioMérieux, Inc.is an automated commercial platform that uses spectrophotometry to determine yeast growth and enables combined fungal identification and minimum inhibitory concentration (MIC) determination (Cuenca-estrella et al., 2010). Candida spp. has unknown

To cite this paper: Kadhim MA, Abdulameer SJ, Al-Dulaimi OGJ, and Al-Azzawi AK (2024). Isolation and Molecular Identification of *Candida albicans* from the Oral cavity of Domestic Chickens using 28S rDNA in Diyala Governorate, Iraq. *J. World Poult. Res.*, 14(2): 124-131. DOI: https://dx.doi.org/10.36380/jwpr.2024.12

susceptibility patterns often and empirical therapy is often used to treat infections (Melhem et al., 2013). The susceptibility testing products available commercially play an important role in treating patients with invasive fungal infections by providing important patient data to guide suitable therapy (Cretella et al., 2016).

The aim of this study was to isolate and identify *C. albicans* from the oral cavity of local chickens utilizing a PCR assay targeting the 28S rDNA region, as well as evaluate the isolated strains for antifungal sensitivity profiles using the commercial Vitek 2 system.

MATERIALS AND METHODS

Ethical approval

The study received approval from the Scientific and Ethical Committee of the College of Veterinary Medicine, University of Diyala, Iraq, with approval number Vet Medicine (300), dated November 2023, signed by M, S, O, and A.

Sample collection

From September 2023 to November 2023, 36 oral cavity samples were collected from domestic chickens in various regions (Muradiya, Razi, Asada , Canaan, and Al-Hadid) of the Diyala governorate in Iraq. The local chickens were mixed breed, different ages and unsex. Some local chickens had visible oral membrane infections at the time of sampling. Samples were collected by gently swabbing the oral membranes and cavities using sterile cotton swabs. Swabs were immediately placed in sterile tubes and transported beside an ice pack to the Microbiology Laboratory at Diyala University in Iraq within an hour after collection.

Sample processing

Upon receipt in the lab, cotton swabs were used to directly inoculate Sabouraud Dextrose Agar plates supplemented with chloramphenicol (SDA-C) to inhibit bacterial growth. Plates were streaked for isolation and incubated at 37°C for up to 72 hours. During this incubation period, the plates were periodically monitored for the development of fungal growth (Matare et al., 2017). Developing colonies were subcultured on fresh SDA-C plates to obtain pure cultures. Gram staining was performed to assess morphology.

Identification of *Candida albicans*

To identify *C. albicans* isolates, all Gram-positive yeast isolates were subjected to germ tube formation by inoculating isolates into serum and incubating at 35°C for up to 3 hours (Sheppard et al., 2008), then visually assessing for generation of germ tubes under a light microscope (Olympus, Japan). Additionally, chlamydospore production was evaluated by spot inoculating corn meal agar plates with isolates, incubating for 48-72 hours at 25°C, and examining under the microscope for distinctive chlamydospores produced by *C. albicans* (Navarathna et al., 2016). Isolates were also inoculated onto CHROMagar *Candida* medium to take advantage of the ability of this differential medium to identify *C. albicans* colonies by their distinctive green color (Sivakumar et al., 2009). Using these three methods, 20 isolates exhibited traits consistent with *C. albicans* identification.

Molecular diagnosis

Fungal genomic DNA was extracted from isolates using a commercial Nucleospin DNA extraction kit (Promega, USA) with the following modifications to the manufacturer's protocol. A single fungal colony was picked with a sterile loop and suspended in 300µl of lysis buffer (1mM EDTA pH8, 10mM Tris, 100mM NaCl, 2% Tween 80, 1% SDS) followed by the addition of 300µl phenol-chloroform (1:1). The mixture was shaken for 5 minutes and centrifuged at 10,000 rpm for 5 minutes. The aqueous supernatant was transferred into a new microcentrifuge tube and an equal volume of chloroform was added. After mixing thoroughly, the solution was centrifuged again at 10,000 rpm for 10 minutes. Next, 500 microliters of 100% ethanol was mixed with the supernatant to precipitate DNA. Tubes were inverted several times before centrifuging again at 10,000 rpm for 7 minutes. Finally, the DNA pellets were air-dried for 10 minutes and resuspended in 100 microliters of TE buffer at pH 8.0 (Mousavi et al., 2007). Extracted DNA was stored at -20°C prior to further analysis. Quality and yield of extracted fungal DNA was assessed by OD260/280 spectroscopic measurements using a NanoDrop. Extracted DNA had OD260/280 ratios ranging from 1.7 to 1.8, indicating high-purity DNA.

PCR

Amplification of the 28S rDNA gene was performed using the following primers that are mentioned in a previous study (Anand et al., 2001).

Forward: 5'-GTGAAATTGTTGAAAGGGAA-3'
Reverse: 5'-GAC TCCTTGGTCCGTGTT-3'

PCR reactions were carried out in a 25µl volume containing 1µl (10pmol) of each primer, 5µl DNA template, 12.5µl Green PCR master mix, and 5.5µl PCR-grade water. Thermal cycling was conducted using a PCR Thermal Cycler (TC-3000, PCR Thermal Cycler, USA) with the following protocol. Initial denaturation at 94°C for 15 minutes, 30 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 1 minute, extension at 72°C for 2 minutes, and final extension at 72°C for 15 minutes.

To analyze the polymerase chain reaction (PCR) products, a 2% agarose gel electrophoresis was performed using ethidium bromide staining. A 50-base pair DNA ladder was run alongside the PCR products as a molecular weight size marker. Gels were visualized under UV light and photographed.

Vitek 2 antifungal susceptibility testing

The Vitek 2 AST-YS07 card was utilized, which contains serial two-fold dilutions of the following antifungals included of Amphotericin B, Micafungin, Fluconazole, Flucytosine, Caspofungin, and Voriconazole. Inoculum suspensions were prepared from 24-hour Sabouraud dextrose agar cultures of *C. albicans* incubated at 37°C. Suspensions were adjusted to a 2.0 McFarland standard and loaded into Vitek 2 cassettes along with sterile polystyrene tubes according to manufacturer's instructions.

Loaded cassettes were placed into the Vitek 2 instrument and ran automatically. The yeast suspensions were diluted appropriately prior to card filling and incubated for 5-17 hours depending on the growth rate of the drug-free control well. Results were reported as minimum inhibitory concentrations (MICs) in µg/mL for each antifungal agent. Interpretive criteria were based on EUCAST 2013 & CLSI 2013 clinical breakpoints used for routine diagnostics as follows: Amphotericin B - Susceptible (S) $\leq$ 1 µg/mL, Resistant (R) $\geq$ 2 µg/mL; Voriconazole - S $\leq$ 0.125 µg/mL, R $\geq$ 0.25 µg/mL; Fluconazole - S $\leq$ 2 µg/mL, Intermediate (I) = 4 µg/mL, R $\geq$ 8 µg/mL; Micafungin - S $\leq$ 0.25 µg/mL, I = 0.5 µg/mL, R $\geq$ 1 µg/mL; Flucytosine - S $\leq$ 4 µg/mL, 8 µg/mL $\leq$ I $\leq$ 16 µg/mL, R $\geq$ 32 µg/mL; Caspofungin - S $\leq$ 2 µg/mL.

RESULTS

Isolation of *Candida albicans*

Of the 36 oral swabs collected from local chickens, 20 (55.6%) yielded isolates identified as *C. albicans* using morphological, cultural, and biochemical characteristics.

Microscopic morphology and cultural characteristics

Gram staining of isolates revealed large, oval purple cells occurring singly or in budding yeast chains. Additionally, wider, tube-like pseudo-hyphae structures were observed in some preparations. When grown on Sabouraud Dextrose agar at 30°C for 48 hours, isolates formed white, smooth, creamy colonies that were circular and concave with entire margins. On corn meal agar incubated at 30°C for 72 hours, distinctive round chlamydospores were visualized terminally along hyphal elements and at intercalary, immediately subtending septa of the hyphae. Chlamydospores were large, thick-walled, refractile structures. Blastoconidia were also observed adjacent to hyphae. Growth on CHROMagar *Candida* medium yielded forest green colonies, consistent with *C. albicans* species identification. Inoculation into serum followed by incubation at 35°C for 3 hours induced the formation of germ tubes, microscopic tubular extensions characteristic of *C. albicans*. This confirmed the identification of this fungal species (Figure 1).

Molecular identification via PCR

The extracted DNA from all isolates was amplified via polymerase chain reaction (PCR) targeting the 28S ribosomal DNA (28S rDNA) region. PCR amplification products obtained using 28S rDNA primers were analyzed by agarose gel electrophoresis and visualized under UV light. Gel electrophoresis and comparison to a size standard were utilized to determine that the DNA isolated from the microbial isolates contained discrete fragments of approximately 260 base pairs. The technique confirms the extracted DNA was of the expected size and has been successfully isolated from the organism samples for further analysis (Figure 2).

Antifungal susceptibility profile of *Candida albicans*

Use a device Vitek 2 Compact system to determine MICs for 6 different antifungal drugs against the yeast that were being tested as shown in Table 1.

As can be seen in Table 1 the results of antifungal susceptibility testing against 20 isolates of the *C. albicans* fungus is presented. The results indicated that all isolates were fully sensitive to the used antifungal agents including Fluconazole, Voriconazole, Caspofungin, Micafungin, and Flucytosine. Regarding Amphotericin B, 19 isolates were sensitive (95%) while one isolate had intermediate sensitivity (5%). There were no resistant isolates to any of the chosen antifungal agents. The results revealed the possible efficacy of all the antifungal agents tested during this study against *C. albicans*.

Table 1. The minimum inhibitory concentration values for antifungals against *Candida albicans*

Antifungal against *C. albicans*	Sensitive		Intermediate		Resistance		MIC (µg/ml) Range
	No. of isolate	S (%)	No. of isolate	I (%)	No. of isolate	R (%)	
Fluconazole	20	100	0	0	0	0	≤ 1
Voriconazole	20	100	0	0	0	0	≤ 0.12
Caspofungin	20	100	0	0	0	0	≤ 0.25
Micafungin	20	100	0	0	0	0	≤ 0.06
Amphotericin B	19	95	1	5	0	0	≤ 0.5
Flucytosine	20	100	0	0	0	0	≤ 1

S: sensitive, *R:resistant; I: Intermediate, No: Number, MIC: minimum inhibitory concentration

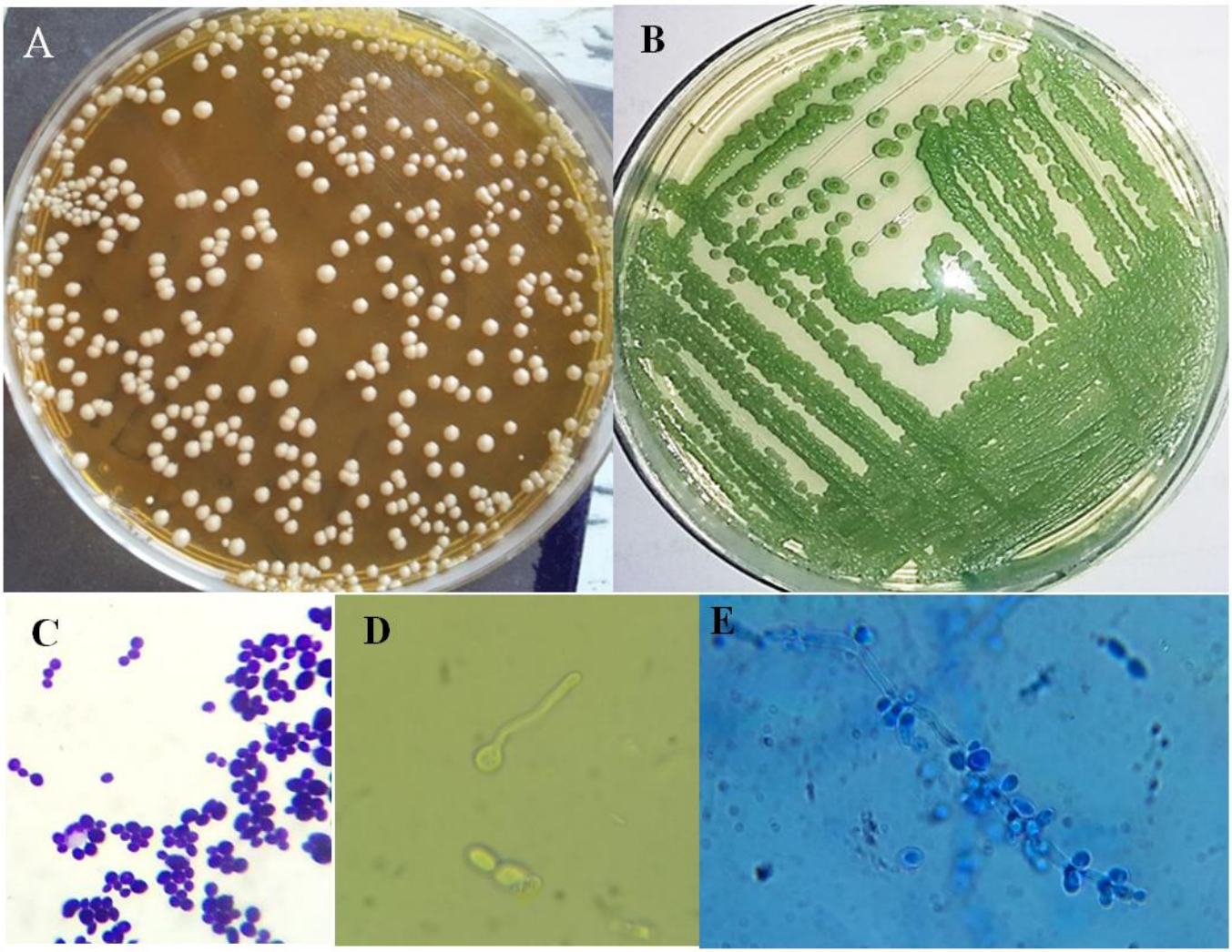


Figure 1. Identification and characterization of *Candida albicans* isolated from local chicken in Diyala Province, Iraq. Growth of *Candida albicans* on Sabouraud Dextrose agar (**A**), *Candida albicans* green colonies on CHROM agar (**B**), Blastoconidia and pseudohyphae of *Candida albicans* stained with gram stain (**C,** 100X), *Candida albicans* germ tube formation (**D**, 100X), *Candida albicans* in corn meal agar showing chlamydospores (**E**).

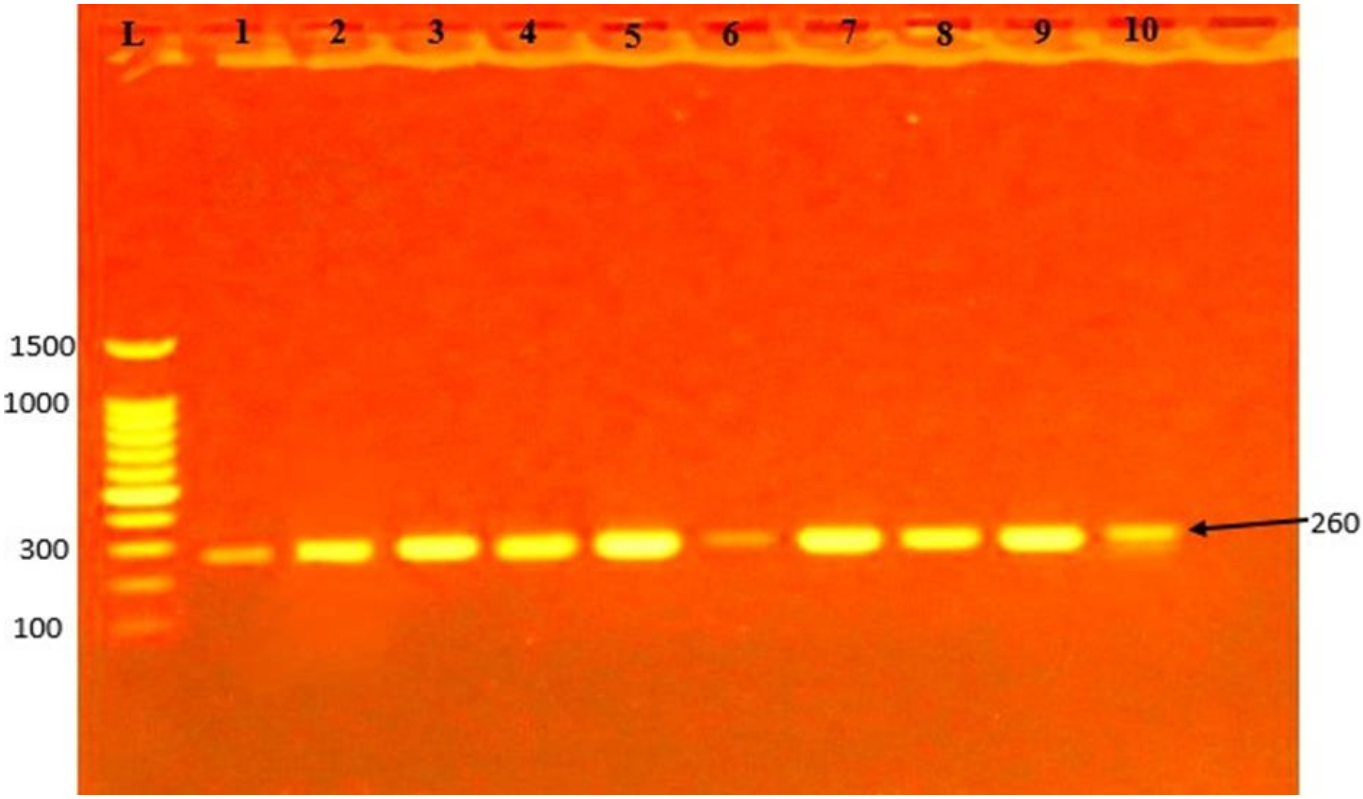


Figure 2. Agarose gel electrophoresis of 28S rDNA PCR products for *Candida albicans* isolated from local chickens under UV transilluminator. **M**: DNA ladder (100 bp – 1500bp). **Lanes**: 28S rDNA bands (260bp) for *Candida albicans*.

DISCUSSION

The study used a comprehensive approach to accurately identify *C. albicans* isolates from chicken oral cavities. The characteristic morphological signs of *C. albicans* were identified during primary examination using the Gram staining method; its cells were large, oval, purple-stained, and occurred singly or in budding yeast chains. This characteristic morphology was also mentioned by Chaffin et al. (1998). The colonies obtained on Sabouraud Dextrose agar, which was the appropriate medium for *C. albicans* and other fungi from the genus *Candida*, also pointed to this species. The culture was white with a smooth, shiny-looking growth. It was creamy and had a circular shape of entire margins; these characteristics were also reported by Hospenthal et al. (2006).

Additionally, the present study noticed that all C. albicans isolates presented distinct features which were separated them from other *Candida* species. Firstly, a positive germ tube test was evident in 2 hours when they were incubated in serum (Sharma et al., 2017). Another unique feature is the presence of chlamydospores when they were cultured on Corn meal agar, the medium also referred to as cell starvation medium (Pincus et al., 2007). In addition, CHROMagar Candida CAC medium was used and all presumptive *C. albicans* isolates yielded typical green colonies (Khadka et al., 2016). The presence of green colonies on CAC, along with the capacity to germinate tubes and chlamydospores formation, are of notable properties for *C. albicans* identification among others *Candida*, in agreement with published work (Souza et al., 2015; Kidd et al., 2016).

The identity of the isolates was molecularly confirmed by a species-specific PCR targeting the 28S rRNA gene, performed with published primers UI and U2 (Sandhu et al., 1995). Owing to the multicopy nature and conservation of fungal ribosomal RNA genes, 28S rRNA PCR effectively and sensitively detects major human and animal fungal pathogens, including *C. albicans* (Ogawa et al., 2012). In this study, all green CAC isolates that tested positive for chlamydospore and germ tube formation contained the 260-bp PCR amplicon specific for *C. albicans*.

The purpose of the current study was to determine whether the local chickens in Diyala, Iraq, were suspected of having *Candida albicans* in their mouths. The majority

of yeast isolates obtained were identified as *C. albicans*, aligning with previous findings by Samaka et al. (2011), Kaab (2013), and Razmyar et al. (2014) that *C. albicans* is the predominant *Candida* species found in avian oral cavity and digestive tract infections (Vieira and Coutinho, 2009). These results are consistent with other studies conducted in different regions, such as the study of Cafarchia et al. (2006), who isolated *C. albicans* from the crop and intestinal contents of poultry in Italy, and Talazadeh et al. (2022), who identified *C. albicans* in the pharyngeal swab and cloacal swab from birds in Ahvaz, Iran.

Antifungal susceptibility profiles of *C. albicans* vet isolates were determined using the VITEK 2 automated system. A study indicated VITEK 2 is an accurate and rapid tool for the characterization of fungal drug resistance and guiding antifungal therapy selection (Mendes et al., 2018). Here, nearly all *C. albicans* isolates exhibited sensitivity to the panel of antifungals tested, contrasting with a previous study conducted on poultry in Turkey, which reported 3 fluconazole-resistant isolates (Samaka et al., 2011) but concurring with broader animal resistance trends (Dalvand et al., 2018). Further accumulation of susceptibility data through wider geographic surveillance studies will help establish appropriate treatment guidelines for infections of animal candidiasis.

CONCLUSION

The combined use of selective culture, species-specific PCR, and antifungal sensitivity profiling on Vitek 2 offers a robust methodology to examine *C. albicans* carriage and drug susceptibility patterns in avian populations. However, further investigation is warranted to identify risk factors driving carriage in poultry flocks and develop evidence-based interventions to mitigate dissemination. A priority for future research should be multidisciplinary studies that elucidate modifiable risks, transmission dynamics, and genetic determinants of drug resistance. Implementing standardized surveillance and biosecurity protocols based on these findings will be paramount to containing outbreaks. With vigilance and proactive management, the threat posed by antifungal-resistant *C. albicans* strains in poultry can be curtailed.

DECLARATIONS

Acknowledgments

The authors would like to express their gratitude to the College of Veterinary Medicine at the University of Diyala for the invaluable logistical support they provided. The authors are also deeply appreciative of the molecular biology laboratory staff for their prompt guidance and assistance throughout this work. Their expertise and contributions were crucial to the completion of this study.

Competing interests

The authors have no conflicts of interest to disclose related to this work.

Authors' contributions

Mahmood Ahmed Kadhim was responsible for conceptualizing and designing the study, as well as collecting local chicken samples. Sarah Jasim Abdul-Ameer, Osama Ghazi Jalil Al-Dulaimi, and Amer Khazaal Al-Azawy contributed to the manuscript by editing and analyzing data. All authors checked the analyzed data, presented findings, and the final draft of the manuscript before submission and publication.

Funding

This work received no specific grant from any funding agency.

Ethical considerations

This study was conducted ethically and in accordance with guidelines for research integrity. Informed consent for participation was obtained from all subjects. The authors adhered to ethical principles related to plagiarism, fabrication, falsification, duplicate publication, and redundancy. All authors have reviewed the final manuscript and confirmed that any potential ethical issues have been avoided.

Availability of data and materials

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

REFERENCES

Anand AR, Madhavan HN, Neelam V, and Lily TK (2001). Use of polymerase chain reaction in the diagnosis of fungal endophthalmitis. Ophthalmology, 108(2): 326-330. DOI: https://www.doi.org/10.1016/S0161-6420(00)00517-0

Cafarchia C, Camarda A, Romito D, Campolo M, Quaglia NC, Tullio D, and Otranto D (2006). Occurrence of yeasts in cloacae of migratory birds. Mycopathologia, 161(4): 229-234. DOI: https://www.doi.org/10.1007/s11046-005-0194-z

Cejudo TG, Gallego AG, Lacasa EC, Aller AI, Romero A, García JP, Andrés GQ, and Martín-mazuelos E (2010). Evaluation of the VITEK 2 system to test the susceptibility of *Candida* spp.,

Trichosporon asahii and Cryptococcus neoformans to amphotericin B, fl ucytosine, fl uconazole and voriconazole: A comparison with the M27-A3 reference method. Medical Mycology, 48(5): 710-719. DOI: https://www.doi.org/10.3109/13693780903473343

Chaffin WL, López-Ribot JL, Casanova M, Gozalbo D, and Martínez JP (1998). Cell wall and secreted proteins of *Candida albicans*: Identification, function, and expression. Microbiology and Molecular Biology Reviews: 62(1): 130-180. DOI: https://www.doi.org/10.1128/MMBR.62.1.130-180.1998

Cretella D, Barber KE, King ST, and Stover KR (2016). Comparison of susceptibility patterns using commercially available susceptibility testing methods performed on prevalent *Candida* spp. Journal of Medical Microbiology, 65(12): 1445-1451. DOI: https://www.doi.org/10.1099/jmm.0.000383

Cuenca-Estrella M, Gomez-Lopez A, Alastruey-Izquierdo A, Bernal-Martinez L, Cuesta I, Buitrago MJ, and Rodriguez-Tudela JL (2010). Comparison of the Vitek 2 antifungal susceptibility system with the clinical and laboratory standards institute (CLSI) and European committee on antimicrobial susceptibility testing (EUCAST) Broth Microdilution reference methods and with the sensititr the sensititre Yeastone and Etest techniques for *in vitro* detection of antifungal resistance in yeast isolates. Applied and Environmental Microbiology, 48(5): 1782-1786. DOI: https://www.doi.org/10.1128/JCM.02316-09

Dalvand A, Katiraee F, Jafari Joozani R, and Shokri H (2018). Genotyping of *Candida albicans* isolated from animals using 25S ribosomal DNA and ALT repeats polymorphism in repetitive sequence. Current Medical Mycology, 4(4): 12-19. DOI: https://www.doi.org/10.18502/cmm.4.4.381

Dhama K, Chakraborty S, Verma AK, Tiwari R, Barathidasan R, Kumar A, and Singh SD (2013). Fungal/mycotic diseases of poultry-diagnosis, treatment and control : A review. Pakistan Journal of Biological Sciences, 16(23): 1626-1640. DOI: https://www.doi.org/10.3923/pjbs.2013.1626.1640

Evertsson U, Monstein HJ, and Johansson AG (2000). Detection and identification of fungi in blood using broad-range 28s rDNA PCR amplification and species-specific hvbridisation. APMIS, 108(5): 385-392. DOI: https://www.doi.org/10.1034/j.1600-0463.2000.d01-73.x

Gade L, Hurst S, Balajee SA, Lockhart SR, and Litvintseva AP (2017). Detection of mucormycetes and other pathogenic fungi in formalin fixed paraffin embedded and fresh tissues using the extended region of 28S rDNA. Medical Mycology, 55(4): 385-395.DOI: https://wwww.doi.org/10.1093/mmy/myw083

Hospenthal DR, Beckius ML, Floyd KL, Horvath LL, and Murray CK (2006). Presumptive identification of *Candida* species other than *C. albicans*, *C. krusei*, and *C. tropicalis* with the chromogenic medium CHROMagar *Candida*. Annals of Clinical Microbiology and Antimicrobials, 5: 1. DOI: https://www.doi.org/10.1186/1476-0711-5-1

Kaab HT (2013). Genotyping of *Candida albicans* isolated from broilers by 25S rDNA analysis. Kufa Journal For Veterinary Medical Sciences, 4(1): 105-111. Available at: https://www.iasj.net/iasj/download/ea95a816a7feac90

Khadka S, Regmi P, Giri S, Shah PK, and Mishra SK (2016). Identification of *Candida* species using CHROM agar. International Journal of Medicine and Biomedical Sciences, 1(3): 10-13. Available at: https://pdfs.semanticscholar.org/0d04/dc914a24003d10317fe05059023eb40b5ab2.pdf

Kidd S, Halliday C, Alexiou H, and Ellis D (2016). Descriptions of medical fungi, 3rd Edition. Adelaide., Australia, pp. 35-37. Available at: https://www.adelaide.edu.au/mycology/ua/media/1596/fungus3-book.pdf

Melhem MSC, Bertoletti A, Lucca HRL, Silva RBO, Meneghin FA, and Szeszs MW (2013). Use of the VITEK 2 system to identify and test the antifungal susceptibility of clinically relevant yeast species. Brazilian Journal of Microbiology, 44(4): 1257-1266. DOI: https://www.doi.org/10.1590/S1517-83822014005000018

Matare T, Nziramasanga P, Gwanzura L, and Robertson V (2017). Experimental germ tube induction in *Candida albicans*: An evaluation of the effect of sodium bicarbonate on morphogenesis and comparison with pooled human serum. BioMed Research International, 2017: 1976273. DOI: https://www.doi.org/10.1155/2017/1976273

Mendes JF, Gonçalves CF, Ferreira GF, Esteves IA, Freitas CH, Villarreal JPV, Mello JRB, Meireles MCA, and Nascente PS (2018). Antifungal susceptibility profile of diferent yeasts isolates from wild animals, cow's milk with subclinical mastitis and hospital environment. Brazilian Journal of Biology, 78(1): 68-75. DOI: https://www.doi.org/10.1590/1519-6984.04916

Mousavi SA, Khalesi E, Shahid Bonjar GH, Aghighi S, Sharifi F, and Aram F (2007). Rapid molecular diagnosis for *Candida* species using PCR-RFLP. Biotechnology, 6(4): 583-587. DOI: https://www.doi.org/10.3923/biotech.2007.583.587

Mugale M, Bhat AA, Gavhane DS, and Bhat SA (2015). Outbreaks of thrush in pigeons in Punjab State of India. Comparative Clinical Pathology, 24(3): 635-638. DOI: https://www.doi.org/10.1007/s00580-014-1958-y

Navarathna DHMLP, Pathirana RU, Lionakis MS, Nickerson KW, and Roberts DD (2016). *Candida albicans* ISW2 regulates chlamydospore suspensor cell formation and virulence *in vivo* in a mouse model of disseminated candidiasis. PloS One, 11(10): e0164449. DOI: https://www.doi.org/10.1371/journal.pone.0164449

Ninet B, Jan I, Bontems O, Léchenne B, Jousson O, Panizzon R, Lew D, and Monod M (2003). Identification of dermatophyte species by 28S ribosomal DNA sequencing with a commercial kit. Journal of Clinical Microbiology, 41(2): 826-830. DOI: https://www.doi.org/10.1128/JCM.41.2.826-830.2003

Nayak N, Satpathy G, Prasad S, Titiyal JS, Pandey RM, and Vajpayee RB (2011). Molecular characterization of drug-resistant and drug-sensitive Aspergillus isolates causing infectious keratitis. Indian Journal of Ophthalmology, 59(5): 373-377. DOI: https://www.doi.org/10.4103/0301-4738.83614

Ogawa M, Sugita S, Watanabe K, Shimizu N, and Mochizuki M (2012). Novel diagnosis of fungal endophthalmitis by broad-range real-time PCR detection of fungal 28S ribosomal DNA. Graefe's Archive for Clinical and Experimental Ophthalmology, 250(1): 1877-1883. DOI: https://www.doi.org/10.1007/s00417-012-2015-7

Osorio C, Fletcher O, Dykstra MJ, Post K, and Barnes HJ (2007). Comb candidiasis affecting roosters in a broiler breeder flock. Avian Diseases, 51(2): 618-622. DOI: https://www.doi.org/10.1637/0005-2086(2007)51[618:CCARIA]2.0.CO;2

Pincus DH, Orenga S, and Chatellier S (2007). Yeast identification-past, present, and future methods. Medical Mycology, 45(2): 97-121. DOI: https://www.doi.org/10.1080/13693780601059936

Razmyar J, Movassaghi AR, Mirshahi A, Eidi S, and Zaeemi M (2014). Osteoarthritis and systemic infection caused by *Candida albicans* in acommon Mynah (*Acridotheres tristis*). Iranian Journal of Veterinary Science and Technology, 6(2): 77-84. DOI: https://www.doi.org/10.22067/veterinary.v6i2.27859

Samaka HM, Al-azzam AH, Al-naqeeb MM, Al-Hatami AO, and Al-Rufai HM (2011). Genotyping of *Candida albicans* isolated from turkeys by 25S intron analysis. 1st Scientific Conference of faculty of Veterinary Medicine-University of Kufa, Iraq, pp. 54-62.

Samour JH and Naldo JL (2002). Diagnosis and therapeutic management of candidiasis in falcons in Saudi Arabia. Journal of Avian Medicine and Surgery, 16(2): 129-132. DOI:

https://www.doi.org/10.1647/1082-6742(2002)016[0129:DATMOC]2.0.CO;2

Sandhu GS, Kline BC, Stockman L, and Roberts GD (1995). Molecular probes for diagnosis of fungal infections. Journal of Clinical Microbiology, 33(11): 2913-2919. DOI: https://www.doi.org/10.1128/jcm.33.11.2913-2919.1995

Sharma K, Chattopadhya U, and Naskar K (2017). Prevalence of *Candida albicans* in raw chicken and mutton meat samples sold in the open markets of Kolkata city of West Bengal. International Journal of Livestock Research, 7(5): 243-249. DOI: http://www.doi.org/10.5455/ijlr.20170328034209

Sheppard DC, Locas MC, Restieri C, and Laverdiere M (2008). Utility of the germ tube test for direct identification of *Candida albicans* from positive blood culture bottles. Journal of Clinical Microbiology, 46(10): 3508-3509. DOI: https://www.doi.org/10.1128/JCM.01113-08

Sivakumar VG, Shankar P, Nalina K, and Menon T (2009). Use ofCHROMagar in the differentiation of common species of *Candida*. Mycopathologia, 167(1): 47-49. DOI: https://www.doi.org/10.1007/s11046-008-9149-5

Souza MN, Ortiz SO, Mello MM, de Oliveira FM, Severo LC, and Goebel CS (2015). Comparison between four usual methods of identification of *Candida species*. Revista do Instituto de Medicina Tropical de Sao Paulo, 57(4): 281-287. DOI: https://www.doi.org/10.1590/S0036-46652015000400002

Talazadeh F, Ghorbanpoor M, and Shahriyari A (2022). Candidiasis in birds (Galliformes, Anseriformes, Psittaciformes, Passeriformes, and Columbiformes): A focus on antifungal susceptibility pattern of *Candida albicans* and non-albicans isolates in avian clinical specimens. Topics in Companion Animal Medicine, 46: 100598. DOI: https://www.doi.org/10.1016/j.tcam.2021.100598

Vieira RG and Coutinho SDA (2009). Phenotypical characterization of *Candida* spp. isolated from crop of parrots (*Amazona* spp.). Pesquisa Veterinária Brasileira, 29(6): 452-456. DOI: https://www.doi.org/10.1590/S0100-736X2009000600002

Zunaina E, Wan Hitam HW, Yean CY, Abdul Rashid HN, Kamarudin B, Zainal Abidin SK, Osman S, Zainuddin AF, and Ravichandran M (2008). Specific detection of fungal pathogens by 18S rRNA gene PCR in microbial keratitis. BMC Ophthalmology, 8: 7. DOI: https://www.doi.org/10.1186/1471-2415-8-7

JWPR
Journal of World's Poultry Research
2024, Scienceline Publication
J. World Poult. Res. 14(2): 132-137, 2024
Research Paper
DOI: https://dx.doi.org/10.36380/jwpr.2024.13
PII: S2322455X2400013-14
ISSN: 2322-455X

Effects of Maize, Millet, and Sorghum as Energy Sources of Diet on Growth Performance of Guinea Fowl

Nouri Brah[1]*, Issa Chaibou[2], Ouseini Moussa Hassan[2], and Abdoul Rachidou Sodo Daka[2]

[1]*Regional Center of Agronomique Research of Maradi, National Institute of Agronomique Research of Niger, BP: 420, Maradi, Niger*
[2]*Livestok Sciences and Technics Department, Dan Dicko Dankoulodo University of Maradi BP: 465, Maradi, Niger*

*Corresponding author's E-mail: brahnouri@yahoo.fr

Received: March 23, 2024, Revised: April 20, 2024, May 25, 2024, Published: June 25, 2024

ABSTRACT

Guinea fowls are more valuable in the market in Niger than chickens; however, their ability to meet consumer demands is limited by low productivity. Therefore, suitable nutrition is an important factor for this productivity. The current study aimed to evaluate the growth performance of guinea fowl using maize, millet, and sorghum as energy sources in the diet. A total of 108 one-day-old unsexed local keets, with an average live weight of 25.5 ± 0.83 g were randomly distributed among three dietary treatments with four replicates per treatment. The keets were reared on the ground with litter at CERRA Maradi, Niger, for an 8-week experimental period, involving 36 keets per treatment and 9 keets per replication. The parameters monitored were feed intake (FI), live weight (LW), average daily gain (ADG), and feed conversion ratio (FCR). After 8 weeks of experimentation, the results indicated that the cereal used in the diet had no statistically significant effect on the keets' FI. Those fed millet-based diets had higher LW, compared to those fed maize and sorghum diets, respectively, however, this difference was not statistically significant. Millet also facilitated a higher ADG, compared to maize and sorghum, although the differences were statistically insignificant. The keets fed sorghum-based had higher FCR, compared to the FCR of the guinea fowls fed maize and millet diet. based on the growth performance assessed in this study, the recommended order for cereals in guinea fowl feed to ensure better growth is millet, followed by maize, and then sorghum.

Keywords: Cereals, Energy source, Growth performance, Keets

INTRODUCTION

Guinea fowl which is indigenous to Africa has been neglected and consigned to the rural areas where it is allowed to scavenge for feed (Amoah et al., 2018). These breeding and feeding conditions contribute to the poor productivity of the birds (Soara et al., 2020). Guinea fowl profitability is hampered by poor nutrition due to a lack of management and feeding guidelines (Tjetjoo et al., 2013). However, nutrition plays a determining role in the success and economic profitability of poultry products (Brah et al., 2015). There are a few formulated diets specifically for guinea fowl feeding. As a result, guinea fowl are fed with commercial broiler and layer diets (Moreki and Seabo, 2012). Cereals are the main source of energy used in poultry feed, with corn being the predominant cereal utilized for this purpose (Ravindran, 2013). However, the extensive use of corn in animal feed poses a challenge, as it contributes to food competition with humans (Teguia and Beynen, 2005). Corn metabolizable energy is used as a reference in the evaluation of other energy sources. Corn provides 3350 Kcal of metabolizable energy per kg of dry matter (NRC, 1994) and contains 11.5% crude protein (Houndonougbo et al., 2009). In Niger, the corn used in poultry feed is imported. However, millet and sorghum are alternatives to maize in poultry feed (Issa et al., 2015; 2016). Millet is a cereal from semi-arid tropical zones (Filardi et al., 2005). Millet provides 3360 Kcal of metabolizable energy per kg of dry matter (NRC, 1994) and contains 14.10% crude protein based on dry matter (Medugu et al., 2010). Sorghum is the cereal expected to replace corn in poultry feed (Etuk et al., 2012). Relative to dry matter, sorghum contains 11.7% crude protein (Issa et al., 2010) and provides 3212 Kcal of metabolizable energy (NRC, 1994). Therefore, this study aimed to evaluate the

To cite this paper: Brah N, Chaibou I, Hassan OM, and Daka ARS (2024). Effects of Maize, Millet, and Sorghum as Energy Sources of Diet on Growth Performance of Guinea Fowl. *J. World Poult. Res.*, 14(2): 132-137. DOI: https://dx.doi.org/10.36380/jwpr.2024.13

growth performance of keets under an intensive management system fed with diets containing maize, millet, and sorghum as energy sources.

MATERIALS AND METHODS

Ethical approval

The experiment was conducted in compliance with current standards for conducting experiments with animals of the National Institute for Agriculture Research of Niger. The guinea fowls were housed in groundnut hulls as beddings. The density was 5/m^2, and heating and lighting were adapted to their recommended living conditions. No injections even the vaccine were given. Human working to ensure hygiene, management, and data collection were equipped with suitable clothing.

Keets and housing

A total of 108 one-day-old local unsexed keets, constituted the biological material of the study. They were obtained by artificial incubation of eggs collected from guinea fowl breeding at the CERRA animal production department in Maradi, Niger. These keets were raised in two phases. A starter phase from the first to the fourth weeks and a grower phase from the fifth to the eighth weeks. The experiment was conducted in an 11.9 m^2 (3.50 m × 3.40 m) poultry house. It was partitioned with small mesh wire into 12 boxes (6 on each side) measuring 0.56 m^2 each. In each box, a 60-watt bulb was positioned 50 cm above the ground to provide heat to the guinea fowl. Throughout the 8-week duration of the experiment, this setup aimed to maintain the temperature inside the poultry house within the range of 30 to 31°C. The humidity level was maintained at 46.6%.

Sanitary and feeding conduct

At the beginning of the experiment, one-day-old keets were introduced to a sugar solution in water (5g/L) for the initial 2 days. Amin'Total, produced by LAPROVET/France (1g/5L), was provided in the drinking water for stress control during the first 5 days. Tetracolivit from LAPROVET/France was administered as an antibiotic in the keet's drinking water at a concentration of 0.5g/L, following the manufacturer's guidelines for 5 days. One day before and the day of weighing keet (4 and 8 weeks old), Amin'Total for stress control was administered at 1g/5L) in keet's drinking water. Corn, millet, sorghum without tannin (IRAT 204), wheat bran, broiler concentrate, peanut meal, bone meal, and peanut oil were used to formulate the experimental diet according to NRC (1994) reference. They were formulated by maintaining the same level (59% at stater and 63% at grower phase) of cereal in the feed and providing at least 2900 kcal of metabolizable energy per kg of dry matter throughout the experiment and 22% and 20% of crude protein in the feed in the start-up and growth phases respectively (Table 1).

Table 1. Ingredient and nutrient composition of 8 weeks experimental local keets' diets

Ingredients (% Dry matter)	Starter phase (1-4 weeks)			Grower phase (5-8 weeks)		
	Maize	**Millet**	**Sorghum**	**Maize**	**Millet**	**Sorghum**
Millet	0	59	0	0	63	0
Maize	59	0	0	63	0	0
Sorghum IRAT 204	0	0	59	0	0	63
Wheat bran	3	3	3	5	5	5
Broiler concentrate	16	18	17	14	14,5	14,25
Peanut meal	19	17	17,5	14,5	14	14
Bone meal	2	2	2	2,75	3	2,75
Peanut oil	1	1	1,5	0,75	0,5	1
TOTAL	100	100	100	100	100	100
Calculated nutritional composition						
ME* (Kcal/KgDM)	2906.98	2931.79	2924.26	2902.59	2911.43	2905.71
Crude Protein (%)	22.3407	22.2053	22.4297	20,.	20.1737	20.463
Crude fiber (%)	3.797	3.724	3.9833	3.739	3.62425	3.933725
Lysine (%)	0.9571	1.1407	0.93475	0.84475	1.0161	0.81305
Methionine (%)	0.3944	0.9052	0.38185	0.35835	0.89135	0.340125
Calcium (%)	1.13	1.2037	1.1688	1.2881	1.3955	1.3072
NPP** (%)	0.5392	0.5748	0.496	0.60215	0.6616	0.55225

*ME: Metabolizable Energy in Kilo calorie per kilogram of dry matter, **NPP: Non Phytate Phosphorus

Experimental design and data recording

The keets were raised on the ground on peanut hull litter during the 8 weeks of the experiment, starting from 1 day old. They were randomly allocated among the 12 boxes, with 9 keets per box. Water and feed were distributed *ad bilitum*. The three dietary treatments (maize, millet, sorghum) were randomly distributed in the boxes with four repetitions per treatment. The experiment parameters monitored included feed intake, live weight, average daily gain, and feed conversion ratio.

Feed intake was evaluated by calculating the difference between the quantities distributed and refusal every day. Within each box, the average feed intake per guinea fowl, expressed in grams (g) per day (d), was determined by dividing the total amount consumed by the number of guinea fowl on that particular day.

At the start of the experiment, the initial keet weight was recorded. At the end of the starter period (4 weeks) and the experiment (8 weeks), all keets from each batch were weighed individually. The average live weight of keet in grams (g) at different phases (start, 4 weeks, and 8 weeks old) was determined by the ratio of the total weight in g and the total number of keet in the same batch. Using weight measurements per period, the average daily gain (ADG) of guinea fowl at 4 and 8 weeks was calculated by taking the ratio of the average gain during a period to the duration in days.

The feed conversion ratio (FCR) was calculated by the ratio between the average amount of feed consumed by the keet over a given period and the average weight gain of this keet corresponding to this period (Gatien et al., 2020).

Statistical analysis

The data collected were entered into Excel 2016. The R software 4.2.1. (2022) was used to carry out the analysis of the variance of biological performances followed by the comparison of the arithmetic means using the Student-Newman-Keuls test to detect the effects of treatments. The means were compared to the 5%, that is for probability values (p value) lower than 0.05, the difference between treatments is considered as significant. Data expressed as mean ± SD.

RESULTS AND DISCUSSION

Feed intake

In the starter millet-based feed and at the grower phases, the sorghum-based feed was better consumed by the keets (Table 2). The group fed by corn had the lowest amount ingested by the keets. However, the difference was not statistically significant ($p > 0.05$). Considering the 8 weeks of the experiment, the keets in sorghum treatment had the greatest feed intake and exceeded the ingestions of feed in maize by 1.96 g/d and millet treatments by 0.17 g/d, without statistically significant difference ($p > 0.05$). The cereal used did not significantly influence the keet feed intake. These feeds have theoretically similar energy concentrations and crude protein (CP) levels. Variations in feed intake of guinea fowl have been reported with feeds varying in their composition, especially in energy and crude protein. An increase in energy density varying from 3050 to 3150 kcal/kg of feed reduced the guinea fowl feed ingestion (Nahashon et al, 2005). Tjetjoo et al. (2013) found that the control feed containing 20% CP in the starter and 18% during the grower phase was significantly less consumed by guinea fowl than feeds based on maize, millet, and sorghum which had 24% CP in the starter and 20% in the grower phase. The amount of feed ingested per keet in this study was greater than the amount reported by Ebegbulem and Asuquo (2018) in rural areas. This could be linked to the CP content because an increase in the ingestion of guinea fowl with the increase in the CP rate from 21 to 25% was reported by Nahashon et al. (2005).

Table 2. Local keet feed intake (g/d) depending on the cereal used for 8 weeks of the experiment

Parameters	Maize	Millet	Sorghum	P-value
Starter (1-4 weeks)	9.73 ± 1.14	10.94 ± 0.5	10.37 ± 0.84	0.25
Grower (5-8 weeks)	19.65 ± 1.36	22.02 ± 2.64	22.94 ± 2.49	0.29
All phases (1-8 weeks)	14.69 ± 5.52	16.48 ± 6.20	16.65 ± 6.93	0.26

Table 3. Local keet live weight (g) depending on the cereal used in 8 weeks of the experiment

Parameters	Maize	Millet	Sorghum	P-value
Initial live weight (1 day)	25.74 ± 1.007	25.03 ± 1.13	25.74 ± 0.38	0.46
Starter phase (4 weeks)	109.95 ± 6.22^{b}	135.75 ± 12.57^{a}	128.10 ± 14.42^{ab}	0.03
Grower phase (8 weeks)	274.88 ± 16.26	336.83 ± 47.49	329.60 ± 44.05	0.09

$^{a, b}$ indicate that the values with the same letters on the same row are not statistically different (P > 0.05).

Table 4. Local keet average daily gain (g/d) depending on the cereal used in 8 weeks of the experiment

Parameters	Maize	Millet	Sorghum	P-value
Starter (1-4 weeks)	3.007 ± 0.24^{b}	3.95 ± 0.42^{a}	3.65 ± 0.52^{ab}	0.02
Grower (5-8 weeks)	5.89 ± 0.50	7.18 ± 1.49	7.19 ± 1.09	0.21
All phases (1-8 weeks)	4.44 ± 0.28	5.56 ± 0.85	5.42 ± 0.78	0.09

$^{a, b}$ indicate that the values with the same letters on the same row are not statistically different (p > 0.05).

Table 5. Local keet feed conversion ratio (kg/kg) depending on the cereal used in 8 weeks of the experiment

Parameters	Maize	Millet	Sorghum	P-value
Starter (1-4 weeks)	3.67 ± 0.46	3.17 ± 0.23	3.47 ± 0.54	0.31
Grower (5-8 weeks)	3.40 ± 0.91	3.60 ± 0.81	3.98 ± 0.34	0.54
All phases (1-8 weeks)	3.53 ± 0.46	3.38 ± 0.48	3.73 ± 0.40	0.58

Live weight

At the start of the experiment, the initial keet weight was similar for all dietary treatments (Table 3). At the end of the starter period, the keets fed the millet-based feed had the highest live weight and exceeded those from the maize by 25.80 g and those from sorghum treatments by 7.65 g. This difference was statistically significant ($p < 0.05$, Table 3). At the end of the experiment (8 weeks old), the keet from the millet treatment also had the highest live weight, compared to the guinea fowl from the maize and sorghum treatments. This difference could be attributed to the level of lysine and methionine in diet which was higher than the levels contained in corn and sorghum-based feeds. Guinea fowl have a better live weight with a lysine content varying from 0.8 to 1.04 in their feed (Portillo Salgado et al., 2022), and feed containing a higher level of methionine improves poultry growth performance (Bunchasak, 2009). The live weights of the guinea fowl obtained were higher than those observed by Ouattara et al. (2016) at 54 days with feeds containing 17.5 to 20% crude protein. It can be due to the protein level in the diet.

Average daily gain

The average daily gain (ADG) of keet was statistically significant at the starter phase between the dietary treatments ($p < 0.05$; Table 4). The millet-based feed induced the greatest ADG at this phase. During the growth phase, the keet ingesting the millet and sorghum-based feeds had similar ADGs (Table 4) and exceeded the guinea fowl in the maize treatment. Considering the experiment period, the keet ingesting the millet-based feed had a higher average ADG and this ADG was higher without significant difference than the average ADG of those consuming the maize-based feed by 1.12 g/d and the sorghum-based feed by 0.14 g/d ($p > 0.05$). This insignificant difference might be due to similarity in crude protein levels that satisfy the nutritional needs regardless of the cereal used in the guinea fowl's diet. The reduction in protein content from 21.48 to 19.11% led to a decrease in weight gain of 5.8% in week 4 (Lombo et al., 2018). However, the increase in protein content of 24 to 25% with the same energy level did not significantly increase the weight gain of guinea fowl (Amoah et al., 2018).

These results of ADG are similar to those found by Sanfo et al. (2015) in a controlled environment, but lower than those reported by Tjetjoo et al. (2013).

Feed conversion ratio

Keets fed with millet-based feed had the lowest FCR followed by those fed with sorghum-based feed in the starter phase. Keets ingested in the maize-based feed had a higher FCR in the starter phase (Table 5). During the growing period, keet that fed maize had the lowest FCR and those fed sorghum had higher FCR. However, this difference was not statistically significant ($p > 0.05$). On average, during the 8 weeks of the experiment, the sorghum-based feed caused a higher FCR of 0.2 from the maize group and 0.35 with those fed millet-based feed. However, this difference was not statistically significant ($p > 0.05$). Tjetjoo et al. (2013) did not report any difference between the FCR induced by the different cereals. However, the control feed containing less crude protein content induced higher FCR than the feeds containing cereals. In addition, Seabo et al. (2011) reported that an increase in FCR was associated with a decrease in the crude protein content in the diet. The FCR obtained in the present study were lower than those reported by Ouattara et al. (2016) in the starter and grower phase of guinea fowl, also lower to those obtained by Tjetjoo et al. (2013) at 16 weeks by using corn, millet and sorghum in keet diet.

CONCLUSION

During the 8 weeks of experimentation, the maize, millet, or sorghum used in feed did not have a statistical effect on keet growth performance. However, the millet-based feed had a greater influence on live weight, and average daily gain and presented the lowest feed conversion ratio than the other feeds. The keet average daily gain induced by the sorghum-based feed was higher than that induced by the maize-based feed during the experimentation period, but the feed conversion ratio obtained with the maize-based feed was better than that recorded with the sorghum-based feed. For a choice of cereal in the diet of guinea fowl, millet would be best indicated for growth performance, followed by maize and sorghum. Further research should be carried out on the effect of cereals on guinea fowl egg production.

DECLARATION

Funding

This research received no external funding.

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 Consideration

All authors have reviewed ethical concerns, such as data fabrication, double publication and submission, redundancy, plagiarism, consent to publish, and misconduct before being published in this journal.

Acknowledgments

The authors would like to thank the National Institute for Agriculture Research of Niger (INRAN) and Dan Dicko Dankoulodo the University of Maradi (UDDM), Niger for creating the conditions for carrying out this experiment. The author also acknowledges the contributions of reviewers of an earlier version of this manuscript who suggested improvements.

Authors' contributions

Nouri Brah, Issa Chaibou, and Ousseini Moussa Hassan contributed to the conceptualization, investigation, data curation, and writing manuscript. Abdoul Rachidou Sodo Daka is involved in data collection and analysis. All authors approved and read the final version of the manuscript

Conflicts of interests

The authors declare no conflict of interest for this article.

REFERENCES

Amoah OK, Nyameasem KJ, Asiedu P, Adu-Aboagye AG, Wallace P, Ahiagbe JMK, and Rhule AWS (2018). Protein and energy requirements for indigenous guinea keets (*Numida meleagris*) in southern Ghana. Ghana Journal of Agricultural Science, 52: 105-111. Available at: https://www.ajol.info/index.php/gjas/article/view/179721

Brah N, Houndonougbo MF, and Issa S (2015). Etapes et méthodes de formulation d'aliment de volaille: Une synthèse bibliographique [Steps and methods of poultry feed formulation: A bibliographical summary]. International Journal of Biological and Chemical Sciences, 9(6): 2924-2031. DOI: http://www.doi.org/10.4314/ijbcs.v9i6.31

Gatien BG, Fatoumata C, and Didier AC (2020). Effet de la densité de mise en charge sur le démarrage des pintadeaux de chair dans la commune de Korhogo [Effect of stocking density on the start of meat guinea fowl in the commune of Korhogo]. Journal of Animal & Plant Sciences, 45(1): 7799-7808. DOI: https://www.doi.org/10.35759/JAnmPlSci.v45-1.4

Bunchasak C (2009). Role of dietary methionine in poultry production. Japan Poultry Science, 46(3): 169-179. Available at: https://www.jstage.jst.go.jp/article/jpsa/46/3/46_3_169/_pdf

Ebegbulem VN and Asuquo BO (2018). Growth performance and carcass characteristics of the black and pearl guinea fowl (*Numida meleagris*) and their crosses. Global Journal of Pure and Applied

Sciences, 24(1): 11-16. DOI: https://www.doi.org/10.4314/gjpas.v24i1.2

Etuk EB, Ifeduba AV, Okata UE, Chiaka I, Okoli IC, Okeudo NJ, Esonu BO, Udedibie ABI, and Moreki JC (2012). Nutrient composition and feeding value of sorghum for livestock and poultry: A review. Journal of Animal Science Advances, 2(6): 510-524. Available at: https://silo.tips/download/nutrient-composition-and-feeding-value-of-sorghum-for-livestock-and-poultry-a-re

Filardi RS, Junqueira OM, Casartelli EM, Laurentiz AC, Duarte KF, and Assuena V (2005). Pearl millet utilization in commercial laying hen diets formulated on a total or digestible amino acid basis. Brazilian Journal of Poultry Science, 7(2): 99-105. DOI: https://www.doi.org/10.1590/S1516-635X2005000200006

Houndonougbo MF, Chwalibog A, and Chrysostome CAAM (2009). Is the nutritional of grains in broiler chickens' diets affected by environmental factors of soybean (*Glycine max*) growing and the variety of maize (*Zea maize*) in Benin?. Livestock Research for Rural Development, 21(2): 22. Available at: http://www.lrrd.org/lrrd21/2/houn21022.htm

Issa S, Jarial S, Brah N, and Harouna L (2016). Are millet and sorghum good alternatives to maize in layer's feeds in Niger, West Africa?. Indian Journal of Animal Sciences, 86(11): 1302-1305. DOI: https://www.doi.org/10.56093/ijans.v86i11.63370

Issa S, Jarial S, Brah N, Harouna L, and Soumana I (2015). Use of sorghum on stepwise substitution of maize in broiler feeds in Niger. Livestock Research for Rural Development, 27(10): 212. Available at http://www.lrrd.org/lrrd27/10/issa27212.html

Issa S, Hancock JD, Tuinstra MR, Brah N, Hassane A, Kapran I, and Kaka S (2010). Le sorgho, un bon substitut du maïs dans les rations des poulets de chair au Niger [Local sorghum as an alternative to maize in broiler diets in Niger]. Communication en Aviculture Familiale, 19(1) : 16-22 Available at: https://www.fao.org/3/aq613t/aq613t.pdf

Lombo Y, Tona K, Talaki E, and Bonfoh B (2018). Effet de l'alimentation sur la croissance des pintadeaux au nord du Togo [Effect of feed on growth of guinea poult in northern Togo]. International Journal of Biological and Chemical Science, 12(5): 2109-2118. DOI: https://www.doi.org/10.4314/ijbcs.v12i5.13

Medugu CI, Kwari ID, Igwebuike J, Nkama I, Mohammed ID, and Hamaker B (2010). Performance and economics of production of broiler chickens fed sorghum or millet as replacement for maize in the semi-arid zone of Nigeria. Agriculture and Biology Journal of North America, 1(3): 321-325 DOI: https://www.doi.org/10.5251/abjna.2010.1.3.321.325

Moreki JC and Seabo D (2012). Guinea fowl production in Botswana. Journal of World's Poultry Research, 2(1): 1-4. Available at: https://jwpr.science-line.com/attachments/article/12/JWPR,%20B1,%202012,%20%201-4.pdf

Nahashon SN, Adefope N, Amenyenu A, and Wright D (2005). Effect of varying metabolizable energy and crude protein concentrations in diets of pearl grey guinea fowl pullets: Growth performance. Poultry Science, 86(5): 973-982. DOI: https://www.doi.org/10.1093/ps/86.5.973

National research council (NRC) (1994). Nutrient requirements of poultry, 9th Revised Edition. National Academy Press., Washington, D. C. pp. 44-70. Available at: https://www.agropustaka.id/wp-content/uploads/2020/04/agropustaka.id_buku_Nutrient-Requirements-of-Poultry_Ninth-Revised-Edition-1994-NRC.pdf

Ouattara S, Bougouma-Yaméogo VMC, Nianogo AJ, and Savadogo B (2016). Influence de la substitution des graines de soja (Glycine max) par celles de niébé (Vigna unguiculta) et du taux de protéines du régime sur les performances des pintadeaux de race locale au Burkina Faso [Influence of the substitution of soybean seeds (Glycine max) with cowpea seeds (Vigna unguiculata) and the protein level of the diet on the performance of local guinea fowl in Burkina Faso]. Revue d'Elevage et de Médecine Vétérinaire des Pays Tropicaux, 69(3): 117-123. DOI: https://www.doi.org/10.19182/remvt.31195

Portillo Salgado R, Bautista Ortega J, Chay-Canul JA, Sánchez-Casanova ER, Segura-Correa CJ, and Cigarroa-Vázquez AF (2022). Factors affecting productive performance of guinea fowl: A review. Tropical and Subtropical Agroecosystemsm, 25(2): 79. DOI: https://www.doi.org/10.56369/tsaes.3861

Ravindran V (2013). Alternative feedstuffs for use in poultry feed formulations. Poultry development review: Poultry feed availability and nutrition in developing countries. FAO., Rome, Italy, pp. 72-75. Available at: http://213.55.90.4/admin/home/Dmu%20Academic%20Resource/Agriculture%20And%20Natural%20Resource/postgraduate/animal%20production/Poultry%20farming.pdf#page=78

Sanfo R, Ouoba Ima S, Salissou I, and Tamboura HH (2015). Survie et performances de croissance des pintadeaux en milieu contrôlé au nord du Burkina Faso [Survival and growth performance of guinea fowl in a controlled environment in northern Burkina Faso]. International Journal of Biological and Chemical Science, 9(2): 703-709. DOI: http://www.doi.org/10.4314/ijbcs.v9i2.11

Seabo D, Moreki JC, Bagwasi N, and Nthoiwa GP (2011). Performance of guinea fowl (NUMIDA MELEAGRIS) fed varying protein levels. Online Journal of Animal Feed Research, 1(6): 255-258. Available at: https://www.ojafr.ir/main/attachments/article/80/OJAFR,%20A43,%20255-258,%202011.pdf

Soara EA, Talaki E, Dayo GK, Oke EO, Belem GMA, and Tona K (2020). Indigenous guinea fowl (*Numida meleagris*) production in West Africa: Inventory, performances and constraints– A review. European Poultry Science, 84: 1-13. DOI: https://www.doi.org/10.1399/eps.2020.303

Teguia A and Beynen AC (2005). Alternative feedstuffs for broilers in Cameroon. Livestock Research for Rural Development, 17(3): 34. Available at: http://www.lrrd.org/lrrd17/3/tegu17034.htm

Tjetjoo US, Moreki CJ, Nsoso JS, and Madibela RO (2013). Growth performance of guinea fowl fed diets containing yellow maize, millet and white sorghum as energy sources and raised under intensive system. Pakistan Journal of Nutrition, 12(4): 306-312. DOI: https://www.doi.org/10.3923/pjn.2013.306.312

JWPR
Journal of World's Poultry Research
2024, Scienceline Publication
J. World Poult. Res. 14(2): 138-145, 2024
Short Communication
DOI: https://dx.doi.org/10.36380/jwpr.2024.14
PII: S2322455X2400014-14
ISSN: 2322-455X

Control of Field Infectious Bronchitis Virus Genotype GI-23 (Variant 2) Using Combined Heterologous Vaccine Genotype Strains GI-13 (1/96) and GI-1 (H120)

Mirel Enache[1], Mihai Pirvulet[1], Guillermo Gonzalez Garcia[2], Mathilde Lecoupeur[2], Higor Cotta[2], and Konstantinos Koutoulis[3*]

[1]*Ceva Sante Animale, 5 Chindiei Street, 040185, Bucharest, Romania*
[2]*Ceva Sante Animale, 10 Avenue de la Ballastière, Libourne, France*
[3]*Department of Poultry Diseases, Faculty of Veterinary Science, School of Health Sciences, University of Thessaly, Karditsa 43100, Greece*

*Corresponding author's E-mail: kkoutoulis@vet.uth.gr

Received: March 10, 2024, Revised: April 15, 2024, Accepted: May 13, 2024, Published: June 25, 2024

ABSTRACT

Infectious bronchitis (IB) is a disease with significant economic impacts both on the costs of control strategies and on productive losses. Various vaccination protocols are applied, depending on homologous or heterologous protection against IB and finding the optimal balance between costs and benefits as a choice by a responsible veterinarian. The current case study aimed to demonstrate the efficacy of a heterologous vaccine combination against field IBV GI-23 (Variant 2) infection when vaccination quality was properly monitored. Two groups, each consisting of six flocks, were examined before and after improving the quality of IB vaccine application in the hatchery. These groups were vaccinated with H-120 and 1/96 vaccine strains for heterologous protection. The study involved field visits, necropsies, serology via ELISA, and oropharyngeal sampling for RT-PCR follow-up activities. Moreover, performance parameters including average body weight, feed conversion ratio, and 7 days plus total mortality were analyzed at the end of the production cycle at 40-45 days of age. Results indicated that the group with enhanced vaccination quality in the hatchery exhibited a significant decrease in IBV titers and an absence of IBV GI-23 field infection. Additionally, there was an improvement in performance data in terms of average body weight, FCR and total mortality. Hatchery vaccination proved to be more controllable and practical compared to traditional on-farm vaccination, ensuring better control of the vaccination process and massive coverage of the farm population.

Keywords: Heterologous protection, Infectious bronchitis, Vaccine strain 1/96, Variant 2

INTRODUCTION

Infectious bronchitis (IB) is a disease that causes major economic losses for the poultry industry worldwide. It has been listed as one of the most harmful diseases for livestock (World Bank; TAFS Forum, 2011). IB is a highly transmittable viral disease caused by an avian coronavirus whose taxonomy has previously been fully described (Coronaviridae family, Orthocoronavirinae subfamily, Gammacoronavirus Genus, and Igacovirus subgenus; Lefkowitz et al., 2018). The genome of IBV is a single-stranded positive-sense RNA and its properties have been extensively analyzed (Boursnell et al., 1987). The IBV's high substitution and recombination rate is similar to other single-stranded RNA viruses (Duffy et al., 2008; Simon-Loriere & Holmes, 2011) and it can lead to the emergence of several genotypes and lineages over time (Valastro et al., 2016). In terms of prevention and population dynamics, live attenuated vaccines are the most effective ones (Jordan, 2017). The most used IB vaccines are live attenuated vaccines, which can mimic the natural infection process of the field viruses with minor post-vaccination reactions and proven efficacy by inducing strong humoral, cellular, and local immunity against closely related strains (Bande, 2015; Bhuiyan et al., 2021). Live vaccines have demonstrated efficacy in widening the conferred protection by combining unrelated IBV vaccine strains to protect against other IBV clusters, an effect which is known as heterologous protection (Cook et al., 1999). Protection with homologous vaccines has also been introduced but their applicability is limited due to interpretation and differentiation of field strains (Legnardi et al., 2022a). Having stated that, several experiments have been performed to explain the mechanism of heterologous or cross-protection vaccination such as assessing kinetics (Tucciarone et al., 2018) and promoting cross-protection

levels by different vaccines through several experimental trials (Franzo et al., 2016; Legnardi et al., 2022b). In such trials, different administration routes and vaccination timings were studied using various assessment methods like quantification of the viral load of the vaccine strains from tracheal swabs (Jackwood, 2009), ciliostasis observation, and challenge virus detection (Tatar-Kis et al., 2014). Among the numerous IBV variant lineages, Genotype I-23 (GI-23) is the most prevalent in the Middle East and Persian Gulf countries, Western Africa, Southern Africa, Turkey, and Eastern Europe, with sporadic detections in Germany and Benelux (Houta et al., 2021). GI-23 was characterized macroscopically and it was found to induce depression, huddling, respiratory symptoms, and diarrhea with a 30% mortality rate 6 days post-infection (dpi) in Specific Pathogen Free (SPF) broiler chickens (Lisowska et al., 2021). Moreover, in regions affected by GI-23, increased virulence and co-infections with other pathogens, such as low-pathogenic avian influenza H9N2, *Escherichia coli*, or *Mycoplasma*, have been reported in commercial poultry farms (Samy and Naguib, 2018). The present case study aimed to demonstrate, through Real-World Evidence analysis, the efficacy of a heterologous vaccine combination against field IBV GI-23 (Variant 2) infection.

MATERIALS AND METHODS

Ethical approval

Since the sampling of the broilers was done during common commercial activities in the farm regulated by national and international laws, ethical review and approval were waived for this study.

Experimental design

Six commercial Ross 308 broiler flocks of 20,000 to 30,000 broilers each with *ad libitum* access to feed and water were investigated in the South-Eastern region of Romania (Ialomița county) from April 2022 to March 2023. Upon the farmer's call, the main symptoms observed in the affected flocks included the general lack of performance, increased mortality, nephritis, watery diarrhea, and wet litter. The diseases including IBV, infectious bursal disease (IBD), Avian rhinotracheitis (ART), Newcastle disease (NDV), *Mycoplasma gallisepticum* (MG), and *Mycoplasma synoviae* (MS) were investigated for differential diagnosis using either RT-PCR or serology (Table 1). Due to local constraints, no access to the hatchery premises was initially allowed; therefore, the quality of application of the IBV vaccine was not assessed until the final investigation stage, when vaccine preparation and application were inspected following Bureau Veritas® Ceva C.H.I.C.K. program recommendations (Franzo et al., 2019). Field, follow-up activities, such as necropsy, serology and RT-PCR, were initiated from the first day of investigation. As a result, two different groups were proposed based on the quality of IB vaccine application in the hatchery: 1) Flocks vaccinated before inspection (group A); 2) Flocks vaccinated after inspection by Bureau Veritas® Ceva C.H.I.C.K. program (group B).

Serology

For both groups, individual blood samples from 100 broilers were collected from all flocks for ELISA analysis by wing puncture from a branchial vein (Kelly and Alworth, 2013) using a small needle, in accordance with all animal welfare protocols between 39 to 42 days of age (doa). After collecting approximately 2 ml of blood from the wing (according to the Biochek® ELISA Kit manufacturers' recommendations, Biochek, Netherlands), individual sera were collected, labeled, and sent for further analysis (Synevovet Str. Industriilor, Nr. 25, comuna Chiajna judetul Ilfov, 077040 Romania).

RT-PCR

Individual oropharyngeal samples for RT-PCR (Tucciarone et al., 2018) were printed on Qiagen® indicating FTA cards (Qiagen, Hulsterweg 82, 5912 PL Venlo, The Netherlands) and sent for further processing to a third-party diagnostics laboratory (De Gezondheidsdienst voor Dieren, Postbus 9, 7400 AA Deventer, The Netherlands) (Table 1).

Table 1. Investigated diseases, methods of investigation, and age of sampling per house in control of field infectious bronchitis virus genotype GI-23 (variant 2) with the combined use of two heterologous vaccines

House	Date	Investigated diseases	Age of sampling (doa)	Serology (n)	RT-PCR (n)
9	19-04-2022	IBV	41	Y (20)	N
8	09-08-2022	IBV, IBD, NDV	42	Y (20)	N
7	03-10-2022	NDV, IBV	42	Y (20)	N
3	22-11-2022	NDV, IBD, IBV, MG, MS, ART	39	Y (20)	Y (4)
3	19-12-2022	IBV	6	N	Y (8)
3	19-01-2023	NDV, IBD, IBV	41	Y (20)	Y (4)

Y: Yes, N: No; Doa: Days of age; n: Number of samples.

Table 2. Vaccination program during the present study.

Vaccine	Manufacturer	Disease	Age (doa)	Site	Route
Cevac® Broiler ND K	Ceva Santé Animale	Newcastle Disease	1	Hatchery	Subcutaneous
Cevac® Transmune IBD		Infectious Bursal Disease	1	Hatchery	Subcutaneous
Cevac® Vitabron L		Infectious Bronchitis	1	Hatchery	Spray
Cevac® IBird™		Infectious Bronchitis	1	Hatchery	Spray
Cevac® New L		Newcastle Disease	10	Farm	Spray
Cevac® New L		Newcastle Disease	20	Farm	Drinking water

doa: Days of age.

Vaccination program

The broiler prophylactic program, techniques, and machinery used for vaccination remained intact throughout the investigational period. The vaccination program included IBD, ND, and IB live vaccines applied both in the hatchery and on the farm. The machine used for IB spray vaccination at day 1 was an Ecat® Spray Cabinet (Ecat-ID, France). Subcutaneous vaccination was done with a Dovac® double V2 injector (Ceva, 2024; Table 2).

Performance parameters

The performance of 9 houses from 6 flocks during 4 consecutive cycles was obtained and correlated with the previous analyses. The collected data included the day of chick placement, age at slaughter, average body weight, Feed Conversion Ratio (FCR), mortality at 7 days of age, and total mortality.

Statistical analysis

Statistical visualizations and tests were performed using Python, v3.10.6.2022. Non-parametrical Wilcoxon-Mann-Whitney (test U of Mann-Whitney) with an alpha-risk at 5% was used, considering the relatively low-performance data points (18 in Group A vs. 18 in Group B).

RESULTS

Serology

The analysis of the titers during the investigational period (April 2022 to March 2023) indicated a wide span of individual titers (Figure 1).

According to the ELISA Kit supplier's interpretation, above 3000 units is the threshold at which field challenges can be considered. The range of titers increased widely above 3000, reaching up to 14000 units in the examined flocks. In addition, a significant difference ($p < 0.05$) in the titers between houses was observed (Figure 2). As stated above, groups A and B were selected according to the maintenance state of the hatchery sprayer cabinet. When comparing the titers of these two groups, a strong significant difference was detected between them, with the group before auditing in the hatchery having significantly higher titers (Figure 3). When analyzing the individual titers in the two groups, most of the individual titers in group A were above that limit (n = 53; 66,3%). In group B, only 4 out of 20 broiler chickens (20%) had titers above the 3000-unit limit (Figure 4).

RT-PCR

The number of RT-PCR results was not significant to compare groups using statistical analysis. RT-PCR yielded positive results for GI-23 (Variant 2) in group A and positive results for GI-13 (1/96) in group B at 6 and 41 days of age (Table 3).

Performance

Performance data of 36 flocks was obtained and analyzed (18 versus 18, Table 4). The null hypothesis (H0) was based on the absence of difference between the two groups, whereas the alternative hypothesis (Ha or H1) was based on a difference between the two groups. Due to the low number of data points, it was not possible to find a statistically significant difference between groups A and B in density, age at slaughter, total mortality, Average Body Weight, and FCR (Figures 5 and Table 5).

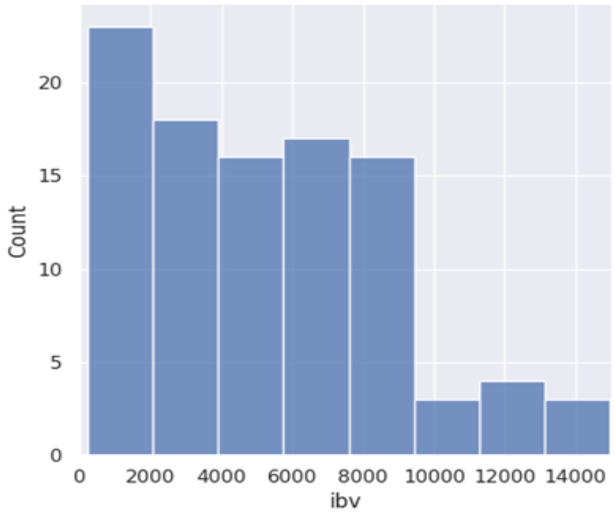


Figure 1. Distribution of ELISA Infectious Bronchitis Virus titers (n= 100) in the flocks (n= 6); in control of field infectious bronchitis virus genotype GI-23 (variant 2) with the combined use of two heterologous vaccine genotype GI-13 (1/96) and GI-1 (H120) strains. Serology was analyzed by Biochek® ELISA IBV kit

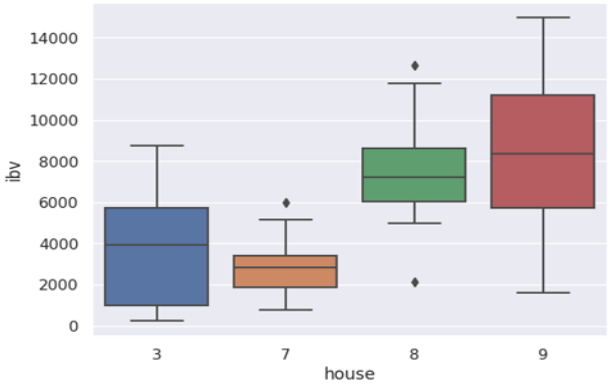


Figure 2. Distribution of ELISA Infectious Bronchitis Virus titers (n=100) in the investigated houses; in control of field infectious bronchitis virus genotype GI-23 (variant 2) with the combined use of two heterologous vaccine genotype GI-13 (1/96) and GI-1 (H120) strains. Biochek® ELISA IBV kit; (test U of Mann-Whitney; $p < 0.05$)

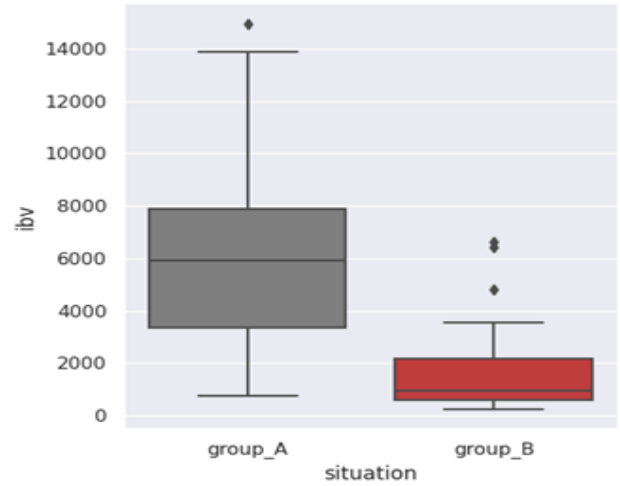


Figure 3. Distribution of ELISA Infectious Bronchitis Virus titers per group before and after auditing in the hatchery (n = 100); in control of field infectious bronchitis virus genotype GI-23 (variant 2) with the combined use of two heterologous vaccine genotype GI-13 (1/96) and GI-1 (H120) strains. Biochek® ELISA IBV kit; (test U of Mann-Whitney; $p < 0.05$)

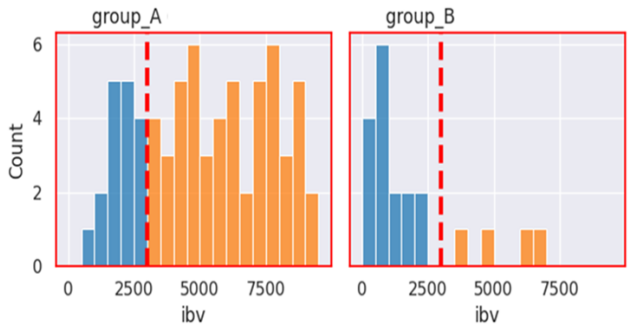


Figure 4. Distribution of individual ELISA Infectious Bronchitis Virus titers per group before auditing (group A) and after auditing (group B) in control of field infectious bronchitis virus genotype GI-23 (variant 2) with the combined use of two heterologous vaccine Genotype GI-13 (1/96) and GI-1 (H120) strains. n = 80 in Group A; n = 20 in group B; Challenge limit = 3000 units.

Table 3. Age of sampling, number, and type of sample in control of field infectious bronchitis virus genotype GI-23 (variant 2) with the combined use of two heterologous vaccine genotype GI-13 (1/96) and GI-1 (H120) strains

House	Group	Date	Investigated Disease	Age of sampling (doa)	RT-PCR sample type (n)	Results
3	A	22-11-2022	IBV	39	Oronasal (1) Caecal tonsils (1) Kidney (2)	GI-23 (Variant 2)
3	B	15-12-2022	IBV	6	Oropharyngeal swabs (8)	GI-13 (1/96)
3	B	19-01-2023	IBV	41	Caecal tonsils (4)	GI-13 (1/96)

n: Number of samples; doa: Days of age

Table 4. Performance of group A (before auditing) and group B (after auditing) in control of field infectious bronchitis virus genotype GI-23 (variant 2) with the combined use of two heterologous vaccine genotype GI-13 (1/96) and GI-1 (H120) strains

House	Placement date	Group	Age at slaughter	ABW	FCR	Mortality 0-7 d (%)	Total mortality (%)	Density (m^2)
1	22/08/2022	Group A	42	2853	1,63	0,32	2,32	21,20
2	18/08/2022	Group A	42	2830	1,62	0,35	2,61	20,61
3	18/08/2022	Group A	43	2932	1,61	0,35	2,75	20,61
4	22/08/2022	Group A	42	2845	1,61	0,43	2,91	21,20
5	22/08/2022	Group A	43	3040	1,62	0,52	3,03	21,20
6	22/08/2022	Group A	42	2808	1,63	0,50	2,65	21,20
7	22/08/2022	Group A	43	2869	1,63	0,54	2,54	21,20
8	22/08/2022	Group A	42	2831	1,64	0,60	2,41	21,20
9	22/08/2022	Group A	43	2855	1,65	0,58	2,68	21,20
1	14/10/2022	Group A	42	2890	1,61	0,41	2,39	20,25
2	14/10/2022	Group A	41	2649	1,66	0,42	4,53	20,25
3	14/10/2022	Group A	41	2646	1,65	0,51	4,71	20,25
4	14/10/2022	Group A	42	2880	1,61	0,51	2,28	20,25
5	17/10/2022	Group A	42	2782	1,59	0,26	2,69	20,86
6	17/10/2022	Group A	42	2802	1,59	0,29	2,68	20,86
7	17/10/2022	Group A	43	2833	1,61	0,29	3,13	20,86
8	17/10/2022	Group A	43	2843	1,62	0,27	2,81	20,86
9	17/10/2022	Group A	43	2732	1,61	0,45	3,43	20,86
1	09/12/2022	Group B	41	2769	1,58	0,46	2,95	20,43
2	09/12/2022	Group B	40	2702	1,56	0,51	2,86	20,43
3	09/12/2022	Group B	41	2781	1,59	0,45	2,90	20,43
4	09/12/2022	Group B	41	2746	1,59	0,40	3,11	20,43
5	09/12/2022	Group B	41	2855	1,59	0,44	3,00	20,43
6	12/12/2022	Group B	44	3026	1,60	0,33	2,81	21,03
7	12/12/2022	Group B	43	2909	1,60	0,32	2,57	21,03
8	12/12/2022	Group B	44	2888	1,60	0,38	2,64	21,03
9	12/12/2022	Group B	43	2775	1,57	0,32	2,81	21,03
1	03/02/2023	Group B	45	3122	1,64	0,52	2,67	20,79
2	03/02/2023	Group B	41	2810	1,62	0,51	2,73	20,79
3	03/02/2023	Group B	42	2855	1,63	0,48	2,63	20,79
4	03/02/2023	Group B	42	2826	1,62	0,58	2,85	20,79
5	03/02/2023	Group B	41	2651	1,62	0,25	1,99	20,79
6	06/02/2023	Group B	43	2855	1,63	0,35	2,26	21,33
7	06/02/2023	Group B	42	2835	1,62	0,61	2,45	21,33
8	06/02/2023	Group B	42	2927	1,62	0,30	1,97	21,33
9	06/02/2023	Group B	43	2868	1,63	0,51	3,03	21,33

ABW: Average body weight at slaughter; FCR: Feed conversion rate; doa: Days of age.

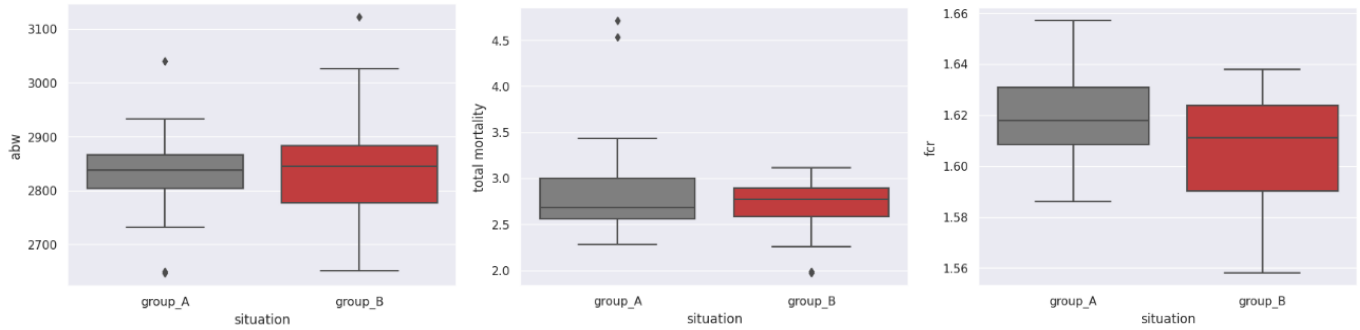


Figure 5. Differences in performance between groups A and B in control of field infectious bronchitis virus genotype GI-23 (variant 2) with the combined use of two heterologous vaccine genotypes GI-13 (1/96) and GI-1 (H120) strains. ABW: Average body weight at slaughter; FCR: Feed conversion rate (test U of Mann-Whitney; $p < 0.05$)

Table 5. Average body weight, FCR, and mortality in control of field infectious bronchitis virus genotype GI-23 (variant 2) with the combined use of two heterologous vaccine genotype GI-13 (1/96) and GI-1 (H120) strains

KPI	Group A: average	Group B: average	Group A: std	Group B: std	p-value
Age at slaughter	42.277778	42.16667	0.669113	1.339447	0.562474
Density (birds/m^2)	20.828889	20.863333	0.376140	0.338509	0.910984
Average body weight	2828.888889	2844.444444	91.861077	110.434022	0.824615
FCR	1.620778	1.606167	0.019907	0.023183	0.136859
Mortality 0-7 d	0.422222	0.428889	0.110536	0.102721	0.849134
Total mortality	2.919444	2.679444	0.683662	0.330285	0.727721

KPI: Key performance indicators; std: Standard deviation.

DISCUSSION

In this field investigation, a thorough analysis of all the predisposing factors influencing disease occurrence was undertaken. However, as previously explained, access to the hatchery was only granted after the analysis of the first three production cycles in 2022. Therefore, the quality of the vaccine application was audited only after that time point. This factor initially compromised the implementation of all necessary mechanisms required to find out the root cause of the issue.

In terms of serology, a wide distribution of titers was observed in the investigated houses. Geometric Mean Titer values indicated a representative difference ($p < 0.05$) between both groups before and after the investigation. However, the consideration that individual IBV-ELISA titers reflect challenging situations on the farm in a more specific way was eminent. The method of considering 3000 ELISA units as the threshold for flocks that suffer IBV-variant challenge was within the recommendations of the ELISA kit manufacturer.

In this sense, the range of individual ELISA-IBV titers spanned from 0 to 15000 ELISA units, with the majority of samples above the 3000 limit, indicating a disease challenge aligned with previous experiences (Cortés et al., 2022). Indeed, the titer differences between groups A and B indicated that this might have been occurring before the failure in the vaccination technique was discovered in the hatchery. The importance of audit control on vaccination quality has been well established previously by Franzo et al. (2019). Once the issue was addressed, the ELISA-IBV titers dropped dramatically, falling further below the 3000-unit threshold. This indicated that GI-23 was under control and confirmed the initial hypothesis and approach to individual serological analysis.

As a confirmation of the previous serological findings, validation of IBV control through RT-PCR was recommended. RT-PCR confirmed GI-23 (Variant 2) clearance in December 2022. The first confirmation of strain GI-13 (1/96) replication was observed at 6 doa via oropharyngeal swabs. This method is an early, useful, and welfare-friendly procedure that addresses vaccination processes directly at the farm level. Final confirmation was achieved via RT-PCR analysis of the caecal tonsils at 41 doa, demonstrating that GI-13 (1/96) outcompeted the GI-23 (Variant 2) field strain detected in the previous flock in November 2022. This confirms the results of previous research works that propose heterologous cross-protection as the only mechanism to control IBV population and genetic drift worldwide, either by direct competitive exclusion or by a strong local immune response that inhibits the replication of the wild IBV strain in the immunized broilers (Franzo et al., 2016; Lisowska et al., 2021). Additionally, heterologous vaccination is not proven to induce actively the escape of wild IBV populations from vaccine-induced immunity over time (Vermeulen et al., 2023). The occurrence of subpopulations within IBV GI-23-based vaccines and the variability featuring different production batches which complicates the differentiation between field and vaccine-derived strains based on sequence analysis alone has also been demonstrated by Legnardi et al. (2022a), thereby presenting another challenge in need of solutions. Generally, IB is accompanied by secondary pathogens that enhance the pathogenicity of the virus and its effects on condemnation rates (Assayag Júnior et al., 2012; Linares et al., 2017). The latter observation was also reflected along with a general impact on performance. However, despite clear trends observed after the improvement of the vaccination process (e.g. mortality and FCR improvement), the number of observations was insufficient to establish a statistically representative difference.

CONCLUSION

Real World Evidence comprises tangible data that enables veterinary professionals to make swift and informed decisions based on well-analyzed field data. In this study, the analysis of field and diagnostic data confirmed that the combination of GI-1 (Mass) and GI-13 (1/96) at day 1 in the hatchery is a highly effective mechanism to protect against the increasing presence of GI-23 (Variant 2) in parts of the world where it is prevalent, making it unnecessary to use a homologous GI-23 vaccine where a Differentiating Infected from Vaccinated Animals (DIVA) strategy is not feasible. Vaccinating in the hatchery is easier to control diseases and more practical to apply than traditional on-farm application. It ensures improving the control of the vaccination process and provides extensive coverage of the farm population, allowing effective protection against several heterologous IBV field strains.

DECLARATIONS

Authors' contributions

Mirel Enache and Mihai Pirvulet contributed to the study design, investigation, and accuracy of the data. Mathilde Lecoupeur, Higor Cotta, Guillermo Gonzalez Garcia, and Konstantinos Koutoulis participated in investigation activities, analysis of the data, writing, reviewing, and editing of the manuscript. All authors checked and approved the final version of the manuscript.

Acknowledgments

The authors would like to thank Ceva Animal Health, Romania, for providing financial support for this study.

Funding

This research was funded by Ceva Animal Health, Romania.

Ethical considerations

The authors confirm that all contributors to this study have reviewed and submitted the manuscript to this journal for the first time.

Availability of data and materials

The original information presented in the study is included in this article. More data can be requested from the corresponding authors.

Conflict of interests

The authors declare that no conflicts of interest exist.

REFERENCES

Assayag Júnior MS, Chacón JL, Rocha PT, and Kuana S (2012). Economic impact of infectious bronchitis in a Brazilian poultry integration system. VII. International Symposium on Avian Corona- and Pneumoviruses and Complicating Pathogens, Rauischholzhausen, Germany, pp. 18-21. Available at: https://www.cabidigitallibrary.org/doi/full/10.5555/20133383893

Bande F, Arshad SS, Bejo MH, Moeini H, and Omar AR (2015). Progress and challenges toward the development of vaccines against avian infectious bronchitis. Journal of Immunology Research, 2015: 424860. DOI: https://www.doi.org/10.1155/2015/424860

Bhuiyan SA, Amin Z, Rodrigues KF, Saallah S, Shaarani S, Sarker S, and Siddiquee S (2021). Infectious bronchitis virus (Gammacoronavirus) in poultry farming: Vaccination, immune response and measures for mitigation. Veterinary Sciences, 8(11): 273. DOI: https://www.doi.org/10.3390%2Fvetsci8110273

Boursnell MEG, Brown TDK, Foulds IJ, Green PF, Tomley FM, and Binns MM (1987). Completion of the Sequence of the genome of the coronavirus avian infections bronchitis virus. Journal of General Virology, 68: 57-77. DOI: https://www.doi.org/10.1099/0022-1317-68-1-57

Ceva Animal Health (2024). Available at: https://poultry.ceva.com/data-and-equipment/hatchery-vaccination/desvac-dovac/

Cook JKA, Orbell SJ, Woods MA, and Huggins MB (1999). Breadth of protection of the respiratory tract provided by different live-attenuated infectious bronchitis vaccines against challenge with infectious bronchitis viruses of heterologous serotypes. Avian Pathology, 28(5): 477-85. DOI: https://www.doi.org/10.1080/03079459994506

Cortés V, Sevilla-Navarro S, García C, Marín C, and Catalá-Gregori P (2022). Seroprevalence and prevalence of infectious bronchitis virus in broilers, laying hens and broiler breeders in Spain. Poultry Science, 101(5): 101760. DOI: https://www.doi.org/10.1016/j.psj.2022.101760

Duffy S, Shackelton LA, and Holmes EC (2008). Rates of evolutionary change in viruses: Patterns and determinants. Nature Reviews Genetics, 9: 267-276. DOI: https://www.doi.org/10.1038/nrg2323

Franzo G, Tucciarone CM, Blanco A, Nofrarías M, Biarnés M, Cortey M, Majó N, Catelli E, and Cecchinato M (2016). Effect of different vaccination strategies on IBV QX population dynamics and clinical outbreaks. Vaccine, 34(46): 5670-5676. DOI: https://www.doi.org/10.1016/j.vaccine.2016.09.014

Franzo G, Swart W, Boyer W, Pasotto D, Ramon G, Koutoulis K, and Cecchinato M (2019). No good vaccination quality without good control: The positive impact of a hatchery vaccination service program. Poultry Science, 99(6): 2976-2982. DOI: https://www.doi.org/10.1016/j.psj.2020.03.017

Houta MH, Hassan KE, Legnardi M, Tucciarone CM, Abdel-Moneim AS, Cecchinato M, El-Sawah AA, Ali A, and Franzo G (2021). Phylodynamic and recombination analyses of avian infectious bronchitis GI-23 reveal a widespread recombinant cluster and new among-countries linkages. Animals, 11(11): 3182. DOI: https://www.doi.org/10.3390/ani11113182

Jackwood MW, Hilt DA, McCall AW, Polizzi CN, McKinley ET, and Williams SM (2009). Infectious bronchitis virus field vaccination coverage and persistence of Arkansas-type viruses in commercial broilers. Avian Diseases, 53(2): 175-183. DOI: https://www.doi.org/10.1637/8465-090308-Reg.1

Jordan B (2017). Vaccination against infectious bronchitis virus: A continuous challenge. Veterinary Microbiology, 206: 137-143. DOI: https://www.doi.org/10.1016/j.vetmic.2017.01.002

Kelly L and Alworth L (2013) Techniques for collecting blood from the domestic chicken. Lab Animal, 42: 359-361. DOI: https://www.doi.org/10.1038/laban.394

Lefkowitz EJ, Dempsey DM, Hendrickson RC, Orton RJ, Siddell SG, and Smith DB (2018). Virus taxonomy: The database of the international committee on taxonomy of viruses (ICTV). Nucleic Acids Research, 46(D1): D708-D717. DOI: https://www.doi.org/10.1093/nar/gkx932

Legnardi M, Cecchinato M, Homonnay Z, Dauphin G, Koutoulis KC, Tucciarone CM, and Franzo G (2022a). Viral subpopulation variability in different batches of Infectious bronchitis virus (IBV) vaccines based on GI-23 lineage: Implications for the field. Virus Research, 319: 198877. DOI: https://www.doi.org/10.1016/j.virusres.2022.198877

Legnardi M, Franzo G, Tucciarone CM, Catelli E, Menandro M, and Cecchinato M (2022b). Effect of changes of vaccination strategies on IBV epidemiology, diagnosis and control: An Italian retrospective study. Veterinaria Italiana, 58(1): 41-45. DOI: https://www.doi.org/10.12834/vetit.2084.11082.2

Linares J, Roh H, Sánchez R, ElAttrache J, and Putnam M (2017). Comprehensive service and diagnostics program and its implementation to elucidate the cause of increased mortality and airsac condemnation in a broiler producing complex: A case report. 66th Western Poultry Disease Conference, pp. 104-105. Available at: https://aaap.memberclicks.net/assets/WPDC/wpdc_2017_proceedings_final.pdf

Lisowska A, Pikuła A, Opolska J, Jasik A, Kycko A, and Domanska-Blicharz K (2021). Virulence properties of GI-23 infectious bronchitis virus isolated in Poland and efficacy of different vaccination strategies. Pathogens, 10(5): 522. DOI: https://www.doi.org/10.3390/pathogens10050522

Samy A and Naguib MM (2018). Avian respiratory coinfection and impact on avian influenza pathogenicity in domestic poultry: Field and experimental findings. Veterinary Sciences, 5(1): 23. DOI: https://www.doi.org/10.3390/vetsci5010023

Simon-Loriere E and Holmes EC (2011). Why do RNA viruses recombine?. Nature Reviews Microbiology, 9(8): 617-626. DOI: https://www.doi.org/10.1038/nrmicro2614

Tatar-Kis T, Kovacs EW, Felfoldi B, Nagy Z, Kiss I, Mato T, and Palya V (2014). Protection achieved by a vaccination programme comprising Mass and 793B type vaccine against recent variant IBV strains circulating in the Middle East. VIII Symposium on Avian Corona and Pneumoviruses and Complicating Pathogens. Rauischholzhausen, Germany, pp. 194-203. Available at: https://www.cabidigitallibrary.org/doi/full/10.5555/20133383910

Tucciarone CM, Franzo G, Berto G, Drigo M, Ramon G, Koutoulis KC, Catelli E, and Cecchinato M (2018). Evaluation of 793/B-like and Mass-like vaccine strain kinetics in experimental and field conditions by real-time RT-PCR quantification. Poultry Science, 97(1): 303-312. DOI: https://www.doi.org/10.3382/ps/pex292

Valastro V, Holmes EC, Britton P, Fusaro A, Jackwood MW, Cattoli G, and Monne I (2016). S1 gene-based phylogeny of infectious bronchitis virus: An attempt to harmonize virus classification. Infection Genetics and Evolution, 39: 349-364. DOI: https://www.doi.org/10.1016/j.meegid.2016.02.015

Vermeulen CJ, Dijkman R, de Wit JJS, Bosch BJ, Heesterbeek JAPH, and Schaik G (2023). Genetic analysis of infectious bronchitis virus (IBV) in vaccinated poultry populations over a period of 10 years. Avian Pathology, 52(3): 157-176. DOI: https://www.doi.org/10.1080/03079457.2023.2177140

World Bank TAFS Forum (2011). World livestock disease atlas: A quantitative analysis of global animal health data (2006-2009). © World Bank, Washington, DC and TAFS Forum, Bern. Available at: https://hdl.handle.net/10986/27118

JWPR
Journal of World's Poultry Research
2024, Scienceline Publication
J. World Poult. Res. 14(2): 146-153, 2024
Research Paper
DOI: https://dx.doi.org/10.36380/jwpr.2024.15
PII: S2322455X2400015-14
ISSN: 2322-455X

Effects of Dried Khat (*Catha edulis*) Leaves as a Natural Feed Additive on the White Leghorn Layers' Performance

Aschalew Girma Asfaw[1], Meseret Girma Abebe[1], Ewonetu Kebede Senbeta[2]*, and Kasech Mulatu[1]

[1]*School of Animal and Range Sciences, Haramaya University, P.O.Box 138, Dire Dawa, Ethiopia*
[2]*College of Veterinary Medicine and Agriculture, Addis Ababa University, P.O.Box 34, Bishoftu, Ethiopia*

*Corresponding author's E-mail: ewonetu2011@gmail.com

Received: March 19, 2024, Revised: April 24, 2024, Accepted: May 10, 2024, Published: June 25, 2024

ABSTRACT

Khat contains many bioactive compounds that are beneficial for chickens' health. Most of the chemical constituents found in Khat are biologically active and are used worldwide for the treatment of many diseases. However, there are few studies on the use of Khat in poultry as a nutrition, antioxidant, and antimicrobial activity. Therefore, the present study evaluated the effects of dried Khat leave (DKL) as a natural feed additive on the White Leghorn layer's performance, serum chemistry, and hematology. A total of 180 White Leghorn chickens, aged 168 days were randomly divided into four groups consisting of 45 chickens in three replications. Thirteen layers and two cocks were assigned to each replication and reared on a deep litter system. The diets of layers in T1, T2, T3, and T4 were supplemented by DKL at proportion of 0%, 0.2%, 0.4%, and 0.6%, respectively. The layers were evaluated for feed intake, body weight gain, egg production, feed conversion ratio, and some blood parameters, such as total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, albumin, globulin, red blood cells, hemoglobin, packed cells volume, white blood cells, and total protein. Feed intake in T1 (103.5 g) was significantly higher than T4 (98.5 g) and T3 (98.9 g). Layers in T1 significantly gained higher body weight than layers in other treatments. The use of DKL in layer chickens' diet did not affect egg production, egg mass, and feed conversion ratio. An increase in the level of DKL in the layers diet significantly reduced the serum high-density lipoprotein, cholesterol, and albumin. The present study showed that chickens that received a higher proportion of dried khat leaf (0.6%) significantly recorded lower levels of low-density lipoprotein and total cholesterol in their blood.

Keywords: Blood analysis, Dried Khat leaf, Layer, White Leghorn

INTRODUCTION

Feed additives are used in small amounts in the ration to improve the productive performance of animals (Embuscado, 2015). However, conventional synthetic feed additives became public health issues because of their traceability in animal products (Wallace et al., 2010). Thus, Europe banned the use of some synthetic additives in animal feed (Nkukwana et al., 2014). These raise questions about finding natural additives (Grashorn, 2010). In Ethiopia, poultry production is mainly constrained by the quality and quantity of feed (Demeke, 2004). Hence, feed additives are used to improve quality by improving the availability of certain nutrients. Feed additives originating from herbs and spices have been also used to improve feed quality in the poultry industry (Guo et al., 2004). Natural feed additives are less toxic and residue-free as compared with synthetic antibiotics (Asfaw et al., 2022).

A dried Khat leaf is a potential natural feed because it contains many bioactive compounds such as alkaloids (cathinone, nor-pseudoephedrine, and nor-ephedrine), terpenoids, flavonoids (quercetin, hesperidin, and

To cite this paper: Asfaw AG, Abebe MG, Senbeta EK, and Mulatu K (2024). Effects of Dried khat (*Catha edulis*) Leaves as a Natural Feed Additive on the White Leghorn Layers' Performance. *J. World Poult. Res.*, 14(2): 146-153. DOI: https://dx.doi.org/10.36380/jwpr.2024.15

naringin), vitamin C, tannins, amino acids, minerals, glycosides, essential oils (limonene, 1-phenyl-1, 2-propanedione, camphor), and beta-carotene (Al-hebshi and Al-ak'hali, 2010) which are chemical constituents used to maintain animal health (Rama et al., 2019). Majority of Khat chemical ingredients are used as antimicrobials and antibiotics (Dai and Mumper, 2010).

The use of dried Khat leaves in animal rations not only reduces the cost of feeds, but also improves antioxidant activities and health, as a stimulant, and as coloring pigments which improve the desired egg yolk color, egg production, quality parameters, and reduced serum cholesterol. For instance, plasma cholesterol, glucose, and triglycerides in rabbits were decreased by the use of Khat leaves in the diet (Al-Habori, 2004). Khat leaf contains Alkaloids and Alkaloid is effective in lowering egg cholesterol (Santoso et al., 2015). Quercetin is among the flavonoids found in Khat leaves and its supplementation in laying hens increases the laying rate, egg production, feed efficiency and protects pathogenic metabolites (Liu et al., 2014). Moreover, Asfaw et al. (2022) noted that the use of dried Khat leaves in the diet of layers had significant effects on egg weight, albumen weight, yolk weight, shell weight, and yolk color parameters.

Despite the presence of many bioactive compounds in Khat leaves, there was no sufficient research information about the utilization of Khat (*Catha edulis*) leaves as natural additives in layers. Therefore, this study evaluated the effects of feeding layers with *Catha edulis* on feed consumption, feed conversion ratio, body weight, egg production, and some blood constituents.

MATERIALS AND METHODS

Ethical approval

All procedures related to animal handling, blood collection, and their routine manipulations were carried out according to animal care guidelines protocols approved by the Institutional Review Board of the College of Agriculture and Environmental Sciences of Haramaya University (CAES, Ethiopia) animals ethics committee with approval number CAES_HU/ECC/9/11/10/20212.

Description of the study site

This experimental study was conducted in the 2022 Gregorian calendar at the Poultry research station of Haramaya University in the eastern part of Ethiopia. The farm is located in the eastern part of Ethiopia 505 Kilometers away from the capital city, Addis Ababa. The study area is characterized by 780 millimeters of mean annual rainfall, 8℃ minimum and 24℃ maximum mean annual temperature, 9°261'N latitudes, 42°3'E longitudes, and an altitude of 1980 meters above sea level.

Sample size and chicken management

White Leghorn chickens were obtained from the Haramaya University (Ethiopia) poultry research center and randomly selected at 168 days of age. This experiment used 180 chickens consisting of one hundred fifty-six hens and twenty-four cocks. The chickens were weighed and randomly assigned into four treatments each consisting of three replications that contained fifteen chickens (thirteen hens and two cocks). The experimental chickens were acclimated for seven days to the ration and the actual period of data collection lasted seventy days. The chickens in each replication were reared in a 4 square meter pen which was covered by litter material of *teff* straw. The necessary inputs such as laying nests, watering and feeding materials, and experimental house were carefully cleaned and disinfected before utilization. The tube feeders were used to provide the experimental diets. Feed was offered to chickens twice a day at 8:00 in the morning and 14:00 in the afternoon on an *ad libtum* basis. Moreover, freshwater was offered every day using fountain drinkers on a free access basis.

Feed ingredients and chemical analysis

Chemical analyses of feeds were done at Haramaya University (Ethiopia) of Animal Nutrition and Soil laboratory. The sample was randomly collected from each feed ingredient (maize grain, noug seed cake, soybean meal, and wheat short) and evaluated for chemical composition following the AOAC (1990) procedure and then the experimental ration was formulated. The kjeldahl procedure was used to determine nitrogen (N) and then crude protein was calculated as N × 6.25. Atomic absorption spectrometry (Germany) was used to determine the calcium and total phosphorous following the AOAC (1996) procedure. The formula described by Wiseman (1987) was used to estimate the total metabolizable energy of each feed ingredient and the experimental ratio. WinFeed software (Cambridge, UK) was used to formulate the experimental diet. The experimental diet consisted of 50% maize grain, 16% wheat short, 15% noug seed cake, 10.5% soybean meal, 0.4% L-lysine HCL, 0.5% commercial vitamin premix, 0.1% Dl-methionine, 0.5% salt, and 7% limestone (Table 1). The diet was formulated to meet the nutrient requirement of layers that

were iso-caloric (2800-2900Kcal ME/kg) and iso-nitrogenous (16-17%) following the NRC (1994) procedure.

Dried khat leaves preparation

A khat leaf that was used as a feed additive was purchased from Awaday town market (Ethiopia) and the leaves were separated from twigs and then dried under shade using plastic sheets. The leaves were pulverized into powder to pass through a 5-millimeter sieve then the powder was mixed with diet. Khat leave powder was added at a rate of 0%, 0.2%, 0.4%, and 0.6% of the experimental diets of T1, T2, T3, and T4, respectively following the procedure described by Asfaw et al. (2022).

Data collection

Feed intake was determined every day by subtracting feed refusal from feed offered and the average was taken for the whole experimental period. The initial and final body weight of chickens were used to determine the individual body weight change while the division of body weight change of layers to the number of days between two consecutive measurements was used to determine the body weight gain of chickens using the formula described by Tullet (1995). The daily egg production rate of each treatment was also calculated following the formula described by Tullet (1995). Qualitative and quantitative data from 144 (one hundred forty-four) eggs were evaluated. Nine eggs per treatment or three eggs per replication were randomly selected every two weeks. Each egg was individually weighed immediately after collection and average egg weight and mass were computed for each replication using the method described by North (1984). The feed intake of chickens in each replication was divided by egg mass to determine the feed conversion ratio (Tullet, 1995). At the end of the experiment, five milliliters of blood samples were collected from the bronchial wing vein of nine hen layers randomly selected from treatments or three layers from each replication. The blood sample was analyzed at Haramaya University's animal physiology and veterinary laboratory (Ethiopia). Ethylenediaminetetraacetic acid (EDTA) and plain tubes were used for blood samples. The serum chemistry (total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, albumin, and globulin) and hematology (red blood cells, hemoglobin, packed cells volume, white blood cells, and total protein) from layers were evaluated using the methods of acid hematin or Sahli's (Weiss et al., 2010) and Neubauer hemocytometer chamber (Dacie and Lewis, 1991). The difference between serum total protein and albumin was used to determine the volume of globulin (Doumas et al., 1981).

Statistical analysis

The research design used in this study was completely randomized. Analysis of variance (ANOVA) was used to analysis of data using the statistical analysis system user's guide of North Carolina, version 9.4. The least significant difference was used to identify the existence of variations among treatment means at $p < 0.05$. The model was used according to the following formula.

$Y_{jk} = \mu + tj + ejk$,

Where Y_{jk} is k^{th} observation taken under treatment j, μ is overall mean, tj is levels of dried khat leave and e_{jk} is random error.

RESULTS AND DISCUSSION

Feed ingredients and ration nutrition values

The chemical composition of feed ingredients and experimental diets are presented in Table 1 and Table 2, respectively. Khat leave was slightly higher in crude protein (CP) than maize but lower than wheat short. The crude fiber (CF) and ash content of dried Khat leave (DKL) is somewhat higher than other dietary ingredients such as maize grain, noug seed cake, soybean meal, and wheat short. DKL's metabolizable energy content is far lower than other ingredients such as maize grain, Noug Seed cake, soybean meal, and wheat short but higher in its calcium and phosphors. On the contrary, a higher CP (12.3%) value was reported by Brhanu and Gebremariam (2019) for Khat. The recorded CP value of khat in this study was almost within the range (10.7-12.6%) that was reported by Mohammed (2005). This variation in CP value might be related to the proportion of leaves maturity and species, leave harvest season, and soil fertility where the Khat plant is grown. The crude fiber content of the diet slightly increased from group T1 to group T4 as the level of DKL increased; this may be due to the slightly higher CF contents of Khat leaves. Consistently, Okorie (2006) noted that the diets formulated using tropical browse plants revealed higher crude fiber (15.56%). In the current study, the diet formulated for all treatments met the iso-caloric and iso-nitrogenous nutrient requirement of layers as recommended by NRC (1994). Layers can adjust their feed consumption to obtain adequate energy when receiving diets ranging in energy from approximately 2500 to 3300 kcal ME per kg of diet (NRC, 1994).

Table 1. Chemical composition of the feed ingredients formulated for White Leghorn layers

Chemical Component	Ingredients				
	Maize Grain	**Noug Seed cake**	**Soybean meal**	**Wheat short**	**Khat leave**
DM (%)	89.0	92.0	91.9	92.2	94.3
CP (%DM)	9.3	31.7	38.2	15.3	10.6
EE (%DM)	5.6	8.1	5.7	4.3	4.8
Ash (%DM)	3.4	7.6	6.4	6.2	10.7
CF (%DM)	4.1	11.4	4.9	5.6	19.6
Ca (%DM)	0.05	0.38	0.19	0.11	0.86
P (%DM)	0.54	0.50	0.65	0.59	1.91
ME(Kcal/kg)	3944.8	3558.9	3632.7	3550.5	2039.8
Chemical Component	**Treatments**				
	T1(0% DKL)	**T2 (0.2% DKL)**	**T3(0.4% DKL)**	**T4 (0.6% DKL)**	
DM (%)	90.9	90.9	91.4	92.1	
CP (%DM)	16.1	16.8	16.1	16.9	
EE (%DM)	3.3	3.5	3.7	4.1	
Ash (%DM)	11.2	11.2	11.3	12.2	
CF (%DM)	8.6	8.8	8.9	8.9	
Ca (%DM)	2.86	2.92	2.88	3.11	
P (%DM)	0.43	0.49	0.53	0.54	
ME (Kcal/kg)	2909.9	2904.2	2896.4	2878.9	

DM: Dry mater; CP: Crude protein; EE: Ether extract; CF: Crude fiber; Ca: Calcium; P: Phosphorus; ME: Metabolizable energy; kcal/kg: Kilo calorie per kilogram, DKL: Dried Khat leaves

Table 2. Performance of layers-fed diets containing different levels of dried Khat leaves

Parameters \ Treatments	T1	T2	T3	T4	SEM	SL
Feed Intake (g/hen /day)	103.5^{a}	100.5^{ab}	98.9^{b}	98.5^{b}	1.08	*
Initial BW (g)	1197.2	1211.0	1229.7	1221.0	12.68	NS
Final BW (g)	1229.0	1225.1	1214.9	1202.1	15.95	NS
Body Wt. Change (g/hen/day)	31.8^{a}	14.1^{ab}	-14.9^{b}	-18.9^{b}	10.34	*
BW gain (g/hen/day)	0.5^{a}	0.2^{ab}	-0.2^{b}	-0.3^{b}	0.15	*
Total Egg /Bird (No)	36.8	33.5	34.5	38.2	4.65	NS
HDEP (%)	50.5	47.9	49.3	54.6	6.54	NS
EM (g/hen/day)	26.4	24.3	25.9	26.8	3.53	NS
FCR (g feed/g egg)	3.9	4.1	3.8	3.6	0.55	NS

^{ab} means within a row with different superscripts are significantly different; * Significant at $p < 0.05$; NS: non-significant at $p > 0.05$; SL: Significant level; SEM: Standard error of the mean; g: Gram; BW: Body weight; HDEP: Hen-day egg production; FCR: Feed conversion ratio; EM: Egg mass (g /hen/day), Wt: Weight

Feed intake

This study result revealed that feed intake was decreased as the dietary inclusion level of DKL increased (Table 2). This might be due to the high concentration of tannins and crude fiber content in Khat leaves. Consistently, Atlabachew et al. (2014) approved the higher tannins (70.2-153 mg tannic acid equivalent/g of dry matter) in their study and associated lower feed intake with tannins because of their bitterness. Uchegbu et al. (2013) also noted decreased feed intake in layers of chicken as the fiber and tannin content of the diet increased. Besides, Buragohain (2018) revealed that the presence of tannin in *Tithonia diversifolia* leaf was attributed to the decreased feed intake and nutrient digestibility in broiler chickens. Brhanu and Gebremariam (2019) reported decreased feed intake in sheep across treatments with an increased inclusion level of Khat. Correspondingly, Abd El-Motaal et al. (2008) reported significantly less feed intake in layer chickens fed diets supplemented with *Eucalyptus* leaves powder as compared to the control group. On the contrary, Cayan and Erener (2015) reported insignificant effects of olive leaf powder use in layers. Lower feed intake of layers in T3 and T4 could also be due to their higher crude fiber content of the ration.

Body weight change and gain

The use of dried khat leaves significantly influenced body weight change and gain ($p < 0.05$, Table 2). The lowest body weight gains were recorded for layers in treatments T3 and T4. This implied decreased body weight gain as DKL increased in layer chickens' diet. This is possibly due to decreased feed intake as DKL increased. Correspondingly, Brhanu and Gebremariam (2019) noted heavier total body weight and average body weight gain in a sheep-fed diet with Khat leave at a higher level (45%) than at a lower level (0-30%).

Egg production, egg mass, and feed conversion ratio

The use of DKL in layer chicken diet did not affect egg production ($p > 0.05$, Table 2). This is consistent with Cayan and Erener (2015) who reported non-significant effects on egg production of layers fed a diet with olive leaves. However, layers in T4 record higher egg numbers and hen-day egg production (HDEP) than T1, T3, and T2. This could be enhanced by the presence of some bioactive compounds such as alkaloids, terpenoids, and flavonoids in the Khat leaves (Atlabachew et al., 2014). Khat leaf has high antioxidant activity that could improve the health status as well as the reproductive and productive performances of chickens. Similarly, Ahmad et al. (2017) noted that egg production remained the same with the supplementation of *Moringa* leaf meals. On the contrary, Abd El-Motaall et al. (2008) noted significantly increased egg number in layers-fed diets supplemented with *Eucalyptus* leaves. The inclusion of DKL in the layers' diet did not affect egg mass and FCR (Table 2). The egg mass and FCR might be related to egg production which was not significantly affected by the use of DKL. Consistently, Paguia et al. (2014) reported that egg mass and FCR remained unchanged by using *Moringa* leaf meal. On the contrary, Liu et al. (2014) noted decreased FCR in a dose-response of quercetin supplementation which is among the bioactive compounds found in Khat leave.

Blood analysis

The results showed that the total serum cholesterol (TSC) was significantly low at a high level of DKL (Table 3, $p < 0.05$). This finding could be associated with the effects of biochemical such as alkaloids, terpenoids, and flavonoids in Khat leaves. Likewise, Mashayekhi et al. (2018) noted decreased serum total cholesterol levels of broilers due to *Eucalyptus* leaf powder supplementation in their diets. On the contrary, Zangeneh and Torki (2011) reported a non-significant effect of olive leaves on the blood cholesterol levels in layer chickens. Serum cholesterol of layers fed neem leaf meal was not significant ($p > 0.05$, Odoh et al., 2016). The chemical constituents found in Khat leaves such as alkaloids are effective in lowering egg cholesterol. This idea is supported by Santoso et al. (2015) who noted the lowered egg cholesterol due to alkaloids. The phenolic compounds (flavonoids, tannins, phenolic acids, terpenes) in Khat leaves could decrease plasma cholesterol in this study. In line with this finding, Al-Habori and Al-Mamary (2004) noted an increased c-AMP concentration due to the stimulating effect of Khat on β-adrenergic receptors by activation of adenylate cyclase and converting ATP to c-AMP. Consequently, c-AMP has a stimulatory effect on lipolysis.

Table 3. Serum chemistry and hematology of layers fed diets containing different levels of dried khat leaves

Parameters \ Treatments	T1	T2	T3	T4	SEM	SL
Total cholesterol (mg/dl)	160.7^{a}	154.3^{ab}	142.0^{bc}	139.1^{c}	4.15	*
HDL (mg/dl)	31.6^{b}	31.8^{b}	33.8^{ab}	35.7^{a}	0.70	*
LDL (mg/dl)	104.5^{a}	99.8^{ab}	95.1^{bc}	93.5^{c}	1.57	*
TP (g/dl)	4.2	4.3	4.6	4.4	0.14	NS
Albumin (g/dl)	1.5^{b}	1.5^{b}	1.6^{a}	1.7^{a}	0.03	**
Globulin (g/dl)	2.7	2.9	2.9	2.8	0.13	NS
RBC (10^{6}/µl)	3.1	3.2	3.3	3.1	0.05	NS
WBC (10^{4}/µl)	6.8^{b}	6.1^{b}	8.0^{a}	7.1^{ab}	0.35	*
Hb (g/dl)	10.6	10.4	10.7	10.6	0.24	NS
PCV (%)	32.2	31.4	32.8	32.0	0.48	NS

^{abc} Means within a row with different superscripts are significantly different; * Significant at $p < 0.05$; **: Significant at $P < 0.05$; NS: Non-significant; RBC: Red Blood Cells; Hb: Hemoglobin; PCV: Packed Cells Volume; WBC: White Blood Cells; TP: Total Protein; HDL: High-Density Lipoprotein; LDL: Low-Density Lipoprotein

The study revealed that T4 was significantly higher in serum HDL cholesterol than in T1 and T2 ($p < 0.05$). This agrees with Santoso et al. (2010) who revealed significantly increased HDL cholesterol concentration in serum with supplementation of alkaloids from *Sauropus androgynuos* leaves in a layer chicken diet. Al-Habori and Al-Mamary (2004) noted increased HDL cholesterol in rabbits due to Khat leave supplementation. On the contrary, Mashayekhi et al. (2018) reported that feeding *Eucalyptus* leaf powder to broiler chickens did not influence their HDL. Significantly lower serum LDL concentration was recorded in T4 as compared with T1 and T2 ($p < 0.05$). This could be due to the phenolic compounds which can decrease the absorption of cholesterol into the blood by adhering to LDL and then inhibit free radicals. This is consistent with the study reported by Brenes and Roura (2010). Besides, decreased blood LDL in rats was reported by the use of an aqueous extract of *Eucalyptus* (Arise et al., 2009). Serum total proteins and globulin were non-significant among treatments ($p > 0.05$). Significantly higher albumin was recorded in T3 and T4 as compared to layers fed on T1 and T2 ($p < 0.05$). However, a non-significant effect of Neem leaves on layers of serum albumin was reported by Odoh et al. 2016 ($p > 0.05$). The change of those parameters in blood revealed that the bioactive components of Khat leaf could activate immune functions such as lymphocyte proliferation, phagocytosis, red blood cells, hemoglobin, and white blood cell counts.

The result showed that the inclusion of DKL in the layers diet did not influence the total red blood cell, hemoglobin, and packed cell volume ($p < 0.05$). Correspondingly, a non-significant effect of *Moringa oleifera* leaf on hemoglobin in broilers was reported by Olugbemi et al. (2010). However, Esonu et al. (2007) reported a significant effect of hematological parameters by the use of neem leaf. The values of RBC fall within the recommended range ($0.5–3.9 \times 10^6/\mu l$) which is the normal value for chickens (Gulland and Hawkey, 1990). Significantly higher white blood cells were recorded in T3 as compared to layers fed on T1 and T2 ($p < 0.05$). Likewise, Mashayekhi et al. (2018) noted increased WBC levels due to *Eucalyptus* leaf powder supplementation in broilers' diets. This might be due to the increased response of layers immune to Khat phenolic compounds which stimulate lymphocyte production.

CONCLUSION

The feed intake of layers in T1 was significantly higher than in T4 and T3. There was a significantly higher body weight change and body weight gain for layers in T1 than in T4 and T3. The study revealed that Khat leaves use in layer diet up to 0.6% significantly reduced feed intake, the body weight of hens, total serum cholesterol, and LDL and significantly increased HDL and WBC. Dried Khat leaves decreased serum LDL from 104.5 in T1 to 93.5 mg/dl in T4. The present study might be used as the ground information of dried Khat leaves effects on layer performance. Thus, further study is needed to identify active chemical compounds in khat leaves.

DECLARATIONS

Funding

This research was funded by Haramaya University and the fund number is HUWG_2020_01_01_27.

Availability of data and materials

All data analyzed and presented in this article are available at the request of the principal and corresponding authors.

Authors' contributions

Aschalew Girma Asfaw conducted experimental research, collected data, analyzed data, and drafted a research report. Meseret Girma Abebe is a major supervisor to the research who commented on the research report. Ewonetu Kebede Senbeta initiated the research idea, drafted the research proposal for a grant, supervised the student as co-advisor, prepared the manuscript, and submitted it. Kasech Mulatu assisted the student during data collection and commented on the manuscript. All authors contributed to the research proposal development and commented on the final manuscript.

Acknowledgments

This research was granted by the Haramaya University research office (HUWG_2020_01_01_27). Therefore, the authors give deep gratitude for the financial support. Besides, Haramaya University staff working on Poultry Farms, Feed processing units, and laboratories are acknowledged by all authors.

Ethical considerations

Ethical issues, such as data fabrication, double publication and submission, redundancy, plagiarism, consent to publish, and misconduct, have been checked by all the authors before submission to this journal.

Competing interests

All authors have no conflict of interest.

REFERENCES

Abd El-Motaal AM, Ahmed AMH, Bahakaim ASA, and Fathi MM (2008). Productive performance and immunocompetence of commercial laying hens given diets supplemented with *Eucalyptus*. International Journal of Poultry Science, 7(5): 445-449. DOI: https://www.doi.org/10.3923/ijps.2008.445.449

Ahmad S, Khalique A, Pasha TN, Mehmood S, Hussain K, Ahmad S, Shaheen MS, Naeem M, and Shafiq M (2017). Effect of *Moringa oleifera* (Lam.) pods as feed additive on egg antioxidants, chemical composition and performance of commercial layers. South African Journal of Animal Science, 47(6): 865-871. DOI: http://www.doi.org/10.4314/sajas.v47i6.14

Al-Habori M and Al-Mamary M (2004). Long-term feeding effects of *Catha edulis* leaves on blood constituents in animals. Phytomedicine, 11(7-8): 639-644. DOI: https://www.doi.org/10.1016/j.phymed.2003.06.004

Al-hebshi NN and Al-ak'hali MS (2010). Experimental gingivitis in male *Khat (Catha edulis*) chewers. Journal International Academy Periodontol, 12(2): 56-62. Available at: https://pubmed.ncbi.nlm.nih.gov/20465033/

Association of official agricultural chemists (AOAC) (1990). AOAC official methods of analysis, 15th Edition. Association of Official Analytical Chemists., Arlington. Available at: https://law.resource.org/pub/us/cfr/ibr/002/aoac.methods.1.1990.pdf

Association of official agricultural chemists (AOAC) (1996). AOAC official method of analysis of the association of official analytical chemists. AOAC International, Arlington.

Arise RO, Malomo SO, Adebayo JO, and Igunnu A (2009). Effects of aqueous extract of *Eucalyptus* globulus on lipid peroxidation and selected enzymes of rat liver. Journal of Medicinal Plants Research, 3(2): 77-81. DOI: https://www.doi.org/10.5897/JMPR.9000508

Asfaw G, Abebe MG, and Senbeta EK (2022). Evaluation of dried Khat (*Catha edulis*) leaf as natural additives on egg quality, embryonic mortality, and chick quality of White Leghorn layers. Journal of the Indonesian Tropical Animal Agriculture, 47(3): 168-176. DOI: https://www.doi.org/10.14710/jitaa.47.3.168-176

Atlabachew M, Bhagwan SC, and Mesfin R (2014). Selected secondary metabolites and antioxidant activity of Khat *(Catha edulis* Forsk) chewing leaves extract. International Journal of Food Properties, 17(1): 45-64. DOI: https://www.doi.org/10.1080/10942912.2011.614367

Brenes A and Roura E (2010). Essential oils in poultry nutrition: Main effects and modes of action. Animal Feed Science and Technology, 158(1-2): 1-14. DOI: https://www.doi.org/10.1016/j.anifeedsci.2010.03.007

Brhanu A and Gebremariam T (2019). Using Khat *(Catha edulis*) leftover meal as feed for sheep: Its implication on feed intake, digestibility and growth. Online Journal Animal Feed Research, 9(5): 191-197. DOI: http://www.doi.org/10.36380/scil.2019.ojafr27

Buragohain R (2018). Growth performance, nutrient utilization, and feed efficiency in broilers fed *Tithonia diversifolia* leaf meal as substitute of conventional feed ingredients in Mizoram. Veterinary World, 9(5): 444-449. DOI: https://www.doi.org/10.14202/vetworld.2016.444-449

Cayan H and Erener G (2015). Effect of olive leaf (*Olea europaea*) powder on laying hens' performance, egg quality and egg yolk cholesterol levels. Asian Australasian Journal of Animal Science, 28(4): 538-543. DOI: https://www.doi.org/10.5713/ajas.14.0369

Dacie JV and Lewis SM (1991). Practical haematology, 7th Edition. Churchil Livingstone., Edinburgh, pp. 54-79.

Dai J and Mumper RJ (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. Molecules, 15(10): 7313-7352. DOI: https://www.doi.org/10.3390/molecules15107313

Doumas BT, Bayso DD, Carter RJ, Peters T, and Schaffer R (1981). Determination of total serum protein. Clinical Chemistry, 27(10): 1642-1643. Available at: https://pubmed.ncbi.nlm.nih.gov/6169466/

Embuscado ME (2015). Spices and herbs: Natural sources of antioxidants– A mini review. Journal of Functional Foods, 18(Part B): 811-819. DOI: http://www.doi.org/10.1016/j.jff.2015.03.005

Esonu B, Opara MN, Okoli IC, Obikaonu HO, Udedibie C, and Iheshiulor OOM (2007). Physiological response of laying bird to neem (Azadirachta indica) leaf meal-based diets: Body weight organ characteristics and haematology. Life Science Journal, 4(2): 37-41. Available at: http://www.ojhas.org/issue18/2006-2-4.htm

Grashorn MA (2010). Use of phytobiotics in broiler nutrition: An alternative to in feed antibiotics. Journal Animal Feed Science, 19: 338-347. Available at: http://www.jafs.com.pl/pdf-66297-7254?filename=7254.pdf

Gulland FMD and Hawkey CM (1990). Avian hematology. Veterinary Annual, 30: 126-136. Available at: https://www.cabdirect.org/cabdirect/abstract/19902206349

Guo FC, Williams BA, Kwakkel RP, Li HS, Li XP, Luo JY, Li WK, and Verstegen MWA (2004). Effects of mushroom and herb polysaccharides, as alternatives for an antibiotic, on the cecal microbial ecosystem in broiler chickens. Poultry Science, 83(2): 175-182. DOI: https://www.doi.org/10.1093/ps/83.2.175

Liu HN, Liu Y, Hu LL, Suo YL, Zhang L, Jin F, Feng XA, Teng N, and Li Y (2014). Effects of dietary supplementation of quercetin on performance, egg quality, cecal microflora populations, and antioxidant status in laying hens. Poultry Science, 93(2): 347-353. DOI: https://www.doi.org/10.3382/ps.2013-03225

Mashayekhi H, Mazhari M, and Esmaeilipour O (2018). Eucalyptus leaves powder, antibiotic and probiotic addition to broiler diets: Effect on growth performance, immune response, blood components and carcass traits. Animal, 12(10): 2049-2055. DOI: https://www.doi.org/10.1017/S1751731117003731

Mohammed I (2005). Effect of feeding different levels of Khat (*Catha edulis*) leftover on feed intake and growth performance of goats fed on sorghum stover. MSc Thesis, Alemaya University of Agriculture, Haramaya, Ethiopia. pp. 10-32.

Nkukwana TT, Muchenje V, Masika PJ, Pieterse E, Hoffman LC, and Dzama K (2014). The effect of *Moringa oleifera* leaf meal supplementation on tibia strength morphology and inorganic contents of broiler chicken. South African Journal of Animal Science, 44(3): 228-239. DOI: http://www.doi.org/10.4314/sajas.v44i3.4

North MO (1984). Commercial chicken production manual, 3rd Edition. Avian Publishing Company Inc., West Connecticut, pp. 134-548.

Available at: https://www.cabdirect.org/cabdirect/abstract/19840180814

National research council (NRC) (1994). Nutrient requirement of poultry, 19th Edition. National Academic Press., Washington D.C. USA. pp. 1-167. Available at: https://www.agropustaka.id/wp-content/uploads/2020/04/agropustaka.id_buku_Nutrient-Requirements-of-Poultry_Ninth-Revised-Edition-1994-NRC.pdf

Odoh IL, Bratte L, and Isikwenu JO (2016). Effects of varying levels of neem (*Azadirachta indica*) leaf meal in layer diets on the haematological and serological indices and faecal bacterial counts of layers. Journal of Natural Sciences Research, 6(15): 64-68. Available at: https://www.iiste.org/Journals/index.php/JNSR/article/view/20168/20572

Okorie KC (2006). Evaluation of leaf meals of *Pentaclethra macrophylla*, *Jacaranda mimosifolia* and *Mucuna pruriens* as feed ingredients in poultry diets. PhD Thesis, Federal University of Technology, Owerri, Nigeria. pp. 1-67.

Olugbemi TS, Mutayobaand SK, and Lekule FP (2010). Effect of *Moringa* (*Moringa oleifera*) inclusion in cassava-based diets fed to broiler chickens. International Journal Poultry, 9(4): 363-367. DOI: https://www.doi.org/10.3923/ijps.2010.363.367

Paguia HM, Paguia RQ, Balba C, and Flores RC (2014). Utilization and evaluation of *Moringa oleifera* L. as poultry feeds. APCBEE Procedia, 8: 343-347. DOI: https://www.doi.org/10.1016/J.APCBEE.2014.03.051

Rama Rao SV, Raju MVLN, Prakash B, Rajkumar U, and Reddy EPK (2019). Effect of supplementing *Moringa* (*Moringa oleifera*) leaf meal and pomegranate (Punicagranatum) peel meal on performance, carcass attributes, immune and antioxidant responses in broiler chickens. Animal Production Science, 59(2): 288-294. DOI: https://www.doi.org/10.1071/AN17390

Santoso U, Suteky T, and Fenita Y (2010). Effects of supplementation of alkaloid and non- alkaloid from *Sauropus androgynus* leaves on egg production and lipid profile in layer chicken. Animal Production, 12(3): 184-189. Available at: https://www.animalproduction.net/index.php/JAP/article/view/290

Santoso U, Fenita Y, and Kususiyah JR (2015). The effect of fermented *Sauropus androgynus* leaf on performance, fat deposition and carcass quality in broiler chicken. International Seminar on Promoting Local Resources for Food and Health, Bengkulu, Indonesia. Available at: https://repository.unib.ac.id/11312/1/018%20U.%20Santoso%20et%20al.pdf

Demeke S (2004). Egg production performance of local and white leg horn hens under intensive and rural household conditions in Ethiopia. Livestock Research for Rural Development, 16(2): 9. Available at: https://lrrd.cipav.org.co/lrrd16/2/deme1602.htm

Tullet SG (1995). Incubation. In: P. Hunton (Editor), World animal science C9, poultry production, the Ontario egg producers' marketing board, Mississaga, Ontario, Canada.

Uchegbu MC, Herbert U, Ogbuewu IP, Nwaodu CH, Esonu BO, and Udedibie ABI (2013). Performance and egg quality characteristics of layers fed diets containing combinations of brewers dried grains, jack bean and cassava root meal. Revista Científica UDO Agrícola, 11(1): 155-160. Available at: https://tspace.library.utoronto.ca/bitstream/1807/45585/1/cg11016.pdf

Wallace RJ, Oleszek W, Franz C, Hahn I, Baser KHC, Mathe A, and Teichmann K (2010). Dietary plant bioactive for poultry health and productivity. British Poultry Science, 51: 461-487. DOI: https://www.doi.org/10.1080/00071668.2010.506908

Weiss M, Schrimpf S, Hengartner MO, Lercher MJ, von Mering C. 2010. Shotgun proteomics data from multiple organisms reveals remarkable quantitative conservation of the eukaryotic core proteome. Proteomics, 10(6): 1297-306. https://doi.org/10.1002/pmic.200900414

Wiseman J (1987). Feeding of non-ruminant livestock. Butterworth and Co. Ltd., pp. 9-15.

Zangeneh S and Torki M (2011). Effects of B-mannanase supplementing of olive included diet on performance of laying hens, egg quality characteristics, humoral and cellular immune response and blood parameters. Global Veterinary, 7: 391-398. Available at: https://idosi.org/gv/GV7(4)11/14.pdf

JWPR
Journal of World's Poultry Research
2024, Scienceline Publication
J. World Poult. Res. 14(2): 154-159, 2024
Research Paper
DOI: https://dx.doi.org/10.36380/jwpr.2024.16
PII: S2322455X2400016-14

ISSN: 2322-455X

Association of Different Body Sizes and Egg Quality Characteristics in White Leghorn Chicken Breed of South Africa

Thobela Louis Tyasi*, Lindiwe Johannah Sathekge, and Victoria Rankotsane Hlokoe

School of Agricultural and Environmental Sciences, Department of Agricultural Economics and Animal Production, University of Limpopo, Private Bag X1106, Sovenga 0727, Limpopo, South Africa

*Corresponding author's E-mail: louis.tyasi@ul.ac.za

Received: March 15, 2024, Revised: April 26, 2024, Accepted: May 16, 2024, Published: June 25, 2024

ABSTRACT

Egg quality could influence the price of the table and hatching eggs. However, the effects of hen's live body weight on egg characteristics are poorly understood. The present study evaluated the influence of body weight (BW) on egg characteristics, such as egg width (EWD), shell weight (SW), egg length (EL), albumen weight (AW), yolk weight (YW), shell surface index (SI), albumen ratio (AR), shell ratio (SR), and yolk ratio (YR) of White Leghorn chicken. A total of 100 White Leghorn chickens at 30 weeks of age were used in the current study of which 300 eggs were collected. Live body weight was classified into three groups namely, small (≤ 195g), medium (196-220g), and large (≥ 221g). The results revealed that the White Leghorn layer's live body weight was positively influenced by the eggshell index and egg length. Additionally, the results indicated that body weight did not affect EW, SW, EWD, SR, YW, AW, AR, and YR. In conclusion, the live body weight influences egg length and shell index in the White Leghorn chicken breed.

Keywords: Chicken, Egg length, Egg weight, Egg width, Shell index

INTRODUCTION

Chickens are an important source of food and money for households, and they also play a significant role in the sociocultural life of rural communities (Qin et al., 2015; Hlokoe et al., 2023). According to Onunkwo and Okoro (2015), the chicken egg functions as the embryo's primary source of nutrition and is a biotic structure for reproduction. The egg characteristics are extremely important in poultry breeding due to their impact on future generations' production performance, as well as the quality and growth of the chicks, breeding performance, and chick performance (Jatoi et al., 2015). Key indicators of egg quality include shell color, weight, thickness, density, albumen height, yolk color, albumen pH, and viscosity (Lee et al., 2016). White Leghorn chickens are mostly used in poultry enterprises for egg production, and they are regarded as one of the highly efficient laying breeds (Ferreira et al., 2022). The egg weight is related to the body weight of a chicken (Jatoi et al., 2015). However, the egg quality is affected by the various body sizes of chickens (Jatoi et al., 2015). The production of poor egg quality results in financial misfortunes, affecting the profit margins (Jatoi et al., 2015). Previous studies have examined the influence of live body weight on the egg characteristics in other poultry species, such as Lohmann White hens (Lacin et al., 2008), Japanese Quails (Jatoi et al., 2015), and guinea fowls (Onunkwo and Okoro, 2015). However, there is a lack of literature on the influence of live body weight on the egg characteristics of the White Leghorn chicken breed. Therefore, the objective of the study was to evaluate the influence of live body weight on the egg characteristics of the White Leghorn chicken breed. The obtained results might assist the chicken farmers in knowing the right live body weight of the chickens that can produce good quality egg characteristics.

To cite this paper: Tyasi TL, Sathekge LJ, and Hlokoe VR (2024). Association of Different Body Sizes and Egg Quality Characteristics in White Leghorn Chicken Breed of South Africa. *J. World Poult. Res.*, 14(2): 154-159. DOI: https://dx.doi.org/10.36380/jwpr.2024.16

MATERIALS AND METHODS

Ethical approval

The University of Limpopo Animal Research and Ethics Committee, in South Africa approved the study (AREC/42/2023:UG).

Study area

The study was conducted at the University of Limpopo Experimental Farm, South Africa. The study area has ambient temperatures that range between 20 and 36ºC in summer (November to January) and between 5ºC and 25ºC during winter (May to July). The University of Limpopo lies at latitude 27.55 ºS and longitude 24.77 ºE. The research area experiences an average yearly rainfall of less than 400 mm (Shabalala et al., 2019). The study was conducted in September 2023.

Experimental animals, management, and experimental design

A total of 100 30-week-old White Leghorn chickens from the University of Limpopo experimental farm were employed in the investigation. The chickens were raised as explained by Alabi et al. (2012). The chickens were raised under an intensive production system. The chicken house was well-ventilated, with curtains opened during the day and closed at night to control the ventilation. The light was provided for 16 hours a day and the temperature in the chicken house ranged from 24 to 32ºC. Wood shavings were used as bedding material. For the hens to produce eggs for the study, they were fed laying mash (Driehoek Feeds, South Africa) and given unlimited access to water. The nutritional constituents of the diet were metabolizable energy (2453.60Kcalkg^{-1}), crude Protein (16%), crude fats and oils (4.3%), crude fibers (4.8%), crude ash (13.6%), calcium (4.3%), phosphorus (0.6%), sodium (0.15%), lysine (0.7%) and methionine (0.35%). The diet was balanced according to NRC (1994). To prevent the hens from catching infectious diseases, Virokill disinfectant (UK) was used to clean the chicken house seven days before the arrival of the chickens. The biosecurity was followed according to Alabi et al. (2012). Mareks' vaccine (Zoetis, South Africa) was administered to the chicks at the hatchery before delivery. Nobilis® CAV P4 (Zoetis, South Africa) vaccine was administered intramuscularly to prevent chicken anemia 6 weeks before the onset of lay. In the current investigation, a completely randomized design was employed.

Eggs collection

A total of 300 eggs were randomly collected from 100 White Leghorn chickens for 3 weeks to measure the physical egg quality traits. A total of 100 eggs per week were randomly selected from the chickens. The eggs were collected in the morning and evening. The collected eggs were taken to the laboratory at room temperature to measure the external and internal egg quality traits.

Measurements of external egg quality and internal egg quality

The external egg characteristics measured from the chickens at different body weights included egg weight (g), egg length (cm), egg width (cm), and shell weight (g). External egg characteristics were measured according to Kgwatalala et al. (2013). Other external egg characteristics such as shell index (%) and shell ratio (%) were computed as described by Markos et al. (2017). The yolk weight (g) and albumen weight (g) were measured as the internal egg characteristics. The procedure introduced by Kgwatalala et al. (2013) was followed to measure the internal egg characteristics. The formulas provided by Ashraf et al. (2016) were used to compute additional internal egg characteristics, such as albumen ratio and yolk ratio. The following formulas were used in this study.

Albumen weight (g) = egg weight – (yolk weight + shell weight)

$$\text{Albumen ratio (\%)} = \frac{\text{Albumen weight}}{\text{Egg weight}} \text{x100}$$

$$\text{Yolk ratio (\%)} = \frac{\text{Yolk weight}}{\text{Egg weight}} \text{x 100}$$

$$\text{Shape index (\%)} = \frac{\text{Egg width}}{\text{Eeg length}} \text{ x 100}$$

$$\text{Shell ratio (\%)} = \frac{\text{Shell weight}}{\text{Egg weight}} \text{ x 100}$$

Measurements of chicken body weight

The BW of 100 chickens was measured using an electronic weighing scale (Medidata®, USA) with a precision of 0.01 g. The chickens were weighed individually on the weighing scale, and the body weights were classified into three namely, small (≤ 195g), medium (196-220g), and large (≥ 221g).

Statistical analysis

For the analysis of data, Statistical Package for Social Sciences IBM SPSS, version 28.0 was employed. For each trait, descriptive statistics were calculated. The association between measured characteristics was examined using Pearson's correlation. The influence of live body weight on egg characteristics was determined using a general linear model (GLM). The least significant difference (LSD) was used for the separation of means. The p-value at 0.05

shows a significant difference. The effect of body weight was computed using the model below (Jatoi et al., 2015):

$Y_{ij} = \mu + S_i + e_{ij}$

Where Y_{ij} is the j^{th} observation in the i^{th} body weight group, μ is the overall mean, S_i is the fixed effect of the i^{th} body weight group and e_{ij} is residual error.

RESULTS

Descriptive statistics

Table 1 displays the summary of the live body weight and egg characteristics. As can be seen, the live body weight of the White Leghorn chickens ranged from 119 g to 259 g. The coefficient of variation ranged from 0.50% to 15.85%.

Correlation matrix

The associations between live body weight and egg characteristics are displayed in Table 2. The findings showed that live body weight had a negatively high remarkable relationship ($p < 0.01$) with yolk weight and positively significantly correlation with albumen ratio ($p < 0.05$). The results also revealed that live body weight had no significant association with egg weight, shell weight, egg width, egg length, shell index, albumen weight, shell ratio, and yolk ratio ($p > 0.05$). Table 2 also showed that egg weight was associated with almost all the traits except with shell index.

Effect of live body weight on egg characteristics

Table 3 shows the findings of the influence of body weight on internal and external egg characteristics. The outcomes displayed that live body weight affected egg length and shell index ($p < 0.05$). The chickens with medium live body weight had the highest egg length, while the chickens with small live body weight had the lowest egg length. The findings further indicated that the shell surface index (SI) of the chickens with small, medium, and large body weight groups was significantly different ($p < 0.05$). The small body weight group had the highest SI, while the medium body weight group had the lowest SI. The outcomes also displayed that live body weight had no effect on egg weight, egg width, shell ratio, shell weight, albumen weight, yolk weight, albumen ratio, and yolk ratio ($p > 0.05$).

Table 1. Body weight and egg characteristics of the White Leghorn chicken breed

Traits	Minimum	Maximum	Mean	SD	CV (%)
Body weight (g)	119	259	211.42	22.13	10.46
Egg weight (g)	48.60	66.84	58.24	3.98	15.85
Egg length (mm)	53.53	63.26	56.95	1.82	3.32
Yolk weight (g)	11.05	23.41	17.94	1.69	2.86
Shell weight (g)	6.33	10.26	7.82	0.77	0.59
Egg width (mm)	40.13	45.28	43.07	1.02	1.04
Albumen weight (g)	23.47	41.68	32.48	3.28	10.73
Shape index (%)	66.04	81.84	75.69	2.70	7.28
Shell ratio (%)	10.87	17.36	13.74	1.25	1.57
Albumen ratio (%)	48.29	68.65	55.70	3.03	9.19
Yolk ratio (%)	18.90	38.26	30.86	2.71	7.33

SD: Standard deviation, CV: Coefficient of variation

Table 2. Correlation between the body weight and egg characteristics of White Leghorn chicken breed

Traits	BW	EW	EL	YW	SW	EWD	ST	AW	SI	SR	AR	YR
BW												
EW	0.13^{ns}											
EL	0.11^{ns}	0.66**										
YW	-0.71**	0.44*	0.29^{ns}									
SW	0.00^{ns}	0.55**	0.35*	0.27^{ns}								
EWD	0.02^{ns}	0.73**	0.19^{ns}	0.44*	0.43*							
AW	0.19^{ns}	0.86**	0.57**	-0.05^{ns}	0.30*	0.55**	0.04^{ns}					
SI	-0.08^{ns}	-0.10^{ns}	-0.76**	0.03^{ns}	-0.02^{ns}	0.49*	0.29^{ns}	-0.13^{ns}				
SR	-0.04^{ns}	0.36*	0.03^{ns}	0.19^{ns}	0.95**	0.40*	0.41*	0.12^{ns}	0.24^{ns}			
AR	0.32*	0.34*	0.23^{ns}	-0.63**	-0.14^{ns}	0.12^{ns}	-0.10^{ns}	0.77**	-0.11^{ns}	-0.24^{ns}		
YR	-0.18^{ns}	-0.31*	-0.20^{ns}	0.72**	-0.13^{ns}	-0.10^{ns}	0.00^{ns}	-0.71**	0.10^{ns}	-0.07^{ns}	-0.93**	1.00

BW: Body weight, EW: Egg weight, EL: Egg length, YW: Yolk weight, SW: Shell weight, EWD: Egg width, AW: Albumen weight, SI: Shell surface index, SR: Shell ratio, AR: Albumen ratio, YR: Yolk ratio, ns: Not significant ($p > 0.05$) *Significant ($p < 0.05$) and **Significant ($p < 0.01$)

Table 3. Effect of live body weight on external and internal egg characteristics of White Leghorn chicken breed

Traits	Small, ≤ 195g (Mean ± SD)	Medium, 196-220g (Mean ± SD)	Large, ≥ 221g (Mean ± SD)
Egg weight (g)	56.84 ± 3.82[a]	58.73 ± 4.07[a]	58.35 ± 3.84[a]
Egg length (mm)	56.09 ± 1.37[b]	57.31 ± 1.82[a]	56.92 ± 1.94[ab]
Shell weight (g)	7.73 ± 0.62[a]	7.85 ± 0.86[a]	7.84 ± 0.71[a]
Egg width (mm)	43.08 ± 1.31[a]	43.09 ± 0.90[a]	43.01 ± 1.02[a]
Shell surface index (%)	76.84 ± 3.00[a]	75.25 ± 2.42[b]	75.62 ± 2.80[ab]
Shell ratio (%)	13.78 ± 1.10[a]	13.69 ± 1.38[a]	13.77 ± 1.15[a]
Yolk weight (g)	17.55 ± 1.41[a]	18.33 ± 1.53[a]	17.53 ± 1.99[a]
Albumen weight (g)	31.55 ± 3.31[a]	32.55 ± 3.15[a]	32.97 ± 3.43[a]
Albumen ratio (%)	55.42 ± 3.29[a]	55.35 ± 2.61[a]	56.44 ± 3.45[a]
Yolk ratio (%)	30.95 ± 2.53[a]	31.27 ± 2.33[a]	30.09 ± 3.28[a]

SD: Standard deviation, [ab] means in the same row with different superscript letters are significantly different ($p < 0.05$).

DISCUSSION

Egg quality contributes to the improved economic price of hatching and table eggs, and the characteristics that define egg quality typically have a genetic basis (Bekele et al., 2022). Pearson's correlation was first used to evaluate the association between live body weight and egg characteristics of the White Leghorn chicken breed. The correlation outcomes displayed that body weight had a negatively high remarkable association with yolk weight, a positive statistical correlation with albumen ratio, and a non-significant correlation with egg weight, egg length, shell weight, egg width, albumen weight, shell surface index, shell ratio, and yolk ratio. According to the study that was conducted by Ojo et al. (2011) in the Japanese quail, it was found that body weight had a positive remarkable relationship with egg length and a negative statistically significant association with egg index. The findings of Ayorinde and Toye (2021) showed that body weight had a negative remarkable correlation with shell thickness and a non-significant association with egg weight. The correlation results imply that increasing the body weight might decrease yolk weight and increase the albumen ratio in White Leghorn chickens. According to Hlokoe et al. (2022), correlated traits are assumed to be controlled by the same genes. However, the results of the current study did not clearly indicate which egg characteristics are directly influenced by body weight, as the correlations only show associations between traits. Therefore, a general linear model was subsequently used to assess the influence of body weight on egg characteristics in the White Leghorn chicken breed. The results indicated that live body weight significantly influenced egg length and shell index. Specifically, chickens with medium live body weight produced eggs with the greatest length, while those with small live body weight produced eggs with the shortest length. The findings further indicated that the SI of the small, medium, and large body weight groups was significantly different. The small body weight group had the highest SI, while the medium body weight group had the lowest SI. The results also indicated that egg weight, shell weight, egg width, shell ratio, albumen ratio, yolk weight, albumen weight, and yolk ratio were not affected by body weight.

The study that was conducted by Lacin et al. (2008) in the Lohmann laying hens reported that shell index was significantly affected by body weight, but body weight had a non-significant effect on yolk ratio which is in line with the current study results. Furthermore, Lacin et al. (2008) reported that the albumen ratio was influenced by body weight, which is not in line with the current study since in the current study body weight had a non-significant effect on the albumen ratio, so the variations may be due to breed differences. Jatoi et al. (2015) in four closed-bred flocks of Japanese quails showed that body weight had a significant effect on yolk ratio which is not in line with the current study. The study also found that body weight had a significant influence on shell weight, egg weight, and shell thickness. Chickens of small and large body weights have higher egg length and shell index than medium ones. The chickens with small, medium, and large body weights perform the same in the following traits egg weight, shell weight, shell ratio, egg width, albumen weight, yolk weight, yolk ratio, and albumen ratio.

CONCLUSION

The study concluded that the live body weight had a negatively high remarkable relationship with yolk weight and positively correlated with albumen ratio. The findings

also indicated that egg weight was associated with almost all the traits except the shell index. The current study indicated that body weight affected some external egg characteristics, namely egg length and shell index. Further studies need to be conducted on the effect of body weight on egg characteristics using more sample size of White Leghorn chickens.

DECLARATIONS

Funding

No available funding was received for the study.

Availability of data and materials

The data is available on request from the corresponding author.

Competing interests

There is no conflict of interest declared by the author.

Ethical considerations

All the authors avoided data fabrication, plagiarism, falsification, misconduct, and double submission/publication and have given consent to publish this article.

Authors' contributions

The initial idea of the study was by Thobela Louis Tyasi and Lindiwe Johannah Sathekge. All the authors contributed to the design of the study. The first draft of the manuscript was written by Lindiwe Johannah Sathekge and Victoria Rankotsane Hlokoe. The manuscript was revised by Thobela Louis Tyasi. The final manuscript was read and approved by all authors.

REFERENCES

Alabi OJ, Ng'ambi JW, Norris D, and Egena SS (2012). Comparative study of three indigenous chicken breeds of South Africa: Body weight and linear body measurements. Agricultural Journal, 7(3): 220-225. Available at: http://repository.futminna.edu.ng:8080/jspui/handle/123456789/4159

Ashraf S, Ashraf M, Akram M, Malik S, and Altaf M (2016). Influence of egg weight on egg quality parameters and growth traits in Ring necked Pheasants in captivity. The Journal of Animal and Plant Sciences, 26(2): 331-338. Available at: https://thejaps.org.pk/docs/v-26-02/05.pdf

Ayorinde KL and Toye AA (2021). Association between body weight and some egg production traits in a strain of commercial layer. Nigerian Journal of Animal Production, 15(1998): 199-125. DOI: http://www.doi.org/10.51791/njap.v15i.1220

Bekele B, Melesse A, Esatu W, and Dessie T (2022). Production performance and egg quality evaluation of indigenous chickens across different agro-ecologies of Southern Ethiopia. Veterinary Integrative Sciences, 20(1): 133-145. DOI: http://www.doi.org/10.12982/VIS.2022.012

Hlokoe VR, Mokoena K, and Tyasi TL (2022). Using multivariate adaptive regression splines and classification and regression tree data mining algorithms to predict body weight of Nguni cows. Journal of Applied Animal Research, 50(1): 534-539. DOI: https://www.doi.org/10.1080/09712119.2022.2110498

Hlokoe VR, Tyasi TL, Mbazima V, and Gunya B (2023). Investigation of egg weight, ovarian follicles morphology and growth differentiation factor9 mRNA expression in Potchefstroom Koekkoek chicken breed. Brazilian Journal of Poultry Science, 25(3): 1-8. DOI: http://www.doi.org/10.1590/1806-9061-2022-1723

Ferreira VHB, Guesdon V, Calandreau L, and Jensen P (2022). White Leghorn and red Junglefowl female chicks use distal and local cues similarly, but differ in persistency behaviours, during aspatial orientation task, behaviour. Behavioural Processes, 200: 104669. DOI: http://www.doi.org/10.1016/j.beproc.2022.104669

Jatoi AS, Sahota AW, Akram M, Javed K, Jaspal MH, Mehmood S, Hussain J, Ishaq HM, and Bughio E (2015). Egg quality characteristics as influenced by different body sizes in four close-bred flocks of Japanese quails (*Coturnix coturnix* japonica). The Journal of Animal & Plant Sciences, 25(4): 921-926. Available at: https://www.cabidigitallibrary.org/doi/pdf/10.5555/20153340310

Kgwatalala PM, Bolebano L, and Nsoso SJ (2013). Egg quality characteristics of different varieties of domesticated helmeted guinea fowl (Numida Meleagris). International Journal of Poultry Science, 12(4): 245-250. DOI: https://www.doi.org/10.3923/ijps.2013.245.250

Lacin E, Yildiz A, Esenbuga N, and Macit M (2008). Effects of differences in the initial body weight of groups on laying performance and egg quality parameters of Lohmann laying hens. Czech Journal of Animal Science, 53(11): 466-471. DOI: http://www.doi.org/10.17221/341-CJAS

Lee MH, Cho EJ, Choi ES, Bang MH, and Sohn SH (2016). The effect of hen age on egg quality in commercial lay. Korean Journal of Poultry Science, 43(4): 253-261. DOI: https://www.doi.org/10.5536/KJPS.2016.43.4.253

Markos S, Belay B, and Astatkie T (2017). Evaluation of egg characteristics of the three indigenous chicken ecotypes kept under farmer's management conditions. International Journal of Poultry Science, 16(5): 180-188. DOI: http://www.doi.org/10.3923/ijps.2017.180.188

NRC (1994). Nutrient requirements of poultry. 9th ed. National Academy Press, Washington, DC. 155 pp.

Ojo V, Ayorinde KL, and Fatoki O (2011). Relationship between body weight and some egg production trait in the Japanese quail. NISEB Journal, 11(1): 89-94. DOI: https://ojs.klobexjournals.com/index.php/nisebj/article/view/161/0

Onunkwo DN and Okoro IC (2015). Predicting egg quality and egg production traits using egg weight and body weight

respectively in three varieties of helmeted guinea fowls in humid tropics. International Journal of Livestock Research, 5(3): 111-121. Available at: https://www.cabidigitallibrary.org/doi/full/10.5555/20153175113

Qin N, Liu Q, Zhang YY, Fan XC, Xu XX, Lv ZC, Wei ML, Jing Y, Mu F, and Xu RF (2015). Association of novel polymorphisms of forkhead box L2 and growth differentiation factor- 9 genes with egg production traits in local Chinese Dagu hens. Journal of Poultry Science, 94(11): 88-95. DOI: https://www.doi.org/10.3382/ps/peu023

Shabalala ZP, Moeletsi ME, Tongwane MI, and Mazibuko SM (2019). Evaluation of in filling methods for time series of daily temperature data: A case study of Limpopo Province, South Africa. Climate, 7(7): 86. DOI: https://www.doi.org/10.3390/cli7070086

JWPR
Journal of World's Poultry Research
2024, Scienceline Publication
J. World Poult. Res. 14(2): 160-171, 2024
Research Paper
DOI: https://dx.doi.org/10.36380/jwpr.2024.17
PII: S2322455X2400017-14
ISSN: 2322-455X

The Safety Evaluation of Novel Bio-based Calcium D-pantothenate Obtained from Recombinant *Escherichia coli* K12 on Growth Performance and Health Status of Broiler Chickens

Guoqing Liu[1, #], Xin Xu[2, #], Lei Zhang[1, *], Cong Li [1], Mengying Li[1], and Honghe Zhang[1]

[1]*Zhejiang NHU Company Ltd., No.4 Jiangbei Road, Yulin Street, Xinchang County, Zhejiang Province, China*
[2]*Key Laboratory of Feed Biotechnology of Ministry of Agriculture and Rural Affairs, Institute of Feed Research, Chinese Academy of Agricultural Sciences, Beijing, China*
[#]*Both authors contributed equally*

*Corresponding author's E-mail: zhanglei@cnhu.com

Received: April 08, 2024, Revised: May 05, 2024, Accepted: May 26, 2024, Published: June 30, 2024

ABSTRACT

The production of traditional synthesized calcium D-pantothenate (D-PA) is accompanied with chemical pollution, therefore, the eco-friendly bio-fermentation technology has received widespread attention. In order to verify the safety of a novel D-PA product produced by genetically engineered bacteria (*Escherichia coli* K12), the authors of the current study investigated the influence of adding D-PA to the diet on growth performance and health status of broiler chickens. A total of 192 day-old healthy Arbor Acres broiler chickens with similar weight (43.21 ± 0.12 g) were randomly divided into 4 treatments with 6 replicates and 8 broiler chickens in each replicate (male and female in half). The *Escherichia coli* K12 was genetically engineered for the production of D-PA. The control group was fed with the basal diet containing 20 mg/kg synthesized D-PA (CT group). The treatments were supplemented with 20 (TCaP1 group, recommended dose group), 100 (TCaP5 group, 5-fold-dose group), and 200 (TCaP10 group, 10-fold-dose group) mg/kg bio-based D-PA product, respectively. The experiment lasted for 42 days and the growth performance and health status of broiler chickens were determined. The results indicated that the addition of 5- and 10-fold doses of bio-based D-PA could increase the average daily weight gain during 22-42 days of age and decrease the feed conversion rate during 22-42 and 1-42 days of age of broilers. There were some differences in white blood cell count, intermediate cell absolute value (MID) count, absolute granulocyte count, absolute lymphocyte count, granulocyte percentage, mean corpuscular volume, red blood cell distribution width-standard deviation, mean platelet volume and serum phosphorus and total bilirubin in different groups, compared with the CT group. Histological observations of the liver, spleen, pancreas, and small intestines showed that the tissue structures of various organs of the broiler chickens fed with the bio-based D-PA were clear, and no abnormal changes such as inflammatory cell infiltration and fibrous tissue hyperplasia were observed in all groups. In summary, dietary supplementation of bio-based D-PA was safe within the 10-fold- dose (200mg/kg) to broiler chickens during 1-42 days.

Keywords: Biological safety evaluation, Broiler chicken, Calcium D-pantothenate, Growth performance, Vitamin B_5

INTRODUCTION

D-calcium pantothenate (D-PA), a white or slightly yellow crystalline powder, is mainly used in feed or the pharmaceutical industry. Pantothenic acid, the precursor of coenzyme A and acyl-carrier protein, is an important water-soluble vitamin which involved in animal growth and development and plays a crucial role in cellular metabolism and the oxidation of fats, carbohydrates, and proteins (Eggersdorfer et al., 2012; Tang et al., 2020a; 2020b). For poultry, dietary supplementation of D-PA can help improve the growth performance, feather quality and decrease the dermatosis and mortality (Hegsted et al., 1949; Beer et al., 1963). Although pantothenic acid is widely distributed in foods and can be synthesized by the intestinal microbiota, it's still necessary to supply additionally in the feed to satisfy the need of fast-growing animals (Wang et al., 2016). Deficiency of pantothenic

acid in animals leads to reduced nutrient utilization, weakened immune system, and various diseases, and it can also reduce the body's tolerance of stresses through hormone synthesis (Tang et al., 2021).

The commercial production of D-PA has currently always relied on developed chemical synthesis routes. There exists highly toxic raw materials and cyanide-containing wastewater pollution in the classical chemical process (Acevedo-Rocha et al., 2019). As people are paying more and more attention to environmental protection and energy shortages, researchers are looking for more environmentally friendly and sustainable methods of the production of D-PA (Leonardi and Jackowski, 2007; Zou et al., 2021). The microbial fermentation method uses natural renewable resources to realize the environmentally friendly and sustainable production of D-PA, which could be the replacement of chemical synthesis routes (Huser et al., 2005; Zhang et al., 2019). Although the report of the toxicity of the traditional synthesized D-PA has not been found, the safety of bio-based D-PA remains unknown, therefore, this experiment investigated the nutritional effects and safety of D-PA produced by biological synthesis methods using genetically engineered bacteria on broiler chickens.

MATERIALS AND METHODS

Ethical approval

All experimental procedures were approved by the Animal Management Committee (in charge of animal welfare) of the Institute of Animal Science, Chinese Academy of Agricultural Sciences (IAS-CAAS, Beijing, China) and performed in accordance with the guidelines. Ethical approval on animal survival was given by the animal ethics committee of IAS-CAAS (approval no.202321, date : 2023.02.16).

Experimental design and treatments

A total of 192 1-day-old healthy Arbor Acres broilers obtained from a local hatchery (Hebei Luanping Huadu Food Co., Ltd, china) with similar weight (43.21 ± 0.12 g) were randomly assigned to 4 treatment groups with 6 replicates per treatment group and 8 chicks per replicate (half hens and half roosters). The experiment was conducted at the experimental farm of IAS-CAAS. The control group was fed with a corn-soybean meal basal diet containing 20 mg/kg of syntheized D-PA (CT group), and the treatments were supplemented with 20 mg/kg (TCaP1 group, recommended dose group, Announcement No. 2625 of the Ministry of Agriculture of the People's Republic of China, 2018), 100 mg/kg (TCaP5 group, 5-fold dose group) or 200 mg/kg (TCaP10 group, 10-fold dose group) bio-based D-PA product, respectively. The experiment lasted for 42 days.

Birds and diets

The bio-based D-PA product (99%) obtained from recombinant *Escherichia coli* K12 (PT06) was provided by Heilongjiang NHU Biotechnology Co., Ltd (China), and there were no genetically engineered bacteria existing in the final D-PA product. The engineered bacteria of recombinant *Escherichia coli* K12 was prepared for the production of D-PA product. This microbial fermentation method uses natural renewable resources - glucose and the recombinant *Escherichia coli* K12 to produce D-PA. The pyruvate and keto-isovalerate are the key precursors of D-PA biosynthesis.

The standard starter diet (1 to 21 days of age) and grower-finisher diet (22-42 days of age) were formulated according to the NRC recommendations (1994) mainly using corn, soybean meal, and the premix. The details of the experimental diets are presented in Table 1. The birds were raised according to the management regulations of Arbor Acres broilers (Aviagen, 2018). The broiler chickens were kept in thermostatically controlled, stainless cages coated with plastic (100 × 50 × 45 cm) and equipped with fiberglass feeders and waterers. Feed and tap water were available *ad libitum*. The temperature in the house was controlled at 33 - 35°C from days 1 - 5, which was gradually reduced every week until 26°C. The house was cleaned and disinfected before the entry of broiler chickens. The broiler chickens were vaccinated against Newcastle disease, infectious bronchitis, avian influenza, and infectious bursal disease vaccines (produced by ShanDong HuaHong Biological Engineering CO.,LTD., china) at 7 days of age.

Sample collection and determination

Broilers were weighed on days 1, 21, and 42 of the experiment. Feed consumption and mortality data were recorded at the end of the experiment. Growth performance indexes such as average daily feed intake (ADFI), average daily weight gain (ADG), feed conversion rate (F/G), and mortality were calculated based on these measurements.

In the study, blood samples were collected from the wing vein broiler chickens on day 42 for testing various blood physiological indexes after 8 hours of fasting. In this regard, 2 mL whole blood samples were preserved using an EDTA anticoagulant tube to test the blood physiological

indexes, including white blood cell count (WBC), granulocyte absolute value (GRA), percentage of granulocyte (GRA%), absolute lymphocyte count (LYM), absolute intermediate cell value (MID), absolute lymphocyte percentage (LYM%), intermediate cell percentage (MID%), red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width-standard deviation (RDW-SD), red blood cell distribution width-coefficient of variation (RDW-CV), platelet count (PLT), platelet crit (PCT), mean platelet volume (MPV), platelet distribution width (PDW) with a TEK-II mini automatic animal blood analyzer (Tecom, China). Another 10 mL blood was collected in a vacuum blood collection tube and placed for 4 h at room temperature. The blood sample was then centrifuged to obtain the serum at 3.500 r/min for 10 min and was stored at -80°C in eppendorf tubes. The serum calcium, phosphorus, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-gamma-glutamyl transpeptidasee, urea nitrogen, creatinine, total bilirubin, lysozyme and serum superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, malondialdehyde, total antioxidant capacity and hydroxy free radical scavenging activity were determined by automatic biochemical analyzer (Hitachi 7600, Japan) using the assay kits (Nanjing Jiancheng Bioengineering Institute, china). Serum immunity parameters (total protein, albumin, globulin, albumin/globulin, immunoglobulin G, immunoglobulin M, immunoglobulin A, complement 3, complement 4, interleukin-1β, tumor necrosis factor α, lysozyme) were determined by enzyme-linked immunoassay using microplate reader (Thermo Multiskan MK3, Finland). Serum hormones (thyroid hormones T3, thyroid hormones T4, insulin) were determined by automatic radioimmunoassay counter (Zonkia GC-2010, China).

After blood sampling 2 broiler chickens from each replicate were dissected (Tang et al., 2021) and the liver, spleen, pancreas, and small intestines were quickly separated, weighted and immersed in 4% paraformaldehyde solution for the determination of organ indexes and observation of organ development and histopathological changes under light microscope (10x, Zhanjing, China). The abnormal structure of organs, proliferation of Kupffer cells, inflammatory cell infiltration, congestion, and fibrous tissue hyperplasia indexes was used to evaluate the health status of broiler chickens (Al-Sultan and Gameel, 2004; Kumar et al., 2009).

Table 1. Composition and calculated nutrient content of basal diets for broiler chickens during 1-42 days of age

Ingredient	**Grower (1-21 days; g/kg)**	**Finisher (22-42 days; g/kg)**
Corn	53.20	57.10
Soybean meal（43%）	38.20	34.00
Soybean oil	4.15	5.00
Dicalcium phosphate	1.70	1.56
Limestone	1.00	0.80
Salt	0.30	0.30
DL- Methionine	0.24	0.14
L- Lysine, 98%	0.21	0.10
Premix[1]	1.00	1.00
Total	100.00	100.00
Nutrition level[2]		
Metabolism（MJ/kg）	12.49	12.89
Crude protein（%）	21.51	20.00
Calcium（%）	1.00	0.87
Available phosphorous（%）	0.46	0.42
Methionine（%）	0.52	0.41
Lysine（%）	1.17	1.00
Tryptophan（%）	0.22	0.20
Threonine（%）	0.64	0.59

[1] Supplied per kilogram of the diet. Vitamin A, 10,000 IU as vitamin A acetate; vitamin D3, 4,500 IU as cholecalciferol; 65 IU of vitamin E; vitamin K, 3.0 mg as menadione sodium bisulfate; thiamine, 2.5 mg as thiamine mononitrate; riboflavin, 6.5 mg; pyridoxine, 3.2 mg as pyridoxine hydrochloride; vitamin B12, 0.03 mg; pantothenic acid, 18 mg as D-calcium pantothenate; niacin, 60 mg; folic acid, 1.9 mg and biotin, 0.25 mg, copper 7.5mg, ferrous 20 mg, manganese 120mg, iodine 1.25mg, selenium 0.3mg.

Statistical analysis

Data were subjected to one-way ANOVA by using the GLM procedures of SAS 9.4 (SAS Inst., Inc., Cary, NC, USA). Cage served as the experimental unit. When one-way ANOVA showed significant ($p < 0.05$) differences among treatments, treatment means were compared using Duncan's method. Data were expressed as "mean ± standard deviation".

RESULTS

Growth performance

The effect of adding D-PA obtained from microbial fermentation method to the diet on the broiler growth performance is shown in Table 2. There were no differences between the CT group and the treatment groups in terms of ADG, ADFI, F/G, and mortality during 1-21 days of age ($p > 0.05$). During 22-42 days of age, there was an increase in ADG in the TCaP5 and TCaP10 group, and an decrease on F/G in the TCaP5 and TCaP10 group compared with the CT group ($p < 0.05$). During days 1-42, the F/G in the TCaP5 and TCaP10 groups was lower than that of the CT group ($p < 0.05$). No difference was observed in ADFI and mortality during 1-21, 22-42, and 1-42 days of age and ADG during 1-42 days of age ($p > 0.05$).

Organ indexes

The effects of dietary supplementation of bio-based D-PA on the broiler organ indexes are shown in Table 3. There was no difference in the organ indexes of the liver, heart, spleen, pancreas, thymus, bursa of Fabricius, muscular stomach, duodenum, jejunum, ileum, and cecum of broilers in different treatments ($p > 0.05$).

Blood physiological parameters

The effects of dietary supplementation of bio-based D-PA on the blood physiological parameters are shown in Table 4. The white blood cell count (WBC), granulocyte absolute value (GRA) and percentage of granulocyte (GRA%) in the TCaP5 group increased compared with that of the CT group or the TCaP10 group ($p < 0.05$), but the percentage of lymphocyte (LYM%) in the TCaP5 group decreased compared with that of the CT group or the TCaP10 group ($p < 0.05$). Intermediate cell absolute value (MID) in the TCaP5 group, mean erythrocyte volume (MCV) in the TCaP1 group, the standard deviation of red blood cell distribution width (RDW-SD) in the TCaP1 group and the TCaP10 group, and mean platelet volume (MPV) in the TCaP1 group increased compared with the CT group ($p < 0.05$).

Serum biochemistry and antioxidant parameters

The effects of dietary supplementation of bio-based D-PA on the serum biochemistry parameters are shown in Table 5. Serum phosphorus level in the TCaP10 group increased compared with that of the TCaP5 group ($p < 0.05$). Serum total bilirubin in the TCaP5 group increased compared with that of the CT group ($p < 0.05$). The effects of dietary supplementation of bio-based D-PA on the serum antioxidant indicators are shown in Table 6. Dietary supplementation of bio-based D-PA had no influence on the activities of serum superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GR), the content of malondialdehyde (MDA), total antioxidant capacity (T-AOC), nitric oxide and Hydroxyl radical (OH) ($p > 0.05$).

Serum hormone parameters

The effects of dietary supplementation of bio-based D-PA on the serum hormone parameters are shown in Table 7. Compared with the CT group, the serum T3, T4, and insulin were not affected in the treatment groups ($p > 0.05$).

Serum immunity parameters

The effects of dietary supplementation of bio-based D-PA on the serum immunity indicators of broilers are shown in Table 8. The serum interleukin-1β level in the TCaP1 group was lower compared with the CT group ($p < 0.05$). The serum lysozyme level in the TCaP5 and TCaP10 group was lower compared with the CT and TCaP1 group ($p < 0.05$). Adding D-PA obtained from the microbial fermentation method to the diet had no influence on serum total protein, albumin, globulin, albumin to globulin ratio, IgG, IgM, IgA, complement-3, complement-4, and tumor necrosis factor-α ($p > 0.05$).

Organ histomorphology

As shown in Figure 1, the clear hepatic lobules from all groups, the uniform hepatocytes, regular hepatic cord arrangements, the round single or dual hepatocyte nuclei with regular morphology, and the clear and visible hepatic sinuses were clear. Compared with the control group, the test groups showed no proliferation of Kupffer cells, inflammatory cell infiltration, or fibrous tissue proliferation in the portal region. As shown in Figure 2, the intact splenic capsules, the abundant white pulp, the

clear margin of the splenic corpuscles, and the obvious germinal centers could be seen in all test groups. No congestion was found in the blood sinus of the red pulp, macrophages were visible in the medullary sinuses and marginal areas, and the red and white pulp structures of the splenic tissues were clear. Compared with the control group, no abnormalities were found in the test groups. As shown in Figure 3, in all the test groups, the delimited pancreatic tissues, the ducts, and blood vessels in the connective tissues of the lobules, the clear structure of pancreatic islet was obvious, and there was no inflammatory cell infiltration in the lobular mesenchyma. As shown in Figure 4, in all the test groups, the clear structure of the duodenal mucosa, the healthy and strong villi, the abundant mucosal epithelial goblet cells, the clear chorionic interstitial, intestinal crypt, and intrinsic layer structures, the visible Paneth cells at the bottom of the crypt was clear, and no inflammation was observed.

Table 2. Effects of dietary supplementation of bio-based D-PA on the broiler chickens' growth performance during 1-42 days of age [1]

	Item	CT	TCaP1	TCaP5	TCaP10	p-value
Day 1-21	ADFI (g/d)	47.34 ± 0.61	46.93 ± 0.24	46.89 ± 0.97	47.19 ± 0.89	0.964
	ADG (g/d)	31.85 ± 1.26	32.60 ± 0.82	33.26 ± 0.49	31.46 ± 1.78	0.689
	F/G	1.50 ± 0.05	1.44 ± 0.03	1.41 ± .02	1.51 ± 0.07	0.352
	Mortality (%)	0	0	0	0	
Day 21	BW (g)	711.88 ± 26.35	727.86 ± 17.24	741.68 ± 10.18	703.75 ± 37.25	0.685
Day 22-42	ADFI (g/d)	142.25 ± 3.60	142.42 ± 3.01	143.69 ± 2.33	141.39 ± 3.02	0.962
	ADG (g/d)	83.05 ± 2.50^{b}	86.71 ± 2.25^{ab}	88.81 ± 0.60^{a}	89.30 ± 0.75^{a}	0.034
	F/G	1.72 ± 0.03^{a}	1.64 ± 0.02^{ab}	1.62± 0.03^{b}	1.58 ± 0.03^{b}	0.019
	Mortality (%)	0	0	0	0	
Day 42	BW (g)	2372.90 ± 74.15	2462.10 ± 54.57	2517.90 ± 11.92	2489.80 ± 41.94	0.244
Day 1-42	ADFI (g/d)	93.03 ± 2.04	92.90 ± 1.52	93.50 ± 1.58	92.53 ± 1.87	0.985
	ADG (g/d)	57.80 ± 2.04	60.47 ± 1.36	61.87 ± 0.30	61.16 ± 1.05	0.194
	F/G	1.64 ± 0.04^{a}	1.58 ± 0.01^{ab}	1.55 ± 0.02^{b}	1.55 ± 0.01^{b}	0.036
	Mortality (%)	0	0	0	0	-

[1] Means within a row with no common superscripts differ ($p < 0.05$), the same as follow. CT: Control group supplemented with 20 mg/kg synthetic calcium D-pantothenate; TCaP1: Treatment supplemented with 20 mg/kg bio-based calcium D-pantothenate; TCaP5: Treatment supplemented with 100 mg/kg bio-based calcium D-pantothenate; TCaP10: Treatment supplemented with 200 mg/kg bio-based calcium D-pantothenate. ADFI: Average daily feed intake; ADG: Average daily weight gain; F/G: feed-to-gain ratio; D-PA: Calcium D-pantothenate.

Table 3. Effects of dietary supplementation of bio-based D-PA on the organ indexes of broiler chickens during 1-42 days of age

Item	CT	TCaP1	TCaP5	TCaP10	p value
Liver	20.70 ± 1.05	20.77 ± 0.91	21.45 ± 1.11	21.29 ± 0.74	0.928
Heart	4.87 ± 0.12	5.02 ± 0.26	4.91 ± 0.22	5.25 ± 0.22	0.596
Spleen	1.11 ± 0.07	1.03 ± 0.06	1.33 ± 0.16	1.42 ± 0.23	0.232
Pancreas	2.08 ± 0.11	2.11 ± 0.11	2.09 ± 0.13	2.31 ± 0.09	0.410
Thymus	3.12 ± 0.36	3.36 ± 0.36	3.34 ± 0.35	4.00 ± 0.42	0.381
Bursa of Fabricius	2.12 ± 0.15	2.24 ± 0.22	2.21 ± 0.19	2.12 ± 0.19	0.958
Muscular stomach	16.58 ± 0.84	16.53± 1.33	17.46 ± 0.91	16.14 ± 0.79	0.813
Duodenum	7.28 ± 0.35	6.79 ± 0.27	6.41 ± 0.27	7.42 ± 0.39	0.180
Jejunum	14.48 ± 0.51	13.32 ± 0.94	12.4 7± 0.39	12.47 ± 0.39	0.326
Ileum	13.28 ± 0.99	12.00 ± 0.87	11.21 ± 0.82	13.45 ± 0.48	0.338
Cecum	6.20 ± 0.39	7.40 ± 0.87	6.60 ± 0.46	7.02 ± 0.81	0.617

CT: Control group supplemented with 20 mg/kg synthesized calcium D-pantothenate; TCaP1: Treatment supplemented with 20 mg/kg bio-based calcium D-pantothenate; TCaP5: Treatment supplemented with 100 mg/kg bio-based calcium D-pantothenate; TCaP10: Treatment supplemented with 200 mg/kg bio-based calcium D-pantothenate.

Table 4. Effects of dietary supplementation of bio-based D-PA on the blood physiological parameters of broiler chickens during 1-42 days of age

Item	CT	TCaP1	TCaP5	TCaP10	*P* value
WBC ($\times10^9$/L)	128.84 ± 2.08^{b}	132.08 ± 1.63^{ab}	134.96 ± 4.35^{a}	129.75 ± 1.78^{b}	0.037
LYM ($\times10^9$/L)	64.59 ± 0.82	63.84 ± 0.74	64.05 ± 0.57	64.73 ± 0.52	0.752
MID ($\times10^9$/L)	18.53 ± 0.33^{b}	18.97 ± 0.30^{ab}	19.58 ± 0.20^{a}	18.79 ± 0.29^{ab}	0.012
GRA ($\times10^9$/L)	45.72 ± 1.88^{b}	49.27 ± 1.18^{ab}	51.33 ± 1.32^{a}	46.23 ± 1.68^{b}	0.043
LYM (%)	50.35 ± 1.00^{a}	48.47 ± 0.58^{ab}	47.62 ± 0.66^{b}	50.13 ± 0.87^{a}	0.030
MID (%)	14.34 ± 0.05	14.32 ± 0.07	14.47 ± 0.05	14.42 ± 0.060	0.260
GRA (%)	35.31 ± 1.00^{b}	37.22 ± 0.57^{ab}	37.92 ± 0.67^{a}	35.45 ± 0.85^{b}	0.033
RBC ($\times10^{12}$/L)	2.01 ± 0.05	1.99 ± 0.03	2.05 ± 0.04	1.98 ± 0.03	0.619
HGB (g/L)	110.42 ± 3.48	112.17 ± 1.61	114.33 ± 1.86	109.67 ± 1.89	0.505
HCT (L/L)	0.18 ± 0.005	0.19 ± 0.003	0.19 ± 0.003	0.18 ± 0.003	0.268
MCV (fL)	90.01 ± 0.79^{b}	93.68 ± 0.59+	91.16 ± 0.80^{b}	90.43 ± 0.69^{b}	0.004
MCH (pg)	54.73 ± 0.51	56.12 ± 0.45	55.64 ± 0.81	55.17 ± 0.64	0.431
MCHC (g/L)	577.75 ± 5.19	569.00 ± 3.25	579.17 ± 4.98	579.25 ± 4.86	0.350
RDW-SD (fL)	27.51 ± 3.29^{b}	34.94 ± 0.54^{a}	30.96 ± 2.64^{ab}	34.88 ± 0.43^{a}	0.019
RDW-CV (%)	23.29 ± 0.60	23.23 ± 0.90	23.29 ± 0.59	23.95 ± 0.40	0.842
PLT ($\times10^9$/L)	31.50 ± 6.17	25.00 ± 2.11	26.42 ± 1.54	26.75 ± 2.07	0.587
PCT (L/L)	0.033 ± 0.006	0.026 ± 0.002	0.028 ± 0.002	0.028 ± 0.002	0.657
MPV (fL)	10.53 ± 0.08^{b}	10.76 ± 0.08^{a}	10.70 ± 0.05^{ab}	10.63 ± 0.05^{ab}	0.017
PDW (fL)	59.23 ± 5.06	70.48 ± 4.67	64.68 ± 4.33	72.59 ± 4.34	0.179

CT: Control group supplemented with 20 mg/kg synthesized calcium D-pantothenate; TCaP1: Treatment supplemented with 20 mg/kg bio-based calcium D-pantothenate; TCaP5: Treatment supplemented with 100 mg/kg bio-based calcium D-pantothenate; TCaP10 : Treatment supplemented with 200 mg/kg bio-based calcium D-pantothenate; WBC: White blood cell count; LYM: Absolute lymphocyte count; MID: Absolute MID cell count; GRA: Absolute granulocyte count; LYM%: Lymphocyte percentage; MID%: MID cell percentage; GRA%: Granulocyte percentage; RBC: Red blood cell count; HGB: Hemoglobin concentration; HCT: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; RDW-SD: Red blood cell distribution width-standard deviation; red blood cell distribution width-coefficient of variation; PLT: Platelet count; PCT: Plateletcrit; MPV: Mean platelet volume; PDW: Platelet distribution width.

Table 5. Effects of dietary supplementation of bio-based D-PA on the serum biochemistry parameters of broiler chickens during 1-42 days of age

Item	CT	TCaP1	TCaP5	TCaP10	*P* value
Ca (mmol/L)	2.68 ± 0.07	2.64 ± 0.06	2.66 ± 0.06	2.58 ± 0.07	0.721
P (mmol/L)	1.79 ± 0.03^{ab}	1.79 ± 0.04^{ab}	1.74 ± 0.03^{b}	1.85 ± 0.03^{a}	0.018
ALT (U/L)	5.27 ± 1.22	2.97 ± 0.47	5.87 ± 0.98	6.12 ± 1.19	0.142
AST (U/L)	469.67 ± 72.29	453.15 ± 56.60	500.10 ± 50.19	601.88 ± 50.87	0.298
ALP (U/L)	125.92 ± 23.44	185.43 ± 43.85	176.28 ± 28.21	104.73 ± 11.35	0.181
GGT (U/L)	18.15 ± 1.32	21.93 ± 1.25	18.55 ± 1.11	17.95 ± 2.39	0.281
BUN (mmol/L)	0.59 ± 0.09	0.49 ± 0.05	0.44 ± 0.20	0.60 ± 0.06	0.318
CREA (μmol/L)	30.18 ± 14.14	24.03 ± 14.26	14.58 ± 5.04	21.05 ± 11.95	0.829
TBiLi (μmol/L)	5.00 ± 0.86^{b}	6.68 ± 0.43^{ab}	7.38 ± 0.38^{a}	6.23 ± 0.73^{ab}	0.015

CT: Control group supplemented with 20 mg/kg synthesized calcium D-pantothenate; TCaP1: Treatment supplemented with 20 mg/kg bio-based calcium D-pantothenate; TCaP5: Treatment supplemented with 100 mg/kg bio-based calcium D-pantothenate; TCaP10: Treatment supplemented with 200 mg/kg bio-based calcium D-pantothenate; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma-gamma-glutamyl transpeptidasee; BUN: Urea nitrogen; CREA: Creatinine; TBiLi: Total bilirubin.

Table 6. The effects of dietary supplementation of bio-based D-PA on the serum antioxidant parameters of Arbor Acres broilers during 1-42 days of age

Item	CT	TCaP1	TCaP5	TCaP10	p value
SOD (U/ml)	134.59 ± 8.68	124.26 ± 9.88	135.59 ± 10.17	108.38 ± 10.52	0.210
CAT (U/ml)	4.63 ± 0.44	5.13 ± 0.17	4.92 ± 0.20	4.85 ± 0.19	0.651
GSH-Px (U/ml)	193.42 ± 5.10	204.35 ± 2.77	195.28 ± 3.87	200.12 ± 4.72	0.281
GR (U/L)	72.35 ± 4.47	61.36 ± 9.60	56.00 ± 4.61	69.67 ± 6.15	0.295
MDA (nmol/ml)	4.00 ± 0.39	4.64 ± 0.44	4.03 ± 0.52	4.88 ± 0.37	0.406
T-AOC (mol/L)	0.59 ± 0.02	0.62 ± 0.03	0.58 ± 0.02	0.57 ± 0.01	0.485
NO (μmol/L)	4.29 ± 0.39	5.08 ± 0.15	4.97 ± 0.26	4.96 ± 0.65	0.514
OH (U/ml)	53.78 ± 1.62	51.64 ± 1.99	48.09 ± 2.29	48.46 ± 2.22	0.189

CT: Control group supplemented with 20 mg/kg synthesized calcium D-pantothenate; TCaP1: Treatment supplemented with 20 mg/kg bio-based calcium D-pantothenate; TCaP5: Treatment supplemented with 100 mg/kg bio-based calcium D-pantothenate; TCaP10: Treatment supplemented with 200 mg/kg bio-based calcium D-pantothenate; SOD: Superoxide dismutase; CAT: Catalase; GSH-Px: Glutathione peroxidase; GR: Glutathione reductase; MDA: Malondialdehyde; T-AOC: Total antioxidant capacity; OH: Hydroxy free radical scavenging activity.

Table 7. The effects of dietary supplementation of bio-based D-PA on the serum hormone parameters of broiler chickens during 1-42 days of age

Item	CT	TCaP1	TCaP5	TCaP10	p value
T3 (ng/ml)	0.50 ± 0.09	0.62 ± 0.07	0.60 ± 0.12	0.52 ± 0.09	0.736
T4 (ng/ml)	17.88 ± 3.11	20.08 ± 1.66	15.14 ± 1.88	16.87 ± 1.74	0.462
INS (MIμ/L)	8.87 ± 0.94	8.12 ± 0.46	8.86 ± 0.45	9.08 ± 0.84	0.789

CT: Control group supplemented with 20 mg/kg synthesized calcium D-pantothenate; TCaP1: Treatment supplemented with 20 mg/kg bio-based calcium D-pantothenate; TCaP5: Treatment supplemented with 100 mg/kg bio-based calcium D-pantothenate; TCaP10: Treatment supplemented with 200 mg/kg bio-based calcium D-pantothenate; T3/T4: Thyroid hormones; INS: Insulin.

Table 8. The effects of dietary supplementation of bio-based D-PA on the serum immune parameters of broiler chickens during 1-42 days of age

Item	CT	TCaP1	TcaP5	TcaP10	p value
TP (g/L)	29.58 ± 1.04	29.03 ± 0.83	29.58 ± 0.87	28.68 ± 0.91	0.873
ALB (g/L)	13.45 ± 0.63	13.17 ± 0.17	13.12 ± 0.28	13.40 ± 0.32	0.906
GLB (g/L)	16.13 ± 0.53	15.87 ± 0.79	16.47 ± 0.70	15.28 ± 0.67	0.660
A/G	0.83 ± 0.03	0.84 ± 0.04	0.80 ± 0.03	0.88 ± 0.03	0.373
IgG (g/L)	8.51 ± 0.59	7.02 ± 0.75	7.75 ± 0.53	7.31 ± 0.62	0.385
IgM (g/L)	1.06 ± 0.07	1.05 ± 0.06	1.06 ± 0.04	1.08 ± 0.06	0.971
IgA (g/L)	1.15 ± 0.07	1.08 ± 0.05	1.11 ± 0.04	1.16 ± 0.05	0.661
C3 (mg/dL)	31.83 ± 1.73	29.19 ± 2.13	32.36 ± 1.11	33.20 ± 2.08	0.451
C4 (mg/dL)	33.82 ± 2.35	31.65 ± 1.63	30.54 ± 1.79	34.53 ± 4.00	0.684
IL-1β (pg/ml)	36.05 ± 5.18^{a}	26.40 ± 0.88^{b}	37.20 ± 3.21^{ab}	28.01 ± 2.30^{ab}	0.031
TNF-α (pg/ml)	65.12 ± 3.06	68.58 ± 4.96	63.53 ± 4.79	63.53 ± 4.79	0.683
LZM (U/ml)	156.45 ± 10.65^{a}	178.48 ± 6.38^{a}	124.20 ± 7.80^{b}	110.75 ±1 0.53^{b}	<0.001

CT: Control group supplemented with 20 mg/kg synthesized calcium D-pantothenate; TCaP1: Treatment supplemented with 20 mg/kg bio-based calcium D-pantothenate; TCaP5: Treatment supplemented with 100 mg/kg bio-based calcium D-pantothenate; TCaP10: Treatment supplemented with 200 mg/kg bio-based calcium D-pantothenate; TP: Total protein; ALB: Albumin; GLB: Globulin; A/G: Albumin/globulin; IgG: Immunoglobulin G; IgM: Immunoglobulin M; IgA: Immunoglobulin A; C3: Complement 3; C4: Complement 4; IL-1β: Interleukin-1β; TNF-α: Tumor necrosis factor α; LZM: Lysozyme.

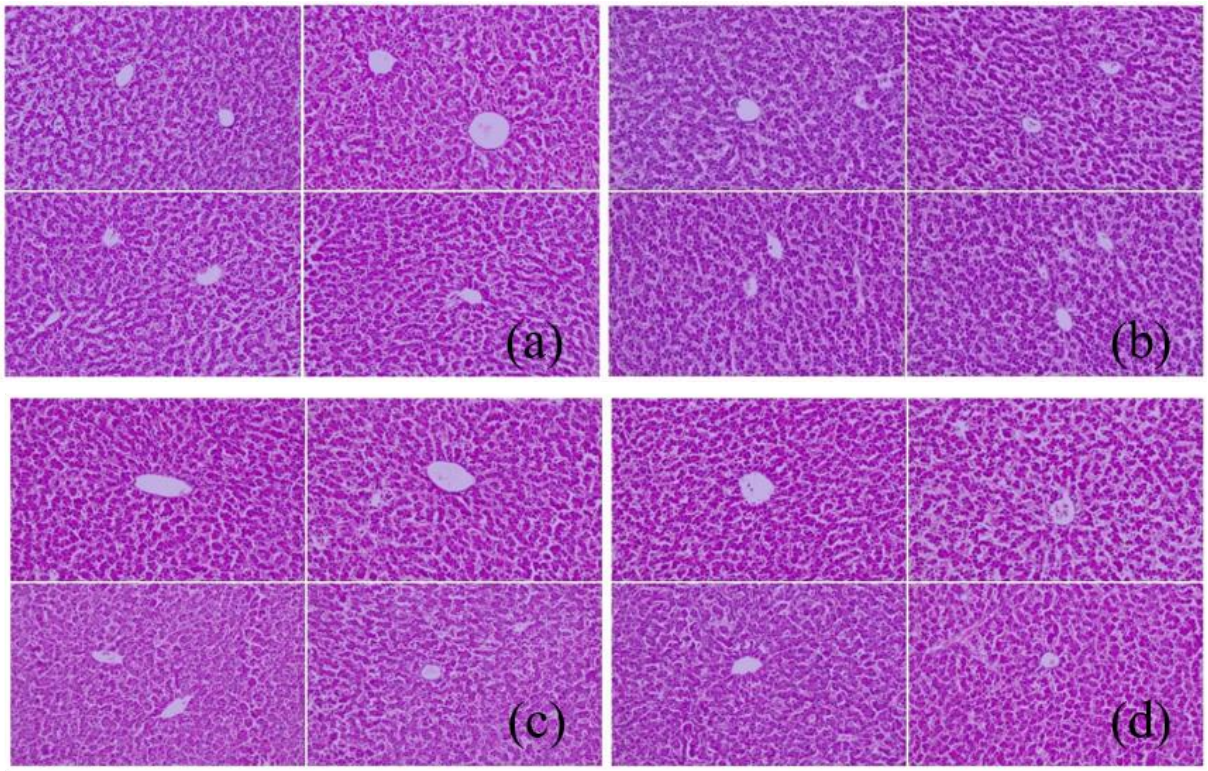


Figure 1. The effects of dietary supplementation of bio-based D-PA on the hepar morphology (×200) broiler chickens during 1-42 days of age. **a:** The control group supplemented with 20 mg/kg synthesized calcium D-pantothenate; **b:** The treatment supplemented with 20 mg/kg bio-based calcium D-pantothenate; **c:** The treatment supplemented with 100 mg/kg bio-based calcium D-pantothenate; **d:** The treatment supplemented with 200 mg/kg bio-based calcium D-pantothenate.

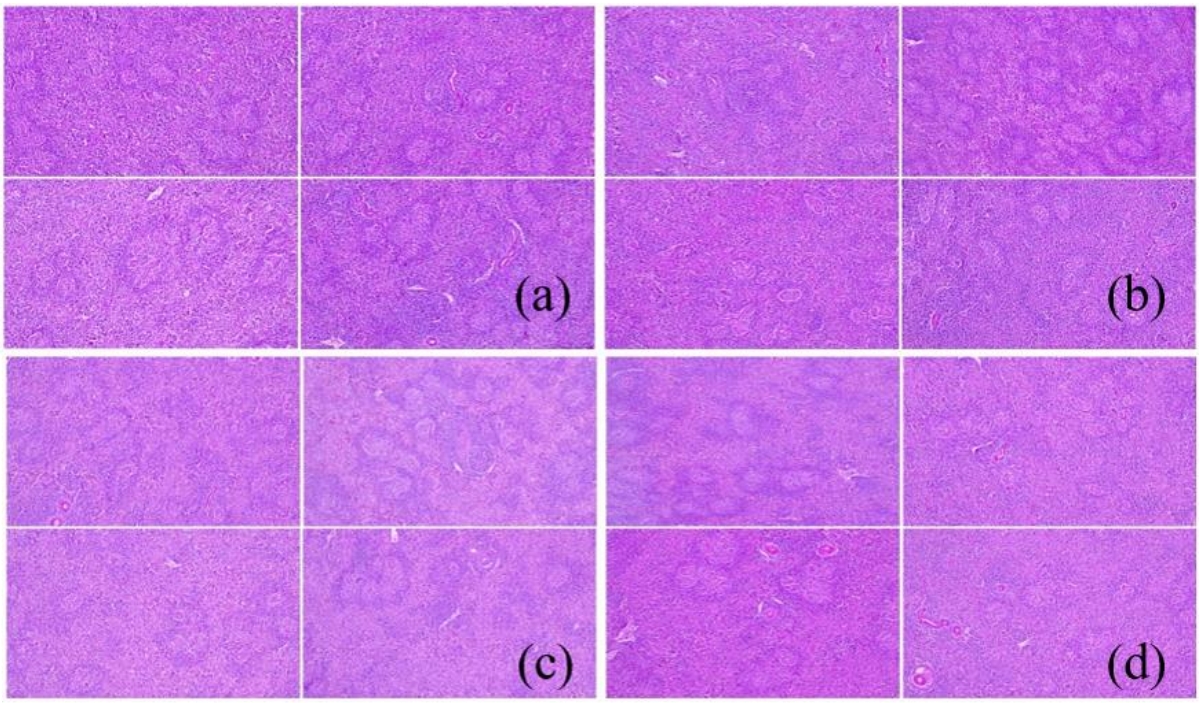


Figure 2. The effects of dietary supplementation of bio-based D-PA on the spleen morphology (×40) of broiler chickens during 1-42 days of age. **a:** The control group supplemented with 20 mg/kg synthesized calcium D-pantothenate; **b:** The treatment supplemented with 20 mg/kg bio-based calcium D-pantothenate; **c:** The treatment supplemented with 100 mg/kg bio-based calcium D-pantothenate; **d:** The treatment supplemented with 200 mg/kg bio-based calcium D-pantothenate.

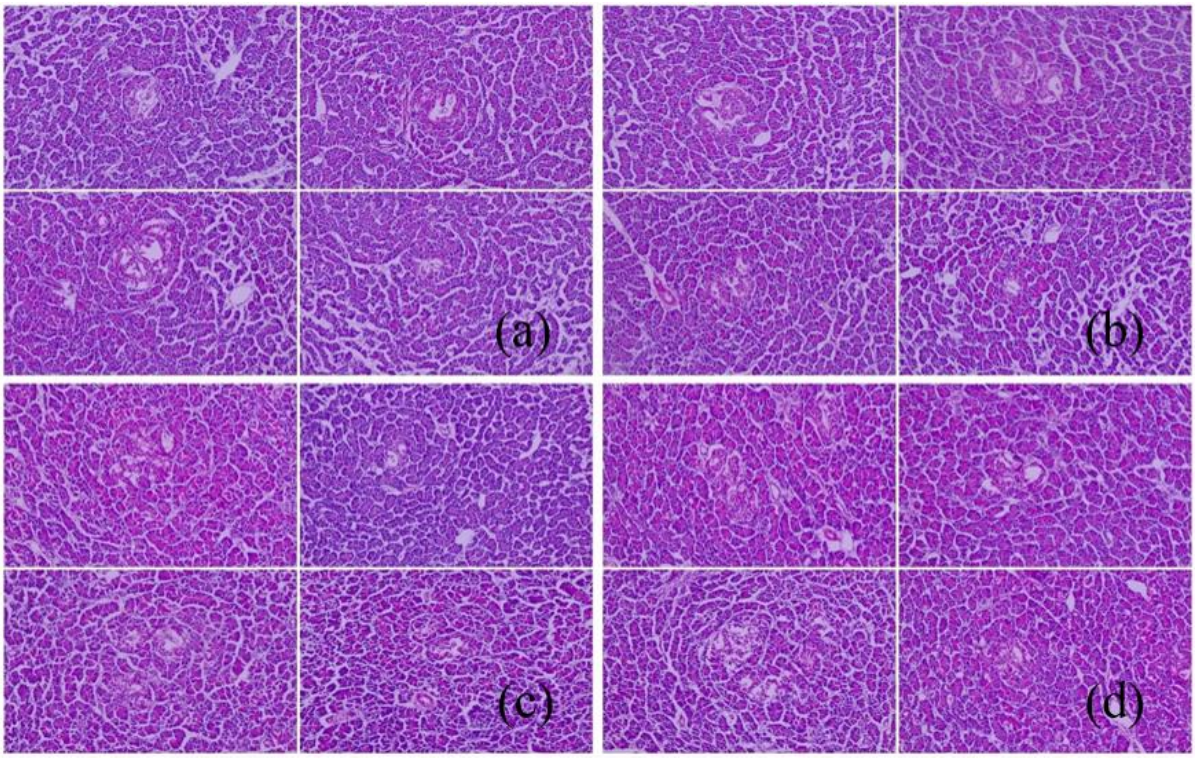


Figure 3. The effects of dietary supplementation of bio-based D-PA on the pancreas morphology(×200) of broiler chickens during 1-42 days of age. **a:** The control group supplemented with 20 mg/kg synthesized calcium D-pantothenate; **b:** The treatment supplemented with 20 mg/kg bio-based calcium D-pantothenate; **c:** The treatment supplemented with 100 mg/kg bio-based calcium D-pantothenate; **d:** The treatment supplemented with 200 mg/kg bio-based calcium D-pantothenate.

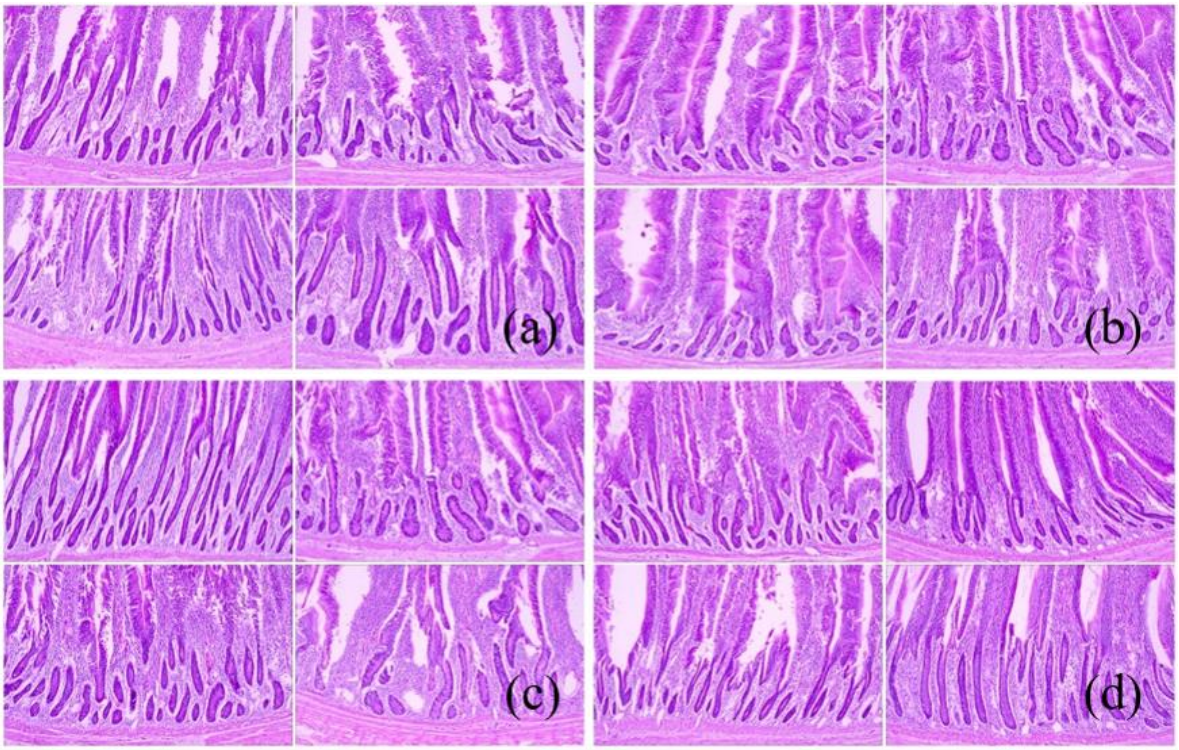


Figure 4. The effects of dietary supplementation of bio-based D-PA on the small intestine morphology (×100) of broiler chickens during 1-42 days of age. **a:** The control group supplemented with 20 mg/kg synthesized calcium D-pantothenate; **b:** The treatment supplemented with 20 mg/kg bio-based calcium D-pantothenate; **c:** The treatment supplemented with 100 mg/kg bio-based calcium D-pantothenate; **d:** The treatment supplemented with 200 mg/kg bio-based calcium D-pantothenate.

DISCUSSION

The chemical synthesis method is the predominant manufacturing route of commercial D-PA product; however, the common chemical synthesis method uses highly toxic raw materials and releases cyanide-containing wastewater. The deteriorating environment and energy deficiency encourage scientists to develop eco-friendly microbial fermentation methods (Postaru et al., 2015; Acevedo-Rocha et al., 2019). The studies available on the toxicity of chemically synthesized D-PA have shown that the redundant pantothenic acid was secreted through urine in about three hours, thus the chemically synthesized D-PA was safe for humans and animals (Spies et al., 1940; Shigeta et al. 1966), however, the safety of D-PA obtained from the microbial fermentation method has not been reported yet.

In the current study, dietary supplementation of 5-fold and 10-fold doses of D-PA obtained from the microbial fermentation method increased the ADG and F/G of broilers during 22-42 days of age and F/G during 1-42 days of age. Similar findings had been reported previously, where dietary deficiency of pantothenic acid led to poor growth performance, and supplementation with D-PA improved growth in broiler chickens, pullets, ducks, and fish (Lepkovsky et al., 1945; Beer et al., 1963; Southern and Baker; 1981; Qian et al., 2015; Tang et al., 2021). However, the optimal requirements of D-PA differed due to the animal species, feed content of pantothenic acid, and the health status of animals. There was no difference in growth performance between the CT group and the TCaP1 group in the current study, indicating that the bio-based D-PA and synthetic D-PA have the same effect of promoting growth.

The blood physiological and biochemical indicators can reflect the nutritional metabolism and health status of poultry, although they vary due to the health status of broilers (Siddon and Tormey, 2019). The former studies indicated that tissue pantothenic acid decreased when diet pantothenic acid deficiency occurred in fish and ducks, thus leading to other functional changes in organs (Qian et al., 2015; Tang et al., 2020a). Tang et al. (2021) reported that pantothenic acid deficiency in duck diets resulted in abnormal glucose metabolism and elevated uric acid content. The effects of dietary supplementation of D-PA on the blood physiological and serum biochemistry parameters, particularly using D-PA obtained from microbial fermentation, were rarely reported.

In the present study, no dose-dependent regularity was observed for WBC, GRA, GRA%, LYM%, MID, MCV, RDW-SD, and MPV in different treatments. The serum antioxidant parameters and hormone parameters were not affected and histological observations of the liver, spleen, pancreas, and small intestines showed that the organs of broiler chickens were in the healthy status. Thus, the differences in blood physiological and biochemical changes may be caused by experimental errors and further studies are need to verify the reason of the blood physiological and biochemical changes. Organ index and histomorphology are important parameters that reflect the impact of different feed treatments on animal development and organ function status (Selim et al., 2021). Pantothenic acid deficiency could cause the metabolic disorders of carbohydrates, lipids, and proteins, thus may affect the organ development and function (Wang et al., 2016; Tang et al., 2021). In the present investigation, the tissue structures of the organs of broiler chickens from the test groups were clear, and no abnormal changes, such as inflammatory cell infiltration and fibrous tissue hyperplasia were observed, indicating that the 10-fold dose supplementation level of the novel bio-based D-PA was safe to broiler chickens. These results in the present study were in consensus with the former reports that pantothenic acid is a water-soluble vitamin that is difficult to store in its original form in the body and almost innocuous to animals (Spies et al., 1940; Shigeta et al. 1966; Wang et al., 2016). Moreover, dietary supplementation of D-PA can improve the growth performance and feather quality while decrease the dermatosis and mortality of poultry (Hegsted et al., 1949; Beer et al., 1963).

CONCLUSION

Under the condition of this experiment, the addition of a novel bio-based D-PA product produced by genetically engineered bacteria (*E. coli* K12) to the diet was safe at the supplementation level of 200 mg/kg without adverse effects on broiler chickens. The application of novel bio-based D-PA products could reduce environmental pollution and promote the growth performance of broilers. In general, the supplementation of the 10-fold recommended dose of D-PA in the diet showed no adverse effect on the growth performance and health status of broilers. Further studies are needed to verify the safety of the novel bio-based D-PA product on other animals.

DECLARATIONS

Authors' contributions

G.L. and X.X. designed the experiment, G.L. and C.L. conducted the experiment, H.Z. completed data analysis; L. Z. supplied resources, M.L. wrote the original draft manuscript, G.L. corrected the manuscript, L.Z. supervised the project. All authors have checked the collected, and analyzed data and agreed on the submission of this article.

Funding

This research received no external funding support.

Availability of data and materials

The data that support the findings of this study are available on reasonable request from the corresponding author.

Acknowledgments

The authors acknowledge the technical assistance of the Institute of Feed Research, Chinese Academy of Agricultural Sciences for supporting the animal experiment and data analysis part of the study.

Conflicts of interests

The authors declare no conflicts of interest.

Ethical considerations

The authors have avoided plagiarism, misconduct, data fabrication/falsification, and double submission/publication and have given consent to publish this article.

REFERENCES

Al-Sultan SI and Gameel AA (2004). Histopathological Changes in the Livers of Broiler Chicken Supplemented with Turmeric (Curcuma longa). International Journal of Poultry Science, 3(5): 333-336. DOI: https://www.doi.org/10.3923/ijps.2004.333.336

Aviagen (2018). Arbor Acres Broiler Management Handbook, Huntsville Alabama, pp. 5-157. Link: https://aviagen.com/assets/Tech_Center/AA_Broiler/AA-BroilerHandbook2018-EN.pdf

Acevedo-Rocha GC, Gronenberg LS, Mack M, Commichau FM, and Genee HJ (2019). Microbial cell factories for the sustainable manufacturing of B vitamins. Current Opinion in Biotechnology, 56: 18-29. DOI: https://www.doi.org/10.1016/j.copbio.2018.07.006

Beer AE, Scott ML, and Nesheim MC (1963). The effects of graded levels of pantothenic acid on the breeding performance of white leghorn pullets. British Poultry Science, 4(3): 243-253. DOI: https://www.doi.org/10.1080/00071666308415501

Eggersdorfer M, Laudert D, Letinois U, McClymont T, Medlock J, Netscher T, and Bonrath W (2012). One hundred years of vitamins-A success story of the natural sciences. Angewandte Chemie International Edition, 51(52): 12960-12990. DOI: https://www.doi.org/10.1002/anie.201205886

Hegsted DM and Riggs TR (1949). The pantothenic acid requirements of chicks receiving a purified diet. The Journal of Nutrition, 37(3): 361-367. DOI: https://www.doi.org/10.1093/jn/37.3.361

Huser AT, Chassagnole C, Lindley ND, Merkamm M, Guyonvarch A, Elisakova V, Patek M, Kalinowski J, Brune I, Puhler A, and Tauch A (2005). Rational design of a *Corynebacterium glutamicum* pantothenate production strain and its characterization by metabolic flux analysis and genome-wide transcriptional profiling. Applied and Environmental Microbiology, 71(6): 3255-3268. DOI: https://www.doi.org/10.1128/ AEM.71.6.3255-3268.2005

Kumar R and Balachandran C (2009). Histopathological changes in broiler chickens fed aflatoxin and cyclopiazonic acid. Veterinarski Arhiv, 79(1): 31-40. Available at: https://hrcak.srce.hr/file/52428

Leonardi R and Jackowski S (2007). Biosynthesis of pantothenic acid and coenzyme A. EcoSal Plus, 2(2): 10-128. DOI: https://www.doi.org/10.1128/ecosalplus.3.6.3.4

Lepkovsky S, Bird FH, Kratzer FH, and Asmundson VS (1945). The Comparative Requirements of Chicks and Turkey Poults for Pantothenic Acid. Poultry Science, 24(4): 335–339. DOI: https://www.doi.org/10.3382/ps.0240335

National research council (NRC) (1994). Nutrient requirement of poultry, 9th Revised Edition. The National Academies Press., Washington, DC. Available at: https://www.agropustaka.id/wp-content/uploads/2020/04/agropustaka.id_buku_Nutrient-Requirements-of-Poultry_Ninth-Revised-Edition-1994-NRC.pdf

Postaru M, Cascaval D, and Galaction AI (2015). Pantothenic acid-applications, synthesis and biosynthesis. The Medical-Surgical Journal, 119(3): 938-943. Available at: https://revmedchir.ro/index.php/revmedchir/article/view/418/373

Qian Y, Li XF, Zhang DD, Cai DS, Tian HY, and Liu WB (2015). Effects of Dietary Pantothenic Acid on Growth, Intestinal Function, Anti-Oxidative Status and Fatty Acids Synthesis of Juvenile Blunt Snout Bream Megalobrama amblycephala. PLOS ONE, 10(3): e0119518. DOI: https://www.doi.org/10.1371/journal.pone.0119518

Selim S, Abdel Megeid NS, Abou-Elnaga MK, and Mahmoud SF (2021). Early nutrition with different diets composition versus fasting on immunity related gene expression and histomorphology of digestive and lymphoid organs of layer type chicks. Animals, 11(6): 1568. DOI: https://www.doi.org/10.3390/ani11061568

Shigeta Y and Shichiri M (1966). Urinary excretion of pantothenic acid and pantethine in human subjects. The

journal of vitaminology, 12(3): 186-191. DOI: https://www.doi.org/10.5925/jnsv1954.12.186

Siddon AJ and Tormey CA (2019). The chemical and laboratory investigation of hemolysis. Advances in Clinical Chemistry, 89:215-258. DOI: https://www.doi.org/10.1016/bs.acc.2018.12.006

Southern LL and Baker DH (1981). Bioavailable Pantothenic Acid in Cereal Grains and Soybean Meal. Journal of Animal Science, 53(2): 403-408. DOI: https://www.doi.org/10.2527/jas1981.532403x

Spies TD, Stanbery SR, Williams RJ, Jukes TH, and Babcock SH (1940). Pantothenic acid in human nutrition. Journal of the American Medical Association, 115(7): 523-524. DOI: https://www.doi.org/10.1001/jama.1940.72810330004010

Tang J, Wu Y, Zhang B, Qi Z, Luo D, Hu J, Huang W, Zhou Z, Xie M, and Hou S (2021). Effects of pantothenic acid supplementation on growth performance, carcass traits, plasma parameters of starter white pekin ducks fed a corn-soybean meal diet. Animals, 11(10): 2872. DOI: https://www.doi.org/10.3390/ani11102872

Tang J, Zhang B, Xue M, Shi W, Wu Y, Feng Y, Huang W, Zhou Z, Xie M, and Hou S (2020a). Pantothenic acid requirement of male White Pekin ducks from hatch to 21 days of age. Animal Feed Science and Technology, 269(1): 114637. DOI: https://www.doi.org/10.1016/j.anifeedsci.2020.114637

Tang J, Zhang B, Liang S, Wu Y, Feng Y, Guo Z, Xing G, Jiao J, Zhou Z, Xie M, and Hou S (2020b). Effects of pantothenic acid on growth performance and antioxidant status of growing male white Pekin ducks. Poultry Science, 99(9): 4436-4441. DOI: https://www.doi.org/10.1016/j.psj.2020.05.021

The ministry of agriculture of the people's Republic of China (2018). Announcement No. 2625. Available at: http://www.moa.gov.cn/nybgb/2018/201801/201801/t20180129_6135954.htm

Wang BW, Zhang X, Yue B, and Ge WH (2016). Effects of pantothenic acid on growth performance, slaughter performance, lipid metabolism, and antioxidant function of *wulong* geese aged one to four weeks. Animal Nutrtion, 2(4): 312-317. DOI: https://www.doi.org/10.1016/j.aninu.2016.07.005

Zhang B, Zhang XM, Wang W, Liu ZQ, and Zheng YG (2019). Metabolic engineering of *Escherichia coli* for D-pantothenic acid production. Food Chemistry, 294: 267-275. DOI: https://www.doi.org/10.1016/j.foodchem.2019.05.044

Zou S, Zhao K, Tang H, Zhang Z, Zhang B, Liu Z, and Zheng Y (2021). Improved production of D-pantothenic acid in Escherichia coli by integrated strain engineering and fermentation strategies. Journal of Biotechnology, 339: 65-72. DOI: https://www.doi.org/10.1016/j.jbiotec.2021.07.014

JWPR
Journal of World's Poultry Research

2024, Scienceline Publication
J. World Poult. Res. 14(2): 172-180, 2024

Review Paper
DOI: https://dx.doi.org/10.36380/jwpr.2024.18
PII: S2322455X2400018-14

ISSN: 2322-455X

Effects of Breeding Systems on Hen Egg Weight: A Meta-Analysis

Thobela Louis Tyasi* and Thabang Sako

School of Agricultural and Environmental Sciences, Department of Agricultural Economics and Animal Production, University of Limpopo, Private Bag X1106, Sovenga, Limpopo 0727, South Africa

*Corresponding author's E-mail: louis.tyasi@ul.ac.za

Received: March 30, 2024, Revised: April 23, 2024, Accepted: May 21, 2024, Published: June 30, 2024

ABSTRACT

In the egg production industry, egg weight is a critical parameter influencing economic viability. The objective of the present study was to determine the effect of cage, free-range, and deep litter breeding systems on hen egg weight using meta-analysis. Articles were searched using Google Scholar, PubMed, ScienceDirect, and Web of Science yielding 175 articles of which 22 articles were included in the present study. Methodological quality was assessed using Joanna Briggs Institute guidelines. A model was used to determine the effect of breeding systems on average hen egg weight. Meta-regression analysis was used to examine the effect of the following moderators, publication year, region, chicken age, and breed. The Cochran's Q test and I^2 statistic were performed for h heterogeneity across used studies. According to the obtained results, there was no significant difference between cage and free-range on average hen egg weight (standardized mean difference (SMD) = 0.08, I^2 = 89%, 95%CI 0.19-0.34). The free-range breeding system had heavier hen egg weight than deep litter (SMD = 0.54, I^2 = 88%, 95%CI 0.01-0.08). The findings also revealed that deep litter and free range had no significant difference in average hen egg weight (SMD = -0.05, I^2 = 87%, 95%CI -0.28-0.17). Meta-regression findings showed that the origin of the used articles, the age of the chickens, and the chicken breed were observed as the main reasons for heterogeneity. This meta-analysis revealed that a free-range breeding system increased the average hen egg weight.

Keywords: Breeding system, Cage, Deep litter, Free-range, Meta-analysis

INTRODUCTION

In commercial egg-laying farming enterprises, success depends on the total number and size of eggs produced (Ojedapo, 2013). According to Ahmad et al. (2019), to reduce the use of cage breeding systems for chicken welfare international regulations have been developed. Therefore, the free-range breeding system gained much attention (Rehman et al., 2017). All the breeding systems used for the egg production industry have their advantages and disadvantages (Samiullah et al., 2017). It is well known that egg quality primarily depends on genetic background, rearing system, and management of birds and it is therefore needed to compare many parameters between rearing systems (Ahammed and Ohh, 2013). Several studies have shown that breeding systems affect the egg quality traits of hens in cage and deep litter systems and that hens reared in cages produce heavier eggs (Ojedapo, 2013), while Dahloum et al. (2018) reported that deep litter produces heavier eggs. Although there are studies that discussed the effect of breeding systems on egg quality traits, to the best of the authors' knowledge no meta-analysis study has examined the effect of breeding systems on hen egg weight. To address this gap in knowledge, the current study aimed to provide evidence using a meta-analysis approach based on the influence of breeding systems (cage, deep litter, and free range) on the hen egg weight. The findings of the current review provide valuable insights that can assist poultry farmers in enhancing average egg weight through the

To cite this paper: Tyasi TL and Sako T (2024). Effects of Breeding Systems on Hen Egg Weight: A Meta-Analysis. *J. World Poult. Res.*, 14(2): 172-180. DOI: https://dx.doi.org/10.36380/jwpr.2024.18

optimal selection of breeding systems (cage, deep litter, and free range).

MATERIALS AND METHODS

Eligibility criteria

Identification of population, intervention, comparison, and outcomes (PICO) components of the research question as explained by Mattos and Ruellas (2015) was performed before conducting the study. The population was defined as "chicken", with an intervention of "rearing system" or "housing system" or "breeding system", a comparison of "cage and deep litter" or "cage and free-range" or "deep litter and free-range" and outcomes of "egg quality traits" or "egg performance" or "external egg quality traits". A preliminary search of the PICO components was conducted before deciding to conduct the meta-analysis.

Literature search

Google Scholar, PubMed, ScienceDirect, and Web of Science were used for searching the literature.

Inclusion criteria

The eligibility criteria for all acquired articles were defined to include studies that investigated the rearing system (housing or breeding system) along with aspects related to egg quality traits, egg performance, or external egg quality traits.

Exclusion criteria

Exclusion criteria were applied to remove duplicate records found across different databases and studies that did not assess the impact of cage, deep litter, and free-range breeding systems on egg quality traits.

Data extraction

The extracted data from the articles included the name of the first author, year of publication, country, species, chicken breed, and sample size.

Statistical analysis

R software version 4.3.1 (The R Foundation for Statistical Computing) using the meta package was used for analysis. The effects of different breeding systems (cage, deep litter, and free-range) on hen egg weight were examined using a random effects model. The Cochran-Q and I^2 statistics were used to test heterogeneity among studies. Forest plots were performed for overall evaluation.

RESULTS

Characterization of included studies

The studies included in the meta-analysis were 22 as indicated in Figure 1. The search findings indicated that one author published two articles in different years (Table 1; Samiullah et al., 2014; 2017). The majority of the studies (n = 13) included in the review focused on exotic chicken breeds, accounting for 59.09% of the total, followed by crossbreeds at 22.73%, indigenous chickens at 13.64%, and studies involving both indigenous chickens and cross breeds at 4.55% (Choudhuri et al., 2014). The chickens ranged from 26 to 78 weeks in terms of age. The sample size used ranged from a minimum of 40 to 4320 eggs. Breeding systems investigated in this study were cages, deep litter, and free-range.

Publication by country

The results indicated that 22 studies were published in different countries worldwide (Figure 2). The majority of the studies originated from Türkiye, with five studies (22.73%), followed by three studies each from India (13.64%) and Pakistan (13.64%). Two studies each were conducted in Poland (9.09%) and Australia (9.09%). Additionally, there was one study each from the Czech Republic (4.55%), Nigeria (4.55%), the Republic of Korea (4.55%), China (4.55%), Algeria (4.55%), Bulgaria (4.55%), and Spain (4.55%).

Publication by year

The findings indicated that all published studies were from 2000 to 2023 (Figure 3). According to the obtained results, According to results, four articles were published in 2009 (18.18%), three in 2018 (13.64%), and two each in 2013, 2014, 2017, and 2020 (each accounting for 9.09%).

The effect of cage and free-range systems on hen egg weight

A total of 13 experiments from 11 studies that compared cage and free-range breeding systems for egg weight were included in the meta-analysis, with cage data used as the experimental group and free-range data as the control group (Figure 4). The results demonstrated no significant difference in egg weight between the cage and free-range systems (Figure 4A). Meta-regression results indicated that the country of the article, the age of the chickens, and the chicken breed were the reasons for heterogeneity (Table 2). Figure 4B shows the publication biases.

The effect of deep litter and free-range on hen egg weight

A total of 13 experiments from 8 studies (Krawczyk and Gornowicz, 2010; Lewko and Gornowicz, 2011; Choudhuri et al., 2014; Samiullah et al., 2017; Ahmad et al., 2019; Popova et al., 2020; Champati et al., 2020; Nayak et al., 2020) were included in the meta-analysis assessing the effect of deep litter and free-range breeding systems on egg weight (Figure 5). The results indicated high heterogeneity, favoring the free-range system. A funnel plot (Figure 5B) was used to evaluate publication bias.

The effect of cage and deep litter on hen egg weight

A total of 15 experiments from 11 studies (Basmacioğlu and Ergül, 2005; Özbey and Esen, 2007; Lichovníková and Zeman, 2008; Roll et al., 2009; Lewko and Gornowicz, 2011; Ahammed and Ohh, 2013; Ojedapo, 2013; Samiullah et al., 2017; Dahloum et al., 2018; Ahmad et al., 2019; Nayak et al., 2020) were used for meta-analysis addressing the effect of cage and deep litter on egg weight (Figure 6). The results indicated no significant difference between the cage and deep litter (Figure 6A). A funnel plot (Figure 6B) was used to predict the publication biases of used articles.

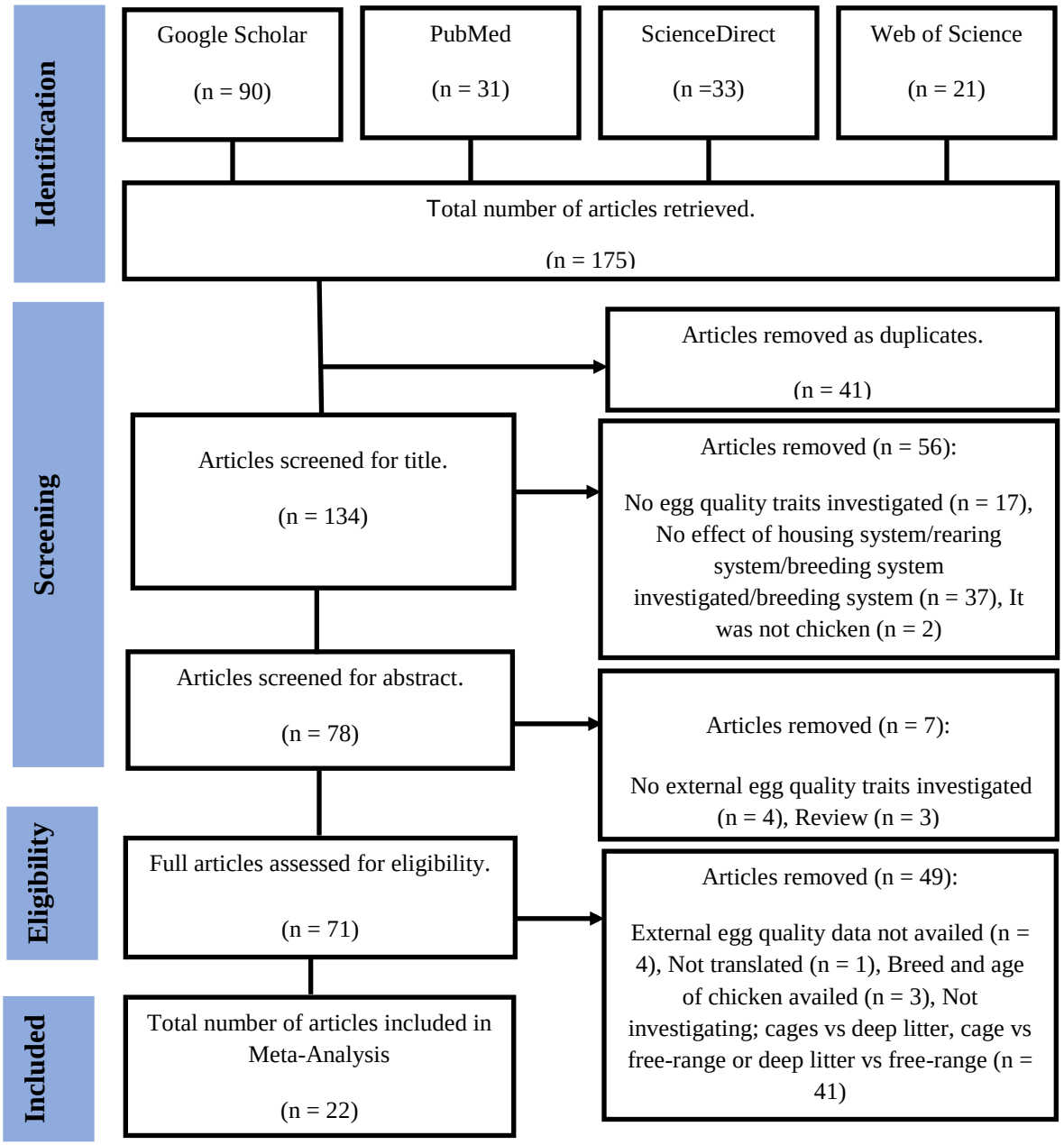


Figure 1. Preferred reporting items for systematic reviews and meta-analyses. The chart detailing the workflow of the selection process of 22 studies about the effects of breeding systems on hen egg weight

Table 1. Characterizations of the selected published articles included in the present study

Authors	Country	Breed	Age (weeks)	Sample size (eggs)	Breeding systems
Ahammed and Ohh (2013)	Republic of Korea	Brown laying pullet (Shaver 579)	30	120	Cage, deep litter
Ahmad et al. (2019)	Pakistan	Rhode Island Red × Naked Neck (RNN), Black Australorp × Naked Neck (BNN) and Naked Neck × Naked Neck (NN)	46	45	Cage, deep litter, free-range
Basmacioğlu and Ergül (2005)	Türkiye	white layers (Babcock-300) and brown layers (IsaBrown)	47	4 320	Cage, deep litter
Baykalir and Simsek (2018)	Türkiye	Bovans White	60	360	Cage, free-range
Champati et al. (2020)	India	Hansli males x Colour synthetic male line (CSML) females	40	50	Deep litter, free-range
Choudhuri et al. (2014)	India	Nicobari and crosses (Nicorock and Nishibari)	50	90	Deep litter, free-range
Dahloum et al. (2018)	Algeria	Adult indigenous Naked neck layers (White layers (WL); Brown layers (BrL) and Black layers (BL))	35	592	Cage, deep litter
Denli et al. (2016)	Türkiye	Lohmann Brown	50	60	Cage, free-range
Islam et al. (2021)	Pakistan	Rhode Island Red (RIR) × Fyoumi (F)	38	800	Cage, free-range
Krawczyk and Gornowicz (2010)	Poland	Polish hybrid layers Messa 45, originating from German “Meister Hybriden” breed	56	240	Deep litter, free-range
Lewko and Gornowicz (2011)	Poland	KA-62, KA-42, KA-68 and KA-48 hybrids derived from crossing strains K-66, K-44 (Rhode Island Red), A-88 and A-22 (Rhode Island White)	34	300	Cage, deep litter, free-range
Lichovníková and Zeman (2008)	Czech Republic	ISA Brown	66	336	Cage, deep litter
Nayak et al. (2020)	India	Vanaraja	26	60	Cage, deep litter, free-range
Ojedapo (2013)	Nigeria	Nera Brown	38	125	Cage, deep litter
Özbey and Esen (2007)	Türkiye	Partridge	38	60	Cage, deep litter
Petek et al. (2009)	Türkiye	Super Nick	36	640	Cage, free-range
Popova et al. (2020)	Bulgaria	Lohmann- Brown Classic	34	40	Deep litter, free-range
Rehman et al. (2017)	Pakistan	Aseel	30	72	Cage, free-range
Roll et al. (2009)	Spain	Isa Brown	78	780	Cage, deep litter
Samiullah et al. (2014)	Australia	Hy-Line Brown	75	540	Cage, free-range
Samiullah et al. (2017)	Australia	Hy-Line Brown	73	180	Cage, deep litter, free-range
Wang et al. (2009)	China	Blue-Shelled	50	120	Cage, free-range

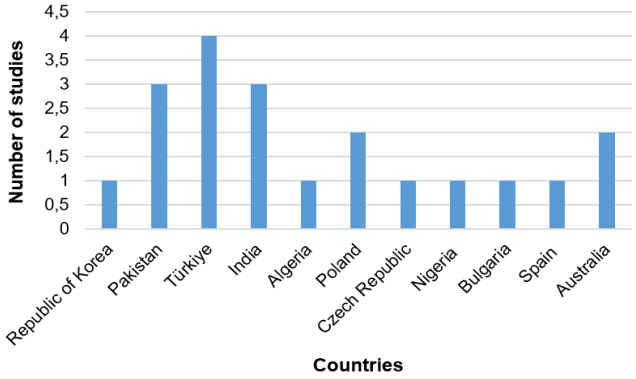


Figure 2. Countries included their published articles

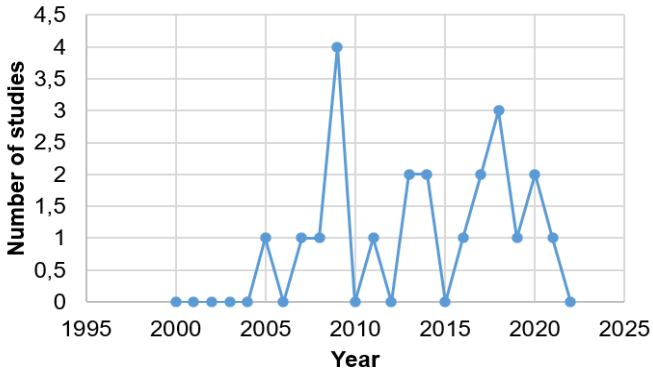


Figure 3. Years of included published studies

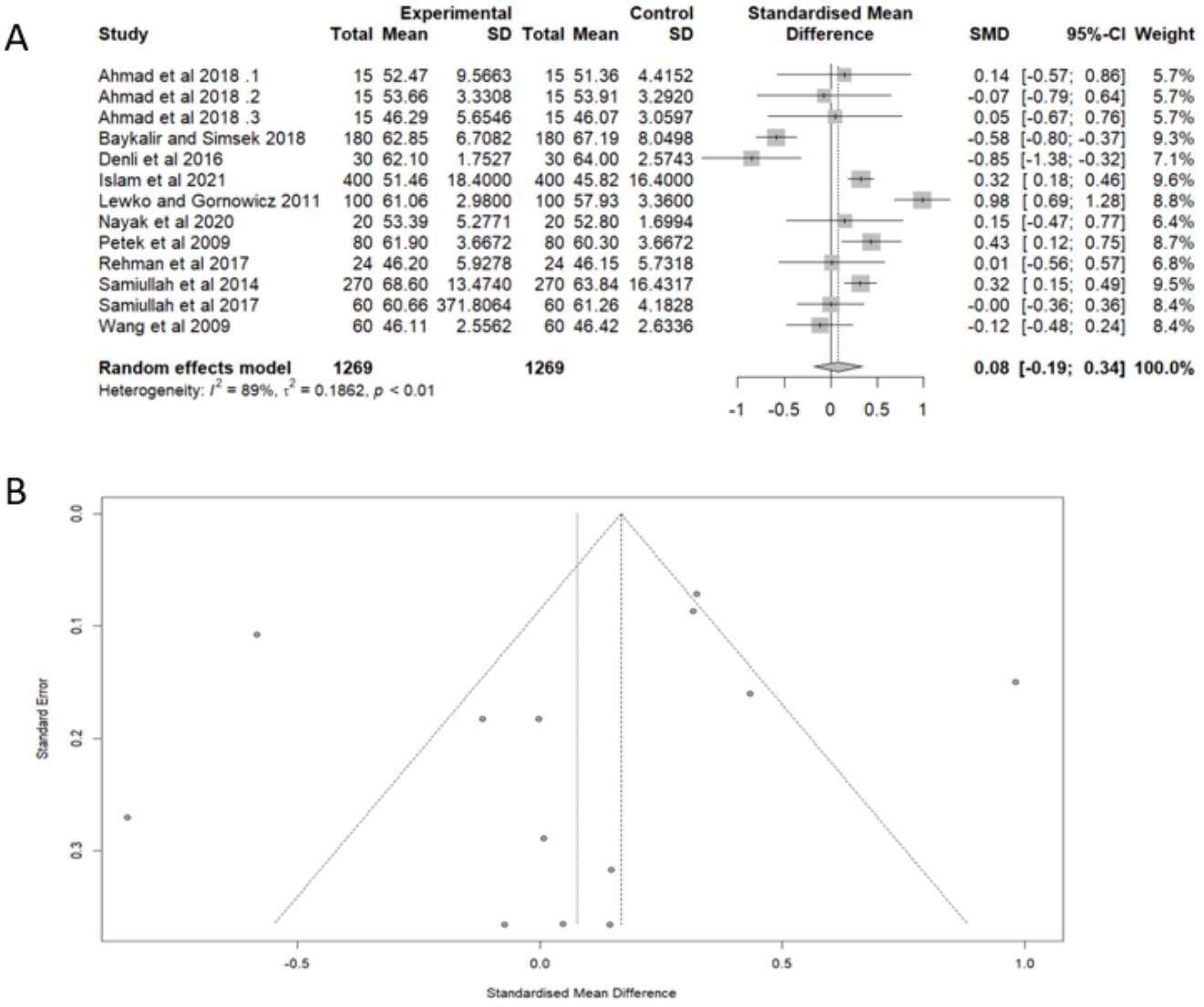


Figure 4. Effects of cage and free-range breeding systems on hen egg weight. **A**: Forest plot. **B**: Funnel plot

To cite this paper: Tyasi TL and Sako T (2024). Effects of Breeding Systems on Hen Egg Weight: A Meta-Analysis. *J. World Poult. Res.*, 14(2): 172-180. DOI: https://dx.doi.org/10.36380/jwpr.2024.18

Table 2. Effect of the year of publication, the origin of the article, chicken age, and chicken breed on included studies on the effect of breeding systems on hen egg weight

Factor	Estimate	Significance
Intercept	- 0.0563	***
Year of publication	- 0.1827	ns
Origin region of publication	0.4460	***
Age of the chicken	- 0.3871	***
Chicken breed	0.4082	***

Significant *** = 0.001, ns = not significant.

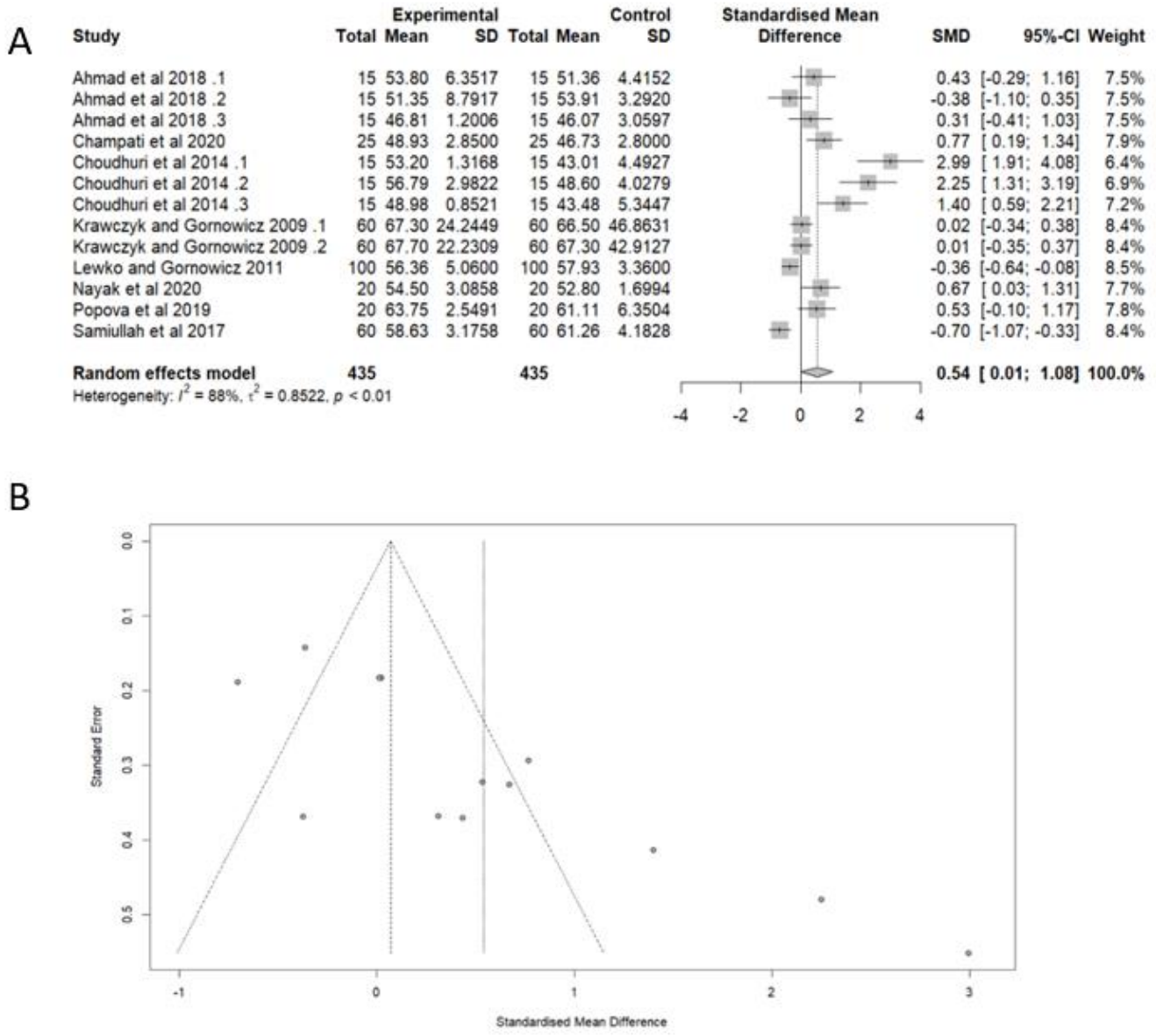


Figure 5. Effects of deep litter and free-range breeding systems on hen egg weight. **A**: Forest plot. **B**: Funnel plot

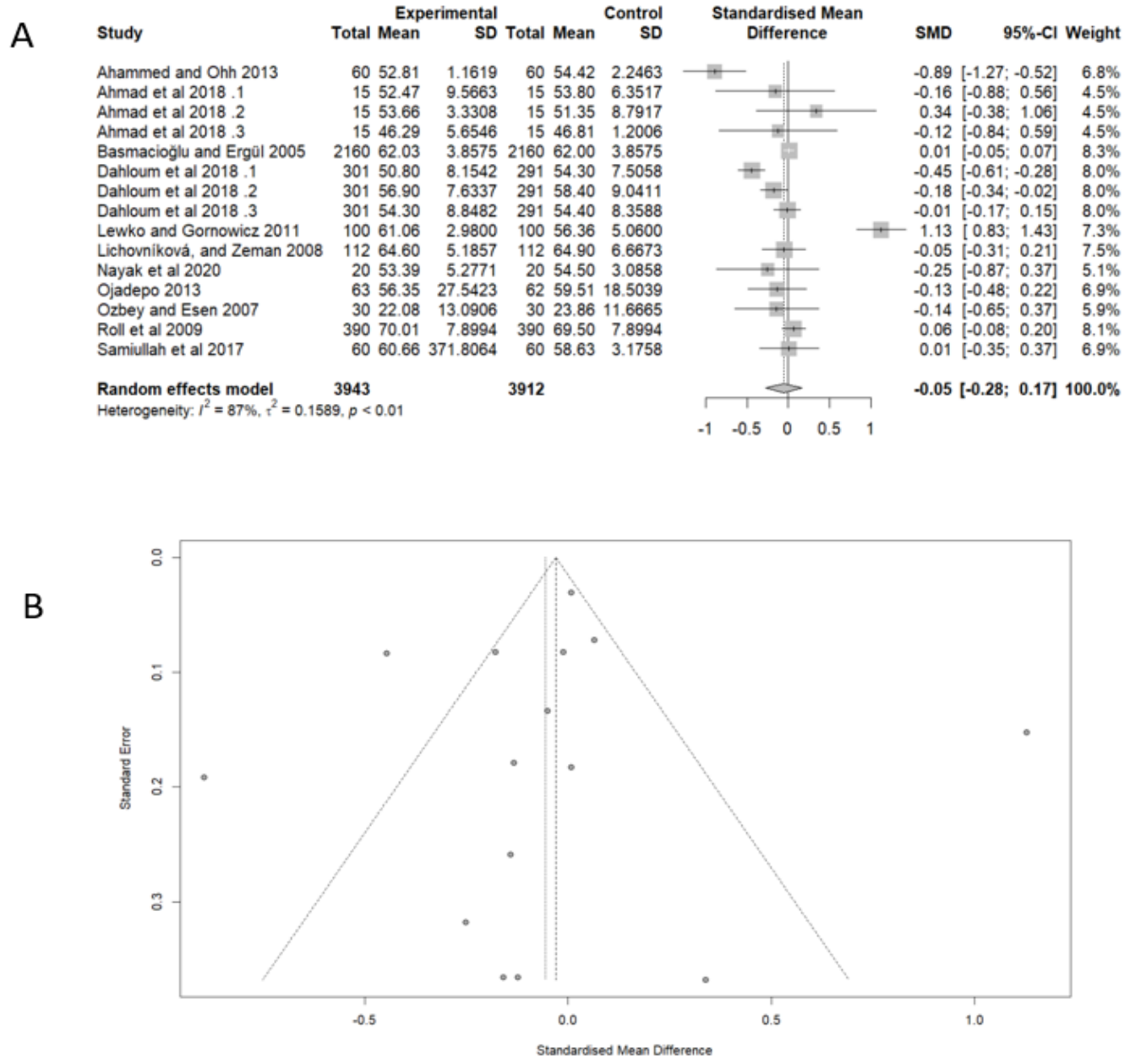


Figure 6. Effects of cage and deep litter breeding systems on hen egg weight. **A**: Forest plot. **B**: Funnel plot

DISCUSSION

Egg weight is one of the vital traits in the egg production industry (Nayak et al., 2020). The study was conducted to determine the effect of breeding systems, such as cage, deep litter, and free-range on hen egg weight using a meta-analysis approach. The results were obtained based on 22 published studies included in the meta-analysis, with the majority of them from Türkiye, Pakistan, and India. The reason might be related to the continent's call for studying the effect of the breeding systems on hen egg weight since Asia is one of the leading continents in egg production (Nayak et al., 2020). The results revealed that there was no significant difference in egg weight between chickens kept in cages and those kept in deep litter and free-range breeding systems. However, a significant difference was found when comparing deep litter and free-range systems, with free-range systems producing heavier eggs. This difference might be because free-range chickens can move freely to scavenge, supplementing their diet beyond the

provided feed. The random effects model applied for comparing the hen egg weight of chickens kept in deep litter and free-range indicated a high heterogeneity and significant difference in hen egg weight. More than 80% of local chickens are kept under the free-range breeding system in rural areas (Msoffe, 2002). According to Nonga et al. (2010), egg weight was largely affected by feeding, age, environmental factors, chicken ecotype, live body weight, and genetic makeup. Ahmad et al. (2019) emphasize that breeding systems influence egg quality traits. Egg weight from the free-range breeding system was influenced by both nutritional factors and ambient microclimate (Sekeroglu et al., 2008). As far as the authors are concerned, this meta-analysis study was the first to compare breeding systems (cage, deep litter, and free range) on hen egg weight. Hence, there were no similar studies for the comparison of the findings. The current study indicated that producers in the egg industry focusing on improving egg weight should look more into production using the free-range breeding system as it produces heavier eggs than cages and deep litter systems. As a benefit of the present findings, this meta-analysis brings conclusive information about the effect of the breeding systems on hen egg weight and selecting the best breeding system when coming to egg production for producing heavier eggs. However, there were some limitations, such as the data synthesis was focused on hen egg weight and outcomes may not be generalized to other egg quality traits, there were disparities in the number of hens, number of eggs, duration of the experiment, and finally the significant heterogeneity was found in the endpoints that may have been caused by different breeds.

CONCLUSION

The results indicated nonsignificant differences in hen egg weight between the cage and deep litter systems and between the cage and free-range breeding systems. However, there were significant differences in average hen egg weight between the deep litter and free-range breeding systems. Specifically, the free-range breeding systems produced heavier eggs compared to both the cage and deep litter systems.

DECLARATIONS

Acknowledgments

The authors acknowledge the University of Limpopo for their financial support.

Authors' contributions

The authors have contributed equally in planning, analyzing, and writing of this review article.

Competing interests

The authors certify that there is no conflict of interest.

Ethical considerations

The ethical issues, such as double publication and submission, data fabrication, plagiarism, redundancy, misconduct and consent to publish have been checked by both authors before publication in this journal.

Availability of data and materials

Data is available by request from the corresponding author.

Funding

The authors acknowledge the University of Limpopo for their financial support.

REFERENCES

Ahammed M and Ohh SJ (2013). Effect of housing systems-barn vs cage on the first phase egg production and egg quality traits of laying pullet. Korean Journal of Poultry Science, 40(1): 67-73. DOI: http://www.doi.org/10.5536/KJPS.2013.40.1.067

Ahmad S, Mahmud A, Hussain J, and Javed K (2019). Productive performance, egg characteristics and hatching traits of three chicken genotypes under free-range, semi-intensive, and intensive housing systems. Brazilian Journal of Poultry Science, 21(2): 1-9. DOI: http://www.doi.org/10.1590/1806-9061-2018-0935

Basmacioğlu H and Ergül M (2005). Research on the factors affecting cholesterol content and some other characteristics of eggs in laying hens the effects of genotype and rearing system. Turkish Journal of Veterinary & Animal Sciences, 29(1): 157-164. Available at: https://journals.tubitak.gov.tr/veterinary/vol29/iss1/26/

Baykalir Y and Simsek UG (2018). Impact of different rearing systems and age on Bovans White layer's performance, egg quality traits and synthesis of heat shock protein 70 kDa. Annals of Animal Science, 18(4): 1045-1060. DOI: https://www.doi.org/10.2478/aoas-2018-0027

Champati A, Samal L, Behura NC, Muduli S, and Popalghat HK (2020). Laying performance and egg quality traits of Hansli x CSML birds under different rearing systems. Journal of Entomology and Zoology Studies, 8(4): 37-39. Available at: https://www.entomoljournal.com/archives/2020/vol8issue4/PartA/8-3-356-573.pdf

Choudhuri NC, Paul G, Kundu A, Kundu MS, De AK, and Ram N (2014). Evaluation of egg quality traits of endangered Nicobari fowl and its crosses under intensive and backyard system of Andaman and Nicobar Islands, India. Veterinary World, 7(9): 693-697. DOI: https://www.doi.org/10.14202/vetworld.2014.693-697

Dahloum L, Yakubu A, and Halbouche M (2018). Effects of housing system and plumage colour on egg quality characteristics of indigenous naked-neck chickens. Livestock Research for Rural Development, 30(12): 206. Available at: http://www.lrrd.org/lrrd30/12/abdul30206.html

Denli M, Bukun B, and Tutkun M (2016). Comparative performance and egg quality of laying hens in enriched cages and free-range systems. Scientific Papers Series D Animal Science, 59: 29-32.

Available at: https://animalsciencejournal.usamv.ro/pdf/2016/Art5.pdf

Islam Z, Sultan A, Khan S, Alhidary IA, Abdelrahman MM, and Khan RU (2021). Impact of varying housing systems on egg quality characteristics, fatty acid profile, and cholesterol content of Rhode Island Red× Fyoumi laying hens. Tropical Animal Health and Production, 53: 1-7. DOI: https://www.doi.org/10.1007/s11250-021-02913-x

Krawczyk J and Gornowicz E (2010). Quality of eggs from hens kept in two different free-range systems in comparison with a barn system. Archiv für Geflügelkunde, 74(3): 151-157. Available at: https://www.european-poultry-science.com/artikel.dll/m09-30mk_gqzdcojxgq4q.pdf

Lewko L and Gornowicz E (2011). Effect of housing system on egg quality in laying hens. Annals of Animal Science, 11(4): 607-616. DOI: https://www.doi.org/10.2478/v10220-011-0012-0

Lichovníková M and Zeman AL (2008). Effect of housing system on the calcium requirement of laying hens and on eggshell quality. Czech Journal of Animal Science, 53(4): 162-168. DOI: https://www.doi.org/10.17221/375-CJAS

Mattos CT and Ruellas ACO (2015). Systematic review and metaanalysis: What are the implications in the clinical practice?. Dental Press Journal of Orthodontics, 20(1): 17-19. DOI: http://www.doi.org/10.1590/2176-9451.20.1.017-019.ebo

Msoffe PLM, Mtambo MMA, Minga UM, Gwakisa PS, Mdegela RH, and Olsen JE (2002). Productivity of natural disease resistance potential of free-ranging local chicken ecotypes in Tanzania. Livestock Research for Rural Development, 14(3): 92-103 Available at: http://lrrd.cipav.org.co/lrrd14/3/msof143.htm

Nayak Y, Samantaray BP, Biswal L, and Nayak Y (2020). Effect of rearing system in production performance and egg quality characteristics of Vanaraja layers (gallus gallus domesticus). International Journal of Modern Agriculture, 9(4): 332-341. Available at: https://modern-journals.com/index.php/ijma/article/view/219

Nonga HE, Kajuna FF, Ngowi HA, and Karimuribo ED (2010). Physical egg quality characteristics of free-range local chickens in Morogoro municipality, Tanzania. Livestock Research for Rural Development, 22(12): 217. Available at: http://www.lrrd.org/lrrd22/12/nong22218.htm

Ojedapo LO (2013). Effect of two housing systems (cages vs deep litters) on external and internal egg characteristics of commercial laying birds reared in derived savanna zone of Nigeria. Transnational Journal of Science and Technology, 3(7): 1857-8047. Available at: http://www.tjournal.org/tjst_july_2013/03.pdf?i=1

Özbey O and Esen F (2007). The effects of different breeding systems on egg productivity and egg quality characteristics of rock partridges. Poultry Science, 86(4): 782-785. DOI: https://www.doi.org/10.1093/ps/86.4.782

Petek M, Alpay F, Cengiz ŞŞ, and Çibik R (2009). Effects of housing system and age on early stage egg production and quality in commercial laying hens. Kafkas Üniversitesi Veteriner Fakultesi Dergisi, 15(1): 57-62. Available at: http://hdl.handle.net/11452/24153

Popova T, Petkov E, Ayasan T, and Ignatova M (2020). Quality of eggs from layers reared under alternative and conventional system. Brazilian Journal of Poultry Science, 22(1): 1-8. DOI: http://www.doi.org/10.1590/1806-9061-2019-1172

Rehman MS, Mahmud A, Mehmood S, Pasha TN, Hussain J, and Khan M (2017). Comparative evaluation of egg morphometry and quality in Aseel hens under different rearing systems. Journal of Applied Poultry Research, 26(3): 401-409. DOI: http://www.doi.org/10.3382/japr/pfx008

Roll VFB, Briz RC, and Levrino GAM (2009). Floor versus cage rearing: effects on production, egg quality and physical condition of laying hens housed in furnished cages. Ciência Rural, 39(5): 1527-1532. DOI: https://www.doi.org/10.1590/S0103-84782009000500034

Samiullah S, Omar AS, Roberts J, and Chousalkar K (2017). Effect of production system and flock age on eggshell and egg internal quality measurements. Poultry Science, 96(1): 246-258. DOI: http://www.doi.org/10.3382/ps/pew289

Samiullah S, Roberts JR, and Chousalkar KK (2014). Effect of production system and flock age on egg quality and total bacterial load in commercial laying hens. Journal of Applied Poultry Research, 23(1): 59-70. DOI: http://www.doi.org/10.3382/japr.2013-00805

Sekeroglu A, Sarica M, Demir E, Ulutas Z, Tilki M, and Saatci M (2008). The effects of housing system and storage length on the quality of eggs produced by two lines of laying hens. Archiv fur Geflugelkunde, 72(3): 106-109. Available at: https://www.european-poultry-science.com/artikel.dll/m07-21mk_gqzdcobtheyq.pdf

Wang XL, Zheng JX, Ning ZH, Qu LJ, Xu GY, and Yang N (2009). Laying performance and egg quality of blue-shelled layers as affected by different housing systems. Poultry Science, 88(7): 1485-1492. DOI: http://www.doi.org/10.3382/ps.2008-00417

JWPR
Journal of World's Poultry Research
2024, Scienceline Publication
J. World Poult. Res. 14(2): 181-195, 2024
Review Paper
DOI: https://dx.doi.org/10.36380/jwpr.2024.19
PII: S2322455X2400019-14
ISSN: 2322-455X

Landmarks in Quail Coccidiosis Research with Special Scrutiny to the Available Egyptian Literature: A Review

Reham M. El Bakrey, Sarah S. Helal, Ahmed A. El Kholy, and Amal A. M. Eid*

Department of Avian and Rabbit Medicine, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Sharkia 44511, Egypt

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

Received: March 23, 2024, Revised: April 25, 2024, Accepted: May 26, 2024, Published: June 30, 2024

ABSTRACT

Quails are an important alternative to chicken production for protein sources, offering many advantages over other poultry species. However, raising quail faces certain challenges, such as a shortage of specified hatcheries and the lack of local markets for quail eggs and meat, particularly in Egypt. In addition, there is less interest in quail's medication and vaccine production. A significant disease affecting the health and productivity of quails is coccidiosis, which is associated with poor feed conversion ratio, lower growth performance, heightened mortality, and high cost of vaccination and treatment. Attention to quail coccidiosis and its clinical forms, diagnosis, morphological characterization, control, and prevention is very critical for improving quail meat and egg production. This review compiles scientific data on quail coccidiosis, with a focus on literature from Egypt, for classification, data analysis, and processing.

Keywords: Anticoccidial, Coccidiosis, *Eimeria*, Egyptian, Morphology characterization, Quail

INTRODUCTION

Coccidiosis is a protozoan disease caused by coccidia of the genus *Eimeria* (Kemp et al., 2013). Over 1800 different species of *Eimeria* invade and infect the digestive tracts of mammals and birds, either wild or domesticated (Haug et al., 2008). When *Eimeria* species target the intestinal tract, they induce a potent inflammatory response and tissue damage, with increased susceptibility to other disease agents, and mortalities in severe cases (Duszynski, 2011). *Eimeria* species have a complex life cycle that involves both intra- and extracellular stages and is completed in a single host due to their high host specificity, in particular *Eimeria (E.) tsunodai*, *E. uzura*, and *E. bateri* in quails (Lu et al., 2021). Each *Eimeria* species replicates to form oocysts in the intestine of the host, which are then released into the environment via feces. Birds ingest sporulated oocysts, which are then transported to the intestine to begin their life cycle (Chapman, 2014).

Several studies have focused on the pathogenesis, pathogenicity, control, and prevention of coccidia in domesticated poultry due to the significant economic losses associated with both subclinical and clinical infections (Nawarathne et al., 2021). Quails, in particular, are considered a viable alternative in poultry production because of their high potential for meat and egg production. Quail farming is a rapidly developing sector worldwide (Lukanov, 2019). In Egypt, with a growing population and an increasing demand for animal proteins, quail breeding has gained attention as a means to boost and expand the production of meat protein (Arafat and Abbas, 2018; Ramadan et al., 2021). Quails are susceptible to several diseases, with coccidiosis being the most significant. This dangerous parasitic disease poses a major threat to the quail industry (Umar et al., 2014). On the other hand, there is limited information available about quail coccidiosis, including its distinct phenotypic and genetic characteristics (Arafat and Abbas, 2018).

This article could provide the existing studies on coccidiosis in quails, emphasizing the disease's distinguishing characteristics and key features. The review focuses particularly on data and results from available literature, with special attention to studies conducted in Egypt.

MATERIALS AND METHODS

In the current review, the available literature of previous international and Egyptian studies (Scopus, PubMed, and Google Scholar) concerned with quail

coccidiosis were carefully reviewed and studied. The related literature was classified and submitted for data breakdown and dispensation. A total of 43 studies were reviewed, including 33 international and 10 Egyptian studies. The collected data encompassed the main characteristics of quail coccidiosis, such as the types of *Eimeria*, clinical findings, gross and histopathological features, diagnostic procedures, and control approaches. The findings from these studies, particularly those from Egypt, were presented in tables and figures, and conclusions were drawn to provide recommendations for stakeholders in the quail industry.

Quails and its products

Quail is a medium-sized bird that belongs to various genera of the family *Phasianidae* (Abd El-Ghany, 2019). Quail production is a short-generation industry with the potential to meet the nutritional and economic needs of developing countries (Ojo et al., 2014). Quail breeding offers numerous advantages, including early sexual maturity, low feed consumption (20-25 g/adult bird/day), high production rate with 300 eggs/year, low mortality rate, highly nutritious meat and eggs, and short generation time (3-4 generations annually, Faitarone et al, 2005; Bashtar et al., 2010; Jatoi et al., 2013). Additionally, they are distinguished by their low startup expenses and small rearing areas (200-250 and 150-200 cm^2 in litter and cage systems, respectively), which suggests a unique trend in poultry production (Shemshadi et al., 2014; Hassan et al., 2017; Yambayamba and Chileshe, 2019).

Quail eggs are inexpensive sources of protein, particularly in developing countries. They are also rich in iron, phosphorus, riboflavin, pantothenic acid, folate, vitamin B12, and selenium (Kalsum et al., 2012). Quail's meat is a healthier choice for people who are health-conscious because it has less fat and calories while offering more moisture and minerals than broiler meat (Wahab, 2002; Tunsaringkarn et al., 2013).

Among the many quail breeds under domestication, the Japanese quail (*Coturnix japonica*) and the Bobwhite quail (*Colinus virginianus*) are the most common species reared in Egypt (Arya et al., 2018; Abd El-Ghany, 2019). Quails have been domesticated in Egypt since ancient times, alongside chickens, ducks, pigeons, and other birds. Quail was a favored food of the ancient Egyptians, as depicted on the walls of many Egyptian temples (Halim et al., 2022). Globally, the breeding of Japanese quails has flourished in aviculture due to the increasing demand for meat and eggs (Berto et al., 2011). The Japanese quail is a migratory bird that inhabits East Asia (Faizullah et al., 2021). Egypt is one of the most significant countries for migrating birds, with at least 300 different species traveling there from all over the world each year (Mazyad et al., 1999). The migratory quail, also called the common quail, travels from Europe to Egypt throughout the autumn (Benskin et al., 2009). The Egyptian northern coast, from Matrouh in the west to the Saini peninsula in the east, as well as the cities of Edko and Rashid, which are districts of the Elbehera governorate near the Mediterranean Sea, is a terminus for many migratory birds, including quails (Waheeb et al., 2022).

Etiology of quail coccidiosis

Coccidiosis is typically a hidden disease in quails that lowers production and growth rate, and increases mortality (Simiyoon et al., 2018). The coccidial infection causes an imbalance in the gut microbiota and impairs digestion and absorption, increasing the chance of contracting another bacterial infection. When more pathogenic bacteria proliferate, the functions of the intestinal mucosal barrier are compromised, and the immune system becomes less capable of recognizing and attacking coccidia. As a result, the infection of coccidia becomes more severe (Lu et al., 2021).

Within the protozoan subgroup of the phylum Apicomplexa, *coccidia* comprises a diverse range of unicellular parasites. The coccidia belongs to the family *Eimeridae*, genus *Eimeria (E.)*, that is unique to a single host species or a group of closely related hosts (Müller and Hemphill, 2013).

Numerous *Eimeria* species have been isolated from various quail species. These include *E. tsunodai*, *E. uzura*, *E. bateri*, and *E. fluminensis* (Norton and Peirce, 1971; Teixeira and Lopes, 2000; 2002; Teixeira et al., 2004; Berto et al., 2013; Al-Zarkoushi and Al-Zubaidi, 2021), as well as *E. taldykurganica* (Svanbaev and Utebaeva, 1973) from Japanese quails. *E. lophortygis* and *E. okanaganensis* were identified in California quails (Liburd and Mahrt, 1970). From mountain quail, *E. crusti*, *E. oreortygis*, and *E. isospora* were detected (Duszynski and Gutierrez, 1981), while *E. conturnicis* and *E. bateri* were identified in grey quail (Chakravarty and Kar, 1947). Moreover, *E. colini* (Fisher and Kelley, 1977), *E. lettyae*, and *E. dispersa* were described from bobwhite quail (Ruff, 1985), and also *E. tahamensis* was described from Arabian quails (Amoudi, 1987; Berto et al., 2013).

In Egypt, *E. tsunodai*, *E. uzura*, *E. bateri* (El-Morsy et al., 2016; Arafat and Abbas, 2018; Hassan et al., 2020; Ramadan et al., 2021; Waheeb et al., 2022), *E. minima* (Arafat and Abbas, 2018), *E. coturniria* (Otify, 1988), as well as *E. colini* and *E. bahli* (Ramadan et al., 2021), were recognized in domesticated Japanese quails.

In migratory quails (*Coturnix coturnix japonica*) trapped during migration season from the El-Behera (Edko and Rashid districts) and Damietta provinces of Egypt, *E. tsunodai*, *E. uzura*, and *E. bateri* were identified (Basiouny et al., 2017; Waheeb et al., 2022), as well as *E. colini* and *E. bahli* (Basiouny et al., 2017).

Table 1 illustrates the available Egyptian literature on the morphological and morphometric characteristics of the oocysts and/or sporocysts of the several *Eimeria* species in quails.

Table 1. The morphological and morphometric features of oocysts and sporocysts of the different *Eimeria* species in quails via the available Egyptian literature

Eimeria species	Quail Species (Common name)	Feature						Reference
		Shape of oocyst	Range of size (L×W) µm	Polar granule	Micropyle	Shape Sporocyst	Shape Stieda body	
E. bateri	*C. Coturnix japonica* (Japanese quail)/ domesticated or migratory	Subspherical or ovoid to ellipsoidal	20-28 × 13-20	+	-	Pear or ovoid shape	Nipple-like	Basiouny et al. (2017); Arafat and Abbas (2018); Ramadan et al. (2021); Waheeb et al. (2022);
E. uzura	*C. Coturnix japonica* (Japanese quail)/ domesticated or migratory	Ovoid to ellipsoidal	18-26 × 13.4-19	+	-	Fusiform or Ovoid to elongate	Crescent or half-moon or a piriform or knob-like	
E. tsunodai	*C. Coturnix japonica* (Japanese quail)/ domesticated or migratory	Subspherical to oval or spherical to ellipsoidal	15-24 × 14-18	+	+/-	Ovoid	Pyriform or nipple-like to triangular	
E. bahli	*C. Coturnix japonica* (Japanese quail)/ domesticated or migratory	Spherical to subspherical	16.7-17.5 × 16.8-17.6	-	+	Oval	Present	Basiouny et al. (2017); Ramadan et al. (2021)
E. colini	*C. Coturnix japonica* (Japanese quail)/ domesticated or migratory	Oval	24.15-24.2 × 20.4-20.6	+	-	Curved fusiform	Present	
E. minima	*C. Coturnix japonica* (Japanese quail)/ domesticated	Spherical to subspherical	15-17 × 15-16	+	-	Ovoid	Nipple-like	Arafat and Abbas (2018)

(L) Length, (W) Width, (*E*) *Eimeria*, (+) present, (-) absent, E: *Eimeria*

To cite this paper: ElBakrey RM, Helal SS, ElKholy AA, and Eid AAM (2024). Landmarks in Quail Coccidiosis Research with Special Scrutiny to the Available Egyptian Literature: A Review. *J. World Poult. Res.*, 14(2): 181-195. DOI: https://dx.doi.org/10.36380/jwpr.2024.19

Life cycle

The life cycle of coccidia mainly consists of exogenous and endogenous stages (Norton and Chard, 1983). During the exogenous phase, the host excretes the unsporulated oocysts, which then undergo sporulation in response to environmental conditions, such as temperature, oxygen, and moisture. The sporulated oocyst contains sporocysts, each of which entails sporozoites. After the host ingests the sporulated oocysts through contaminated food and water, the endogenous stage begins inside the host, which involves asexual (schizogony) and sexual (gametogony) reproduction (Dalloul and Lillehoj, 2005; Gilbert et al., 2011; Quiroz-Castañeda and Dantán-González, 2015). During this stage, the sporulated oocysts are exposed to digestive enzymes, and excystation of oocysts occurs in the gizzard. The sporozoites are released then invade the epithelial cells, and develop into trophozoites.

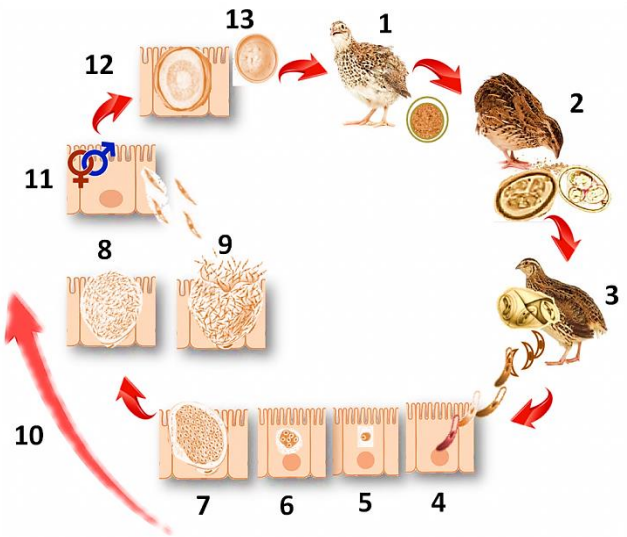


Figure 1. The life cycle of *Eimeria* in quails. 1: Shedding the mature unsporulated oocyst from quail. 2: Quail ingest the sporulated oocyst of *Eimeria bateri* and *Eimeria tsunodai*. 3: Releasing the sporozoites. 4: Invading the sporozoites into the epithelial cells. 5: Trophozoites. Both 6 and 7: Immature schizont. 8: Mature schizont. 9: Ruptured schizont and releasing merozoites. 10: Several asexual generations (schizogony). 11: Performing macrogametes (female) and microgametes (male) and occurring fertilization. 12: Developing oocysts. 13: Releasing the oocyst from the epithelial cells (modified from Conway and McKenzie, 2007)

The schizont begins replicating asexually, producing thousands of first-generation merozoites from each schizont.

Once the schizogony cycle is completed, the merozoites infect newly created epithelial cells in the intestinal lumen after the host cells are destroyed. Asexual reproduction occurs over several generations. Following that, the parasite replicates sexually and produces both macrogametes and microgametes. After macrogametes and microgametes fertilize each other to create zygotes, the zygote grows into an oocyst, which is then released into the environment along with fecal droppings (Ferguson et al., 2003; Shirley et al., 2005; Quiroz-Castañeda and Dantán-González, 2015). In this study, a diagram is designated by the authors using some individual parts from Conway and McKenzie (2007) to illustrate the life cycle of quail coccidiosis (Figure 1).

Clinical signs and gross pathological lesions

Several studies report the clinical findings in the quails infected with coccidia, and the Japanese quail is one of the most studied species. Under field conditions, mixed *Eimeria* species infections in quails are more common (Zoroaster et al., 2024). The most common clinical signs detected in the naturally infected quail include a lack of appetite, depression, anemia, emaciation, ruffled feathers, uncoordinated movements, diarrhea sometimes mixed with blood, and loss of weight, in addition to decreased egg production in laying quails (Teixeira et al., 2004; Simiyoon et al., 2018). These signs were more severe in young quails than in adults, which were more susceptible to coccidiosis infection (Teixeira et al., 2004). The pathological lesions vary depending on the type and location of *Eimeria*. According to Umar et al. (2014), cecal ballooning without any bloody exudate in the lumen is the primary pathological lesion in Japanese quails with a mixed *Eimeria* spp. infection. Two species of coccidia, *E. tsunodai* and *E. bateri* were shown to exhibit inflammatory changes in the cecum during post-mortem examination. These changes include dilated intestinal lumen, bloody intestinal contents, and mucosal lesions in Japanese quails (Sokół et al., 2015). The same cecal lesions were observed by Anbarasi et al. (2016) and Simiyoon et al. (2018).

In Egypt, abnormal intestines filled with bloody fecal material, as well as thickening of the intestinal mucosa with hemorrhage, were recorded in affected domesticated and migratory quails with a mixed infection of *E. bateri*, *E. uzura*, and *E. tsunodai* (Waheeb et al., 2022). The infection rates of various *Eimeria* species found in naturally infected domesticated or migratory quails in the Egyptian field are shown in Figures 2 and 3.

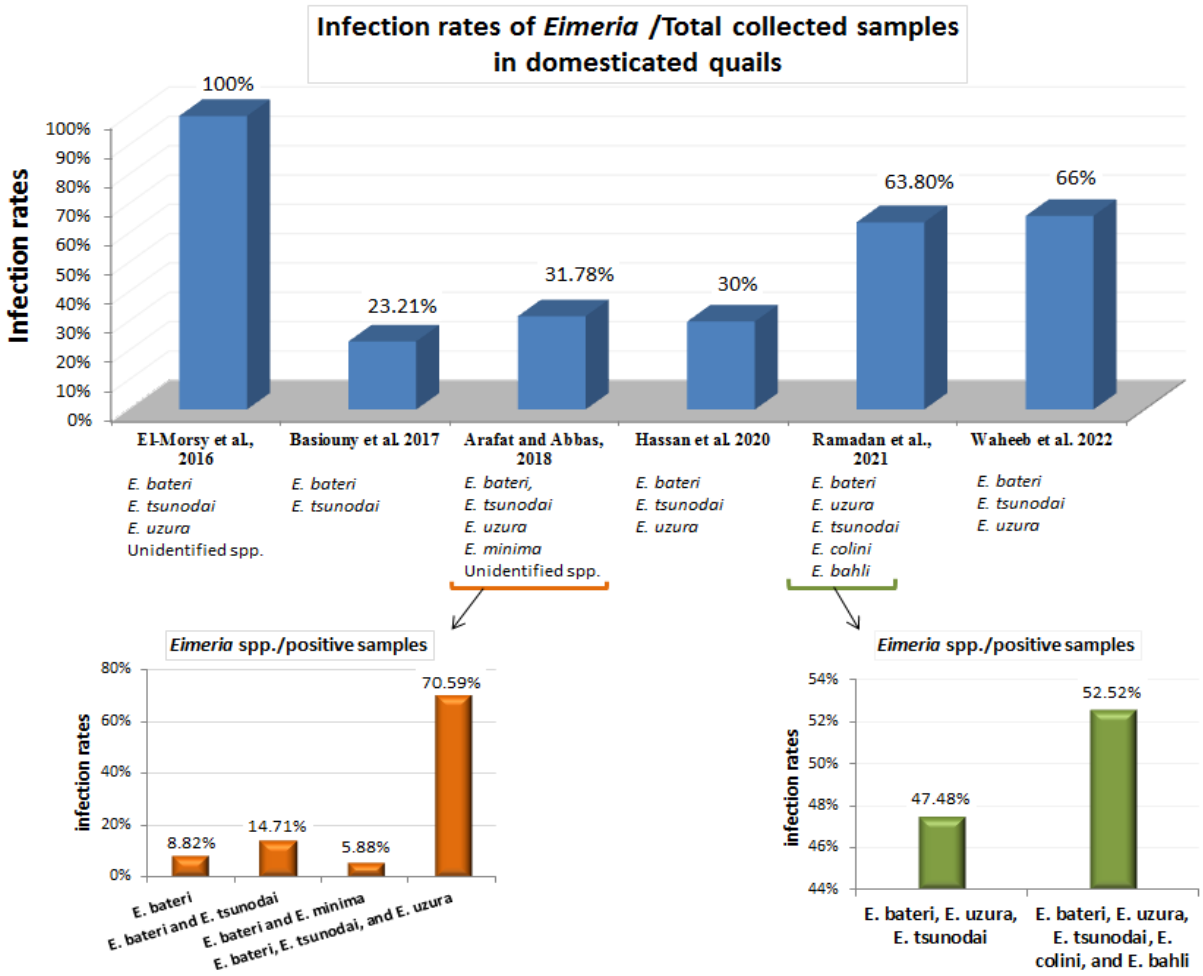


Figure 2. The infection rates of *Eimeria* species in naturally infected domesticate quail farms in Egypt. **Upper panel:** Infection rate (%) of *Eimeria* infection to the total collected samples either investigated individual quails or farms); **Lower panel:** Infection rate (%) of different *Eimeria* species to the positive samples

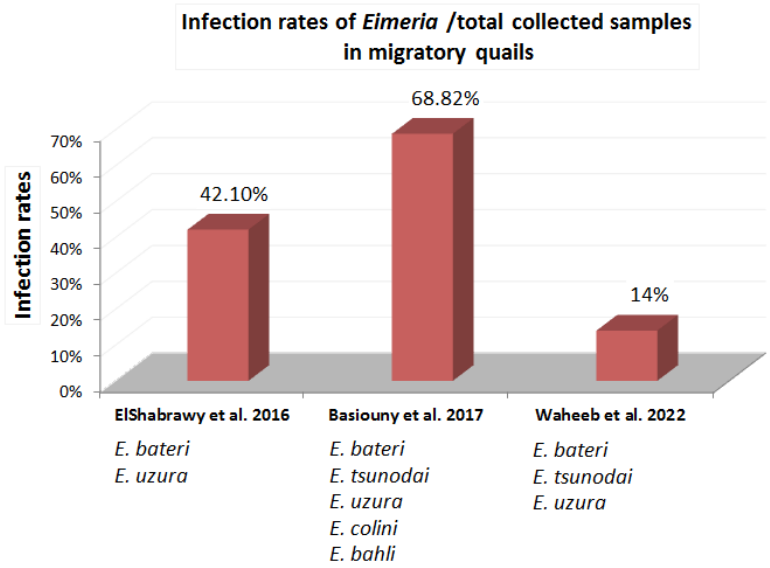


Figure 3. The infection rates of different *Eimeria* spp. in naturally infected migratory quails in Egypt

Regarding the experimental studies conducted on certain *Eimeria* species, anorexia, mild loss of weight, and softening of feces have been detected in the young Japanese quails experimentally infected with *E. bateri* (Norton and Pierce, 1971). Tsunoda and Muraki (1971) reported low pathogenicity of *E. uzura* in Japanese quails experimentally infected with 1×10^5 oocysts, and diarrhea and anemia were observed with no mortality.

Ruff and Wilkins (1987) investigated the effect of various doses of *E. lettyae* on bobwhite quails of different ages. They found that in 5-day-old bobwhites, a dose of 5×10^5 oocysts led to mortality rates ranging from 25% to 43%, while in 18-day-old bobwhites, there were no mortalities observed. In 5-day-old and 18-day-old bobwhites, a dose of 1×10^6 oocysts resulted in mortality rates of up to 100% and 83%, respectively. Body weight gain was significantly reduced in 5- and 18-day-old bobwhites infected with 1×10^5 and 5×10^5 oocysts or greater. Bobwhite inoculated with 5×10^5 oocysts or more exhibited typical signs of coccidiosis, including listlessness, droopiness, and anorexia with watery intestinal contents that were sometimes noticed. However, *E. lettyae* infection in mature bobwhite quails did not result in mortality; rather, it led to reduced egg production and fertility.

Under the investigations conducted in Egyptian studies, Arafa and Nasef (2004) recorded bloody diarrhea, low weight gain, and a mortality rate of 24% in infected Japanese quails with coccidia. El-Morsy et al. (2016) detected ruffled feathers, depression, decreased appetite, emaciated breast muscle, and bloody diarrhea in the Japanese quails experimentally infected with 4.1×10^4 oocysts of *E. tsunodai*. Additionally, severely enlarged and thickened mucosa of two ceca, a bloody cecal core, and ballooning were the most prominent lesions. On the other hand, Arafat and Abbas (2018) studied the pathogenicity of *E. bateri* in Japanese quails that were infected with various doses (10^2, 10^3, 10^4, and 10^5) of sporulated oocysts. They indicated that there were variable degrees of diarrhea, intestinal gross lesions, low weight gain, and food conversion rate (FCR) depending on the inoculated dose. The most severe signs and lesions were recorded in the quails infected with 10^4 and 10^5 doses of oocysts. Additionally, mortalities were recorded within 10% and 16.67% in groups inoculated with 10^4 and 10^5 oocysts, respectively. Emaciation, bloody diarrhea, and mortality rate reached 32% in Japanese quails experimentally infected with mixed oocysts of *E. bateri*, *E. uzura*, *E. tsunodai*, *E. colini*, and *E. bahli.* Additionally, observations revealed bloody cores and ballooning in the two ceca (Ramadan et al., 2021). There are variations in the signs, lesions, and severity, as well as the difference in the infection rate of the *Eimeria*, which could be attributed to the species of *Eimeria*, the oocyst infectious dose, the health status of birds, the type of rearing, and the environmental conditions. All data, diagnostic tools, obtainable clinical signs, and post-mortem lesions of natural and experimental infection with different *Eimeria* species in quails through the available Egyptian literature are demonstrated in Tables 2 and 3.

Histopathology lesions

The infection with *Eimeria* spp. mainly induces pathological changes in the intestine. Developmental stages of *Eimeria* spp. are mostly found in the duodenum, jejunum, and ileum. Commonly observed changes include severe necrotic enteritis, thickening of the epithelial cells, massive erosion in the small intestine, and hypertrophy of the villi with crypt enlargement (Teixeira and Lopes, 2002; Teixeira et al., 2004; Simiyoon et al., 2018; Al-Zarkoushi and Al-Zubaidi, 2021). Additionally, there is notable enterocyte degeneration and necrotic modifications, with enlarged cells occasionally containing parasitophorous vacuoles of protozoal developmental stages observed within intestinal villi. Furthermore, the parasitophorous uninucleated the epithelial cells and released free merozoites from enterocytes, primarily in crypts. The goblet cells in the crypt-mucosal epithelium and the spaces between the villus epithelial cells were filled with more mucin (Al-Zarkoushi and Al-Zubaidi, 2021). Moreover, significant inflammatory cell infiltration, including eosinophils, extending into the lamina propria and submucosa of the caecum, occasionally reaching the muscular coat and serosa, along with the presence of granulocytes and mononuclear cells, has been documented (Teixeira et al., 2004; Al-Zarkoushi and Al-Zubaidi, 2021). Furthermore, the caecum indicates an accumulation of micro- and macrogametes in the submucosa, as well as the desquamation of surface epithelium, lamina propria, and parasite vacuoles in the mucosal epithelium (Al-Zarkoushi and Al-Zubaidi, 2021).

Generally, the development stages of the *Eimeria* and the distraction in the epithelium cells of the intestinal mucosa and submucosa result in maldigestion and malabsorption accordingly, leading to economic losses due to weight loss and decreased productivity in the quail industry (Teixeira et al., 2004; Al-Zarkoushi and Al-Zubaidi, 2021). Within accessible Egyptian publications, several studies conducted field or experimental investigations utilizing microscopic examination as one of the diagnostic methods (Tables 2 and 3). Waheeb et al. (2022) detected hyperplasia of epithelial cells, desquamation of intestinal villi, and necrosis of intestinal epithelium alongside different developmental stages of parasites in naturally infected migratory and domesticated quails with *E. tsunodai*, *E. uzura*, and *E. bateri*. Additionally, severe intestinal inflammatory reactions with infiltration of eosinophilic and denuded villi, and severe damage of the cecal mucosa with cystic dilation of the submucosal gland of the cecal tonsil were observed as microscopic intestinal lesions in experimentally infected Japanese quails with sporulated oocysts of *Eimeria* spp. (Nasr El Deen et al., 2021).

Table 2. Diagnose of natural infection with different *Eimeria* species in domesticated and migratory quails in the available Egyptian literature

Total No. of investigated quails or farms	Species	Location	Methods of detection	Identified *Eimeria* spp.	Signs and lesions	Microscopic lesions	Reference
27 farms	Domesticated Japanese quail	Al-Dakahlia and Kafr El-Sheikh governorates	• Direct smear from fecal contents • Floatation technique under light microscope • Morphometric identification was done by using a calibrated ocular micrometer	*E. bateri* *E. tsunodai* *E. uzura* unidentified *Eimeria* species	—	—	El-Morsy et al. (2016)
190 live quails	Migratory quails (*Coturnix coturnix*)	Matrouh governorate	• Direct fecal smear • Sporulation of *Eimeria* oocysts	*E. bateri* *E. uzura*	• All birds were apparently healthy	—	ElShabrawy et al. (2016)
205 live quails	Domestic farm (n= 112) Migratory (*Coturnix coturnix japonica*) (n= 93)	The farm's ones from Sharkia governorate. Migrant quails from Rashid and Damietta cities.	• Direct fecal smear • Concentration floating method • Sporulation of *Eimeria* oocysts	**Migrant quails;** *E. bateri* *E. tsunodai* *E. uzura* *E. colini* *E. bahli* **Domestic quails;** *E. bateri* *E. tsunodai*	—	—	Basiouny et al. (2017)
107 examined farms	Young broiler (n= 71) Adult layer (n= 36)	Dakahlia, Damieta (North Delta), and Port Said (North coast), Egypt	• Simple and sugar flotation technique • The shape indices (length/width) of the sporulated oocysts (morphologically identified)	Four identified *Eimeria* spp *E. bateri*, *E. tsunodai*, *E. uzura*, and *E. minima* and unidentified *Eimeria* species	—	—	Arafat and Abbas (2018)
100 live quails	Domesticated quails	Assiut and El-menia governorates	• Sporulation of *Eimeria* oocysts with morphological differentiation • Unstained wet mount technique • Concentration technique	*E. bateri* *E. tsunodai* *E. uzura*	• Thickened intestinal wall	—	Hassan et al. (2020)
900 birds	Domesticated Japanese quail	Kalioubia governorate	• Morphological characteristics • Morphometric characteristics (dimensions) of oocysts	*E. bateri, E. uzura, E. tsunodai, E. colini, and E. bahli*	—	—	Ramadan et al. (2021)
100 live quails	Domesticated (n= 50) Migratory (*Coturnix coturnix japonica*) (n= 50)	El-Behera governorate (Edko and Rashid districts)	• Direct fecal smear • Simple floating method • Sporulation of *Eimeria* oocysts • Histopathology	*E. bateri* *E. tsunodai* *E. uzura*	• Abnormal intestine filled with bloody faecal material • Thickening of the intestinal mucosa with hemorrhage	• Hyperpalasia of epithelial cells with presence of different developmental stages of parasites (shizonts, macrogamets, and microgametes). • Desquamation of intestinal villi and necrosis of intestinal epithelium	Waheeb et al. (2022)

n: Number of quails collected from each species, —: Not mentioned.

To cite this paper: ElBakrey RM, Helal SS, ElKholy AA, and Eid AAM (2024). Landmarks in Quail Coccidiosis Research with Special Scrutiny to the Available Egyptian Literature: A Review. *J. World Poult. Res.*, 14(2): 181-195.
DOI: https://dx.doi.org/10.36380/jwpr.2024.19

Table 3. . Diagnose of experimental infection with *Eimeria* species in domesticated Japanese quails in the Egyptian literature

Type of infectious *Eimeria*	Dose of infectious sporulated oocyst (Route)	Rearing system	Age of challenge	Experiment parameter	Clinical signs of positive control	Post mortem lesions of positive control	Microscopic lesions	Reference
A field strain of quail's intestinal coccidiosis (Japanese quails)	6×10^4 (Orally)	Cages	21 days of age	• Mortality percentage • Body weight score • Total oocyst output	• Mortalities (24%) • Bloody diarrhea • Low weight gain • High lesion = 90 and intestinal *Eimeria* score reached to 4.6±0.9	—	—	Arafa and Nasef (2004)
E. tsunodai (Japanese quail)	4.1×10^4 (Intra crop)	Isolated sterilized wire floored rearing cages	14 days of age	• Clinical signs • Mortalities • Lesion scoring • Total oocyst output • Weight gain and FCR measurements	• Signs appeared at the 4^{th} day post-infection • General signs of illness as ruffled feather, depression, huddling together, decreased appetite, emaciated breast muscle, and knife edged keel bone • Bloody diarrhea • Mortalities reached to 23.3%	• Severely enlarged two cecae with thickened mucosa • Bloody cecal core and ballooning	—	ElMorsy et al. (2016)
E. bateri (Japanese quail)	1ml of 10^2, 10^3, 10^4, and 10^5 sporulated oocysts (Orally)	Strict isolator/ wire floor cages	28 days of age	• Weight gain • FCR • Mortality • Severity of diarrhea (fecal score) • Intestinal lesion scores	• Diarrhea, low weight gain, and adverse effect on FCR varied in inoculated quails • More severe in groups infected with 10^4 and 10^5 • Mortalities were recorded only in 10^4 (10%) and 10^5 (16.67%)	• Gross lesion of the upper (duodenum and jejunum), lower (ileum), and cecum were different according to the dose level • More severe gross lesion in groups infected with 10^4 and 10^5	—	Arafat and Abbas (2018)
Mixed oocysts of *E. bateri*, *E. uzura*, *E. tsunodai*, *E. bahli* and *E. colini* (Japanese quail)	10^3 (—)	Isolated room	—	• Oocyst counting • Sporulation percentage • clinical signs • Body weight and mortalities. • Intestinal lesion	• General signs of illness • Mortalities 32% • Bloody diarrhea • Emaciation	• Bloody cecal core • Enlarged two cecai with ballooning	—	Ramadan et al. (2021)
Sporulated oocysts of *Eimeria* spp (Japanese quails)	4.1×10^4 (Intra crop)	—	14 days of age	• Histopathology • *Eimeria* oocyst count	—	—	• Severe intestinal inflammatory reaction with denuded villi and eosinophilic infiltration • Severe damage of the cecal mucosa caused by the proliferation of the parasites, meronts growth, and release of the merozoites with cystic dilation of the cecal tonsil submucosal gland	Nasr El Deen et al. (2021)

—: Not mentioned, FCR: Feed conversion ratio

Methods of diagnosis

Given the frequent occurrence of mixed infections in the field (Zoroaster et al., 2024), accurate differentiation between the different species of *Eimeria* remains challenging but necessary to obtain a prompt therapeutic or preventive intervention, particularly when the most dangerous species are circulating on the farm (Zoroaster et al., 2024).

Currently, the identification of *Eimeria* at the species level in quails relies on clinical and anatomopathological findings, coupled with the morphological characterization of mature oocysts and sporocysts using direct smear and floatation techniques under the light microscope (Duszynski and Wilber, 1997; Zoroaster et al., 2024), and morphometric characterization using a calibrated ocular micrometer (Henddrix and Robinson, 2012). These previously mentioned diagnostic methods were nearly used by all researchers to identify *Eimeria* spp. in quails (ElMorsy et al., 2016; Arafat and Abbas, 2018; Hassan et al., 2020; Ramadan et al., 2021; Waheeb et al., 2022). Only specialized laboratories with well-trained staff members can perform such time-consuming procedures (Zoroaster et al., 2024).

Previously, molecular tools were not commonly employed in diagnosing *Eimeria* species in quails due to limited information about the molecular characterization of the *Eimeria* species in quails, as well as the lack of available sequences in public databases (AL-Zarkoushi and AL-Zubaidi, 2022). In 2011, PCR-specific primers were specified and constructed against the internal transcribed spacer region 1 (ITS-1) of the ribosomal RNA gene to determine the prevalence of the different *Eimeria* spp. in captive game birds, such as northern bobwhite quails (Gerhold et al., 2011a). Analyses by PCR have targeted either the 18S rRNA (AL-Zarkoushi and AL-Zubaidi, 2022; Zoroaster et al., 2024) or the internal transcribed spacers (ITS1-5.8rRNA-ITS2) regions (Zoroaster et al., 2024). Moreover, the phylogenetic analysis of the 18S rRNA gene was performed on oocyst populations separately isolated from naturally infected Japanese quails (AL-Zarkoushi and AL-Zubaidi, 2022; Zoroaster et al., 2024). The nucleotide sequences of the 18s rDNA genes revealed the presence of seven genotypes of *Eimeria* spp. in Japanese quails (AL-Zarkoushi and AL-Zubaidi, 2022), while Zoroaster et al. (2024) inferred the potential presence of *E. uzura* based on their findings. Thus, molecular techniques have been pivotal in discerning the various genotypes of *Eimeria* species in animals.

Control and prevention

Trials of using anticoccidial drugs in quails

Several strategies for coccidiosis control include farm-level management techniques, vaccines, and natural and traditional anticoccidials (Shivaramaiah et al., 2014). To effectively manage coccidiosis in quail farms, appropriate control measures should be implemented, such as preventing water spills, maintaining high stocking density, disposing of litter regularly and hygienically, and enhancing hygiene standards (Umar et al., 2014).

Anti-coccidial medications, which prevent the sexual and asexual reproduction of *Eimeria* spp., are the main method of coccidiosis treatment (Odden et al., 2018). Using coccidiostats in feed or adding coccidiocidal drugs to the water were the most effective ways to control coccidiosis. Sokół et al. (2014) confirmed that Toltrazuril with different doses (7, 14, and 24.5 mg/kg body weight) could be an effective treatment of quail coccidia, but this effectiveness varied according to the species of coccidia and the parasitic developmental stages. Toltrazuril eliminates *E. bateri* and causes a high reduction in the number of *E. tsunodai* oocysts in the naturally infected Japanese quails.

In a study by Ruff et al. (1987) involving bobwhite quail infected with a mixed inoculum of *E. dispersa* and *E. lettyae* at a dose of 10^6 sporulated oocysts, the efficacy of salinomycin, amprolium, and monensin in preventing coccidiosis was examined. Based on body weight gains, the study found that both monensin and salinomycin were the most effective treatments for preventing coccidiosis. Monensin additionally reduced the number of parasites in the duodenum, while salinomycin decreased parasite numbers in both the duodenum and ileum at comparable rates. Furthermore, both anticoccidial drugs exhibited a reasonable safety margin in bobwhite quail. In contrast, amprolium was found to be ineffective in preventing coccidiosis (Ruff et al., 1987).

Furthermore, Gerhold et al. (2011b) detected that clopidol (125 ppm), decoquinate (30 ppm), diclazuril (1 ppm and 2 ppm), lasalocid (120 ppm), narasin (36 ppm), nicarbazin (36 ppm), robenidine (33 ppm), sulfadimethoxine/ormetoprin (125/75 ppm), and zoalene (150 ppm) have excellent to good efficacy with reducing lesion and fecal scores as well as improving weight gain and FCR in northern bobwhites experimentally infected with *E. lettyae*. However, monensin (90 ppm), salinomycin (60 ppm), semduramicin (25 ppm), or a combination of roxarsone and semduramicin were found to provide low protection. Amprolium (250 ppm),

roxarsone (50 ppm), and zoalene (125 ppm) proved to be ineffective in controlling coccidia.

Several studies were conducted in Egypt to evaluate the efficacy of different anticoccidial drugs. In a study by El-Morsy et al. (2016), the efficacy of salinomycin and diclazuril as coccidia prophylactic feed additives was investigated in Japanese quails experimentally infected with *E. tsunodai* at a dose of 4.1×10^4 sporulated oocysts. The study also evaluated amprolium plus ethopabate and toltrazuril as coccidia water medicaments. The results indicated that water medicaments were significantly more effective compared to feed additive anticoccidials. Additionally, the mortality rate was low in groups treated with amprolium plus ethopabate, and toltrazuril had the least effect on the sporulation of oocysts.

Some studies evaluated the efficacy of natural products as an alternative anticoccidial to control quail coccidiosis and reached variable conclusions according to the kind of products, doses, duration, *Eimeria* spp., and quail species used in these experiments (Ahmadov et al., 2014; Asghar et al., 2020).

Among the Egyptian investigations on alternative anticoccidials, Nasr El Deen et al. (2021) examined alternative anticoccidials and compared the effectiveness of probiotics (products containing *Bacillus subtilis*, *Pediococcus pentosaceus*, *Pediococcus acidilactici*, *Lactobacillus acidophilus*, and *Saccharomyces cerevisiae*) and toltrazuril in treating coccidiosis in Japanese quails. The probiotics can be utilized as a possible substitute anticoccidial and effectively treat coccidiosis by reducing the quantity of *Eimeria* oocysts, minimizing the negative effects of free radicals, and increasing the levels of IFN-γ and IL-2 in the cecum. Ramadan et al. (2021) investigated the efficacy of Propolis and neem as natural anticoccidial products, compared to a chemical anticoccidial drug as amprol (amprolium hydrochloride and ethopabate), against the challenge of mixed infection of *E. bateri*, *E. uzura*, *E. tsunodai*, *E. colini*, and *E. bahli* in Japanese quails. The natural and chemical anticoccidial products reduced the symptoms, mortalities, intestinal lesions, and oocysts shedding. On the other hand, propolis achieved the highest body weight gain and the lowest percentage of oocyst sporulation in infected quails. The different trials that evaluated the efficacy of various chemical and alternative (herbal/probiotic) anticoccidials in Egyptian articles are mentioned in Table 4.

Experiments of immunization in quails' coccidiosis

Coccidiosis is usually controlled using live vaccines. The basic component of all vaccines prepared for poultry is sporulated oocysts from several species. There are various techniques for administering vaccines to chickens, such as spraying and applying gel droplets in diet or drinking water (Jenkins et al., 2012; Awad et al., 2013; Jenkins et al., 2013). To the authors' knowledge, a specific vaccine for *Eimeria* species in quail has not yet been produced or manufactured in Egypt. Available literature shows limited trials or investigations for immunization quails infected with coccidia.

In an attempt to immunize northern bobwhite quail at the age of two days, Gerhold et al. (2010) administered 100 or 1000 oocysts orally using a pipette. Four weeks after vaccination, 1×10^6 *E. lettyae* was given to the immunized quails as a challenge. Immunized quail showed a 50% lower FCR, fewer gross intestinal and cecal lesions, roughly 99.7% fewer oocysts, and decreased signs of diarrhea. Elmorsy et al. (2021a) found that the immunization with a 100-oocyst dose of *E. bateri*, *E. uzura*, and *E. tsunodai* separately at 2 days of age in Japanese quails yielded better results against a high-dose challenge, which was 4×10^4 oocysts of *E. tsunodai* and 1×10^5 oocysts of *E. bateri* and *E. uzura* at 2 weeks post-immunization. Moreover, Elmorsy et al. (2021b) evaluated the efficacy of immunization with a low dose of live sporulated cysts of different abovementioned respective *Eimeria* species separately in the Japanese quail, compared to the efficacy of amprolium plus sulphaquinoxaline. Depending on clinical signs, mortality, weight gain, FCR, oocyst output, lesion score, and hematological parameters, immunization against any isolated species achieved the best results regarding all tested parameters compared to amprolium plus sulphaquinoxaline.

In Egypt, Arafat and Abbas (2018) conducted an experiment where 2-day-old Japanese quails were challenged with 1×10^5 sporulated oocysts of *E. bateri* at 30 days old. They found that oral immunization with either 100 or 1000 sporulated oocysts of *E. bateri* reduced diarrhea, intestinal lesions, and oocyst production while also improving weight gain and FCR.

Table 4. Treatment trials of quail coccidiosis using different types of anticoccidial medications either chemical or alternative (herbal and probiotic)

Medication	Dose (Con.)	Route	Duration	Quail species	Infected		Parameters	Judgment	Reference
					***Eimeria* spp.**	**Dose (Age of infection)**			
Salinomycin	1 kg/ton (60 ppm)	Ration	48 hours before infection till 21 days post-infection	Japanese quail	*E. tsunodai*	4.1×10^4 (14 days)	• Clinical signs • Mortality rate • Lesion score • Body parameters (Body Weight, Body Weight Gain, and Feed Conversion Rate) • Count oocysts	• Diclazuril showed better results than salinomycin all tested parameters except both were showed the same lesion score with lower oocyst output in salinomycin • Amprolium and ethopabate had better results than toltrazuril in all tested parameters except the mortality rate was the same. • Coccidial water treatments were found to be more effective than prophylactic feed additives. • Toltrazuril had the lowest effect on sporulation of oocysts.	El-Morsy et al. (2016)
Diclazuril	200 gm/ ton (0.5%)	Ration	48 hours before infection till end exp.						
Amprolium and ethopabate	1 ml/liter (—)	Drinking water	5 days post-infection						
Toltrazuril	1ml /litter (25 ppm)	Drinking water	48 hours post-infection						
Amprol (amprolium hydrochloride and ethopabate)	20%	Drinking water	6^{th} day post-infection	Japanese quail	Mixed infection of *E. bateri, E. uzura, E. tsunodai, E. colini, and E. bahli*	10^3 (—)	• Oocysts counting • Sporulation percentage • Clinical signs • Intestinal lesion • Body weight • Mortalities	• Amprolium, Propolis, and neem had effect in reduction the counts of oocyst, signs, mortality rate, and inflammatory intestinal lesions. • Propolis had the highest effect in increasing the body gain, and declined the percentage of *Eimeria* oocyst sporulation in infected quails	Ramadan et al. (2021)
Propolis	20%	Drinking water							
Neem extract	20%	Drinking water							
Probiotic (Gro-2-max)	1 gm/liter	Drinking water	a day till 28 days old	Japanese quails	Sporulated oocysts of *Eimeria* spp.	4.1×10^4 (14 days)	• Blood biochemical analysis • Antioxidant enzyme activities • Immunological parameters (inflammatory markers; Cecal interferon-gamma (IFN-γ) and interleukin-2 (IL-2) using ELISA kits • Histopathology • *Eimeria* oocysts count	• Probiotic relatively minimize the oocysts shedding • Probiotic improvement in the cecal IFN-γ & IL-2 and antioxidant enzymes, which reduces the damage caused by free radicals	Nasr El Deen et al. (2021)
Toltrazuril	1 ml/ liter (25ppm)	Drinking water	16 days of age for 2 consecutive days						

Con: concentration, —: Not mentioned

To cite this paper: ElBakrey RM, Helal SS, ElKholy AA, and Eid AAM (2024). Landmarks in Quail Coccidiosis Research with Special Scrutiny to the Available Egyptian Literature: A Review. *J. World Poult. Res.*, 14(2): 181-195.
DOI: https://dx.doi.org/10.36380/jwpr.2024.19

CONCLUSION

Reviewing the available literature on quail coccidiosis has indicated a range of symptoms from subclinical to clinical. Consequently, quail farms must be routinely examined to detect the infection and overcome its adverse consequences on quail productivity. The conventional tools used in detection and identification need to be developed due to the presence of an unknown *Eimeria* species in many studies. Thus, the molecular technique is a probable tool that needs to be introduced in the identification of unknown species besides the traditional tools. Due to the high prevalence of coccidia among quail farms, its control and prevention should be taken into consideration. There is an emerging need to find alternatives for chemical anticoccidial drugs as they have adverse effects on animal and human health. Further research into alternative anticoccidials and vaccinations should be conducted.

DECLARATIONS

Funding

There was no funding source for this study.

Availability of data and materials

All the data supporting this study are present in the article. Any additional information needed is obtainable from the corresponding author upon justifiable request.

Authors' contributions

Amal A. M. Eid, Reham M. El Bakrey, and Sarah S. Helal were involved in the conception and design. Amal A. M. Eid, Reham M. El Bakrey, Sarah S. Helal, and Ahmed A. El Kholy carried out data collection and drafted the manuscript. Reham M. El Bakrey designated the figures. All authors read and approved the final edition of the manuscript.

Ethical considerations

The manuscript was examined by the authors for signs of plagiarism, permission to publish, misconduct, fraud or data manipulation, duplicate publication or submission, or redundancy.

Competing interests

The authors did not disclose any potential conflicts of interest.

REFERENCES

Abd El-Ghany WA (2019). A comprehensive review on the common emerging diseases in quails. Journal of World's Poultry Research, 9(4): 160-174. DOI: https://www.doi.org/10.36380/jwpr.2019.20

Ahmadov EI, Topciyeva ShA, Hasanova JV, and Namazova AA (2014). Effects of herbal plants on ducks and quail infected with *Eimeria* species. Journal of Entomology and Zoology Studies, 4(4): 1150-1152. Available at: http://www.entomoljournal.com/archives/2016/vol4issue4/PartL/4-4-32-471.pdf

AL-Zarkoushi MMF and AL-Zubaidi MTS (2022). Molecular study of *Eimeria* species in quail birds (*Coturnix coturnix japonica*) in Thi-Qar Province, Southern Iraq. Indian Journal of Forensic Medicine and Toxicology, 16(1): 1674. DOI: https://www.doi.org/10.37506/ijfmt.v16i1.17809

Al-Zarkoushi MM and Al-Zubaidi MTS (2021). Epidemiological, morphological, and histopathological study of quail coccidiosis in Thi-Qar Province, Iraq. The Iraqi Journal of Veterinary Medicine, 45(1): 69-74. DOI: https://www.doi.org/10.30539/ijvm.v45i1.1045

Amoudi MA (1987). *Eimeria tahamensis* N. Sp. (Apicomplexa: *Eimeriidae*) from the Arabian quail (*Coturnix delegorguei Arabica*). The Journal of Protozoology, 34(4): 455-456. DOI: https://www.doi.org/10.1111/j.1550-7408.1987.tb03214.x

Anbarasi P, Ponnudurai G, Senthilvel K, Puvarajan B, and Arulmozhi A (2016). A note on incidence of coccidiosis in Japanese quail (*Coturnix coturnix japonica*). Indian Veterinary Journal, 93(2): 29-31. Available at: http://krishikosh.egranth.ac.in/handle/1/68354

Arafa AEA and Nasef SA (2004). Some studies on the effect of different strains of *Eimeria* Spp. in quails experimentally infected with *E. Coli*. Kafrelsheikh Veterinary Medical Journal, 2(1): 107-115. DOI: https://www.doi.org/10.21608/KVMJ.2004.112394

Arafat N and Abbas I (2018). Coccidia of Japanese quail: From identification, prevalence, infection, and immunization. The Journal of Parasitology, 104(1): 23-30. DOI: https://www.doi.org/10.1645/17-109

Arya K, Gupta R, and Saxena VL (2018). Quail survey: Elaborative information and its prospects. Research Journal of Life Science, Bioinformatics, Pharmaceutical and Chemical Sciences, 4(4): 197-209. DOI: https://www.doi.org/10.26479/2018.0404.16

Asghar M, Durrani UF, Hussain R, Matloob K, Mahmood AK, Anees M, and Oneeb M (2020). Comparative efficacy of Amprolium, Garlic oil (*Allium sativum*) and Ginger oil (*Zingiber officinale*) against coccidiosis in common quail (*Coturnix coturnix*). Journal of the Hellenic Veterinary Medical Society, 71(3): 2273-2278. DOI: https://www.doi.org/10.12681/jhvms.25072

Awad AM, El-Nahas AF, and Abu-Akkada SS (2013). Evaluation of the protective efficacy of the anticoccidial vaccine Coccivac-B in broilers, when challenged with Egyptian field isolates of *E. tenella*. Parasitology Research, 112(1): 113-121. DOI: https://www.doi.org/10.1007/s00436-012-3112-6

Bashtar AR, Abdel-Ghaffar F, Al-Rasheid KA, Mehlhorn H, and Al Nasr I (2010). Light microscopic study on *Eimeria* species infecting Japanese quails reared in Saudi Arabian farms. Parasitology Research, 107: 409-416. DOI: https://www.doi.org/10.1007/s00436-010-1881-3

Basiouny AA, Mohamed SMN, and Reham GAA (2017). Prevelance and morphological identification of *Eimeria* species in quails. Egyptian Veterinary Medical Society of Parasitology Journal, 13(1): 55-63. Available at: https://evmspj.journals.ekb.eg/article_37467_7a1e79bb566b60e4ae3ea000e5930ea3.pdf

Benskin CM, Wilson K, Jones K, and Hartley IR (2009). Bacterial pathogens in wild birds: A review of the frequency and effects of infection. Biological Reviews, 84(3): 349-373. DOI: https://www.doi.org/10.1111/j.1469-185X.2008.00076.x

Berto BP, Flausino W, McIntosh D, Teixeira-Filho WL, and Lopes CW (2011). Coccidia of new world passerine birds (Aves: *Passeriformes*): A review of *Eimeria* Schneider, 1875 and Isospora Schneider, 1881 (Apicomplexa: *Eimeriidae*). Systematic Parasitology, 80: 159-204. DOI: https://www.doi.org/10.1007/s11230-011-9317-8

Berto BP, Borba HR, Lima VM, Flausino W, Teixeira-Filho WL, and Lopes CG (2013). *Eimeria* spp. from Japanese quails (*Coturnix japonica*): New characteristic features and diagnostic tools. Pesquisa Veterinaria Brasileira, 33(12): 1441-1447. DOI: https://www.doi.org/10.1590/S0100-736X2013001200008

Chakravarty M and Kar B (1947). A study on the coccidia of Indian birds. Proceedings of the Royal Society of Edinburgh, 62(Pt 3): 225-233. Available at: https://pubmed.ncbi.nlm.nih.gov/18898344/

Chapman HD (2014). Milestones in avian coccidiosis research: A review citing articles via. Poultry Science, 93(3): 501-511. DOI: https://www.doi.org/10.3382/ps.2013-03634

Conway DP and McKenzie ME (2007). Poultry coccidiosis: Diagnostic and testing procedures, 3rd Edition. John Wiley & Sons, pp. 1-12. DOI: https://www.doi.org/10.1002/9780470344620

Dalloul RA and Lillehoj HS (2005). Recent advances in immunomodulation and vaccination strategies against coccidiosis. Avian Diseases, 49(1): 1-8. DOI: https://www.doi.org/10.1637/7306-11150R

Duszynski DW (2011). *Eimeria*. Encyclopedia of life sciences. John Wiley and Sons, Ltd., Chichester. DOI: https://www.doi.org/10.1002/9780470015902.a0001962.pub2

Duszynski DW and Wilber PG (1997). A guideline for the preparation of species descriptions in the *Eimeriidae*. Journal of Parasitology, 83: 333-336. DOI: https://www.doi.org/10.2307/3284470

Duszynski DW and Gutiérrez RJ (1981). The coccidia of quail in the United States. Journal of Wildlife Diseases, 17(3): 371-379. DOI: https://www.doi.org/10.7589/0090-3558-17.3.371

El-Morsy MA, Abou El-Azm KI, and Awad SS (2016). Efficacy of some anticoccidial drugs on experimentally induced cecal coccidiosis (*E. tsunodai*) in Japanese quails. Egyptian Journal of Veterinary Sciences, 47(2): 165-177. DOI: https://www.doi.org/10.21608/ejvs.2017.3591

Elmorsy MA, Das M, Senapati SK, Jena GR, Mishra S, Panda SK, Kundu AK, and Kumar D (2021a). Efficacy of Immunization of Japanese quail (*Coturnix coturnix japonica*) against the challenge with different *Eimeria* species. Indian Journal of Animal Research, 1: 10. DOI: https://www.doi.org/10.18805/IJAR.B-4437

Elmorsy MA, Das M, Senapati SK, Jena GR, Panda SK, Kundu AK, Mishra S, and Kumar D (2021b). Efficacy of immunization compared to an anticoccidial drug combination in the management of challenged coccidiosis in Japanese quail. Veterinary Parasitology, 295: 109451. DOI: https://wwww.doi.org/10.1016/j.vetpar.2021.109451

ElShabrawy N, Abu-Elnaga T, Gouda A, and Abdel Aal A (2016). Prevalence of some enteric parasitic infections in migratory quails (*Coturnix coturnix*). Suez Canal Veterinary Medical Journal, 21(1): 27-36. DOI: https://www.doi.org/10.21608/SCVMJ.2016.62745

Faitarone ABG, Pavan AC, Mori C, Batista LS, Oliveira RP, Garcia EA, Pizzolante CC, Mendes AA, and Sherer MR (2005). Economic traits and performance of Italian quails reared at different cage stocking densities. Revista Brasileira de Ciências Avícolas, 7: 19-22. DOI: https://www.doi.org/10.1590/S1516-635X2005000100003

Faizullah JS, Taj K, Ud Din Z, and Akbar Khan M (2021). Migratory Japanese quail (*Coturnix coturnix japonica*) as a host and carrier for coccidiosis and ascaridiasis. Journal of Veterinary Sciences and Dairy & Poultry Research, 1: 1-10. Available at: https://scientificeminencegroup.com/articles/Migratory-Japanese-Quail.pdf

Ferguson DJ, Belli SI, Smith NC, and Wallach MG (2003). The development of the macrogamete and oocyst wall in *Eimeria Maxima*: Immuno-light and electron microscopy. International Journal of Parasitology, 33: 1329-1340. DOI: https://www.doi.org/10.1016/s0020-7519(03)00185-1

Fisher JW and Kelley GL (1977). The sporulated oocyst of *Eimeria colini* sp. n. from the bobwhite quail, *Colinus virginianus*. The Journal of Parasitology, 63(2): 200-202. DOI: https://www.doi.org/10.2307/3280036

Gerhold RW, Fuller AL, Beckstead RB, and McDougald LR (2010). Low-dose immunization of northern bobwhites (*Colinus virginianus*) with *Eimeria lettyae* provides protection against a high-dose challenge. Avian Diseases, 54(4): 1220-1223. DOI: https://www.doi.org/10.1637/9403-052510-Reg.1

Gerhold RW, McDougald LR, and Beckstead RB (2011a). Construction of PCR primers to detect and distinguish *Eimeria* spp. in northern bobwhites and a survey of *Eimeria* on game bird farms in the United States. Journal of Parasitology, 97(5): 892-895. DOI: https://www.doi.org/10.1645/GE-2816.1

Gerhold RW, Fuller AL, Lollis L, Parr C, and McDougald LR (2011b). The efficacy of anticoccidial products against *Eimeria* spp. in northern bobwhites. Avian Diseases, 55(1): 59-64. DOI: https://www.doi.org/10.1637/9572-101310-Reg.1

Gilbert ER, Cox CM, Williams PM, McElroy AP, Dalloul RA, Ray WK, Barri A, Emmerson DA, Wong EA, and Webb Jr KE (2011). *Eimeria* species and genetic background influence the serum protein profile of broilers with coccidiosis. PLoS ONE, 6(2): e14636. DOI: https://www.doi.org/10.1371/annotation/e9373e8a-b316-49c6-b33f-f49557453b48

Halim SM (2022). A comparative study between the representation of quails in ancient Egyptian and Byzantine art. Annal of General Union of Arab Archaeologists, 25(25): 61-96. DOI: https://www.doi.org/10.21608/cguaa.2022.113746.1099

Hassan AM, Mohammed DA, Hussein KN, and Hussen SH (2017). Comparison among three lines of quail for egg quality characters. Science Journal of University of Zakho, 5(4): 296-300. DOI: https://www.doi.org/10.25271/2017.5.4.413

Hassan AK, Naeem EV, and Soliman MA (2020). Investigation the prevalence of common parasitic infections in farmed quails in Upper Egypt. SVU-International Journal of Veterinary Sciences, 3(2): 38-50. DOI: https://www.doi.org/10.21608/SVU.2020.31915.1058

Haug A, Gjevre AG, Thebo P, Mattsson JG, and Kaldhusdal M (2008). Coccidial infections in commercial broilers: Epidemiological aspects and comparison of *Eimeria* species identification by morphometric and polymerase chain reaction techniques. Avian pathology, 37(2): 161-170. DOI: https://www.doi.org/10.1080/03079450801915130

Henddrix CM and Robinson E (2012). Diagnostic Parasitology for Veterinary Technicians, 4th Edition. Mosby Elsevier., Missouri, United States, pp. 232-236. Avilable at: https://www.vet-ebooks.com/diagnostic-parasitology-for-veterinary-technicians-4th-edition/

Jatoi AS, Sahota AW, Akram M, Javed K, Hussain J, Mehmood S, and Jaspal MH (2013). Response of different body weights on blood serum chemistry values in four close-bred flocks of adult Japanese quails (*Coturnix coturnix japonica*). The Journal of Animal & Plant Sciences, 23(1): 35-39. Available at: http://www.thejaps.org.pk/docs/v-23-1/06.pdf

Jenkins MC, Parker C, Klopp S, O'Brien C, Miska K, and Fetterer R (2012). Gel-bead delivery of *Eimeria* oocysts protects chickens against coccidiosis. Avian Diseases, 56(2): 306-309. DOI: https://www.doi.org/10.1637/9940-092111-Reg.1

Jenkins MC, Parker C, O'Brien C, Persyn J, Barlow D, Miska K, and Fetterer R (2013). Protecting chickens against coccidiosis in floor pens by administering *Eimeria* oocysts using gel beads or spray

vaccination. Avian Diseases, 57(3): 622-626. DOI: https://www.doi.org/10.1637/10516-022213-Reg.1

Kalsum U, Soetanto H, Achmanu A, and Sjofjan O (2012). Influence of a Probiotic containing *Lactobacillus* fermentum on the laying performance and egg quality of Japanese quails. International Journal of Poultry Science, 11(4): 311-315. Available at: http://www.pjbs.org/ijps/fin2108.pdf

Kemp LE, Yamamoto M, and Soldati-Favre D (2013). Subversion of host cellular functions by the apicomplexan parasites. FEMS Microbiology Reviews, 37(4): 607-631. DOI: https://www.doi.org/10.1111/1574-6976.12013

Liburd EM and Mahrt JL (1970). *Eimeria lophortygis* n. sp. and *E. okanaganensis* n. sp. (Sporozoa: *Eimeriidae*) from California quail *Lophortyx californicus* in British Columbia. The Journal of Protozoology, 17(2): 352-353. DOI: https://www.doi.org/10.1111/j.1550-7408.1970.tb02384.x

Lu C, Yan Y, Jian F, and Ning C (2021). Coccidia-microbiota interactions and their effects on the host. Frontiers in Cellular and Infection Microbiology, 11: 751481. DOI: https://www.doi.org/10.3389/fcimb.2021.751481

Lukanov H (2019). Domestic quail (*Coturnix japonica domestica*), is there such farm animal?. World's Poultry Science Journal, 75(4): 547-558. DOI: https://www.doi.org/10.1017/S0043933919000631

Mazyad SA, Morsy TA, Fekry AA, and Farrag AM (1999). Mites infesting two migratory birds, *Coturnix c. coturnix* (quail or Simmaan) and *Sturnus v. vulgaris* (starling or zarzuur) with reference to avian zoonosis. Journal of the Egyptian Society of Parasitology, 29(3): 745-761. Available at: https://pubmed.ncbi.nlm.nih.gov/12561915/

Müller J and Hemphill A (2013). *In vitro* culture systems for the study of apicomplexan parasites in farm animals. International Journal of Parasitology, 43(2): 115-124. DOI: https://www.doi.org/10.1016/j.ijpara.2012.08.004

Nasr El Deen N, Ismail S, and Kaser A (2021). Comparative Study on the Effect of a probiotic and toltrazuril for controlling coccidiosis in Japanese quails (*Coturnix japonica*). Zagazig Veterinary Journal, 49(4): 400-413. DOI: https://www.doi.org/10.21608/zvjz.2021.97319.1158

Nawarathne SR, Yu M, and Heo JM (2021). Poultry coccidiosis-A concurrent overview on etiology, diagnostic practices, and preventive measures. Korean Journal of Poultry Science, 48(4): 297-318. DOI: https://www.doi.org/10.5536/KJPS.2021.48.4.297

Norton CC and Chard MJ (1983). The oocyst sporulation time of *Eimeria* species from the fowl. Parasitology, 86(2): 193-198. DOI: https://www.doi.org/10.1017/S0031182000050368

Norton CC and Pierce MA (1971). The life cycle of *Eimeria bateri* (Protozoa, *Eimeriidae*) in the Japanese quail *Coturnix coturnix japonicum*. Journal of Protozoology, 18(1): 57-62. DOI: https://www.doi.org/10.1111/j.1550-7408.1971.tb03280.x

Odden A, Enemark HL, Ruiz A, Robertson LJ, Ersdal C, Nes SK, Tømmerberg V, and Stuen S (2018). Controlled efficacy trial confirming toltrazuril resistance in a field isolate of ovine *Eimeria* spp. Parasites and Vectors, 11(1): 394. DOI: https://www.doi.org/10.1186/s13071-018-2976-4

Ojo V, Fayeye TR, Ayorinde KL, and Olojede H (2014). Relationship between body weight and linear body measurements in Japanese quail (*Coturnix coturnix japonica*). Journal of Science Research, 6(1): 175-183. DOI: http://www.doi.org/10.3329/jsr.v6i1.16368

Otify YZ (1988). Prevelance and the differential morphological status of oocysts of *Eimeria* species infecting quails (*Coturnix coturnix*) in Egypt. Journal of the Egyptian Veterinary Medical Association, 48(2): 265-269.

Quiroz-Castañeda RE and Dantán-González E (2015). Control of avian coccidiosis: Future and present natural alternatives. BioMed Research International, 2015: 430610. DOI: https://www.doi.org/10.1155/2015/430610

Ramadan M, Elmadawy R, and Tolba I (2021). Coccidiosis in Japanese quails (*Coturnix coturnix japonica*) in Kalioubia governorate: Prevalence and treatment trials. Benha Veterinary Medical Journal, 40(2): 131-136. DOI: https://www.doi.org/10.21608/bvmj.2021.68178.1375

Ruff MD (1985). Life cycle and biology of *Eimeria lettyae* sp. n. (Protozoa: *Eimeriidae*) from the northern bobwhite, *Colinus virginianus* (L.). Journal of Wildlife Diseases, 21(4): 361-370. DOI: https://www.doi.org/10.7589/0090-3558-21.4.361

Ruff MD and Wilkins GC (1987). Pathogenicity of *Eimeria lettyae* Ruff, 1985 In the northern bobwhite (*Colinus virginianus L.*). Journal of Wildlife Diseases, 23(1): 121-126. DOI: https://www.doi.org/10.7589/0090-3558-23.1.121

Ruff MD, Wilkins GC, and Chute MB (1987). Prevention of coccidiosis in bobwhites by medication. Poultry Science, 66(9): 1437-1445. DOI: https://www.doi.org/10.3382/ps.0661437

Shemshadi B, Ranjbar BS, and Mirakhori M (2014). Study on parasitic infections of quails in Garmsar, Iran. International Journal of Advanced Biological and Biomedical Research, 2(2): 262-266. Available at: https://www.sid.ir/EN/VEWSSID/Jpdf/57000020140202.pdf

Shirley MW, Smith AL, and Tomley FM (2005). The biology of avian *Eimeria* with an emphasis on their control by vaccination. Advanced Parasitololgy, 60: 285-330. DOI: https://www.doi.org/10.1016/S0065-308X(05)60005-X

Shivaramaiah C, Barta JR, Hernandez-Velasco X, Téllez G, and Hargis BM (2014). Coccidiosis: Recent advancements in the immunobiology of *Eimeria* species, preventive measures, and the importance of vaccination as a control tool against these Apicomplexan parasites. Veterinary Medicine Research and Reports, 5: 23-34. Available at: https://www.tandfonline.com/doi/epdf/10.2147/VMRR.S57839

Simiyoon L, Arulmozhi A, and Balasubramaniam GA (2018). Pathology of caecal coccidiosis in Japanese quails (*Coturnix coturnix japonica*). International Journal of Science and Environmental Technology, 7(1): 299-302. Available at: http://www.ijset.net/journal/2047.pdf

Sokół R, Gesek M, Ras-Norynska M, and Michalczyk M (2014). Toltrazuril (BaycoxR) treatment against coccidiosis caused by *Eimeria* sp. in Japanese quails (*Coturnix coturnix japonica*). Polish Journal of Veterinary Sciences, 17(3): 465-468. DOI: https://www.doi.org/10.2478/pjvs-2014-0067

Sokół R, Gesek M, Raś-Noryńska M, Michalczyk M, and Koziatek S (2015). Biochemical parameters in Japanese quails *Coturnix coturnix japonica* infected with coccidia and treated with Toltrazuril. Polish Journal of Veterinary Sciences, 18(1): 79-82. DOI: https://www.doi.org/10.1515/pjvs-2015-0010

Svanbaev SK and Utebaeva MK (1973). Coccidial infections of Phasianus colchicus mongolicus and Coturnix coturnix in Kasakhstan. Akademii Nauk Kazakhskoi SSR, Seriya Biologischeskikh Nauk, 1: 62-68. Available at: http://www.k-state.edu/parasitology/worldcoccidia/GALLIFORMES

Teixeira M and Lopes C (2002). Species of the genus *Eimeria* (apicomplexa: *Eimeriidae*) from Japanese quails (*Coturnix japonica*) in Brazil and *E. fluminensis* for the preoccupied *E. minima* of this quail. Revista Brasileira de Ciência Veterinária, 9(1): 53-56. DOI: http://www.doi.org/10.4322/rbcv.2015.350

Teixeira M and Lopes CWG (2000). *Eimeria miniman*. sp. (Api-complexa: *Eimeriidae*) from the Japanese quail (*Cuturnix cuturnix japonica*) in Brazil. Revista Brasileira de Ciência Veterinária, 7(3): 157-158. DOI: http://www.doi.org/10.4322/rbcv.2015.203

Teixeira M, Teixeira Filho WL, and Lopes CWG (2004). Coccidiosis in Japanese quails (*Coturnix japonica*): Characterization of a naturally

occurring infection in a commercial rearing farm. Brazilian Journal of Poultry Science, 6: 129-134. DOI: https://www.doi.org/10.1590/S1516-635X2004000200010

Tsunoda K, and Muraki Y (1971). A new coccidium of Japanese quails: *Eimeria uzura* sp. Nov. Japanese Journal of Veterinary Science, 33(5): 227-235. Available at: https://www.cabidigitallibrary.org/doi/full/10.5555/19722268494

Tunsaringkarn T, Tungjaroenchai W, and Siriwong W (2013). Nutrient benefits of quail (*Coturnix Coturnix japonica*) eggs. International Journal of Scientific and Research Publications, 3(5): 2250-3153. Available at: https://www.ijsrp.org/research-journal-0513.php

Umar HA, Lawal IA, Okubanjo OO, and Wakawa AM (2014). Morphometric identification, gross and histopathological lesions of *Eimeria* species in Japanese quails (*Coturnix coturnix japonica*) in Zaria, Nigeria. Journal of Veterinary Medicine, 2014: 451945. DOI: https://www.doi.org/10.1155/2014/451945

Wahab MA (2002). Quails could reduce protein deficiency in poor countries. World. Poultry, 18(6): 39. Available at: https://www.poultryworld.net/poultry/quails-could-reduce-protein-deficiency-in-poor-countries/

Waheeb H, Menshawy S, Mahmoud S, Otify Y, and AbouLaila M (2022). Prevalence and scanning electron microscope of some parasites infecting domesticated and migratory quails from Edko and Rashid districts, El-Behera governorate, Egypt. Damanhour Journal of Veterinary Sciences, 7(2): 28-34. DOI: https://www.doi.org/10.21608/djvs.2022.236994

Yambayamba KE and Chileshe PC (2019). Effect of increased photoperiod on feed intake, egg production and egg size in Japanese quail (*Coturnix japonica*) under Zambian conditions. EC Veterinary Science, 4(5): 334-342. Available at: https://ecronicon.net/assets/ecve/pdf/ECVE-04-00109.pdf

Zoroaster A, Singh Y, Marchiori E, Cullere M, Dotto G, Franzo G, and di Regalbono AF (2024). Differential diagnosis of *Eimeria* species in farmed Japanese quails (*Coturnix japonica*). Poultry Science, 103(3): 103418. DOI: https://www.doi.org/10.1016/j.psj.2023.103418

JWPR
Journal of World's Poultry Research
2024, Scienceline Publication
J. World Poult. Res. 14(2): 196-203, 2024
Research Paper
DOI: https://dx.doi.org/10.36380/jwpr.2024.20
PII: S2322455X2400020-14
ISSN: 2322-455X

Enterobacteriaceae Antibiotic Resistance Identification in Slender-billed Gull Species Migrating to Libya

Aya Mansour[1], Ehab Sharif[2,7], Abdulmajid Hamhoom[3,7], Ibrahim Eldaghayes[4,7], Khaled Etayeb[5,7], Abdulnaser Dayhum[6,7], and Abdulwahab Kammon[2,7]*

[1]*Department of Microbiology, Faculty of Science, Zintan University, Zintan, Libya*
[2]*Department of Poultry and Fish Diseases, Faculty of Veterinary Medicine, University of Tripoli, Libya*
[3]*National Center for Animal Health, Tripoli, Libya*
[4]*Department of Microbiology, Faculty of Veterinary Medicine, University of Tripoli, Libya*
[5]*Department of Zoology, Faculty of Science, University of Tripoli, Libya*
[6]*Department of Preventive Medicine, Faculty of Veterinary Medicine, University of Tripoli, Libya*
[7]*National Research Center for Tropical and Transboundary Diseases, Zintan, Libya*
*Corresponding author's email: a.kammon@nrcttd.ly

Received: March 29, 2024, Revised: April 24, 2024, Accepted: May 21, 2024, Published: June 30, 2024

ABSTRACT

The Libyan coast has various types of wetlands that are passed by many migratory birds throughout the year, however, studies on bacterial isolation from these birds in Lybia are scarce. The present study aimed to isolate enteropathogenic bacterial species from the seagulls that migrated to the Libyan coast as well as identification of the antibiotics that are resistant to these bacteria. A total of 50 fresh fecal samples were collected from slender-billed gulls in January 2023 at Farwa Island near the city of Zuwara, in Western Libya. Bacteria were isolated by conventional culturing method, identified using the Enterosystem 18R, and antibiotic susceptibility tests were conducted on the isolated bacteria. The results revealed the isolation of 46 bacteria, but only 32 of them were identified using biochemical tests. These identified bacteria belong to six species of Enterobacteriacae, namely *Citrobacter* (*C.*) *freundii*, *Pantoea* (*P.*) *agglomerans*, *Escherichia (E.) coli*, *Enterobacter (En.) cloacae*, *Serratia liquifaciens*, and *Proteus mirabilis*, with percentages of 53.125%, 31.25%, 6.25%, 3.125%, 3.125%, and 3.125%, respectively. All isolated bacteria were 100% sensitive to gentamicin (10 µg) and ciprofloxacin (5 µg). The highest resistance result was observed against the antibiotic cefoxitin (30 µg), with both *C. freundii* (5 samples) and *P. agglomerans* (4 samples). Resistance was observed in 5 samples of *C. freundii* and 4 samples of *P. agglomerans* out of 11 samples. Resistance to antibiotics, such as azithromycin (15 µg), ceftriaxone (30 µg), and ampicillin (10 µg), was also noted in a few isolates. The results indicated that *C. freundii* was the most antibiotic-resistant bacterial species isolated in this study. The highest multiple antibiotic resistance index was demonstrated by bacteria *C. freundii*, *P. agglomerans*, and *En. cloacae*, with a value of 0.33 for each of them. In conclusion, slender-billed gulls carry multi-drug-resistant bacteria. The study recommends the implementation of a national program to survey antibiotic-resistant bacteria, determine their prevalence, and assess the presence of antibiotic residues in animal-derived food. Furthermore, the present study advises expanding scientific studies on risk analysis, and antibiotic alternatives in migratory birds.

Keywords: *Citrobacter freundii*, Enterobacteriaceae, Multiple antibiotic resistance, Prevalence, Slender-billed gull

INTRODUCTION

Enterobacteriaceae members are among the most important types of bacteria naturally found in the human and animal digestive tract. Some of them can also be pathogenic (Brenner et al., 2005). They are responsible for various diseases, including intestinal inflammation, food poisoning, urinary tract infections, and other illnesses in both humans and animals (Tilahun et al., 2021; Zaitsev et al., 2022). This family includes several genera, such as *Escherichia coli*, *Klebsiella*, *Shigella*, *Salmonella*, *Enterobacter*, and others. One of Enterobacteriaceae's key characteristics is being Gram-negative rods, easy to grow on simple media, motile, and with noticeable biochemical activity. Some of them also produce endotoxins (Brenner et al., 2005).

Wild birds are among the most important reservoirs and potential carriers of antibiotic-resistant bacteria (Bonnedahl and Järhult, 2014). Many antibiotic-resistant bacteria have been isolated from wild birds, and several studies have shown that wild birds can spread antibiotic-resistant bacteria through long-distance migration to distant areas. These bacteria can also transfer from birds to humans and vice versa, posing a significant threat (Bonnedahl and Järhult, 2014). Even if these bacteria are not pathogenic themselves, they can transfer antibiotic resistance to pathogenic bacteria, which is a concerning issue. Therefore, the study of antibiotic resistance in wild birds, especially migratory ones, is of great importance (Bonnedahl and Järhult, 2014). These birds can travel hundreds of kilometers and spread disease-causing agents and antibiotic resistance across different regions and continents (Martín-Maldonado et al., 2022).

The survival instinct encourages many birds to migrate, taking routes such as coasts, mountain ranges, valleys, rivers, beaches, and other paths. Although bird migration is a fascinating natural phenomenon, it poses inevitable risks to public and animal health due to direct or indirect contact between birds and humans or can lead to the transmission of antibiotic-resistant bacteria (Georgopoulou and Tsiouris, 2008). Approximately 5 billion migratory wild birds fly across continents twice every year, which may lead to a global transfer and spread of many diseases (Elsohaby et al., 2021).

The Libyan coast, in particular, has various types of wetlands, such as saline marshes, lagoons, lakes, and islands, with approximately 50 observatories for migratory birds in Libyan territory. These areas provide good shelters and feeding sites for migratory birds during their journeys between Eurasia and Africa. The winter census of migratory birds in Libya for the year 2022 revealed the presence of 2.169 individuals of the Slender-billed Gull species (Etayeb et al., 2023). Antibiotic-resistant bacteria have been isolated and identified in both resident and migratory gulls worldwide, and some studies have suggested that gulls can serve as carriers of antibiotic-resistant bacteria, distributing them through aquatic environments (Zeballos-Gross et al., 2021). However, confirming this remains challenging and incomplete, requiring further studies, and there is a lack, or perhaps an absence, of studies targeting the isolation of bacteria from migratory birds in wetlands in Libya. Therefore, this study was conducted to isolate and identify enteric bacterial species from the Slender-billed Gull, a migratory bird to Farwa Island, and to investigate and identify antibiotic resistance bacteria among these isolated enteric bacteria.

MATERIALS AND METHODS

Ethical approval

The Animal Care and Use Committee, Faculty of Veterinary Medicine, University of Tripoli (Lybia) has approved this research study following protocols of animal welfare.

Sampling

Fifty fresh fecal samples from 50 migratory Slender-billed gull species were collected during January 2023 at Farwa Island 40 Km west of Zwara City in Western Libya, close to the Libyan border with Tunisia. Following monitoring and identification of the migrated flock by a specific telescope (Kite spotting scope, Belgium), the place of the flock is determined and visited immediately for sampling of fresh feces. The samples were collected using sterilized cotton swabs with care (not to touch the soil), labeled from Z1 to Z50, and stored in a refrigerated container. On the same day, the samples were then transported to the Microbiology Laboratory at the Faculty of Science in Zintan University (Lybia) for bacterial isolation, identification, and antibiotic susceptibility testing.

Culture

Bacteria were isolated by culturing the samples in Peptone water (BD, USA), and incubated at 37℃ for 24 hours. Since salmonella was highly expected, enrichment and selective media were used. 1 ml of peptone water was then transferred to 9 ml of Rappaport- Vassiliadis (RV) liquid medium (Park Scientific Limited, UK), and incubated at 42℃ for 24 hours. A loopful of RV medium was then streaked on Salmonella-Shigella Agar (SSA) and for isolation of other enterobacteria, a loopful of cultured peptone water was streaked on MacConkey Agar (Bio Tec Diagnostics, UK) and incubated at 37℃ for 24 hours. The isolated bacteria were Gram-stained and examined for morphological characteristics under a microscope (Optech, Germany). The Enterosystem 18R, which includes 20 biochemical tests was used for identifying the isolated bacteria (Liofilchem, Italy) as per the manufacturer's instructions.

Antibiotic susceptibility test

The Bauer-Kirby method was used to determine antibiotics' resistance. A swab from the isolated bacterial colonies was transferred to sterilized glass tubes containing 5 ml of peptone water and incubated in an incubator at 37°C for 24 hours. Muller-Hinton Agar

(Himedia, USA) was used to cultivate the bacteria following the disk diffusion technique. Antibiotic discs namely Gentamicin (10 µg), Ciprofloxacin (5 µg), Azithromycin (15 µg), Ceftriaxone (30 µg), Ampicillin (10 µg), and Cefoxitin (30 µg) were used. A single colony from each isolate was cultured in Nutrient broth (BD, USA) and then incubated at 37℃ for 24 hours. The cultures were then streaked on Muller-Hinton Agar. By using sterile forceps, antibiotic discs were placed on the agar. Following the incubation time for 24 hours at 37°C (Bauer et al., 1966), the inhibition zones were measured and the results were recorded in millimeters. MAR index was calculated using the formula: "A/B," where "A" is the number of antibiotics resisted by the isolated bacteria, and "B" is the total number of antibiotics tested in this study (Krumperman, 1983).

RESULTS

In this study, 50 fecal samples were collected from migratory seagulls in Libyan territories, specifically at Farwa Island near the city of Zwara, in January 2023. These seagulls belong to the Slender-billed gull (*Chroicocephalus genei)* species.

Bacterial isolation and identification

Following the culturing of the samples on various media, a total of 46 bacteria were isolated, and only 32 of them were identified using biochemical tests (14 isolates were not identified using the Enterosystem 18R). These identified bacteria belong to six species of the family Enterobacteriaceae, namely *Citrobacter (C.) freundii*, *Pantoea (P.) agglomerans* (formerly known as *Enterobacter agglomerans*), *E. coli*, *Enterobacter (En.) cloacae*, *Serratia liquifaciens*, and *Proteus mirabilis*, at levels of 53.125% (17), 31.25% (10), 6.25% (2), 3.125% (1), 3.125% (1), and 3.125% (1), respectively (Table 1).

Table 1. Name, number, and percentage of isolated and identified bacteria from feces of Slender-billed gull species in Lybia

Name of bacteria	No. of identified bacteria	Percentage (%)
Citrobacter freundii	17	53.125
Pantoea agglomerans	10	31.25
E. coli	2	6.25
Enterobacter cloacae	1	3.125
Serratia liquifaciens	1	3.125
Proteus mirabilis	1	3.125
Total	32	100

Antibiotic susceptibility testing

The results in Table 2 indicated that all isolated bacterial species in this study were 100% sensitive to the antibiotics gentamicin (10 µg) and ciprofloxacin (5 µg).

Regarding bacterial resistance in this study, the highest resistance record was observed against the antibiotic cefoxitin (30 µg), with both *C. freundii* and *P. agglomerans* being the most resistant. Resistance was observed in 5 samples of *C. freundii* and 4 samples of *P. agglomerans* out of 11 samples. Resistance was also noted in a few isolates to antibiotics such as azithromycin (15 µg), ceftriaxone (30 µg), and ampicillin (10 µg, *Enterobacter cloacae*, *Serratia liquifaciens*, and *Proteus mirabilis*). Table 3 shows the result of the multiple antibiotic resistance (MAR) index in which the calculation reveals indicators of multiple antibiotic resistance. The highest recorded indicator was demonstrated by bacteria *C. freundii*, *P. agglomerans*, and *En. cloacae*, with a value of 0.33 for each of them.

Table 2. Number and bacteria percentage regarding the antibiotics susceptibility tests in identified bacteria isolated from feces of Slender-billed gull species in Lybia

Antibiotic	Antibiotic Code	Sensitive	Intermediate	Resistant	
				No. and (percentage) of bacteria	Bacterial species
Gentamicin	CN	32 (100%)	0	0	None
Ciprofloxacin	CIP	32 (100%)	0	0	None
Azithromycin	AZM	27 (84.4%)	3 (9.4%)	2 (6.3%)	*Citrobacter freundii*
Ceftriaxone	CRO	27 (84.4%)	4 (12.5%)	1 (3.1%)	*Enterobacter cloacae*
Ampicillin	AMP	27 (84.4%)	3 (9.4%)	2 (6.3%)	*Pantoea agglomerans* *Proteus mirabilis*
Cefoxitin	FOX	18 (56.3%)	3 (9.4%)	11 (43.4%)	*Citrobacter freundii* *Pantoea agglomerans* *Enterobacter cloacae* *Serratia liquifaciens*

No: Number.

Table 3. Multiple antibiotic resistance index in identified bacteria isolated from feces of Slender-billed gull species in Lybia

No.	Bacterial species	Resistance to antibiotics	MAR Index
1	*Citrobacter freundii*	AZM, FOX	0.33
2	*Enterobacter agglomerans*	AMP, FOX	0.33
3	*Enterobacter cloacae*	CRO, FOX	0.33
4	*Serratia liquifaciens*	FOX	0.17
5	*Proteus mirabilis*	AMP	0.17

AZM: Azithromycin (15 µg), FOX: Cefoxitin (30 µg), AMP: Ampicillin (10 µg), CRO: Ceftriaxone (30 µg), MAR: Multiple antibiotic resistance

DISCUSSION

In the current study, a total of 46 bacteria were isolated from fecal samples of slender-billed gull birds, and only 32 of them were identified using biochemical tests namely, the Enterosystem 18R, consisting of 20 tests, was employed for the identification of enteropathogenic bacterial species. Previous studies that utilized this system for identifying enteropathogenic bacterial species have shown success rates of at least 90% (Piccolomini et al., 1991; Bissong et al., 2017).

In this study, 17 bacteria of the *C. freundii* species were isolated, accounting for 53.125% of the total isolates. This finding aligns with a study conducted in France (Vittecoq et al., 2022), as well as studies in Egypt (Nabil et al., 2020) and Portugal (Fournier et al., 2022). However, it differs from the results of studies conducted in Italy (Russo et al., 2021) and South America (Liakopoulos et al., 2016), where the prevalence of these species was at a low level. *C. freundii* is known to play a significant role in opportunistic infections and is associated with neonatal meningitis, where mortality rates in neonates can reach 25-50% (Badger et al., 1999). It also causes urinary tract and respiratory infections in humans (Wanger et al., 2017). *C. freundii* was also isolated from diseased domestic ducks in Bangladesh (Ahmed et al., 2023), in which the isolated bacteria showed multiple drug resistance to some antibiotics such as gentamicin and ciprofloxacin and the index of multiple antibiotic resistance ranged from 0.07 to 0.79.

Ten (31.25%) *P. agglomerans* bacteria were isolated from slender-billed gull in the current study, which is a gram-negative aerobic *bacillus* and a member of the family Enterobacteriaceae. The bacterial genus *Pantoea* can be isolated from the environment including indoor dusts of animal sheds, plants, and soil (Andersson et al., 1999; Monier and Lindow, 2005). This bacterium can be either pathogen or commensal causing secondary infections. The *P. agglomerans* is the most common species of the genus *Pantoea* isolated from humans, which may cause soft tissue or bone/joint infections following penetrating trauma by vegetation (Ulloa-Gutierrez et al., 2004). Cruz et al. (2007) reported the isolation of *P. agglomerans* from the urine and urinary tract of 4 children. The *P. agglomerans* was most associated with penetrating trauma by vegetative material and catheter-related bacteremia (Cruz et al., 2007). In a study conducted by Giorgio et al. (2018), *P. agglomerans* was isolated from *Muscicapa striata* migratory birds. However, there is a lack of information on the isolation of *P. agglomerans* from cloacal swabs or fecal samples of slender-billed gull species.

In the current study, two isolates (6.25%) of *E. coli* were identified. A recent study conducted in Poland on strains of wild birds (including Mallards, white-tailed eagles, common buzzards, Eurasian sparrow hawks, Eurasian tawny owls, mute swans, little bitterns, little owls, short-eared owl, great spotted woodpecker, lesser spotted woodpecker, European green woodpecker, bohemian waxwing, western capercaillie, grey heron, and Eurasian golden oriole) yielded important results, as *E. coli* bacteria were isolated from 32 samples out of 34, and the results reached were as follows including resistance to tetracycline (50%), ciprofloxacin (46.8%), gentamicin (34.3%), and ampicillin (28.1%) was frequently demonstrated, and approximately 31.2% of *E. coli* showed a multidrug resistance phenotype (Nowaczek et al., 2021). In Bangladesh, *E. coli* were isolated and identified from a total of 66 fecal matter samples from migratory birds (Islam et al., 2021). The diseases caused by *E. coli* in humans include urinary tract infections, hospital-acquired pneumonia, gastrointestinal infection, meningitis, and sepsis (Sarowska et al., 2019). However, migratory birds were found as reservoirs of multi-drug resistant (MDR) *E. coli* isolates that can carry virulence genes of avian pathogenic *E. coli* (APEC-associated), which can contribute to developing human and animal diseases (Islam et al., 2021).

Enterobacter cloacae was also isolated and identified in the current study. It is a Gram-negative bacterium, that

can be aerobic or anaerobic, and under the microscope, they are rod-shaped with rounded ends (Buckle, 2016). It is a common pathogen in hospitals, capable of producing a variety of infections, such as pneumonia, urinary tract infections, and septicemia (Annavajhala et al., 2019). The *En. cloacae* have shown resistance to multiple drugs, such as aminoglycosides, fluoroquinolones, third-generation cephalosporins, and carbapenems (Liu et al., 2021). In a study conducted on common wild birds in Europe, bacterial species, including *En. cloacae*, showed significant frequent resistance to antibiotics, and multiple resistance to three or more groups of antibiotics (Giacopello et al., 2016).

Serratia liquifaciens and *Proteus mirabilis* were also isolated and identified. *S. liquifaciens* is a bacillus bacterium with rounded ends, Gram-negative, facultatively anaerobic, motile, and positive for the catalase test. One of the common types of infections they cause is blood-borne infections caused by contaminated red blood cells (Harvey et al., 2015). In a study conducted in Egypt on 20 quails, the results showed the presence of many types of intestinal bacteria, including *S. liquefaciens*, at a prevalence level of 2.3% (Othman et al., 2023). *Proteus mirabilis* is a Gram-negative intestinal bacterium, a motile bacillus, positive for the urease test, negative for lactose and indole, and produces hydrogen sulfide (Schaffer and Pearson, 2015). It is the second most common cause of urinary tract infections after *E. coli*, especially in patients with kidney stones (Mo et al., 2022). In a study conducted in the Messina region in Italy on common European wild birds, 83 strains of intestinal bacteria were isolated, including the genus *Proteus mirabilis*. The isolates showed frequent antibiotic resistance, and multiple resistance to three or more groups of antibiotics (Giacopello et al., 2016). Machado et al. (2018) have studied free-living grey-breasted parakeets and isolated many bacteria of the genera *Escherichia*, *Proteus*, *Citrobacter*, *Pantoea*, *Klebsiella*, *Enterobacter*, *Morganella*, *Hafnia*, *Enterobacter*, and *Serratia*in which the most common isolated bacteria were *E. coli*, *Proteus mirabilis, and Proteus vulgaris*, with percentages of 36.1%, 26.4%, and 8.3%, respectively. They found that these bacteria were resistant to azithromycin and tetracycline, whereas *E. coli* was presenting multidrug resistance.

Isolated bacterial species in this study were 100% sensitive to gentamicin and ciprofloxacin. Gentamicin injections are used to treat severe bacterial infections such as meningitis, bloodstream infections, abdominal infections, pneumonia, skin and bone infections, joint infections, and urinary tract infections in humans (Chaves and Tadi, 2023). Ciprofloxacin is a well-known broad-spectrum antibiotic that is used to treat many bacterial infections, such as uncomplicated urinary tract infections, respiratory infections (including pneumonia), skin infections, and bone infections (Thai et al., 2023). These results provide some reassurance and are consistent with a study by Young et al. (2018), that suggested a low gentamicin consumption is associated with a low resistance level, emphasizing the need for national antibiotic rotation strategies since antibiotic susceptibility test is not routinely used and broad-spectrum antibiotics are being prescribed. However, a study in Catalonia on a group of wild birds revealed that *C. freundii* bacteria exhibited multidrug resistance, including resistance to fluoroquinolones, tetracyclines, sulfonamides, and aminoglycosides, including gentamicin (Darwich et al., 2019).

Regarding bacterial resistance in this study, the highest resistance record was observed against the antibiotic cefoxitin, with both *C. freundii* and *P. agglomerans* being the most resistant. Resistance was observed in 5 samples of *C. freundii* and 4 samples of *P. agglomerans* out of 11 samples. Resistance was also noted in a few isolates to antibiotics such as azithromycin, ceftriaxone, and ampicillin. The results indicate that *C. freundii* was the most antibiotic-resistant bacterial species isolated in this study. It is known that *C. freundii* is capable of transferring antibiotic resistance genes between its strains, and studies suggest that the acquisition of resistance genes, such as beta-lactamase genes or sul1 and sul2 genes, from external sources, such as the environment or other bacteria, can lead to resistance to multiple drugs (Ahmed et al., 2023). The *P. agglomerans* ranked second in antibiotic resistance in the current study. This bacterium can carry multiple resistance genes on its plasmids, including ESBL genes (Raphael and Riley, 2017). These bacteria are associated with plants and are not a common human pathogen. However, they can cause opportunistic infections resulting from injuries from plant materials or as healthcare-acquired infections, mainly affecting individuals with compromised immune systems (Dutkiewicz et al., 2016). A study conducted in Spain on wild birds showed that all the strains isolated, including *P. agglomerans*, exhibited resistance to at least one of the antibiotics used (Tardón et al., 2021).

Calculation of the multiple antibiotic resistance (MAR) index revealed that the highest recorded indicator was demonstrated by bacteria *C. freundii*, *P. agglomerans*, and *En. cloacae*, with a value of 0.33 for each of them. The acquisition of antibiotic resistance among bacterial

species is a possible occurrence, and it happens through various methods, including vertical and horizontal gene transfer. However, the conjugative transfer of plasmids carrying resistance genes among bacterial species is considered one of the most important mechanisms for resistance transfer in bacteria (Tao et al., 2022). There are many mechanisms that bacteria may develop in order to resist antibiotics including changes in drug targets, prevention of cell entry, elimination through efflux pumps, or drug inactivation. In order to select the most effective antibiotics to treat multidrug-resistant bacteria would be to understand and predict the patterns of resistance (Chiș et al., 2022).

Libya is characterized by diverse natural landscapes that lead to a wide variety of ecosystems. Based on this, it has been classified into two environmental regions including a northern region consisting of two parts (the coastal plain and mountainous regions in the north, and the central region, which is a pre-desert area); and a southern region representing the desert with some oases and mountains (Bundy, 1976; Isenmann et al., 2016). Most of these areas host migratory birds in varying numbers, especially the areas along the Mediterranean Sea coast, where the diversity of wetlands, water bodies, and the Mediterranean climate create favorable conditions that attract migratory birds from Europe to Africa in the early winter and vice versa when they return to their habitats usually in the spring (Lehikoinen et al., 2019).

The team responsible for monitoring and census of migratory birds, affiliated with the Department of Zoology at the Faculty of Science at the University of Tripoli, Libya, conducts annual monitoring of migratory bird species in collaboration with the Libyan Bird Society. In the winter of 2012, a total of 29,314 birds belonging to 69 species of water birds were counted. Relatively, the number of sites surveyed in 2012 was fewer than in previous survey years. Most of the birds belonged to seven species of gulls (Etayeb et al., 2012). In March 2014, the team monitored and counted birds in the navigation area, which is a salt marsh by a channel from the sea throughout the year and is characterized by rainfall during the winter. This area was classified as nationally important for birds such as the Black-winged Stilt, Great Cormorant, Dunlin, Greater Flamingo, Shoveler, and Teal. The monitoring and census resulted in the observation of 47 species, with a total of 1.966 birds of all species recorded during this study (Benyezza et al., 2017). The winter census of migratory birds in Libya for the year 2022 showed the presence of 2.169 birds of the Slender-billed Gull species (Etayeb et al., 2023).

CONCLUSION

In light of these results, Slender-billed gulls play a role in the spread of potentially pathogenic and antibiotic-resistant agents. Libyan citizens may be at risk of antibiotic-resistant bacteria through direct contact, especially during migratory wild bird hunting seasons. Resistance can also be transferred to domestic wild birds and birds raised for commercial purposes. The study recommends the implementation of a national program to survey antibiotic-resistant bacteria, determine their prevalence, and assess the presence of antibiotic residues in animal-derived food, ensuring they comply with acceptable levels. Furthermore, the study advises expanding scientific studies on risk factors and possible antibiotic alternatives used in wild and commercial birds.

DECLARATION

Funding

This study received no funding.

Acknowledgments

The authors expressed extreme thanks to the members of the Libyan Society for Birds and the staff of Bisida Society for the protection of Farwa Island and Lagoon for their support during the fieldwork.

Authors' contributions

Abdulmajid Hamhoom, Ehab Sharif, Ibrahim Eldaghayes, Khaled Etayeb, and Abdulnaser Dayhum contributed to the sampling, data collection, and revision of the manuscript. Aya Mansour and Abdulwahab Kammon did the laboratory analysis and writing up the manuscript. All authors read and approved the final edition of the manuscript.

Availability of data and materials

All data are available in the manuscript. Any extra data needed can be provided by the corresponding author upon reasonable request.

Competing interests

The authors have declared that there are no competing interests in this study.

Ethical considerations

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

REFERENCES

Ahmed T, Islam MS, Haider N, Elton L, Hasan B, Nuruzzaman M, Rahman MT, Kabir SML, and Khan MSR (2023). Phenotypic and genotypic characteristics of antimicrobial resistance in citrobacter freundii isolated from domestic ducks (Anas platyrhynchos domesticus) in Bangladesh. Antibiotics, 12(4): 769. DOI: https://www.doi.org/10.3390/antibiotics12040769

Andersson AM, Weiss N, Rainey F, and Salkinoja-Salonen MS (1999). Dust-borne bacteria in animal sheds, schools and children's day care centres. Journal of Applied Microbiology, 86(4): 622-34. DOI: https://www.doi.org/10.1046/j.1365-2672.1999.00706.x

Annavajhala MK, Gomez-Simmonds A, and Uhlemann AC (2019). Multidrug-resistant *Enterobacter cloacae* complex emerging as a global, diversifying threat. Frontiers in Microbiology, 10: 44. DOI: https://www.doi.org/10.3389/fmicb.2019.00044

Badger JL, Stins MF, and Sik Kim K (1999). Citrobacter freundii invades and replicates in human brain microvascular endothelial cells. Infection and Immunity, 67(8): 4208-4215. DOI: https://www.doi.org/10.1128/iai.67.8.4208-4215.1999

Bauer AW, Kirby WM, Sherris JC, and Turck M (1966). Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology, 45(4): 493-496. DOI: https://www.doi.org/10.1093/ajcp/45.4_ts.493

Benyezza E, Shanan T, Berbash A and Etayeb K (2017). The diversity of aquatic birds and breeding of some species in Al-Mallaha, Tripoli. Vogelwelt, 137: 143-148. Available at: https://uot.edu.ly/downloadpublication.php?file=b8EHiSXY11221679955488_pub.pdf

Bissong MEA, Mbah C, Tatsing Foka F, and Kamga HL (2017). Spectrum of uropathogens and antimicrobial susceptibility in clinically diagnosed cases of urinary tract infection in the Bamenda regional hospital, Cameroon. American Journal of Health Research, 5(2): 19-24. DOI: https://www.doi.org/10.11648/j.ajhr.20170502.11

Bonnedahl J and Järhult JD (2014). Antibiotic resistance in wild birds. Upsala Journal of Medical Sciences, 119(2): 113-6. DOI: https://www.doi.org/10.3109/03009734.2014.905663

Brenner DJ, Krieg NR, Staley JT, and Garrity GM (2005). Bergey's manual of systematic bacteriology, 2nd Edition. Vol. II. The Proteobacteria, part C, Springer., New York. Available at: https://link.springer.com/book/10.1007/0-387-29298-5

Buckle J (2016). Clinical aromatherapy: Essential oils in healthcare, 3rd Edition. Elsevier., Amsterdam, The Netherlands. Available at: https://shop.elsevier.com/books/clinical-aromatherapy/buckle/978-0-7020-5440-2

Bundy G (1976). The birds of Libya: An annotated check-list. BOU check-list No. 1. London, UK, British Ornithologists' Union. Available at: https://www.abebooks.com/first-edition/BIRDS-LIBYA-annotated-check-list-Bundy-Graham/31649468553/bd

Chaves BJ and Tadi P (2023). Gentamicin. StatPearls Publishing., Treasure Island (FL). Available at: https://www.ncbi.nlm.nih.gov/books/NBK557550/

Chiș AA, Rus LL, Morgovan C, Arseniu AM, Frum A, Vonica-Țincu AL, Gligor FG, Mureșan ML, and Dobrea CM (2022). Microbial resistance to antibiotics and effective antibiotherapy. Biomedicines, 10(5): 1121. DOI: https://www.doi.org/10.3390/biomedicines10051121

Cruz AT, Cazacu AC, and Allen CH (2007). Pantoea agglomerans, a plant pathogen causing human disease. Journal of Clinical Microbiology, 45(6): 1989-1992. DOI: https://www.doi.org/10.1128/JCM.00632-07

Darwich L, Vidal A, Seminati C, Albamonte A, Casado A, López F, Molina-López RA, and Migura-Garcia L (2019). High prevalence and diversity of extended-spectrum β-lactamase and emergence of OXA-48 producing *Enterobacterales* in wildlife in Catalonia. PLoS One, 14(8): e0210686. DOI: https://www.doi.org/10.1371/journal.pone.0210686

Dutkiewicz J, Mackiewicz B, Lemieszek MK, Golec M, and Milanowski J (2016). Pantoea agglomerans: A mysterious bacterium of evil and good. Part IV. Beneficial effects. Annals of Agricultural and Environmental Medicine, 23(2): 206-222. DOI: https://www.doi.org/10.5604/12321966.1203879

Elsohaby I, Samy A, Elmoslemany A, Alorabi M, Alkafafy M, Aldoweriej A, Al-Marri T, Elbehiry A, and Fayez M (2021). Migratory wild birds as a potential disseminator of antimicrobial-resistant bacteria around Al-Asfar Lake, Eastern Saudi Arabia. Antibiotics, 10(3): 260. DOI: https://www.doi.org/10.3390/antibiotics10030260

Etayeb K, Galidana A, Berbash A, Eisa A, Al-Kordi A, Al-Helali E, Abuhajar M, Alswyeb A, Abdulqader H, Azabi N et al. (2023). Results of the eighteenth winter waterbird census in Libya in 2022. Open Veterinary Journal, 13(4): 407-418. DOI: https://www.doi.org/10.5455/OVJ.2023.v13.i4.2

Fournier C, Poirel L, Despont S, Kessler J, and Nordmann P (2022). Increasing trends of association of 16S rRNA methylases and carbapenemases in *Enterobacterales* clinical isolates from Switzerland, 2017-2020. Microorganisms, 10(3): 615. DOI: https://www.doi.org/10.3390/microorganisms10030615

Harvey AR, Basavaraju SV, Chung KW, and Kuehnert MJ (2015). Transfusion-related adverse reactions reported to the National Healthcare Safety Network Hemovigilance Module, United States, 2010 to 2012. Transfusion, 55(4): 709-718. DOI: https://www.doi.org/10.1111/trf.12918

Georgopoulou I and Tsiouris V (2008). The potential role of migratory birds in the transmission of zoonoses. Veterinaria Italiana, 44(4): 671-677. Available at: https://pubmed.ncbi.nlm.nih.gov/20411494/

Giacopello C, Foti M, Mascetti A, Grosso F, Ricciardi D, Fisichella V, and Lo Piccolo F (2016). Antimicrobial resistance patterns of Enterobacteriaceae in European wild bird species admitted in a wildlife rescue center. Veterinaria Italiana, 52(2): 139-144. DOI: https://www.doi.org/10.12834/VetIt.327.1374.2

Giorgio A, De Bonis S, Balestrieri R, Rossi G, and Guida M (2018). The isolation and identification of bacteria on feathers of migratory bird species. Microorganisms, 6(4): 124. DOI: https://www.doi.org/10.3390/microorganisms6040124

Isenmann P, Hering J, Brehme S, Essghaier M, Etayeb K, Bourass E, and Azafzaf H (2016). Oiseaux de Libye - birds of Libya. SEOF, 302 pp. Available at: https://www.nhbs.com/birds-of-libya-oiseaux-de-libye-book

Islam MS, Nayeem MMH, Sobur MA, Ievy S, Islam MA, Rahman S, Kafi MA, Ashour HM, and Rahman MT (2021). Virulence determinants and multidrug resistance of *Escherichia coli* isolated from migratory birds. Antibiotics, 10(2): 190. DOI: https://www.doi.org/10.3390/antibiotics10020190

Krumperman PH (1983). Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. Applied Environmental Microbiology, 46(1): 165-170. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC239283/

Lehikoinen A, Lindén A, Karlsson M, Andersson A, Crewe T, Dunn E, Gregory G, Karlsson L, Kristiansen V, Mackenzie S et al. (2019). Phenology of the avian spring migratory passage in Europe and North America: Asymmetric advancement in time and increase in duration. Ecological Indicators, 101: 985-991. DOI: https://www.doi.org/10.1016/j.ecolind.2019.01.083

Liakopoulos A, Olsen B, Geurts Y, Artursson K, Berg C, Mevius DJ, and Bonnedahl J (2016). Molecular characterization of extended-spectrum-cephalosporin-resistant Enterobacteriaceae from wild kelp gulls in South America. Antimicrobial Agents and Chemotherapy, 60(11): 6924-6927. DOI: https://www.doi.org/10.1128/aac.01120-16

Liu S, Huang N, Zhou C, Lin Y, Zhang Y, Wang L, Zheng X, Zhou T, and Wang Z (2021). Molecular mechanisms and epidemiology of carbapenem-resistant Enterobacter cloacae complex isolated from Chinese patients during 2004-2018. Infection and Drug Resistance, 2021: 3647-3658. DOI: https://www.doi.org/10.2147/IDR.S327595

Machado DN, Lopes ES, Albuquerque AH, Horn RV, Bezerra WGA, Siqueira RAS, Lopes IT, Nunes FP, Teixeira RSC, and Cardoso WM (2018). Isolation and antimicrobial resistance profiles of Enterobacteria from nestling grey-breasted parakeets (Pyrrhura Griseipectus). Brazilian Journal of Poultry Science, 20(1): 103-110. DOI: https://www.doi.org/10.1590/1806-9061-2017-0551

Martín-Maldonado B, Rodríguez-Alcázar P, Fernández-Novo A, González F, Pastor N, López I, Suárez L, Moraleda V, and Aranaz A (2022). Urban birds as antimicrobial resistance sentinels: White storks showed higher multidrug-resistant *Escherichia coli* levels than seagulls in Central Spain. Animals, 12(19): 2714. DOI: https://www.doi.org/10.3390/ani12192714

Mo L, Wang J, Qian J, and Peng M (2022). Antibiotic sensitivity of proteus mirabilis urinary tract infection in patients with urinary calculi. International Journal of Clinical Practice, 2022: 7273627. DOI: https://www.doi.org/10.1155/2022/7273627

Monier JM and Lindow SE (2005). Aggregates of resident bacteria facilitate survival of immigrant bacteria on leaf surfaces. Microbial Ecology, 49(3): 343-352. DOI: https://www.doi.org10.1007/s00248-004-0007-9

Nabil NM, Erfan AM, Tawakol MM, Haggag NM, Naguib MM, and Samy A (2020). Wild birds in live birds markets: Potential reservoirs of enzootic avian influenza viruses and antimicrobial resistant enterobacteriaceae in northern Egypt. Pathogens, 9(3): 196. DOI: https://www.doi.org/10.3390/pathogens9030196

Nowaczek A, Dec M, Stępień-Pyśniak D, Urban-Chmiel R, Marek A, and Różański P (2021). Antibiotic resistance and virulence profiles of *Escherichia coli* strains isolated from wild birds in Poland. Pathogens, 10(8): 1059. DOI: https://www.doi.org/10.3390/pathogens10081059

Othman B, Talat D, and Ibrahim M (2023). Individual samples from quail harboring diverse bacterial populations and different serotypes of *Escherichia coli*. Damanhour Journal of Veterinary Sciences, 10(1): 17-24. DOI: https://www.doi.org/10.5455/djvs.2022.125360.1067

Piccolomini R, Di Girolamo A, Catamo G, Cellini L, Allocati N, and Ravagnan G (1991). Enterosistem 18-R: Description and comparative evaluation with conventional methods for identification of members of the family Enterobacteriaceae. Journal of Clinical Microbiology, 29(10): 2300-2304. DOI: https://www.doi.org/10.1128/jcm.29.10.2300-2304.1991

Raphael E and Riley LW (2017). Infections caused by antimicrobial drug-resistant saprophytic Gram-negative bacteria in the environment. Frontiers in Medicine, 4: 183. DOI: https://www.doi.org/10.3389/fmed.2017.00183

Russo TP, Pace A, Varriale L, Borrelli L, Gargiulo A, Pompameo M, Fioretti A, and Dipineto L (2021). Prevalence and antimicrobial resistance of enteropathogenic bacteria in yellow-legged gulls (*Larus michahellis*) in Southern Italy. Animals, 11(2): 275. DOI: https://www.doi.org/10.3390/ani11020275

Sarowska J, Futoma-Koloch B, Jama-Kmiecik A, Frej-Madrzak, Ksiazczyk M, Bugla-Ploskonska B, and Choroszy-Krol I (2019). Virulence factors, prevalence and potential transmission of extraintestinal pathogenic *Escherichia coli* isolated from different sources: Recent reports. Gut Pathogens, 11: 10. DOI: https://www.doi.org/10.1186/s13099-019-0290-0

Schaffer JN and Pearson MM (2015). Proteus mirabilis and urinary tract infections. In: M. A. Mulvey, D. J. Klumpp, A. E. Stapleton (Editors), Urinary tract infections: Molecular pathogenesis and clinical management, 2nd Edition. pp. 1-39. DOI: https://doi.org/10.1128/microbiolspec.uti-0017-2013

Tao S, Chen H, Li N, Wang T, and Liang W (2022). The spread of antibiotic resistance genes *in vivo* model. Canadian Journal of Infectious Diseases and Medical Microbiology, 2022: 3348695. DOI: https://www.doi.org/10.1155/2022/3348695

Tardón A, Bataller E, Llobat L, and Jiménez-Trigos E (2021). Bacteria and antibiotic resistance detection in fractures of wild birds from wildlife rehabilitation centres in Spain. Comparative Immunology, Microbiology and Infectious Diseases, 74: 101575. DOI: https://www.doi.org/10.1016/j.cimid.2020.101575

Thai T, Salisbury BH, Zito PM (2023). StatPearls. StatPearls Publishing., Treasure Island (FL). Available at: https://www.ncbi.nlm.nih.gov/books/NBK535454/

Tilahun M, Kassa Y, Gedefie A, and Ashagire M (2021). Emerging carbapenem-resistant enterobacteriaceae infection, its epidemiology and novel treatment options: A review. Infection and Drug Resistance, 14: 4363-4374. DOI: https://www.doi.org/10.2147/IDR.S337611

Ulloa-Gutierrez R, Moya T, and Avila-Aguero ML (2004). Pantoea agglomerans and thorn-associated suppurative arthritis. Pediatric Infectious Disease Journal, 23(7): 690. DOI: https://www.doi.org/10.1097/00006454-200407000-00025

Vittecoq M, Brazier L, Elguero E, Bravo IG, Renaud N, Manzano-Marín A, Prugnolle F, Godreuil S, Blanchon T, Roux F et al. (2022). Multiresistant Enterobacteriaceae in yellow-legged gull chicks in their first weeks of life. Ecology and Evolution, 12(6): e8974. DOI: https://www.doi.org/10.1002/ece3.8974

Wanger A, Chavez V, Huang R, Wahed A, Dasgupta A, and Actor JK (2017). Microbiology and molecular diagnosis in pathology: A comprehensive review for board preparation, certification and clinical practice, 1st Edition. Elsevier Science Publishing Co. Inc., USA. Available at: https://shop.elsevier.com/books/microbiology-and-molecular-diagnosis-in-pathology/wanger/978-0-12-805351-5

Young Ah Kim, Yoon Soo Park, Taemi Youk, Hyukmin Lee, and Kyungwon Lee (2018). Correlation of aminoglycoside consumption and amikacin- or gentamicin-resistant pseudomonas aeruginosa in long-term nationwide analysis: Is antibiotic cycling an effective policy for reducing antimicrobial resistance?. Annals of Laboratory Medicine, 38(2): 176-178. DOI: https://www.doi.org/10.3343/alm.2018.38.2.176

Zaitsev SS, Khizhnyakova MA, and Feodorova VA (2022). First case report of detection of multidrugresistant enterobacter hormaechei in clinical sample from an aborted ruminant. Microorganisms, 10(5): 1036. DOI: https://www.doi.org/10.3390/microorganisms10051036

Zeballos-Gross D, Rojas-Sereno Z, Salgado-Caxito M, Poeta P, Torres C, and Benavides JA (2021). The role of gulls as reservoirs of antibiotic resistance in aquatic environments: A scoping review. Front Microbiology, 12: 703886. DOI: https://www.doi.org10.3389/fmicb.2021.703886

JWPR
Journal of World's Poultry Research
2024, Scienceline Publication
J. World Poult. Res. 14(2): 204-210, 2024
Research Paper
DOI: https://dx.doi.org/10.36380/jwpr.2024.21
PII: S2322455X2300021-13
ISSN: 2322-455X

Pharmacokinetic Characteristics of Diclazuril in Japanese Quails (*Coturnix japonica*) and Domestic Pigeons (*Columba livia*)

Sara T. Elazab [1,*], Iqra Zafar[2,3], and Nahla S. Elshater[4]

[1]*Department of Pharmacology, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt*
[2] *National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan*
[3]*Livestock and Dairy Development Department, Veterinary Research Institute, Lahore, Pakistan*
[4]*Animal Health Research Institute, Agriculture Research Center, Giza- Dokki, 12618, Egypt*

Received: *March 12, 2024, Revised: April 21, 2024, Accepted: May 23, 2024, Published: June 30, 2024*

ABSTRACT

Coccidiosis, caused by the protozoan *Eimeria*, is a significant disease in poultry farms worldwide, leading to substantial economic losses. Triazines, benzene-aceto-nitrile derivatives, are widely employed in the field of veterinary healthcare to combat the hazardous impacts of protozoan parasite infestation comprising coccidiosis. The current research was designed to investigate the pharmacokinetic profile of diclazuril, a member of triazines, in Japanese quails (*Coturnix japonica*) and domestic pigeons (*Columba livia*) following single oral administration at 0.3 mg/kg body weight. 78 Quails (male: female, 1:1, 7 weeks old) and 78 pigeons (male: female, 1:1, 4 weeks old) were randomly divided into 13 groups for each species (n=6 birds/ group). Plasma samples were obtained at various time intervals (at time 0 [preceding diclazuril administration], and 0.5, 1, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours after diclazuril administration) to determine its concentration utilizing high-performance liquid chromatography (HPLC). The non-compartmental approach was applied to assess the pharmacokinetic parameters via the aid of WinNonlin 8.3 software. In quails and pigeons, the peak plasma concentrations were 5.35 and 9.14 µg/mL attained at 8 hours, respectively. Additionally, the elimination half-lives ($T_{1/2\lambda z}$) were 30.74 and 26.48 hours, and the area under the plasma concentration-time curve from time zero to the last sample ($AUC_{0\text{-last}}$) values were 155.67 and 343.57 µg h/mL, respectively. The mean residence time was 30.71 hours in quails and 39.68 hours in pigeons. Diclazuril exhibited favorable pharmacokinetic characteristics after oral administration at a dose of 0.3 mg/kg in quails and pigeons. However, to adjust the dosage regimen for curing coccidiosis, a future study is warranted to determine the clinical efficacy against coccidia infection. Moreover, further investigation is needed to evaluate the tissue residues and calculate the withdrawal time of diclazuril in quails and pigeons.

Keywords: Diclazuril, High-performance liquid chromatography, Japanese quail, Pharmacokinetic, Pigeon

INTRODUCTION

Avian coccidiosis, a parasitic disease caused by apicomplexan protozoan of the genus *Eimeria*, is one of the most serious diseases affecting poultry, causing significant economic losses worldwide (Blake and Tomely, 2014). It causes massive injury in the intestinal epithelial lining of the bird, resulting in impaired feed conversion and growth retardation. The clinical signs of avian coccidiosis may be hidden or manifested by weakness, diarrhea, the presence of blood or mucus in the feces, loss of appetite, reduced egg production, and increased mortality (Chapman, 2003; Christaki et al., 2004). The principal approach for controlling coccidiosis, besides rigorous hygiene and biosecurity techniques, is via the administration of the appropriate dose of anticoccidial therapy (Kadykalo et al., 2018). Triazines are extensively utilized in the veterinary field to combat the deleterious effects of protozoan parasites including coccidiosis (Stock et al., 2018).

To cite this paper: Elazab ST, Zafar I, and Elshater NS (2024). Pharmacokinetic Characteristics of Diclazuril in Japanese Quails (*Coturnix japonica*) and Domestic Pigeons (*Columba livia*). *J. World Poult. Res.*, 14(2): 204-210. DOI: https://dx.doi.org/10.36380/jwpr.2024.21

Diclazuril (2,6-dichloro-a-[4-chlorophenyl]-4-[4,5-dihydro-3,5-dioxo-1,2,4-triazin-2{3H}yl] benzeneacetonitrile), belongs to triazine family, is a chemical compound derived from the benzeneacetonitrile class that is developed successfully as an anticoccidial remedy for sheep, poultry, and rabbits (Hu et al., 2009). This compound exhibited a potent action against all pathogenic *Eimeria* species affecting poultry (Conway et al., 2002, Gadelhaq et al., 2017). Although the actual mechanism of the antiprotozoal action has not been fully elucidated yet, diclazuril has been claimed to perform its anticoccidial activity by attacking the sexual and asexual stages of *Eimeria* (Zhou et al., 2010; Wang et al., 2013, El-Ashram et al., 2019). Moreover, prior researchers have indicated that diclazuril may accomplish its anticoccidial action by suppressing serine/ threonine protein phosphatase type 5 expression (Zhou et al., 2013).

The pharmacokinetics of diclazuril have been described in several species such as cattle (Dirikolu et al., 2022), horses (Pusterla et al., 2023), rabbits (Hu et al., 2009), and chickens (Mortier et al., 2005; Zhang et al., 2020). Nevertheless, so far as the authors know, the pharmacokinetic features of diclazuril in quails and pigeons have not been studied and documented yet. Therefore, the purpose of the present study was to assess the pharmacokinetic behavior of diclazuril in Japanese quails (Coturnix japonica) and domestic pigeons (*Columba livia*) after single oral administration.

MATERIALS AND METHODS

Ethical approval

All procedures incorporating birds were reviewed and approved by the Research Ethics Committee of the Faculty of Veterinary Medicine, Mansoura University, Egypt (Approval No. R/139).

Chemicals

In this experiment, diclazuril (0.5% solution, Shandong Luxi Animal Medicine Share Co., Shandong, China) was obtained. The diclazuril standard, N, N-dimethylformamide (DMF), and tetrabutylammonium hydrogen sulfate were supplied from Sigma Aldrich Co. (St. Louis, MO. USA). Hexane, Acetonitrile, and methanol were bought from Thermo Fisher Scientific (Waltham, MA, USA). Acetic acid was provided by Merck (Darmstadt, Germany). All chemicals utilized in this work were of high-performance liquid chromatography analytical grade. The Milli–Q system (Waters Corp., Milford, MA. USA) was employed to obtain Purified water.

Animals

Quails

Seventy-eight clinically healthy adult Japanese quails (Coturnix japonica, male: female, 1:1), weighing 180 ± 10 g, were obtained from the Faculty of Agriculture, Mansoura University, Egypt. They were allotted into 13 groups (n= 6 birds/group/cage) and were offered medication-free ration and had unrestricted access to water. The quails underwent a 14-day acclimatization period before the initiation of the trial.

Pigeons

Seventy-eight adult healthy pigeons (*Columba livia*, 250 ± 10 g, male: female, 1:1) were procured from a pigeon farm (Dakahlia Governorate, Egypt) and were enrolled into 13 groups (n=6/group) and were kept in cages (one group (6 birds/cage). Medication-free diet and water were supplied during the study. A period of two weeks was considered for the pigeons to adapt to their surroundings before the commencement of the investigation

Experimental design

Quails and pigeons were divided into 13 groups for each species (n=6). All quails and pigeons received a single oral dose of 0.3 mg diclazuril/kg body weight (EPMAR, 2013; Said et al., 2019) directly into the crop employing a 1-cc, 26 G syringe. Each bird was subjected to blood sampling only once (the amount of blood sample was not more than 1% of body weight). According to the method of Turk et al. (2021), blood samples from various groups were withdrawn from the right brachial vein (1mL from each bird) using an insulin syringe (a 26-gage,½-inch needle) at time 0 (preceding diclazuril administration), and 0.5, 1, 4, 8, 12, 24, 48, 72, 96, 120, 144, 168 h post drug administration (n = 6 birds of each species/time point, (Hunyadi et al., 2015; Zhang et al., 2020). After centrifugation of blood samples at 2000 x g for 15 minutes, plasma was preserved at −20 ˚C for further investigation.

Analysis of diclazuril in plasma samples

Standards and plasma specimen preparation

A solution of the diclazuril reference standard in DMF was prepared (1mg/ml). Then, it was diluted utilizing blank plasma collected either from quails and pigeons as a diluent to prepare diclazuril calibration

standards at concentrations of 0.025,0.05, 0.1, 0.25, 0.5, 1, 2.5, 5, 10 µg/mL.

Plasma samples were prepared as reported by Dirikolu et al. (1999). Briefly, the solid phase extraction (SPE) column (Bond Elut C18, 500 mg, 3 or 6 ml; Varian) was treated with 2 ml methanol followed by 2 ml of 0.1 M phosphate buffer (pH 6.0). The sample was pulled gradually through the column. The column was washed with 2 ml of 0.1 M phosphate buffer (pH 6.0), then 2 ml of 1.0 M acetic acid, and finally with 2 ml hexane. It was kept to dry for 5- 10 min after every wash. 4 ml elution solution consisted of methanol: HCl (conc); 95:5 was added to the column. The elute was collected and the solvent was evaporated at 40 ^{0}C under a nitrogen stream. The residue was reconstituted with 100 µl DMF. After vortex and sonication, 100 µl of water was added, and the resuspension process was repeated. 20 µL of the sample were introduced into the HPLC system.

Chromatographic condition

Following the technique of Dirikolu et al. (1999), the levels of diclazuril in plasma samples were evaluated. The HPLC Agilent Series 1200 quaternary gradient pump, Series 1200 autosampler, Series 1200 UV VIS detector adapted at 280 nm, and HPLC 2D Chemstation software (Hewlett-Packard, Les Ulis, France) were employed. Chromatographic separation was accomplished with the aid of Phenomenex C18 column (5 µm, 150 mm x 4.6 mm). The mobile phase comprised of solvent A (80% [0.5% ammonium acetate, 0.01 M tetrabutylammonium hydrogen sulphate in water]: 20% acetonitrile) and solvent B (80% methanol, 20% acetonitrile, A: B, 46:54 v/v). The flow rate was 1 ml/min. The retention time was 13.7 min. The validation of the HPLC analytical assay was performed by evaluating recovery, sensitivity, precision, and linearity (Table 1). The linearity of the method was identified ($R^2 > 0.99$) in the range of 0.025–10 µg/ml plasma. The lower limits of detection and quantification of diclazuril were 0.008 and 0.025 µg/ml.

Pharmacokinetic analysis

The mean plasma concentration of diclazuril for every sampling time point was estimated for each species of bird (quails and pigeons). The non-compartmental approach was applied to analyze the mean concentrations of diclazuril utilizing the WinNonlin 8.3 software (Certara, USA) (Dirikolu et al., 2022). The area under the plasma concentration-time curve ($AUC_{0\text{-last}}$) assessed employing the linear up/log down trapezoidal method, the elimination half-life (T1/2λz), mean residence time (MRT), volume of distribution scaled by bioavailability (Vz_F_obs), clearance divided by bioavailability (Cl_F_obs) were among the pharmacokinetic parameters calculated. The values of the peak plasma concentration (C_{max}) and the time needed to achieve C_{max} (T_{max}) were identified from the data on the plasma concentration-time plot.

Table 1. Validation parameters of the high-performance liquid chromatography technique used for measuring diclazuril in plasma samples of Japanese quails (*Coturnix japonica*) and domestic pigeons (*Columba livia*) after its administration at a level of 0.3 mg/kg of body weight

Matrix	Average recovery (%)	Intra-day RSD (%)	Inter-day RSD (%)	LOD (µg/mL)	LOQ (µg/mL)
Quails' plasma	102.91 ± 4.87	2.06	2.65	0.008	0.025
Pigeons' plasma	99.48 ± 8.08	4.43	3.72	0.008	0.025

Data for recovery are elucidated as mean ± Standard deviation, LOQ: Limit of quantification, LOD: Limit of detection, RSD: Relative standard deviation. Intra-day RSD and Inter-day RSD % (n = 6, 0.025µg/mL). Average recovery % (utilizing spiked concentrations in the range of 0.025–10µg/mL in triplicate investigation).

RESULTS

No noticeable side effects from diclazuril were recorded in experimental birds throughout the study. The plasma concentration-time plots of diclazuril after being administered once at 0.3 mg/kg to quails and pigeons are illustrated on a semilogarithmic graph in Figure 1. The plasma levels of diclazuril were higher than the LOQ (0.025µg/ml) up to 168 h post-administration in quails and pigeons. The plasma concentration versus time curves revealed that quails had lower drug concentrations relative to pigeons. Table 2 demonstrates the pharmacokinetic features of diclazuril in quails and pigeons. The C_{max} values of diclazuril were identified to be 5.35 and 9.14 µg/mL at 8 h after oral administration in quails and pigeons, respectively. Diclazuril was eliminated with elimination half-lives ($T_{1/2\lambda z}$) of 30.74 and 26.48 h in quails and pigeons, respectively. The $AUC_{0\text{-last}}$ of the drug was 155.67 µg *h/mL in quails and 343.57 µg *h/mL in pigeons. The calculated MRT values in quails and pigeons were 30.71 and 39.68 h, respectively.

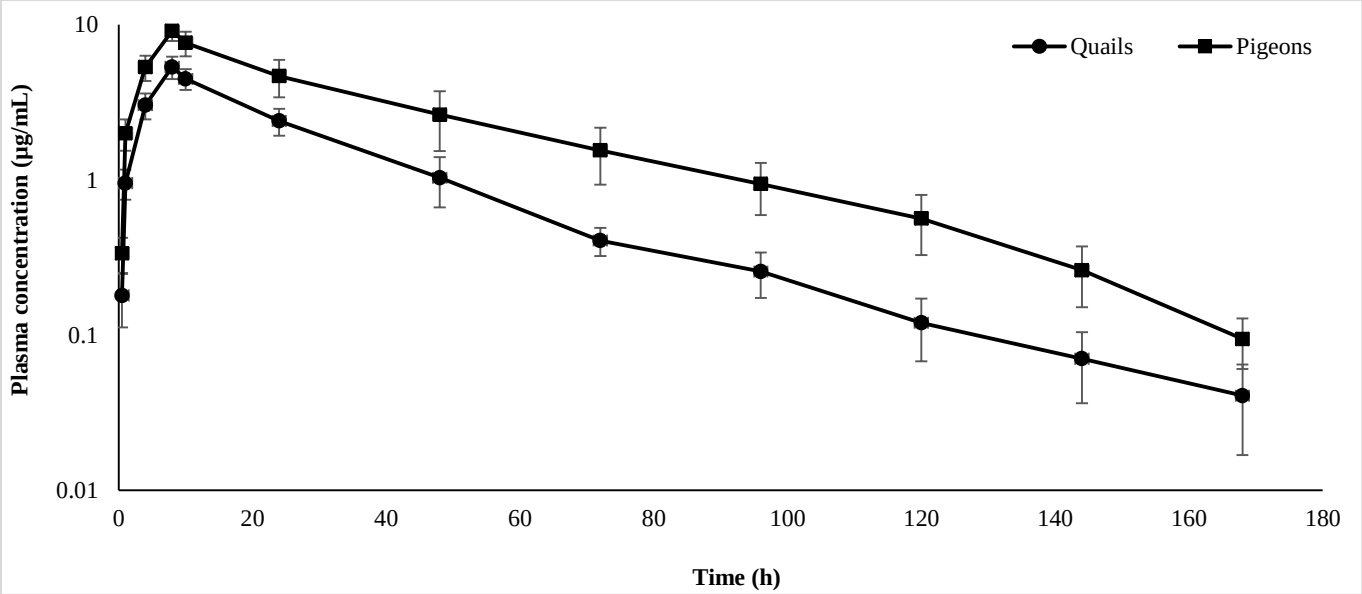


Figure 1. Mean plasma concentrations of diclazuril in Japanese quails (*Coturnix japonica*) and domestic pigeons (*Columba livia*) after a single oral administration at a dose of 0.3 mg/kg. Values are shown as mean ± SD (n=6)

Table 2. Pharmacokinetic parameters of diclazuril following its single oral administration in Japanese quails (*Coturnix japonica*) and domestic pigeons (*Columba livia*)

Parameters	Japanese quails	Domestic pigeons
C_{max} (µg/mL)	5.35	9.14
T_{max} (h)	8.00	8.00
λz (1/h)	0.022	0.026
$T_{1/2}$ λz (h)	30.74	26.48
$AUC_{0\text{-}last}$ (µg*h/mL)	155.67	343.57
Vz_F_ obs (ml/kg)	84.51	33.01
Cl_F_obs (ml/hr/kg)	1.91	0.86
MRT (h)	30.71	39.68

C_{max}: Maximum concentration in plasma; T_{max}: Time to achieve maximum concentration; λz: The first order rate constant; $T_{1/2\ \lambda z}$: Elimination half-life; $AUC_{0\text{-}last}$: Area under the plasma concentration-time curve from 0 to last time; Vz_F_obs: Volume of distribution scaled by bioavailability; Cl_F_obs: Clearance divided by bioavailability; MRT: Mean residence time.

DISCUSSION

Dilcazuril is effective against intestinal *Eimeria* species in avians, all pathogenic *Eimeria* species affecting poultry and turkeys, intestinal and hepatic coccidiosis in rabbits, and toxoplasmosis in mice, and possesses extended-spectrum anticoccidial activity in many other mammalian species (El- Banna et al., 2005; Kotra, 2007; Vereecken et al., 2012; Noack et al., 2019). Limited data are available concerning the pharmacokinetic features of diclazuril in quails and pigeons. The evaluation of the pharmacokinetic characteristic designated that diclazuril was quickly absorbed following oral ingestion in quails and pigeons, with peak plasma level achieved at 8 hours (T_{max} was 8 hr in both species). This finding is consistent with the report of Dirikolu et al. (2022) who demonstrated rapid absorption of diclazuril after oral administration in cattle at 2.2 mg/kg with T_{max} of 8 h. Similarly, Giorgi et al. (2010) recorded a T_{max} of 9.4 h for diclazuril in lambs following a single oral administration at 5mg/kg. The C_{max} of diclazuril in quails and pigeons were 5.35 and 9.14 µg/ml, respectively. The C_{max} value in quails was comparable to that announced for horses who received diclazuril orally at 2.2 mg/kg (4.2 µg/mL, Dirikolu, 2001). In contrast, the C_{max} values in quails and pigeons were less than that revealed in rabbits administered diclazuril at 10

mg/kg (16.42 µg/ml, Hu et al., 2009). Meanwhile, they were higher than those observed in chickens who received diclazuril at 0.5 mg/kg (21.6 ng/mL, Zhang et al., 2020), and in sheep and lambs who received diclazuril at 5 mg/kg (0.9 and 1.3 µg/mL, Giorgi et al., 2010). These variations may be owed to species and dose differences.

Moreover, the current study declared that the $T_{1/2\lambda z}$ of diclazuril in quails and pigeons were 30.74 and 26.48 h, respectively. These findings were relatively similar to those of Zhang et al. (2020) and Giorgi et al. (2010) who reported that the values of $T_{1/2\lambda z}$ of diclazuril were 37.6, and 27.3 h in chickens administered diclazuril orally at 1 mg/kg, and lambs received diclazuril at 5 mg/kg, respectively. On the contrary, the $T_{1/2\lambda z}$ found in rabbits (9.23 h, Hu et al., 2009) was shorter than that recorded in this research for quails and pigeons. Furthermore, in this research, the quails and pigeons had longer MRT (30.71 and 39.68 h, respectively) than that revealed by Hu et al. (2009) in rabbits (10.41 h). Conversely, The MRT announced for horses (113.6 h) by Dirikolu (2001) was longer than that computed in this study. The Vz_F_ obs for diclazuril was 84.51 mL/kg in quails and 33.01 mL/kg in pigeons. To the best of the authors' knowledge, no data are documented about the Vz of diclazuril in other species.

CONCLUSION

Diclazuril displayed favorable pharmacokinetic properties after oral administration at a dose of 0.3 mg/kg in quails and pigeons. Nevertheless, to determine the appropriate dosage regimen for treating coccidiosis in clinical practice, future study is required to assess the clinical effectiveness against coccidial infection. In addition, further research is warranted to evaluate the residues in tissues and estimate the withdrawal period of diclazuril in pigeons and quails.

DECLARATIONS

Acknowledgments

We appreciate the effort exerted by the staff members of the Animal Health Research Institute Giza-Dokki, in investigating the concentrations of diclazuril in plasma samples utilizing HPLC.

Funding

No funding resources for this study

Authors' contributions

Sara T. ELazab; Conceptualized the idea and methodology, conducted the experiment, analyzed the data, and wrote the draft and final manuscript. Iqra Zafar contributed to the data analysis and review of the manuscript. Nahla S. Elshater performed the laboratory analysis of plasma samples using HPLC and reviewed the draft of the manuscript. All authors have read and approved the final manuscript.

Availability of data and materials

The data sets generated for this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare no conflict of interest.

Ethical consideration

All authors confirmed that the research adheres to ethical issues such as avoiding plagiarism, getting permission before publishing, avoiding misconduct, preventing data fabrication or falsification, refraining from double publication or submission, and avoiding redundancy.

REFERENCES

Blake DP and Tomley FM (2014). Securing poultry production from the ever-present *Eimeria* challenge. Trends in Parasitology, 30(1): 12-19. DOI: https://www.doi.org/10.1016/j.pt.2013.10.003

Conway DP, Mathis GF, and Lang M (2002). The use of diclazuril in extended withdrawal anticoccidial programs: Immunity to *Eimeria tenella* challenge after drug withdrawal. Poultry Science, 81(3): 353-355. DOI: https://www.doi.org/10.1093/ps/81.3.353

Chapman HD (2003). Origins of coccidiosis research in the fowl-The first fifty years. Avian Diseases, 47(1): 1-20. DOI: https://www.doi.org/10.1637/0005-2086(2003)047[0001:OOCRIT]2.0.CO;2

Christaki E, Florou-Paneri P, Giannenas I, Papazahariadou M, Botsoglou NA, and Spais AB (2004). Effect of a mixture of herbal extracts on broiler chickens infected with *Eimeria tenella*. Animal Research, 53(2): 137-144. DOI: https://www.doi.org/10.1051/animres:2004006

Dirikolu L, Lehner F, Nattrass C, Bentz B, Woods W, Carter W, Karpiesiuk W, Jacobs J, Boyles J, Harkins J et al. (1999). Its identification and detection and preliminary pharmacokinetics. Veterinary Pharmacology and Therapeutics, 22(6): 374-379. DOI: http://www.doi.org/10.1046/j.1365-2885.1999.00232.x

Dirikolu L (2001). Detection, quantification and pharmacokinetics of triazine- based antiprotozoal agents for the treatment of equine protozoal myeloencephalitis. PhD Dissertation, University of Kentucky, USA.

Dirikolu L, Lehner AF, and Tobin T (2022). Plasma concentrations of diclazuril following oral administration of diclazuril and diclazuril sodium salt to cattle. Journal of

Veterinary Pharmacology and Therapeutics, 45(4): 392-401. DOI: https://www.doi.org/10.1111/jvp.13062

El-Banna HA, El-Bahy MM, El-Zorba HY, and El-Hady M (2005). Anticoccidial efficacy of drinking water soluble diclazuril on experimental and field coccidiosis in broiler chickens. Journal of Veterinary Medicine Series A, 52(6): 287-291. DOI: https://www.doi.org/10.1111/j.1439-0442.2005.00727.x

El-Ashram S, Aboelhadid SM, Arafa WM, Gadelhaq SM, and Abdel-Razik ARH (2019). Protective potential of diclazuril-treated oocysts against coccidiosis in layer chicks. Veterinary Parasitology, 273: 105-111. DOI: https://www.doi.org/10.1016/j.vetpar.2019.08.010

European public MRL assessment report (EPMAR) (2013). Diclazuril (extension to poultry), European agency. committee for medicinal products for veterinary use. Commission implementing regulation (EU), No. 115/2013, pp. 1-8. Available at: https://www.ema.europa.eu/en/documents/mrl-report/eprinomectin-extention-ovine-species-european-public-maximum-residue-limit-assessment-report-epmar-cvmp_en.pdf

Gadelhaq S, Arafa W, Dahshan AH, and Abolhadid S (2017). Using of diclazuril in attenuation of *Eimeria* species for induction of protective immunity against coccidiosis in layer chicks. Assiut Veterinary Medical Journal, 63(155): 101-108. DOI: https://www.doi.org/10.21608/avmj.2017.170969

Giorgi M, Niccolini A, Soldani G, and Martelli F (2010). Pharmacokinetic study of diclazuril in pre-ruminant and ruminant lambs. Israel Journal of Veterinary Medicine, 65(2): 62-67. Available at: https://www.semanticscholar.org/paper/Pharmacokinetic-study-of-diclazuril-in-pre-ruminant-Giorgi Niccolini/ec3413c5298039b5fd5fc84944c90499290ad313?fbclid=IwAR1NXZXcHVSeGT2sUSB-9P5IZKswOAHmKLjBix1NQxFFb8-PDNPCVQjJuLo

Hu LD, Liu C, and Xu W (2009). Pharmacokinetics of diclazuril after oral administration of clinical doses to rabbits. Journal of Chinese Pharmaceutical Sciences, 18(3): 273-277. Available at: https://caod.oriprobe.com/articles/25941101/Pharmacokinetics_of_diclazuril_after_oral_administ.htm

Hunyadi L, Papich MG, and Pusterla N (2015). Pharmacokinetics of a low dose and FDA-labeled dose of diclazuril administered orally as a pelleted topdressing in adult horses. Journal of Veterinary Pharmacology and Therapeutics, 38(3): 243-248. DOI: https://www.doi.org/10.1111/jvp.12176

Kadykalo S, Roberts T, Thompson M, Wilson J, Lang M, and Espeisse O (2018). The value of anticoccidials for sustainable global poultry production. International Journal of Antimicrobial Agents, 51(3): 304-310. DOI: https://www.doi.org/10.1016/j.ijantimicag.2017.09.004

Kotra LP (2007). Toxoplasmosis. X pharm: The comprehensive pharmacology reference, pp. 1-6. DOI: https://www.doi.org/10.1016/B978-008055232-3.60934-1

Mortier L, Daeseleire E, Huyghebaert G, Grijspeerdt K, and Peteghem CV (2005). Detection of residues of the coccidiostat diclazuril in poultry tissues by liquid chromatography− Tandem mass spectrometry after withdrawal of medicated feed. Journal of Agricultural and Food Chemistry, 53(4): 5-911. DOI: https:/www.doi.org/10.1021/jf048468z

Noack S, Chapman HD, and Selzer PM (2019). Anticoccidial drugs of the livestock industry. Parasitology Research, 118: 2009-2026. DOI: https://www.doi.org/10.1007/s00436-019-06343-5

Pusterla N, Vaala W, Bain FT, Chappell DE, Craig B, Schneider C, Barnett DC, Gaughan E, and Papich MG (2023). Pharmacokinetics of a FDA-labeled dose of diclazuril administered orally once weekly to adult horses. Journal of Equine Veterinary Science, 120: 104183. DOI: https://www.doi.org/10.1016/j.jevs.2022.104183

Said AA, El-Nabtity SM, El-Aziz AMA, and Elassal E I (2019). Residues of anticoccidial drug (diclazuril) in different broiler tissues by high performance liquid chromatography. Advances in Animal and Veterinary Sciences, 7(s2): 19-25. DOI: https://www.doi.org/10.17582/journal.aavs/2019/7.s2.19.25

Stock ML, Elazab ST, and Hsu WH (2018). Review of triazine antiprotozoal drugs used in veterinary medicine. Journal of Veterinary Pharmacology and Therapeutics, 41(2): 184-194. DOI: https://www.doi.org/10.1111/jvp.12450

Turk E, Tekeli IO, Corum O, Durna Corum D, Kirgiz FC, Cetin G, Atessahin DA, and Uney K (2021). Pharmacokinetics of meloxicam, carprofen, and tolfenamic acid after intramuscular and oral administration in Japanese quails (*Coturnix coturnix Japonica*). Journal of Veterinary Pharmacology and Therapeutics, 44(3): 388-396. DOI: https://www.doi.org/10.1111/jvp.12972

Vereecken M, Lavazza A, De Gussem K, Chiari M, Tittarelli C, Zuffellato A, and Maertens L (2012). Activity of diclazuril against coccidiosis in growing rabbits: Experimental and field experiences. World Rabbit Science, 20(4): 223-230. DOI: https://www.doi.org/10.4995/wrs.2012.1232

Wang C, Han C, Li T, Yang D, Shen X, Fan Y, Xu Y, Zheng W, Fei C, Zhang L et al. (2013). Nuclear translocation and accumulation of glyceraldehyde-3-phosphate dehydrogenase involved in diclazuril-induced apoptosis in *Eimeria tenella* (*E. tenella*). Veterinary Research, 44(1): 29. DOI: https://ww.doi.org/10.1186/1297-9716-44-29

Zhang M, Qiu J, Shu X, Tang X, Sha X, Wu L, Fan J, Zeng D, He R, Zhang W et al. (2020). Pharmacokinetics, activity, and residue elimination of R-and S-diclazuril in broiler chickens. Journal of Agricultural and Food Chemistry, 68(33): 8987-8995. DOI: https://www.doi.org/10.1021/acs.jafc.0c03091

Zhou BH, Wang HW, Wang XY, Zhang LF, Zhang KY, and Xue FQ (2010). *Eimeria tenella*: Effects of diclazuril treatment on microneme genes expression in second-generation merozoites and pathological changes of caeca in parasitized chickens. Experimental Parasitology, 125(3): 264-270. DOI: https://www.doi.org/10.1016/j.exppara.2010.01.022

Zhou BH, Wang HW, Zhao ZS, Liu M, Yan WC, Zhao J, Zhang Z, and Xue FQ (2013). A novel serine/threonine protein phosphatase type 5 from second-generation merozoite of *Eimeria tenella* is associated with diclazuril-induced apoptosis. Parasitology Research, 112(4): 1771-1780. DOI: https://www.doi.org/10.1007/s00436-013-3336-0

To cite this paper: Elazab ST, Zafar I, and Elshater NS (2024). Pharmacokinetic Characteristics of Diclazuril in Japanese Quails (*Coturnix japonica*) and Domestic Pigeons (*Columba livia*). *J. World Poult. Res.*, 14(2): 204-210. DOI: https://dx.doi.org/10.36380/jwpr.2024.21

JWPR
Journal of World's Poultry Research
2024, Scienceline Publication
J. World Poult. Res. 14(2): 211-218, 2024
Research Paper
DOI: https://dx.doi.org/10.36380/jwpr.2024.22
PII: S2322455X2400022-14
ISSN: 2322-455X

Evaluation of *Salmonella Enteritidis* Isolated from Layer Hens and Murine Fecal Pellets in Poultry Farms of Libya

Imad Benlashehr[1], Khaled Elmasri[1], Abdulwahab Kammon[1,2]*, and Abdulatif Asheg[1]

[1]*Department of Poultry and Fish Diseases, Faculty of Veterinary Medicine, University of Tripoli, P. O. Box 13662, Tripoli, Libya*
[2]*National Research Center for Tropical and Transboundary Diseases, Alzintan, Libya*

*Corresponding author's E-mail: a.kammon@uot.edu.ly

Received: March 28, 2024, Revised: April 22, 2024, Accepted: May 20, 2024, Published: June 30, 2024

ABSTRACT

The rodents play a significant role in the transmission of *Salmonella* between farms and regions. The present study aimed to compare the virulence of *Salmonella enteritidis* isolated from fecal samples of laying hens and murine within the same poultry house but different regions in Libya using Vivo-quantitative measurement of invasiveness (chicken intestinal loop model). A total of 540 cloacal swabs from laying hens (Hy-line brown chickens) aged 36 weeks and 200 batches of murine fecal pellets were collected from the same poultry house at Gaser Bin Gisher and Furnag regions in Libya. The samples were passed on pre-enrichment broth (Buffered Peptone Water) and enrichment broths (Rappaport Vassiliadis, Selenite broth, and tetrathionate), then the samples were cultured onto Xylose Lysine Deoxycholate agar, brilliant green agar, *Salmonella* Shigella agar, and Hektoen enteric agar. Single colonies were selected and stained by gram stain and tested biochemically using analytical profile index (API) 20 tests. *Salmonella enteritidis* was isolated from all the collected samples. The invasion of *Salmonella enteritidis* isolated from laying hens and murine feces was significantly higher in the anterior inoculation position compared to the posterior position of jejunum in both regions. The account of *Salmonella enteritidis* isolated from laying feces of hens and murine at Gaser Bengasher region was significantly higher than that isolated from the AlFurnge region. In the present study, the rodents act only as mechanical transmitters without affecting *Salmonella* invasiveness capacity. Furthermore, the invasion of *Salmonella enteritidis* depends on the inoculation position in the jejunum. Moreover, the invasiveness variation of *Salmonella enteritidis* isolated from the Gaser Bengasher and AlFurnge regions could be attributed to the presence of different *Salmonella* strains in the studied area. *Salmonella enteritidis* isolated from poultry and murine in the current study was sensitive to gentamicin, ciprofloxacin, and enrofloxacin and resistant to doxycycline, chloramphenicol, sulfafurazol, and ampicillin.

Keywords: Invasiveness, Layer chicken, Murine infestation, *Salmonella enteritidis*

INTRODUCTION

Salmonella enteritidis belongs to the Enterobacteriaceae family and it is a facultative intracellular bacteria. *Salmonella* has More than 2600 different serovars, which are divided based on host adaptation into non-host-specific serovars (ubiquitous serovars) that cause potential infections in humans and animals such as *Salmonella Enteritidis* (SE) and *Typhimurium*, and host-restricted serovars, such as *Salmonella Gallinarum* (SG) and *Salmonella Pullorum* (SP, Odoch et al., 2017; Xiong et al., 2018;Sreekantapuram et al., 2021). Fowl typhoid in chickens due to infection by *Salmonella Gallinarum* (SG) and *Salmonella Pullorum* (SP) causes potential clinical disease with high mortality in all ages, and the surviving chicken can carry the *Salmonella* for the rest of its life (Wigley et al., 2001; Eriksson et al., 2018; Berhanu and Fulasa, 2020). The factors, such as flagella, capsule, plasmids, and adhesion systems, are responsible for virulence variation of *Salmonella* pathogenesis between hosts, including adhesins, invasions, fimbriae, hemagglutinins, exotoxins, and endotoxins, type 3 secretion systems and *Salmonella* pathogenicity island system which located in chromosomes or plasmids (Daigle, 2008; Sabbagh et al., 2010). These factors control *Salmonella* colonization in the host intestine and cross host-defense-mechanisms as GIT microbial population, gastric acidity, and enzymes as proteases (Foley et al., 2008; 2013; Kaur and Jain, 2012; Yue and Schifferli, 2013). *Salmonella* is generally presented mainly in the

digestive tracts of humans, animals, and avian hosts. Therefore, the presence of *Salmonella* in water, environment, and food is due to fecal contamination (Yue and Schifferli, 2013; Mezal et al., 2014). The termination of *Salmonella* from poultry farms is a difficult task in the presence of natural carriers, such as rodents, wild animals, insects, and human traffic. All those factors increase *Salmonella* persistence in animal farms (Lawson et al., 2014; Brobey et al., 2017; Zamora-Sanabria and Molina Alvarado, 2017). Previous studies indicated that the different pathogenicity effects of *Salmonella* serovars are related to gene mutations, gene transfer, and genome degradation (Rabsch et al., 2002; Kisiela et al., 2012). The present study aimed to compare the virulence of *Salmonella enteritidis* isolated from fecal samples of laying hens and murine within the same poultry house. The study considered different regions in Libya using Vivo-quantitative measurement of invasiveness (chicken intestinal loop model).

MATERIALS AND METHODS

Ethical approval

All the ethical standards for animal welfare and the experiments are done in experimental units in the Department of Poultry and Fish Diseases, Faculty of Veterinary Medicine, the University of Tripoli, Libya under full-authorized staff. The Ethical Approval Committee Code Number is POU.505-2022/SA.

Sampling

Between February 2022 and June 2022, a total of 540 cloacal swabs from Hy-line brown laying hens aged 36 weeks were collected from poultry houses at Gaser Bin Gisher and Furnag regions in Libya. A total of 200 fecal pellets samples were collected from live rodents (Meriones spp.) by insulated Tomahawk traps inside the poultry houses as described by Kilonzo et al. (2013).

Isolation of bacteria from fecal samples

The fecal samples were pre-enrichment with Buffered Peptone Water (BPW; Oxoid CM0509, 1: 4) and incubated aerobically at 37ºC for 24 hours. An amount of 0.1 ml of pre-enriched samples was added into Rappaport-Vassiliadis (Oxoid CM866) as the selective enrichment medium. The mixture was then incubated aerobically at a temperature of 42ºC for 24 hours. The enriched samples were streaked onto Xylose- Lysine-Desoxycholate agar (XLD; Oxoid CM469) and incubated aerobically at 37ºC for 24 (Aabo et al., 2002, Kilonzo et al., 2013, Irfan et al., 2015). According to Aabo et al. (2000), the isolate was identified by using the analytical profile index (API) 20 (BIOMÉRIEUX, 2011- France). The experimental design was conducted on nine lying hens divided into three replicate groups.

Invasiveness

The two *Salmonella enteritidis* isolated from poultry and murine at the poultry farms and one *Salmonella* reference strain (POULVAC, *Salmonella Typhimurium* Vaccine, Live Culture, USA) were inoculated separately. Loop positions included three parts, the anterior part, the intermediate part, and the posterior part of the jejunum per chicken. After 2 hours, gentamicin was injected and left for 1 hour to kill non-invading bacteria. The bacterial counts (CFU/ ml) of homogenate mucosa tissue at diameter (42-mm^2) were used to express *Salmonella* invasiveness throughout the study using log^{10}.

Antibiotic sensitivity test

Antibiotic susceptibility of isolated bacteria against seven antibiotic substances of veterinary significance was determined by a disc diffusion test (Bauer et al., 1966). *In vitro* antimicrobial susceptibility was screened on Mueller-Hinton agar (MHA- Oxoid, Hampshire, UK) which was incubated at 37℃ for 24 hours. At the end of the incubation period, antibiotic inhibition zones were measured by a measuring caliber.

Statistical analysis

The statistical analysis was done using the GraphPad Prism Version-5 software (California-USA), and one-way analysis following Tukey's Multiple Comparison Test was used (p values less than 0.05 were considered significant).

RESULTS

In the present study, the *Salmonella enterica* serovar enteritidis was isolated from feces of laying hens and murine fecal pellets in the same poultry house at Al-Furnge region and Gaser Bengasher regions in Libya in all samples (Table 1). The invasion of the reference strain (as control) *Salmonella Typhimurium* was quite similar without any significant differences between the three inoculation parts in jejunum during all experiments ($p > 0.05$). The prevalence of *Salmonella enteritidis* in laying hens and murine feces was significantly higher in the anterior inoculation position of the jejunum compared to the intermediate and posterior inoculation positions of the jejunum, as indicated in Table 1 ($p < 0.05$). Notably, the

account (log^{10} CFU) of *Salmonella enteritidis* isolated from laying hens and murine at the Gaser Bengasher region was significantly higher than AlFurnge region during the experiment ($p < 0.05$). The accounts of *Salmonella enteritidis* isolated from poultry at Gaser Bengasher region and insulated in the jejunum were 5.3, 4.6, and 4.7 CFU/ ml in anterior, intermediate, and posterior positions, respectively. The accounts of *Salmonella enteritidis* isolated from murine were 5.7, 5.1, and 4.6 CFU/ ml in anterior, intermediate, and posterior positions, respectively (Table 1). However, at the Alfurnage region, the accounts of *Salmonella enteritidis* isolated from poultry anterior, intermediate, and posterior positions of the jejunum, were 4.5, 4.3, and 4.2 CFU/ ml, respectively. Whereas, the account of *Salmonella enteritidis* isolated from murine at the same region in anterior, intermediate, and posterior positions were 4.5, 4.4, and 4.0 CFU/ ml, respectively (Table 1).

Salmonella enteritidis isolated from poultry and murine in the current study was sensitive to gentamicin, ciprofloxacin, and enrofloxacin and resistant to doxycycline, chloramphenicol, sulfafurazol, and ampicillin (Tables 2 and 3).

Table 1. Evaluation of two *Salmonella* isolates from the field and one *Salmonella* reference strain inoculated separately in three loop positions from the anterior part to the posterior part of the jejunum per chicken

Loop site of inoculation	Furnage region			Gaser Bengasher region		
	SEL	SEM	R.S	SEL	SEM	R.S
L1-R1	4.47	4.51	5.8	5.6	5.7	5.17
L1-R2	4.48	4.55	5.21	5.2	5.8	5.1
L1-R3	4.47	4.5	5.16	5	5.7	5.11
Average L1 log^{10} CFU	4.5*	4.5*	5.4	5.3 ***	5.7	5.1
L2-R1	4.27	4.31	5.11	5	5	5.11
L2-R2	4.22	4.5	5.15	4.1	5.1	5.8
L2-R3	4.34	4.5	5.11	4.82	5.1	5.9
Average L2 log^{10} CFU	4.3	4.4	5.1	4.6	5.1	5.6
L3-R1	4.19	3.9	5.2	4.68	4.7	4.92
L3-R2	4.15	4	5	4.82	4.61	5.2
L3-R3	4.12	4	4.9	4.7	4.57	4.92
Average L3 log^{10} CFU	4.2	4.0	5	4.7	4.6	5.0
Average overall log^{10} CFU	4.3*	4.3*	5.2	4.9**	5.2**	5.3

SEL: *Salmonella Entritidis* (layer), SEM: *Salmonella Entritidis* (murine), RS: Reference strain (*S. Typhimurium*), L1: Anterior loop of jejunum, L2: Intermediate loop of jejunum, L3: Posterior loop of jejunum, R: Replication. Values within a column lacking a common superscript differ at $p < 0.05$. Values within a row carrying two and three stars (**,***) are significantly different from values carrying only one star (*) at $p < 0.05$. The bacterial counts (CFU/ ml) of homogenate mucosa tissue were expressed in log^{10}

Table 2. The antibiotics sensitivity test for *Salmonella enteritidis* isolated from poultry in Lybia

Antibiotic	Standard inhibition zone			*Salmonellla enteritidis* isolated from poultry	
	Resistant	Intermediate	Sensitive	Inhibition zone	Response
Doxycycline 30 ug	< 8	8-12	> 18	5 mm	Resistant
Enrofloxacin 5 ug	< 8	8-12	> 12	22 mm	Sensitive
Chloramphenicol 30 ug	< 16	16-21	> 20	10 mm	Resistant
Sulfafurazol 100 ug	< 11	11-15	> 15	8 mm	Resistant
Ampicillin 10 ug	< 13	14-16	> 17	9 mm	Resistant
Gentamycin 30 ug	< 11	11-15	> 15	8 mm	Sensitive
Ciprofloxacin 10 ug	< 16	16-21	> 21	25mm	Sensitive

Table 3. Antibiotics sensitivity test for *Salmonella enteritidis* isolated from murine In Lybia

Antibiotic	Standard inhibition zone			*Salmonellla enteritidis* isolated from poultry	
	Resistant	Intermediate	Sensitive	Inhibition zone	Response
Doxycycline 30 ug	< 8	8-12	> 18	6 mm	Resistant
Enrofloxacin 5 ug	< 8	8-12	> 12	17 mm	Sensitive
Chloramphenicol 30ug	< 16	16-21	> 20	9 mm	Resistant
Sulfafurazol 100 ug	< 11	11-15	> 15	7 mm	Resistant
Ampicillin 10 ug	< 13	14-16	> 17	9 mm	Resistant
Gentamycin 30 ug	< 11	11-15	> 15	20 mm	Sensitive
Ciprofloxacin 10 ug	< 16	16-21	>21	24 mm	Sensitive

DISCUSSION

Throughout the study, all three inoculation sites in the jejunum indicated equal invasion results for the reference strain (*Salmonella Typhimurium*). There is a lack of data about the isolation of *Salmonella enterica* serovar *enteritidis* from the feces of laying hens and murine in Libya. However, Lawson et al. (2014), Brobey et al. (2017), and Zamora-Sanabria and Molina Alvarado (2017) isolated the *Salmonella* from intestines or feces of rodents, wild animals, and wild birds respectively. The virulence of *Salmonella* could be attenuated or strengthened depending on environmental exposure, mutation, and gastric acidity of reservoir hosts (Sabbagh et al., 2010; Yue et al., 2013; Zamora-Sanabria and Molina Alvarado, 2017). In the present study, the effects of some factors such as phage type and mutations on the virulence of *Salmonella* are not significantly obtained. However, a role in insignificant differences between the invasion of *Salmonella enteritidis* isolated from layer and murine are found. The decline in *Salmonella enteritidis* total counts between anterior to the posterior inoculation loop during the experiment in laying hens and murine isolates agrees with a previous study by Aabo et al. (2000; 2002). Aabo et al. (2000; 2002) reported an 8.5-fold decline in $\log^{10}$ CFU of total *Salmonella* counts between the anterior and the posterior inoculation loop. The significantly high account of *Salmonella enteritidis* isolated from laying hens and murine at the Gaser Bengasher region compared to the AlFurng region could be explained by the presence of different virulence strains of *Salmonella* in the studied area. This result is compatible with the previous study by Asheg et al. (2003) that demonstrated the adhesion, colonization, and migration of *Salmonella enteritidis* in the intestinal tract of chickens depending on the dose of the bacteria administered. According to Asheg et al. (2023), the presence of different virulence strains of *Salmonella* in the South and West of Tripoli could be due to differences in antibiotic resistance of *Salmonella* isolated from slaughterhouses in the South, West, and East of Tripoli –Libya.

Additionally, the current study considered the result of the antibiotic sensitivity test, especially after the emergence of strains resistant to multiple antibiotics as *salmonellosis* surveillance has been described all over the world, making control and treatment (Brisabois et al., 1997).

The results of the antibiotic sensitivity test in Libya by Beleid (1993) indicated that the tested isolates, including *Salmonella enteritidis*, were susceptible to ampicillin, sulfafurazol, chloramphenicol, enrofloxacin and doxycycline. However, the present result revealed that gentamycin was the most effective drug followed by enrofloxacin, and marked resistance of the isolates to ampicillin, sulfafurazol, chloramphenicol, and doxycycline. The comparison of the obtained result of the current study with Beleid's (1993) findings shows suciptibility of isolated salmonella to enrofloxacin. However, antimicrobial resistance of salmonella to specific kinds of antibiotics were recorded during the past 26 years. Recently, Asheg et al. (2023) reported resistance of *Salmonella enteritidis* isolated from broilers at slaughterhouses to sulfamethazon/trimethoprim, ciprofloxacin, trimethoprim, gentamycin, doxytetracyclin, amoxycillin/clavanic acid, and ampicillin, in percentages of 41%, 45%, 48%, 69%, 69%, 76%, and 100%, respectively.

Notably, a previous study indicated that plasmid-borne ampicillin resistance is associated with the attenuation of serovar enteritidis (Ridley et al., 1996).

The observed marked resistance of both *Salmonella enteritidis* and *Salmonella Newport* isolates in the present study is considered to be a biological indicator for the presence of multi-drug resistant bacteria. It has been reported in several countries (Arlet et al., 2006; Cobbold et al., 2006; Egorova et al., 2007; Pławińska-Czarnak et al., 2022), and it is considered a serious problem among both food animals and humans (Zhao et al., 2001; Gupta et al., 2003; Devasia et al., 2005; Poppe et al., 2006; Egorova et al., 2008). This finding is a concern for surveillance and environmental control organisms since the increase in antimicrobial resistance has limited the potential uses of antibiotics for the treatment of infections in humans and animals (Angulo et al., 2004). The total of methicillin-resistant Staphylococcus infections in U.S. hospitals and communities has increased from 2.4% in 1975 to 29% in 1991 (Panlilio et al., 1992). However, in 2013, the average percentage of hospitals reporting HA-MRSA in the U.S. was 61.5% (Fukunaga et al., 2016).

In addition, the recent emergence in Africa and Europe, mainly in turkey flocks of *Salmonella Kentucky* (CipR) resistant to ciprofloxacin (Le Hello et al., 2013) which is highly pathogenic and highly resistant to antibiotics reminds that the combat is never-ending.

CONCLUSION

The obtained results indicated that rodents could be active mechanical transmitters of *Salmonella* in poultry farms especially in the studied area. Furthermore, the resistance of isolated *Salmonella* to broad-spectrum antibiotics needs more attention thus further research is highly recommended to determine the extent of the problem in the suspected areas and to find the best solutions for controlling *Salmonella* isolates that resistance to broad-spectrum antibiotics from farm-to-fork.

DECLARATIONS

Acknowledgments

The National Animal Health Services' Dr. Elforjany Kraim is much appreciated by the authors for his assistance in serotyping *Salmonella*

Authors' contributions

Dr. Imad Benlashehr contributed to the database, data gathering, and the manuscript's preparation. Dr. Kaled Elmasry also completed the data analysis and manuscript preparation. The primary and secondary supervisors for the study's conduct were the doctors Abdulatif Asheg and Abdulwahb Kammon. The final edition of the manuscript has been reviewed by all authors and approved for publication in the current journal.

Funding

This study had no funding source.

Availability of data and materials

The current publication contains all of the study data, and the accompanying author can provide further details upon request.

Ethical considerations

The ethical concerns of plagiarism, permission to publish, misconduct, data fabrication and falsification, double publishing, submission, and redundancy have all been reviewed by the authors.

Competing interests

The authors have proclaimed that no contending interest exists

REFERENCES

Aabo S, Christensen JP, Chadfield MS, Carstensen B, Jensen TK, Bisgaard M, and Olsen JE (2000). Development of an *in vivo* model for the study of intestinal invasion by *Salmonella enterica* in chickens. Infection and Immunity, 68(12): 7122-7125. DOI: http://www.doi/10.1128/IAI.68.12.7122-7125.2000

Aabo S, Christensen JP, Chadfield MS, Carstensen B, Olsen JE, and Bisgaard M (2002). Quantitative comparison of intestinal invasion of zoonotic serotypes of *Salmonella enterica* in poultry. Avian Pathology, 31(1): 41-47. DOI: https://www.doi.org/10.1080/03079450120106615

Angulo F, Nargund V, and Chiller T (2004). Evidence of an association between use of antimicrobial agents in food animals and anti-microbial resistance among bacteria isolated from humans and the human health consequences of such resistance. Journal of Veterinary Medicine B Infectious Diseases and Veterinary Public Health, 51(8-9): 374-379. DOI: https://www.doi.org/10.1111/j.1439-0450.2004.00789.x

Arlet G, Barrett TJ, Butaye P, Cloeckaert A, Mulvey MR, and White DG (2006). *Salmonella* resistant to extended-spectrum cephalosporins: prevalence and epidemiology. Microbes and Infection, 8(7): 1945-

1954. DOI: https://www.doi.org/10.1016/j.micinf.2005.12.029

Asheg AA, Otman MF, Benlashehr IA, Kraim EF, Almashri RA, and Kammon AM (2023). Prevalence of *Salmonella* in poultry slaughterhouses located in Tripoli, Libya. Open Veterinary Journal, 13(5): 638-644. DOI: https://www.doi.org/10.5455/OVJ.2023.v13.i5.17

Asheg AA, Levkut M, Revajová V, Ševčíková Z, Kolodzieyski L, Pistl J, and Pilipčinec E (2003). Spreading of *Salmonella* enteritidis in the cecum of chickens. Folia Microbiolgia, 48: 277-279. Available at: https://www.link.springer.com/article/10.1007/BF02930969

Bauer AW, Kirby WMM, Sherris JC, and Turk M (1966). Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology, 45(4_ts): 493-496. DOI: https://www.doi.org/10.1093/ajcp/45.4_ts.493

Beleid AM (1993). Salmonellosis in some poultry farms in Libya. M.V.Sc. Thesis, Faculty of Veterinary Medicine, Al- Fateh University, Tripoli, Libya.

Berhanu G and Fulasa A (2020). *Pullorum* disease and fowl typhoid in poultry. British Journal of Poultry Sciences, 9(3): 48-56. Available at: https://www.idosi.org/bjps/9(3)20/1.pdf

Brisabois A, Cazin I, Breuil J, and Collatz E (1997) Surveillance of antibiotic resistance in *Salmonella*. Eoroserveillance, 2(3): 19-20. DOI: https://www.doi.org/10.2807/esm.02.03.00181-en

Brobey B, Kucknoor A, and Armacost J (2017). Prevalence of Trichomonas, *Salmonella*, and Listeria in wild birds from Southeast Texas. Avian Diseases, 61(3): 347-352. DOI: https://www.https://www.doi.org/doi.org/10.1637/11607-020617

Fukunaga BT, Sumida WK, Taira DA, Davis JW, and Seto TB (2016). Hospital-acquired methicillin-resistant *Staphylococcus aureus* bacteremia related to medicare antibiotic prescriptions: A state-level analysis. Hawaii Journal of Medicine and Public Health, 75(10): 303-309. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5056633/

Cobbold RN, Rice DH, Davis MA, Besser TE, and Hancock DD (2006). Long-term persistence of multi-drug-resistant *Salmonella enterica* serovar Newport in two dairy herds. Journal of the American Veterinary Medical Association, 228(4): 585-591. DOI: https://www.doi.org/10.2460/javma.228.4.585

Daigle F (2008). Typhi genes expressed during infection or involved in pathogenesis. Journal of Infection in Developing Countries, 2(6): 431-437. DOI: https://www.doi.org/10.3855/jidc.157

Devasia RA, Varma JK, Whichard J, Gettner S, Cronquist AB, Hurd S, Segler S, Smith K, Hoefer D, Shiferaw B et al. (2005). Antimicrobial use and outcomes in patients with multidrug-resistant and pansusceptible *Salmonella* Newport infections, 2002-2003. Microbial Drug Resistance, 11(4): 371-377. DOI: https://www.doi.org/10.1089/mdr.2005.11.371

Egorova S, Kaftyreva L, Grimont PA, and Weill FX (2007). Prevalence and characterization of extended-spectrum cephalosporin-resistant nontyphoidal *Salmonella* isolates in adults in Saint Petersburg, Russia (2002-2005). Microbial Drug Resistance, 13(2): 102-107. DOI: https://www.doi.org/10.1089/mdr.2007.712

Egorova S, Timinouni M, Demartin M, Granier SA, Whichard JM, Sangal V, Fabre L, Delauné A, Pardos M, Millemann Y et al. (2008). Ceftriaxone-resistant *Salmonella enterica* serotype Newport, France. Emerging Infectious Diseases, 14(6): 954-957. DOI: https://www.doi.org/10.3201/eid1406.071168

Eriksson H, Söderlund R, Ernholm L, Melin L, and Jansson DS (2018). Diagnostics, epidemiological observations and genomic subtyping in an outbreak of *Pullorum* disease in non-commercial chickens. Veterinary Microbiology, 217: 47-52. DOI: https://www.doi.org/10.1016/j.vetmic.2018.02.025

Foley SL, Johnson TJ, Ricke SC, Nayak R, and Danzeisen J (2013). *Salmonella* pathogenicity and host adaptation in chicken-associated serovars. Microbiology and Molecularbiology Reviews, 77(4): 582-607. DOI: https://www.doi.org/10.1128/mmbr.00015-13

Foley SL, Lynne AM, and Nayak R (2008). *Salmonella* challenges: Prevalence in swine and poultry and potential pathogenicity of such isolates. Journal of Animal Science, 86(14): E149-E162. DOI: https://www.doi.org/10.2527/jas.2007-0464

Gupta A, Fontana J, Crowe C, Bolstorff B, Stout A, Van Duyne S, Hoekstra MP, Whichard JM, Barrett TJ, and Angulo FJ (2003). National antimicrobial resistance monitoring system pulsenet working group. Emergence of multidrug-resistant *Salmonella enterica* serotype Newport infections resistant to expanded-spectrum cephalosporins in the United States. The Journal of Infectious Diseases, 188(11): 1707-1716. DOI: https://www.doi.org/10.1086/379668

Irfan AM, Sudhir KK, and Sunil M (2015). Isolation, serotype diversity and antibiogram of *Salmonella enterica* isolated from different species of poultry in India. Asian Pacific Journal of Tropical Biomedicine, 5(7): 561-567. DOI: https://www.doi.org/10.1016/j.apjtb.2015.03.010

Kaur J and Jain SK (2012). Role of antigens and virulence factors of *Salmonella enterica* serovar Typhi in its pathogenesis. Microbiological Research, 167(4): 199-210. DOI: https://www.doi.org/10.1016/j.micres.2011.08.001

Kilonzo C, Li X, Vivas EJ, Jay-Russell MT, Fernandez KL, and Atwill ER (2013). Fecal shedding of zoonotic food-borne pathogens by wild rodents in a major agricultural region of the central California coast. Applied and Environmental Microbiology, 79(20): 6337-6344. DOI: https://www.doi.org/10.1128/AEM.01503-13

Kisiela DI, Chattopadhyay S, Libby SJ, Karlinsey JE, Fang FC, Tchesnokova V, Kramer JJ, Beskhlebnaya V, Samadpour M, Grzymajlo K et al. (2012). Evolution of *Salmonella enterica* virulence via point mutations in the fimbrial adhesin. PLoS Pathogenes, 8(6): e1002733. DOI: https://www.doi.org/10.1371/journal.ppat.1002733

Lawson B, De Pinna E, Horton RA, Macgregor SK, John SK, Chantrey J, Duff JP, and Kirkwood JK (2014). Epidemiological evidence that garden birds are a source of human salmonellosis in England and Wales. PLoS One, 9(2): e88968. DOI: https://www.doi.org/10.1371/journal.pone.0088968

Le Hello S, Harrois D, Bouchrif B, Sontag L, Elhani D, Guibert V, Zerouali K, and Weill FX (2013). Highly drug-resistant *Salmonella enterica* serotype Kentucky ST198-X1: A microbiological study. Lancet Infectious Diseases, 13(8): 672-679. DOI: https://www.doi.org/10.1016/S1473-3099(13)70124-5

Mezal EH, Sabol A, Khan MA, Ali N, Stefanova R, and Khan AA (2014). Isolation and molecular characterization of *Salmonella enterica* serovar Enteritidis from poultry house and clinical samples during 2010. Food Microbiology, 38: 67-74. DOI: https://www.doi.org/10.1016/j.fm.2013.08.003

Odoch T, Wasteson Y, L'Abée-Lund T, Muwonge A, Kankya C, Nyakarahuka L, Tegule S, and Skjerve E (2017). Prevalence, antimicrobial susceptibility and risk factors associated with non-typhoidal *Salmonella* on Ugandan layer hen farms. BMC Veterinary Research, 13(1): 365. DOI: https://www.doi.org/10.1186/s12917-017-1291-1

Panlilio AL, Culver DH, Gaynes RP, Banerjee S, Henderson TS, Tolson JS, Martone WJ, and National Nosocomial Infections Surveillance System (1992). Methicillin-resistant *Staphylococcus aureus* in U.S. hospitals, 1975-1991. Infection Control and Hospital Epidemiology, 13(10): 582-586. DOI: https://www.doi.org/10.1086/646432

Pławińska-Czarnak J, Wódz K, Kizerwetter-Świda M, Bogdan J, Kwieciński P, Nowak T, Strzałkowska Z, and Anusz K (2022). Multi-drug resistance to *Salmonella* spp. when isolated from raw meat products. Antibiotics, 11(7): 876. DOI: https://www.doi.org/10.3390/antibiotics11070876

Poppe C, Martin L, Muckle A, Archambault M, McEwen S, and Weir E (2006). Characterization of antimicrobial resistance of *Salmonella* Newport isolated from animals, the environment, and animal food products in Canada. Canadian Journal of Veterinary Research, 70(2): 105-114. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1410721/

Rabsch W, Andrews HL, Kingsley RA, Prager R, Tschäpe H, Adams LG, and Bäumler AJ (2002). *Salmonella enterica* serotype *Typhimurium* and its host adapted variants. Infection and Immunity, 70(5): 2249-2255. DOI: https://www.doi.org/10.1128/iai.70.5.2249-2255.2002

Ridley AM, Punia P, Ward LR, Rowe B, and Threlfall EJ (1996). Plasmid characterization and pulsed-field electrophoretic analysis demonstrate that ampicillin-resistant strains of *Salmonella* enteritidis phage type 6a are derived from Salm. enteritidis phage type 4. Journal of Applied Bacteriology, 81(6): 613-618. DOI: https://www.doi.org/10.1111/j.1365-2672.1996.tb03555.x

Sabbagh SC, Forest CG, Lepage C, Leclerc JM, and Daigle F (2010). So similar, yet so different: Uncovering distinctive features in the genomes of *Salmonella* enterica serovars *Typhimurium* and *Typhi*. FEMS Microbiology Letters, 305(1): 1-13. DOI: https://www.doi.org/10.1111/j.1574-6968.2010.01904.x

Sreekantapuram S, Berens C, Barth SA, Methner U, and Berndt A (2021). Interaction of *Salmonella gallinarum* and *Salmonella enteritidis* with peripheral leucocytes of hens with different laying performance. Veterinary Research, 52(1): 123. DOI: https://www.doi.org/10.1186/s13567-021-00994-y

Wigley P, Berchieri A, Page KL, Smith AL, and Barrow PA (2001). *Salmonella enterica* serovar persists in splenic macrophages and in the reproductive tract during persistent, disease-free carriage in chickens. Infection and Immunity, 69: 7873-7879. DOI: https://www.doi.org/10.1128/iai.69.12.7873-7879.2001

Xiong D, Song L, Pan Z, and Jiao X (2018). Identification and discrimination of *Salmonella enterica* serovar *Gallinarum* biovars *Pullorum* and *Gallinarum* based on a one-step multiplex PCR assay. Frontiers in Microbiololgy, 9: 1718. DOI: https://www.doi.org/10.3389/fmicb.2018.01718

Yue M and Schifferli DM (2013). Allelic variation in *Salmonella*: An underappreciated driver of adaptation

and virulence. Frontiers in Microbiology, 4: 419. DOI: https://www.doi.org/10.3389/fmicb.2013.00419

Zamora-Sanabria R and Molina Alvarado A (2017). Preharvest *Salmonella* risk contamination and the control strategies. Current topics in *Salmonella* and Salmonellosis. InTech Open., London. pp. 193-213. DOI: http://www.doi.org/10.5772/67399

Zhao S, White DG, McDermott PF, Friedman S, English L, Ayers S, Meng J, Maurer JJ, Holland R, and Walker RD (2001). Identification and expression of cephamycinase bla (CMY) genes in *Escherichia coli* and *Salmonella* isolates from food animals and ground meat. Antimicrobial Agents and Chemotherapy, 45(12): 3647-3650. DOI: https://www.doi.org/10.1128/AAC.45.12.3647-3650.2001

JWPR
Journal of World's Poultry Research
2024, Scienceline Publication
J. World Poult. Res. 14(2): 219-235, 2024
Research Paper
DOI: https://dx.doi.org/10.36380/jwpr.2024.23
PII: S2322455X2400023-14
ISSN: 2322-455X

Molecular Characterization of Newcastle Disease Virus Genotype VII.1.1 from Egyptian Mallard Ducks with Nervous Manifestations

Mahmoud Ibrahim[1]*, Mohamed A. Wahba[2], and Nahed Yehia[3]

[1]*Department of Birds and Rabbit Medicine, Faculty of Veterinary Medicine, University of Sadat City, 32958, Menoufiya, Egypt*
[2]*Egyptian Company for Biological & Pharmaceutical Industries (Vaccine Valley), 6th October City, 12511, Giza, Egypt*
[3]*Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Agricultural Research Center, Dokki, Giza, 12618, Egypt*

*Corresponding author's E-mail: mahmoud.jameel@vet.usc.edu.eg

Received: March 23, 2024, Revised: April 20, 2024, Accepted: May 16, 2024, Published: June 30, 2024

ABSTRACT

In Egypt, Newcastle disease virus (NDV) strains of genotype VII are known to be mild in domestic waterfowl and considered reservoirs. This is the first report for the detection of NDV GVII.1.1 from ducks showing severe clinical signs with high mortalities and nervous manifestations, additionally, isolation of NDV and molecular characterization for full HN and F genes were performed. In the current study, 16 backyard mallard duck flocks showing severe nervous signs with high mortalities were investigated by real-time RT-PCR using primers specific for the Fusion gene of NDV and matrix gene for avian influenza virus (AIV). Fourteen duck flocks tested positive for AIV and only two flocks tested positive for NDV infection. NDV was isolated from the trachea and brain of the same duck from each flock then full HN and F genes were sequenced. The phylogenetic analysis of the F and HN genes indicated that these strains were clustered with NDV genotype VII 1.1. The F gene had a specific mutation that cluster them in a new branch with with A11T in the signal peptide, N30S, T324A, and 480K in the hydrophobic heptad repeat (HRc) compared to the Lasota strain. The duck strains of NDV isolated from the brain had N294K in the hydrophobic heptad repeat-b (HRb) of F protein compared to the strains isolated from the trachea of the same duck, which may have a role in crossing the blood-brain barrier. The HN protein had a specific mutation that clustered them in a new branch with mutations of A4V, R15K in the cytoplasmic region, A28T in the transmembrane domain, and S76L in the HRa. In addition, HN protein had A50T, S54R T232N, P392S, and T443V, and multiple mutations were detected in the neutralizing epitopes specific to strains in the present study (N120G, K284R, S521T) that can alter virus antigenicity. The current study indicated the continuous evolution of NDV strains from genotype VII circulating in Egypt with increasing pathogenicity in ducks. The present findings demonstrated the urgent need for the vaccination of ducks and geese with killed NDV vaccines to reduce economic losses due to virus infection and prevent transmission to chickens helping in ND control in Egypt.

Keywords: F gene, Genotype VII 1.1, Mallard duck, Newcastle disease virus, Protein

INTRODUCTION

Newcastle disease (ND) is a contagious viral disease which detected in a wide range of bird species causing devastating economic impacts worldwide. Control of the disease relies on vaccination combined with the implementation of biosecurity measures to reduce virus shedding and decrease economic losses from infection. However, the continuous genetic evolution of the virus negatively affects the efficacy of the available commercial vaccines (Moustapha et al., 2023).

The disease is caused by virulent avian orthoavulavirus 1 (AOAV-1), belonging to the

Paramyxoviridae family (Rima et al., 2019), the virus was previously referred as avian paramyxovirus-1 (APMV-1). The virus is enveloped with a negative sense, non-segmented RNA coding six genes (3-N-P-M-F-HN-L-5), which translated into eight proteins with two important surface glycoproteins; haemagglutinin-neuraminidase (HN) and fusion (F) protein (Steward et al., 1993). Important components for the virus's entry and exit from host cells are the HN and F proteins. Hemolysis, cell fusion, and virus entrance are all highly influenced by the F protein (Morrison, 2003).

The clinical signs observed in ND virus infection varied from mild infection to severe clinical symptoms (sometimes mortality reaches 100%) according to the virus virulence and bird species (Jindal et al., 2009). Also, coinfections, immune status, age, health, and environmental conditions affect the disease severity. Newcastle disease viruses are categorized regarding their pathogenicity in the host into velogenic (showing severe clinical signs with high mortality), mesogenic (showing respiratory manifestations, rarely nervous signs), lentogenic (ranging from subclinical to mild respiratory signs), and asymptomatic enteric (No clinical signs, Cattoli et al., 2011; Miller and Koch, 2013).

For decades, waterfowl represented the natural reservoirs of NDVs with unnoticed infection or only mild clinical signs when infected by either lentogenic or velogenic NDVs (Alexander and Senne, 2008; Dimitrov et al., 2016). In Egypt, muscovy ducks were shown to be excellent carriers for NDV-genotype VIId and efficiently transmit NDV to broiler chickens in contact (Elbestawy et al., 2019). However, it has been documented that NDVs can spread from chickens to wild birds, and then wild birds can transmit the virus to other countries (Xiang et al., 2017).

During the last several years, ND outbreaks in domestic waterfowl with obvious clinical manifestations have been reported frequently (Dai et al., 2013). Interestingly, the pathogenicity of one duck and one chicken NDV strain isolated from China was studied (Meng et al., 2018), and the results showed that the mortality reported for NDV chicken isolates in 4-week-old ducks was 70% compared to 20% mortalities caused by NDV duck isolate, both strains were belonging to NDV genotype VII(1.1). This can be explained by Hidaka et al. (2021), who found that consecutive circulation of NDV chicken strain (9a5b strain) in domestic waterfowl can result over time in more virulent strains for chickens and waterfowl. Moreover, outbreaks of ND (NDV/duck/Jiangsu/JSD0812/2008) in laying duck flocks in China were reported with a 70% drop in egg production and mortalities up to 50% (Liu et al., 2015).

In the current study; for the first time in Egypt velogenic NDV GVII.1.1 was isolated from mallard ducks showing severe clinical signs, nervous manifestations, and high mortality. Moreover, molecular characterization of full F and HN genes of the NDV strains isolated from trachea and brain tissues was performed.

MATERIAL AND METHODS

Ethical approval

Ethical approval for this study was given by the Institutional Animal Care and Use Committee under the University of Sadat City No. VUSC-004-1-24.

Sampling

Trachea and brain were collected from 16 backyard duck flocks showing respiratory, enteric, and nervous signs with high mortalities and were examined during the winter of 2023 from Menoufiya governorate. One duck with nervous signs from each flock was humanly euthanized then packed and transported on ice to the birds and rabbit medicine department, faculty of veterinary medicine, university of Sadat City, Menoufiya, Egypt. Post-mortem lesions were recorded like severe congestion in the liver, spleen, pancreas, kidney and intestine then trachea and brain from the same duck collected and processed separately (total 16 trachea and 16 brain samples were collected). The clinical signs and postmortem lesions are shown in (Figure 1). Tissue samples were homogenized, suspended in sterile phosphate-buffered saline (PBS) (with penicillin 2000 units/mL), freezing and thawing three times then clarified by centrifugation at 1500 rpm for 30 minutes at 4^{o}C (WHO, 2002).

Molecular detection of the causative agent by rRT-PCR

The QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) was used to extract RNA from 16 tracheal tissue suspensions in accordance with the manufacturer's recommendations. Real-time reverse transcriptase polymerase chain reaction (rRT-PCR) was performed using Quantitect probe RT-PCR kit (Qiagen, Inc. Valencia CA, USA) with specific primers for matrix (M) gene of avian influenza virus (AIV) (Spackman et al. 2003), and M gene of NDV (Wise et al., 2004), sequences of primers and probes were listed in Table 1.

Virus isolation

The trachea and brain tissue suspensions of two mallard duck flocks that tested positive for NDV by rRT-PCR were used for ND virus isolation. The clarified tissue suspensions were filtered by 0.2 µm bacteriological filter

then 0.2 ml was injected in the allantoic cavity of 10-day-old SPF embryonated chicken eggs (for each sample five eggs were inoculated). Eggs were incubated at 37 ˚C and candling was performed for successive 3 days after allantoic cavity inoculation, then at the end of the third-day eggs were chilled to 4 ◦C, then the allantoic fluids were tested by hemagglutination test (HA). Briefly, two-fold serial dilution for the allantoic fluid in 50 µl PBS was performed then HA activity was tested by the addition of 50 µl of 1% washed RBCs after 20-30 minutes of incubation at room temperature.

Amplification of fusion and hemagglutinin-neuraminidase genes by RT-PCR

Two primer sets were used for each gene to be amplified into two segments. Primer's sequence and cycling protocols for F gene amplification were carried out according to Munir et al. (2010), and for HN gene amplification was carried out according to Kiani et al. (2021). Reverse transcription-polymerase chain reaction (RT-PCR) was performed using COSMO RT-PCR Master Mix (Willowfort, Birmingham, UK). Size-specific PCR products for each gene were separated by gel electrophoresis and Specific DNA bands were purified for sequencing using the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany).

Fusion and hemagglutinin-neuraminidase gene sequencing

In separate reactions, use forward and reverse primers with each specific DNA fragment. The sequencing procedure was carried out using a cycle sequencing kit, big dye terminator v3.1 (Applied Biosystems, Foster City, California, USA), according to the manufacturer's instructions.

Fusion and hemagglutinin-neuraminidase genes sequence analysis

Using the Clustral W alignment approach of BIOEDIT software (Hall, 1999), the sequences obtained during the study were contrasted with Egyptian field strains and reference strains from various countries that were listed in the GenBank database (NCBI). The maximum likelihood technique in MEGA 6 software was used to generate the phylogenetic trees (Tamura et al., 2013). The percentage of similarity between the strain sequences used in this study and other sequences that have been published and made available in the NCBI database was calculated using Lasergene software (version 7.2; *DNASTAR*, Madison, WI).

The Expasy database, PyMOL software, and SWISS-MODEL were used to simulate the three-dimensional (3D) structure of the F and HN genes (Waterhouse et al., 2018). According to Gupta and Brunak (2016), N-linked glycosylation was detected using NetNGlyc 1.0 Server.

Table 1. Primers used for detection of matrix gene of avian influenza and F gene of Newcastle disease virus

Virus	Gene	Name	Sequence	Reference
AI	M	Sep1	AGATGAGTCTTCTAA CCGAGGTCG	Spackman et al. (2003)
		Sep2	TGCAAAAACATCTTC AAGTCTCTG	
		Sepro	[FAM]TCAGGCCCC CTCAAAGCCGA [TAMRA]	
NDV	M	M-F	5'AGTGATGTGCTCGGACCTTC3'	Wise et al. (2004)
		M-R	5'CCTGAGGAGAGGCATTTGCTA3'	
		M-probe	5'[FAM] TTCTCTAGCAGTGGGACAGCCTGC [TAMRA]3'	

AI: Avian influenza, NDV: Newcastle disease virus.

RESULTS

Virus identification and isolation

By testing the tracheal suspensions of the 16 duck flocks by rRT-PCR; two backyard mallard duck flocks only tested positive for NDV While the remaining 14 flocks tested positive for AIV. The first flock that tested NDV positive was sampled in January 2023 and was 2 weeks old with 70% mortality and the second flock sampled in February 2023 was 4 weeks old with 55% mortality.

Four samples (2 tracheae and 2 brains) of the NDV-positive flocks were inoculated in a total of 20 eggs (5 for each sample) resulting in embryonic death between 36-48 hrs post-inoculation indicating virulent virus. The 4 isolates were tested HA positive with HA titer of 256 HAU. The allantoic fluids were tested by rRT-PCR confirmed that the causative agent in the 4 samples was NDV while the assay gave negative results for the avian influenza virus.

Sequence and phylogenetic analysis of fusion and hemagglutinin-neuraminidase genes

The F and HN gene segments for the four identified and isolated NDV strains (two strains isolated from the

trachea and two strains isolated from the brain) were successfully amplified and sequenced. The obtained sequences were submitted with an accession number to the GeneBank at the National Center for Biotechnology Information (NCBI), and listed in Table 2.

The nucleotide alignment of the complete F and HN gene sequences and the phylogenetic tree of the duck isolates were recognized as a new branch in the genotype VII 1.1 (Class II) as shown in figures 2, 3, 4, and 5.

The amino acid (A.A) identity of the complete F and HN protein sequence compared to other reference strains, vaccines, and Egyptian strains. The two isolates had high similarity and close relatedness 98.5-99.3% and 96.4-96.8% for F and HN genes with Chinese strains related to VIIJ, respectively, and 96.7-99.3% and 97.3-98.2% with other Egyptian strains for F and HN gene, respectively (Figures 6 and 7).

The duck strain had high A.A. identity 96-96.2% and 94.5% with vaccine strains that cluster with VIId (NDV-KBNP-C4152R2L) Korean vaccine for F and HN gene, respectively, and 87.8-88.5% and 86.2-86.4% with classic vaccine strain (Lasota, clone 30 and VG/GA [Avinew]) genotype II for F and HN, respectively and 90.7-90.9% and 89.1% with D26/76 vaccine genotype I for F and HN gene, respectively (Figures 6 and 7).

Table 2. List of duck Newcastle disease virus strains names, origin, and gene bank accession numbers

Strain Name	Sample origin	F	HN
Avian-orthoavulavirus-1-Duck-Egypt-MN1-2023	Trachea	PP182340	PP182344
Avian-orthoavulavirus-1-Duck-Egypt-MN2-2023	Brain	PP182341	PP182345
Avian-orthoavulavirus-1-Duck-Egypt-MN3-2023	Trachea	PP182342	PP182346
Avian-orthoavulavirus-1-Duck-Egypt-MN4-2023	Brain	PP182343	PP182347

F: Fusion, HN: Hemagglutinin-neuraminidase

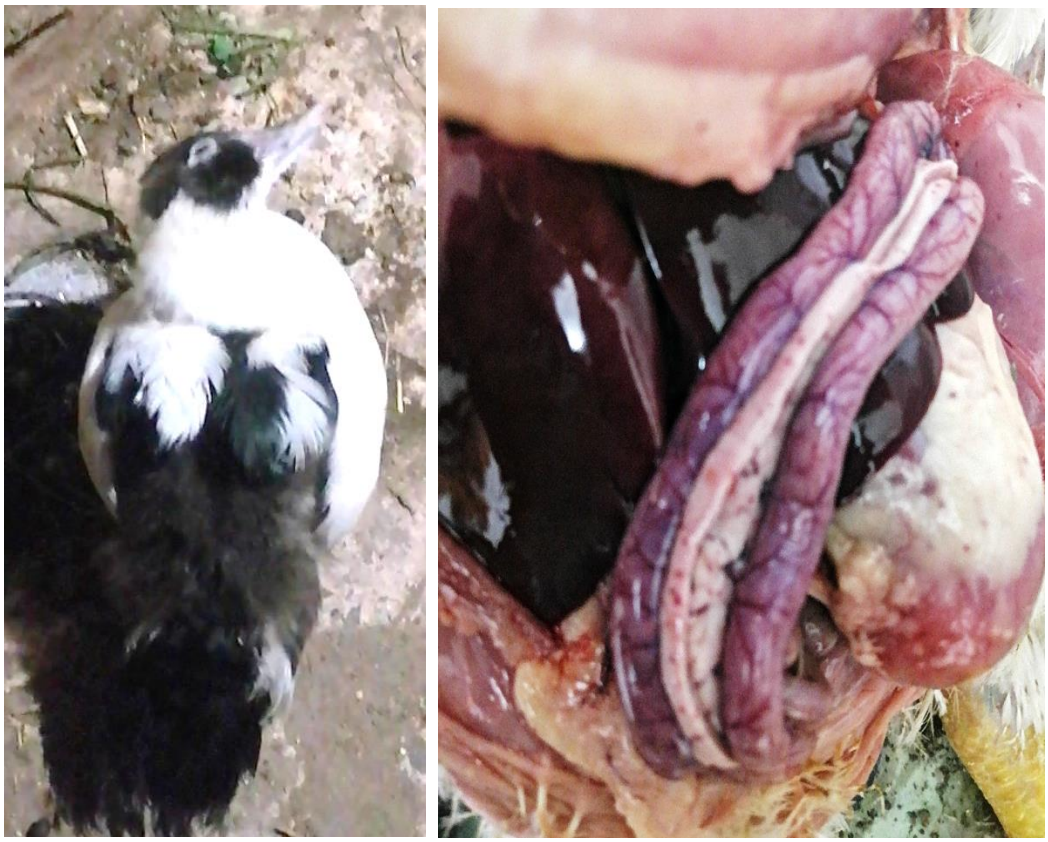


Figure 1. Clinical signs and post-mortem lesions observed in the liver, pancreas, and intestine of a 4-weeks-old mallard duck in February 2023

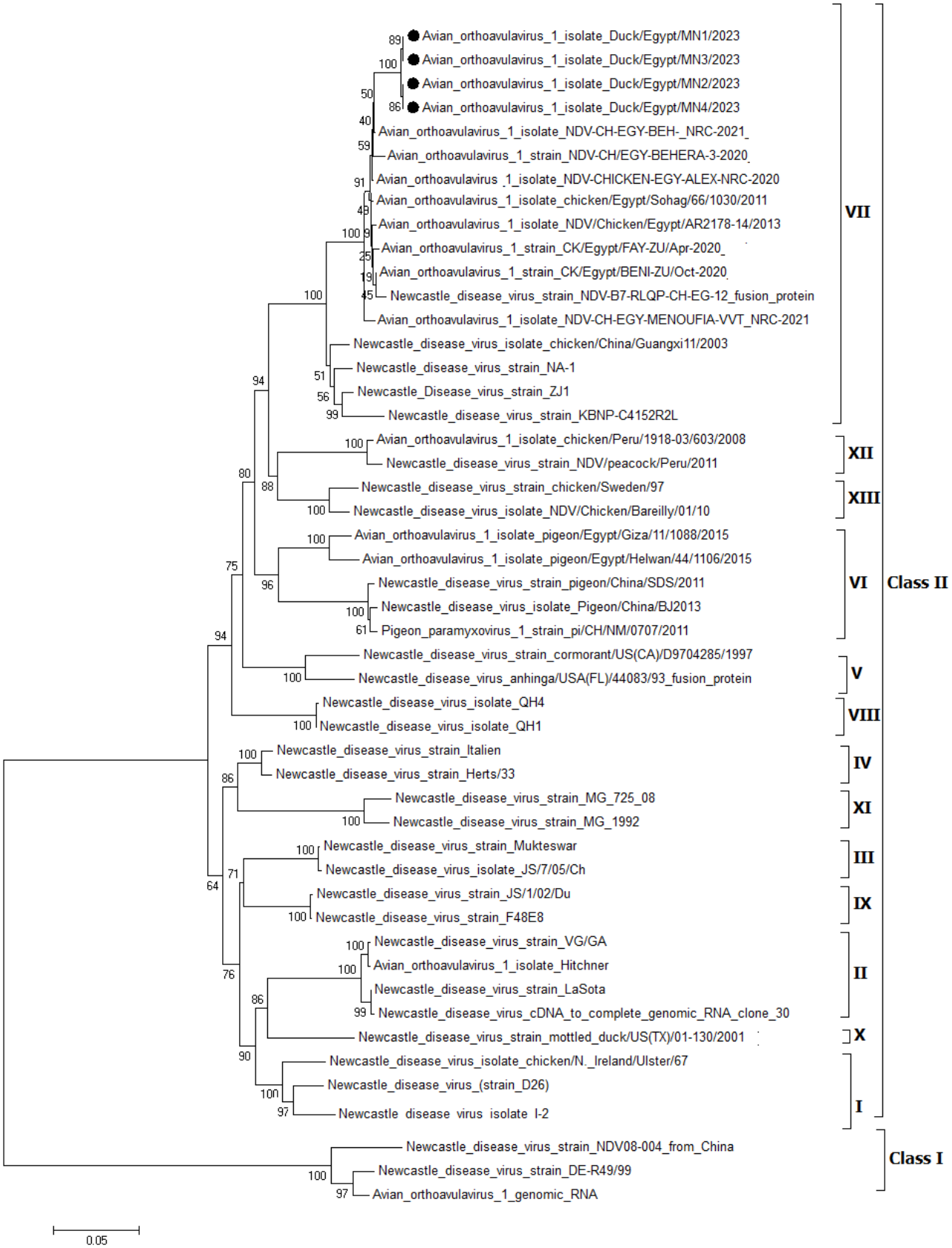


Figure 2. Phylogenetic tree based on nucleotide sequences of the fusion gene using reference sequences representing different genotypes. The black bullets indicated the mallard duck Newcastle disease virus strains isolated in this study

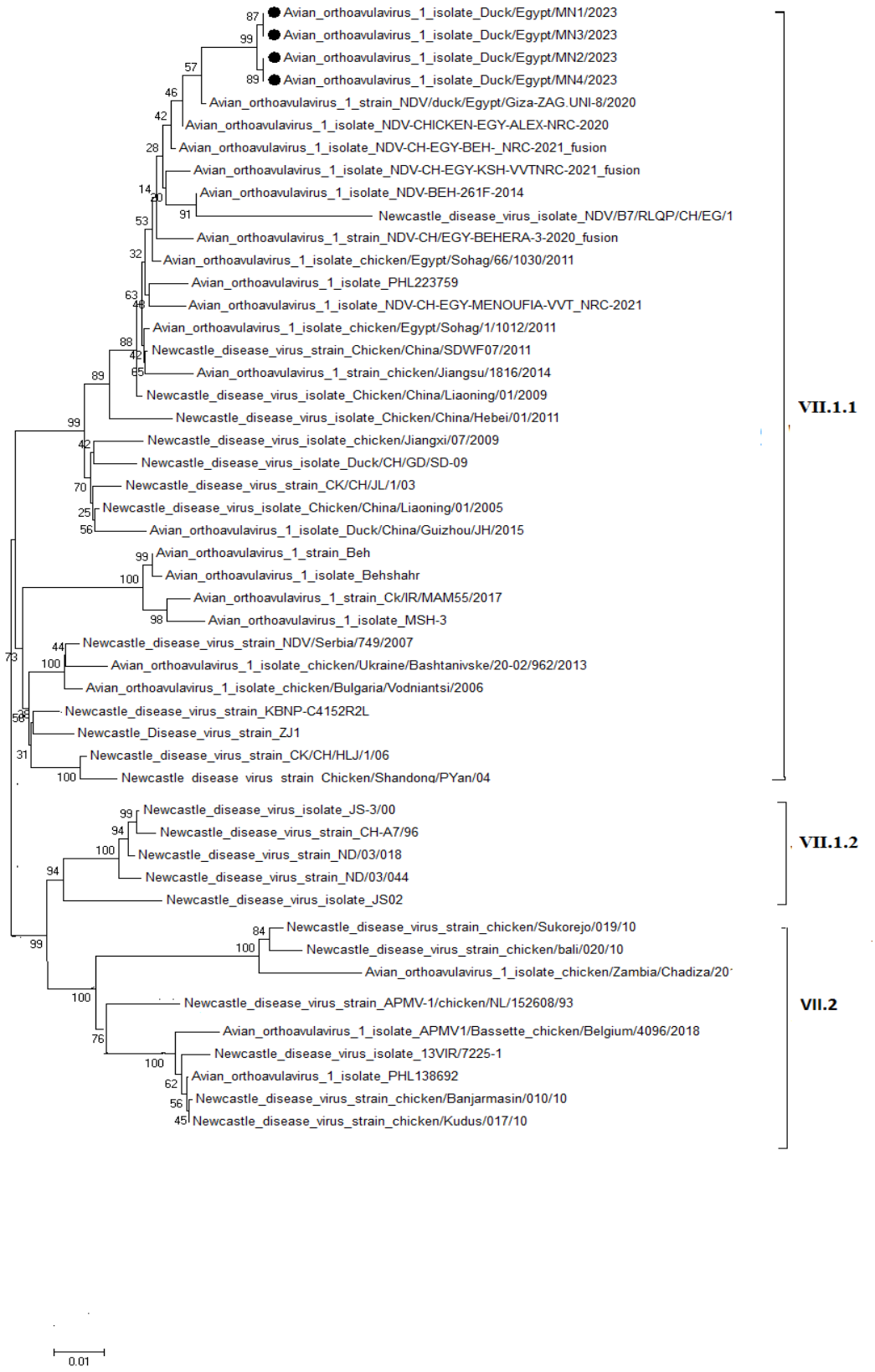


Figure 3. Phylogenetic tree based on nucleotide sequences of the fusion gene using reference strains for Newcastle disease virus GVII. The black bullets indicated the mallard duck Newcastle disease virus strains isolated in this study

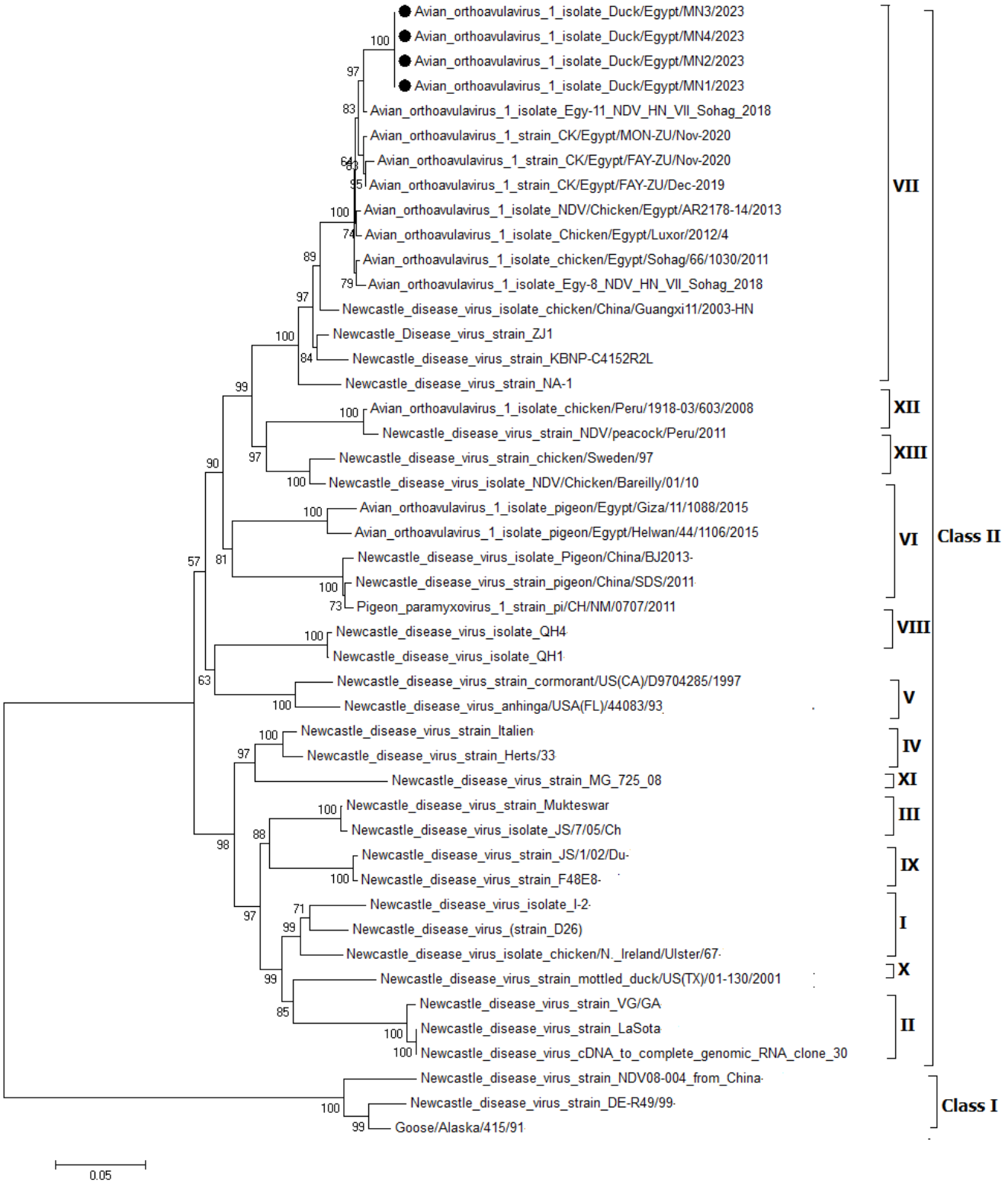


Figure 4. Phylogenetic tree based on nucleotide sequences of the hemagglutinin-neuraminidase gene using reference sequences representing different genotypes. The black bullets indicates the mallard duck Newcastle disease virus strains isolated in this study

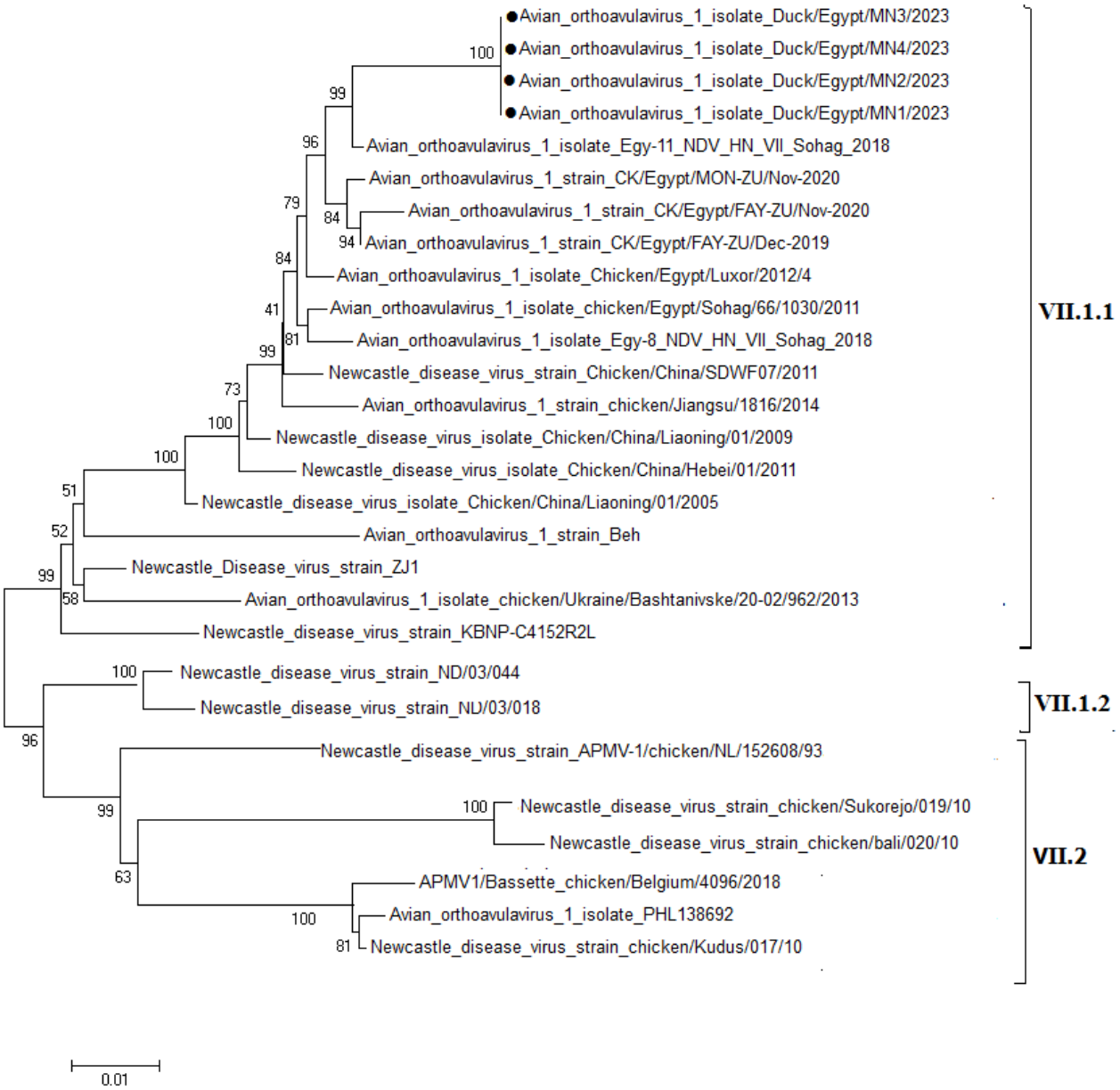


Figure 5. Phylogenetic tree based on nucleotide sequences of the hemagglutinin-neuraminidase gene using the strains and reference strains for Newcastle disease virus GVII. The black bullets indicate the mallard duck Newcastle disease virus strains isolated in this study

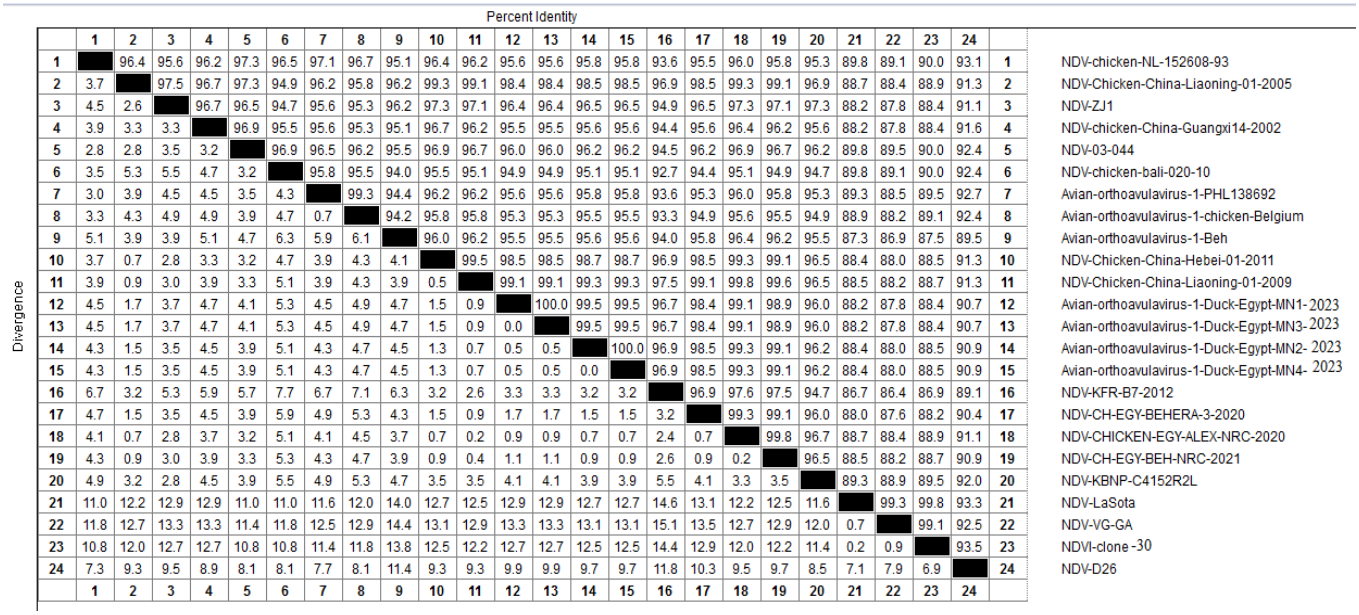


Figure 6. Amino acid identity percent of the obtained sequences of fusion gene from mallard duck and representative global Newcastle disease virus strains plus the commercial vaccinal strains

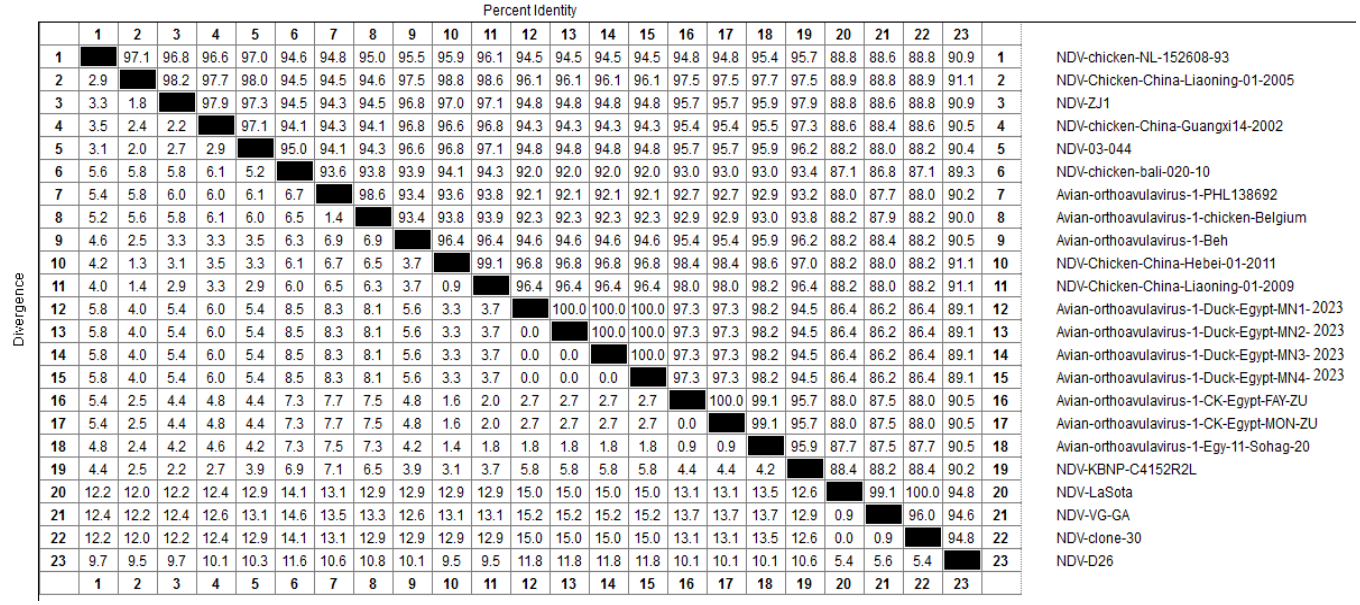


Figure 7. Amino acid identity percent of the obtained sequences of the hemagglutinin-neuraminidase gene from mallard duck and representative global Newcastle disease virus strains plus the commercial vaccinal strains

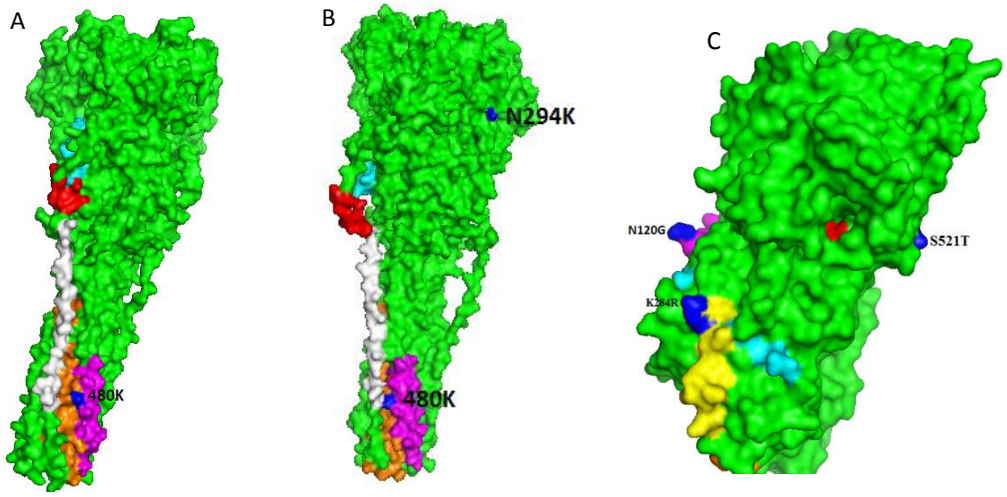


Figure 8. Three-dimensional structure of fusion and hemagglutinin-neuraminidase protein of the mallard duck Newcastle disease virus. **A** and **B**: Three-dimensional structure for fusion protein showing cleavage site (Red), fusion peptide (gray), HRa (orange), HRb (cyan), and HRc (Magnet). The mutation in the HR region (blue). The template protein was obtained by submitting the amino acid sequence of the fusion protein of Avian-orthoavulavirus-1-Duck-Egypt-MN1-2023 and Avian-orthoavulavirus-1-Duck-Egypt-MN2-2023, respectively. **C**: Three-dimensional structure for hemagglutinin-neuraminidase protein showed IDE1 (red), IDE2 (yellow), IDE3 (magnet), IDE4 (cyan), and IDE5 (orange). The mutation in the neutralizing epitopes (blue). The template protein was obtained by submitting the amino acid sequence of the hemagglutinin-neuraminidase protein of Avian-orthoavulavirus-1-Duck-Egypt-MN1-2023

Mutation analysis of fusion gene

All strains in this study had more than 52 amino acid mutations compared with Lasota strains in the F gene as that had been detected in the Egyptian strains. Compared with the lasota strain, The F gene of all strains had specific characteristics of 14 nucleotide new mutations with sense amino acid mutations at A11T in the signal peptide and T324A. Also, they had 480K in the HRc as lasota strains but different than other strains of genotype VII.1.1. The NDV-Duck-Egypt-MN1-2023 and NDV-Duck-Egypt-MN3-2023 that isolated from trachea isolates had specific addition mutations; L15F in the signal peptide and D97E in the F2 subunit. The NDV-Duck-Egypt-MN2-2023 and NDV-Duck-Egypt-MN4-2023 that were isolated from the brain had N294K in the hydrophobic heptad repeat-b (HR_b).

The distinctive characteristics of genotype VII were found in the conserved amino acids in the F gene of all isolates at positions K101 and V121 of the fusion (F) protein, and the cleavage site resembled NDV (RRQKR/F) as NDV genotype VII. Comparing them to the Lasota strain, the fusion protein's functionality depends on seven neutralizing epitopes, which were preserved at locations D72, E74, A75, R78, A79, 157SIAATNEAVHEVT171, and L343. Additionally, 10 amino acid cysteine sites were preserved at positions C76, C199, C338, C347, C362, C370, C394, C399, C401, and C424.

Mutation analysis of the HN gene

All strains in this study had more than 52 A.A. mutations compared with lasota strains as in the Egyptian strains. Compared with the lasota strain, the HN gene had a specific 16 nucleotide new mutation with multiple sense A.A. mutations at A4V, R15K in the cytoplasmic region, A28T in the transmembrane domain, S76L in the HRa. also, it had A50T, S54R T232N, P392S, T443V. There was no difference between the strains isolated from the brain and trachea tissues.

The HN gene of all strains in this study contained seven conserved neutralizing epitopes (193 to 201 [site 23]; 345 to 353 [site 1,14], 494 [site 12], 513 to 521 [site 2,12], and 569 [site 2]) compared with Lasota strain except E347K, S494D, I514V as other Egyptian strains and S521T that specific to strains in this study. In addition, all strains in this study had five (IDEs) neutralizing epitopes, the IDE1 was conserved with Lasota strains. Compared with the Lasota strain, the IDE2 had V288T, T290V, and G293K. The IDE3 had A118E, W123C, I127V. IDE4 had

E256G and IDE5 had V329A, Y340H, D342N, E347K as other Egyptian strains. The N120G and K284R in IDE3 and IDE2 had characteristics in all strains in this study. Also, additional mutations in the neutralizing epitope were detected at F156Y, Y203H, N263K, G495E, and A155V in all Egyptian strains.

All strains of this study shared three essential residues for receptor recognition at positions 174 (R), 401 (E), 416 (R), and 526 (Y), as well as the sialic acid-binding site (NRKSCS) at positions 234–239. In addition, all strains exhibited conservation of the 13 cysteine residues located at positions 123, 172, 186, 196, 238, 247, 251, 344, 455, 461, 465, 531, and 542 when compared to Lasota strains.

Glycosylation sites

All isolates' F gene glycosylation motifs indicated that residues 85, 191, 366, 447, 471, and 541 were the six possible locations for N-linked glycosylation. A study of the glycosylation motif in the HN gene across all isolates identified five possible glycosylation sites at amino acid locations (119, 341, 433, 481, and 508).

The three-dimension structure

The three-dimensional structure of the NDV trachea and brain stains was represented by the full fusion protein, which also clarified the substitution residues in the various active domains (HR1, HR2, cleavage site, and fusion peptides region) when compared to the Lasota strain. (Figure 8 A and B). The NDV strains isolated in this study were represented by the full HN protein, whose three-dimensional structure modeling demonstrated IDE1-IDE5 antigenic epitope and clarified the substitution residues in the neutralizing epitope specific to all strains of this study when compared to the Lasota strain (Figure 8 C).

DISCUSSION

Newcastle disease was a highly contagious avian disease that generated significant financial losses for the global poultry industry. Newcastle disease virus, also referred to as orthoavulavirus-1 or avian paramyxovirus serotype 1 (APMV-1), was presently categorized under the genus Orthoavulavirus of subfamily Avulavirinae in the family Paramyxoviridae of order Mononegaviriales (Rima et al., 2019). The virus encoded six structural proteins: large polymerase protein (L), fusion protein (F), matrix protein (M), phosphoprotein (P), nucleoprotein (NP), and hemagglutinin-neuraminidase (HN). It was a single-stranded RNA virus that was enclosed and had a negative sense genome (Steward and others, 1993). Newcastle disease virus infection and antigenicity depend on surface proteins known as fusion (F) and haemagglutinin-neuraminidase (HN) proteins (de Leeuw, 2005; Kim et al., 2011).

The amino acid sequence motif of the fusion protein's protease cleavage site and the capacity of particular cellular proteases to break this protein were linked to the molecular bases of NDV pathogenicity. Phylogenetically, NDV may be divided into two classes: class 1 and class 2. Class 1 has only one genotype while Class 2 has 21 genotypes till now (2.I-2.XXI, Dimitrov et al., 2019). Major populations of class 1 viruses were apathogenic and originated in aquatic wild birds (Kim et al., 2007), while class 2 viruses were those that cause epidemics in poultry and usually were highly pathogenic with continuous evolution over time (Miller et al., 2010).

Genotype VII was widely distributed globally causing serious economic losses and imposing great risk to the international poultry industry. Newcastle disease virus VII was subdivided into three subgenotypes comprise genotype VII: VII.1.1 (previously containing subgenotypes VIIb, VIId, VIIe, VIIj, and VIIl), subgenotype VII.1.2 (previously referred to as sub-genotype VIIf), and subgenotype VII.2 (previously containing subgenotypes VIIa, VIIh, VIIi, and VIIk; Dimitrov et al., 2019).

The NDV endemic situation in Egypt threatens the country's poultry industry since new cases continue to arise despite widespread routine vaccination programs that have been implemented in commercial poultry farms. Newcastle disease virus genotype VII has been reported from Egypt in the last few years (Radwan et al., 2013), in particular, Sub-genotype VII.1.1 (Nagy et al., 2020; AbdElfatah et al., 2021). Furthermore, Egyptian researchers have reported cases of co-infection with avian influenza viruses and infectious bronchitis (Moharam et al., 2019), this might affect on the effectiveness of the ND vaccination programs and complicate the ND control.

Newcastle disease virus is known to infect a minimum of 241 bird species belonging to 27 out of 50 bird orders. Waterfowl, including ducks and geese, are typically thought of as natural NDV transporters or reservoirs that exhibit few or non-existent clinical symptoms when infected with viruses, even the most pathogenic for chickens (Alexander and Senne, 2008).

This study was the first report for NDV genotype VII.1.1 isolation and characterization from mallard ducks that showed severe clinical signs and a high mortality rate in Egypt during the winter of 2023. The duck flocks showed highly pathogenic H5 avian influenza with similar

signs and lesions like nervous manifestations, respiratory and enteric signs, and a high mortality rate 55-70%, with sever congestion and swelling of parenchymatous organs like liver, spleen, and kidney (Figure 1). Testing tracheas from 16 duck flocks by rRT-PCR using NDV and AIV-specific primers, only 2 flocks tested positive for NDV, and the remaining 14 flocks were tested positive for AIV. Then NDV was successfully isolated from the trachea and brain of these two NDV-positive flocks then allantoic fluids from the first egg passage were positive for NDV by rRT-PCR and negative for influenza as previously detected in breeder duck flocks in China was reported with drop in egg production by about 70% and mortalities up to 50% (Liu et al., 2015), also high pathogenicity in experimentally infected ducks was reported (Dai et al., 2013). These observations should be considered by duck consultants and producers in Egypt to put in mind the NDV infection for differential diagnosis with HPAI H5 outbreaks in ducks and highlight the need for duck vaccination, especially with NDV-killed vaccines to control the disease.

The phylogenetic analysis of F and HN genes of NDV strains isolated in this study belonged to class II genotype VII 1.1 in new branch as previously described in other studies (Eid et al., 2022; Ragab et al., 2022; Sallam et al., 2022) with high identity percent with Chinese strains (98.5-99.3% and 96.4-96.8%) and other Egyptian strains (96.7-99.3% and 97.3-98.2%) for F and HN gene respectively. The NDV strains isolated in this study were closely related to each other, according to sequence analysis of F and HN, and there has been a modest new branch within genotype VIIj (Figures 6 and 8), this is probably because the mutations in fusion and HN genes in the strains in this study. This finding indicated the high evolution rate of NDV and reflected the complicated situation in the poultry industry with the highly increased rate of backyard rearing of multiple bird species through all Egyptian governorates.

The F protein of NDV was carrying structures with important functional roles like fusion peptide, signal peptide cleavage sites, and B-cell mediated antibody response. The fusion peptide (FP), located between positions 117 and 136 aa in the F1 subunit, three hydrophobic heptad repeat (HR) domains (HRa, 143–185 aa, HRb, 268–299 aa, and HRc, 471–500 aa), the transmembrane (TM) domain (501–522 aa), and the cytoplasmic tail (from 523–553 aa) are crucial for the pathogenicity and infectivity of the virus (Sergel-Germano et al., 1994). Newcastle disease virus strains isolated from the trachea and brain of duck had over 52 mutations in amino acids when compared with the Lasota strain as other Egyptian strains which can be reflected in the immunogenicity of these strains producing heterologous immunity (Selim et al., 2018). With specific mutations in all strains in this study in the F gene clustered in the new branch, mutations were detected in the signal peptide at A11T and T324A specific strains in this study.

Interestingly, the amino acid mutation was observed at N294K in the HRb in the *F* protein of brain strains NDV-Duck-Egypt-MN2-2023 and NDV-Duck-Egypt-MN4-2023 compared to the NDV-Duck-Egypt-MN1-2023 and NDV-Duck- Egypt-MN3-2023 strains isolated from trachea of the same duck. The N294K in the $HR_{b\ is}$ expected to modify F protein receptor binding affinity to receptors on nervous cells, these mutations might be an important factor for the virus to cross the blood-brain barrier and adapt to brain tissue indicating the major importance of the *F* gene for NDV adaptation (Sergel et al., 2001), it needs further experimental study. In addition, the 480K in the HRc was detected in strains isolated in this study also as Lasota strain but different from other NDV GVII strains that is deemed to be characteristic to NDV genotype VII.1.1 duck strains that may alter the fusion activity of the virus (Sergel et al., 2001), this may have a role in the adaptation of the virus and its increased virulence to ducks. Additional research is necessary to examine the impact of these mutations on the virus's pathogenicity.

When compared to the Lasota strain, the entire fusion protein portrayed the three-dimensional structure of the NDV trachea and brain staining and made the substitution residues in the several active domains (HR1, HR2, cleavage site, and fusion peptides region) (Morrison, 2003). By merging the viral envelope with the plasma membrane, the host cell proteases influenced this proteolytic cleavage, which increased the virus's capacity to infect the host cell (Lamb and Parks, 2007). Two pairs of various basic amino acids were discovered when the amino acid sequences of the F protein were aligned: F phenylalanine amino acid at position 117 and R/K at locations 112 to 116. These locations matched the virulent strain RRQKRF's cleavage site motif. According to Wang et al. (2017), the presence of Q in the motif of the virulent strain RRQKRF enhanced and magnified its pathogenic potential. The conserved amino acids at positions K101 and V121 of the F protein, which were unique to genotype VII, were also present in all strains. This discovery aligned with the findings of previous studies (Lien et al., 2007).

No amino acid mutations were detected in the antigenic epitope of F protein for strains isolated in this

study, the neutralizing epitopes contained in fusion protein are necessary for antibody binding and the diversity of antigens (Qin et al., 2008). Viral glycoprotein structure and function, which impacted viral tropism, infectivity, and antigenicity, were influenced by the glycosylation process (Aguilar et al., 2006; Eichler et al., 2006). Modifications at one of these two N-glycosylation residues might be crucial for the facilitation of fusion (McGinnes et al., 2001). The NDV strains isolated in this study had conserved six glycosylation sites as previously found (Selim et al., 2018). Cysteine residues were essential for protein folding because they created disulfide bonds, which gave the protein structural stability (McLellan et al., 2013). In all strains examined in this investigation, there were ten consistent cysteine residues found in the NDV fusion as previously recorded (Selim et al., 2018).

Newcastle disease virus's surface glycoprotein HN has several functions related to viral tropism and pathogenicity (Huang et al., 2004). The stalk region, globular head, transmembrane region, and cytoplasmic domain make up the *HN* protein (Ferreira et al., 2004). It can attach to cell surface receptors that contain sialic acid and has the neuraminidase (NA) activity that is required to prevent viral self-agglomeration. Furthermore, HN stimulated the fusion activity of the *F* protein, which mediated both cell-to-cell and virus-to-cell fusion (Melanson and Iorio, 2004). What's more, during NDV infection, HN, a significant protective antigen, could stimulate the production of antibodies that neutralize the virus (Kim et al., 2009; Yan et al., 2009).

It was found that they possessed over fifty-two amino acid mutations in the HN protein when comparing the two strains isolated from the trachea and brain of domestic duck with the Lasota strain, so antibodies produced against the Lasota vaccine will be heterologous to these circulating strains that could be failed to neutralize them (Selim et al., 2018). Specific mutations in all strains in this study in the HN gene clustered them in new branches with no difference between strains isolated from the trachea and brain (Figures 7 and 8).

Although the HN protein's structure and functions could be impacted by mutations in its transmembrane and stalk domains (McGinnes et al., 1993), the cytoplasmic tail was important for replication, and the species-specific phenotypes (Kim et al., 2011). The HN in all strains detected from ducks in this study had a transmembrane mutation in A28T, also, A4V, and R15K in the cytoplasmic domain that could affect viral fusion (Kim et al., 2009). In addition, S76L in the HRa, and mutations at A50T, S54R, T232N, P392S, and T443V were also detected. These reported amino acid mutations could be considered species adaptive mutations and could have a role in increased virus virulence in ducks. We need further research to study the effect of these mutations on the pathogenicity of the virus.

Seven antigenic sites were previously discovered, consisting of residues 193-201 (site 23), 345-353 (sites 1 and 14), and residues 494, 513 to 521, and 569 (sites 12 and 2) in the C-terminal domain (Iorio et al., 1991). Jin et al. (2021) have employed the PepSCan technique to identify the NDV, HN protein's immuno-dominant epitopes (IDEs). These were the IDE numbers: IDE1 (554-568aa), IDE2 (283-297aa), IDE3 (119-133aa), IDE4 (242-256aa), and IDE5 (328-342aa, Jin et al., 2021). Neutralizing epitopes have been shown to undergo amino acid modification, which may result in neutralizing escape variants and help create antigenic epitopes (Hu et al., 2010). Nineteen amino acid mutations in the HN neutralizing epitopes were indicated in Egyptian strains as previously described by Naguib et al. (2021), in addition to three amino acid mutations (N120G, K284R, S521T) were detected specific to NDV strains of this study that can alter the virus antigenicity. The mutations reported in F and HN proteins overformed novel NDV strains with variable antigenicity and transmission of these viruses from ducks back to chickens can escape the immunity produced by used commercial vaccines in chickens (Elbestawy et al., 2023).

According to Chen et al. (2001), an essential membrane protein that is glycosylated through the N-link is the HN glycoprotein. The initiation and maintenance of protein folding into its physiologically active conformation, protein stability and solubility, intracellular transport of the proteins to different subcellular compartments and the cell surface, and the antigenicity and immunogenicity of the proteins were just a few of the many glycoprotein properties that were impacted by N-linked glycosylation (Quinones-Kochs et al., 2002). According to Panda et al. (2004), the loss of a single glycosylation site modified the pathogenicity of NDV. According to the findings of Cattoli et al. (2010), the Newcastle disease virus strains examined in this investigation possessed five putative glycosylation sites (residues 119, 341, 433, 481, and 508) as VII, NDV.

Comparative to vaccines used in Egypt and strains isolated in this study, we detected low similarity percent 87.8-88.5% and 86.2-86.4% between vaccines related to genotype II (Lasota, clone 30 and VG/GA) and ranged between 90.7-90.9% and 89.1% with D26/76 vaccine (genotype I) for F and HN gene respectively as previously

reported by Xue et al. (2017). The genotype VII vaccine (KBNPC415R2L) used in Egypt has an interesting nucleotide identity range of 96-96.2% and 94.5% with the F and HN genes protein sequence, suggesting a higher probability of protection with this type of vaccine as previously described (Abd El-Hamid et al., 2020). These observations indicated that genotype-matched vaccine with VII strains in NDV vaccination programs is a must now in Egypt and any country that has a similar situation.

In conclusion, this was the first report of outbreaks of NDV genotype VII.1.1 in domestic mallard ducks with severe clinical signs, nervous manifestations, respiratory and enteric signs, and high mortalities during the winter season of 2023 in Egypt. The full gene sequence for F and HN genes revealed significant evolution of NDVs isolated from ducks compared to circulating VII strains in other species specifically chickens and vaccine strains, especially of genotypes II and I. These A.A. mutations recorded in *F* and HN proteins clustered them in new branches that could be the result of the extensive circulation of the virus in different Egyptian poultry over a long period, and also, could be due to specific signature mutations for duck adaptation. Interestingly, we reported specific amino acid mutations between NDV strains isolated from brain strains compared to trachea strains from the same duck, which might play the main role in virus adaptation to brain tissue and crossing the blood-brain barrier but further experimental studies were required. Based on the results obtained in this study, vaccination of duck flocks in Egypt should be considered in future ND control strategies to reduce economic losses to duck producers, decrease shedding to other poultry flocks, especially chickens, and control NDV evolution in the country.

DECLARATIONS

Authors' contributions

Mahmoud Ibrahim contributed in collecting samples, isolation, and identification of. Mohamed Wahba performed RT-PCR for F and HN genes. Nahed Yehia made the sequence and phylogenetic analysis. M Ibrahim and N Yehia wrote the darft of the manuscript, and revised it before submission. All authors checked and confirmed all data and the last draft of the manuscript before submission to the journal.

Acknowledgments

The authors would like to thank the Faculty of Veterinary Medicine, University of Sadat City for providing facilities and support for this study.

Funding

This study was funded in part by the Egyptian Company for Biological & Pharmaceutical Industries (Vaccine Valley), Egypt.

Ethical considerations

The authors confirm that all authors have reviewed and submitted the manuscript to this journal for the first time.

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.

Conflict of interests

The authors have not declared any conflict of interest.

REFERENCES

Abd El-Hamid HS, Shafi ME, Albaqami NM, Ellakany HF, Abdelaziz NM, Abdelaziz MN, Abd El-Hack ME, Taha AE, Alanazi KM, and Elbestawy AR (2020). Sequence analysis and pathogenicity of avian Orthoavulavirus 1 strains isolated from poultry flocks during 2015-2019. BMC Veterinary Research, 16(1): 253. Available at: https://link.springer.com/article/10.1186/s12917-020-02470-9

AbdElfatah KS, Elabasy MA, El-khyate F, Elmahallawy EK, Mosad SM, El-Gohary FA, Abdo W, Al-Brakati A, Seadawy MG, Tahoon AE et al. (2021). Molecular characterization of velogenic Newcastle disease virus (Sub-Genotype VII.1.1) from wild birds, with assessment of its pathogenicity in susceptible chickens. Animals, 11(2): 505. DOI: https://www.doi.org/10.3390/ani11020505

Aguilar HC, Matreyek KA, Filone CM, Hashimi ST, Levroney EL, Negrete OA, and Su SV (2006). N-glycans on Nipah virus fusion protein protect against neutralization but reduce membrane fusion and viral entry. Journal of Virology, 80(10): 4878-4889. DOI: https://www.doi.org/10.1128/jvi.80.10.4878-4889.2006

Alexander DJ and Senne DA (2008). Newcastle disease, other avian paramyxoviruses, and pneumovirus infections. In: Y. M. Saif, A. M. Fadly, J. R. Glisson, L. R. McDougald, N. K. Nolan, D. E. Swayne (Editors), Diseases of poultry, 12th Edition. Blackwell Publishing., Ames, IA. pp. 75-115.

Cattoli G, Fusaro A, Monne I, Molia S, Le Menach A, Maregeya B, Nchare A, Bangana I, Maina AG, N'Goran Koffi JN et al. (2010). Emergence of a new genetic lineage of Newcastle disease virus in West and Central Africa: Implications for diagnosis and control (2010). Veterinary Microbiology, 142(3-4): 168-176. DOI: https://www.doi.org/10.1016/j.vetmic.2009.09.063

Cattoli G, Susta L, Terregino C, and Brown C (2011). Newcastle disease: A review of field recognition and current methods of laboratory detection. Journal of Veterinary Diagnostic Investigation, 23(4): 637-656. DOI: https://www.doi.org/10.1177/1040638711407887

Chen L, Gorman JJ, McKimm-Breschkin J, Lawrence LJ, Tulloch PA, Smith BJ, Colman PM, and Lawrence MC (2001). The structure of the fusion glycoprotein of Newcastle disease virus suggests a novel paradigm for the molecular mechanism of membrane fusion. Structure, 9(3): 255-266. DOI: https://www.doi.org/10.1016/S0969-2126(01)00581-0

Dai Y, Liu M, Cheng X, Shen X, Wei Y, Zhou S, Yu S, and Ding C (2013). Infectivity and pathogenicity of Newcastle disease virus strains of different avian origin and different virulence for mallard ducklings. Avian Diseases, 57: 8-14. DOI: http://www.doi.org/10.1637/10298-070212-Reg.1

de Leeuw OS, Koch G, Hartog L, Ravenshorst N, and Peeters BPH (2005). Virulence of Newcastle disease virus is determined by the cleavage site of the fusion protein and by both the stem region and globular head of the haemagglutinin–neuraminidase protein. Journal of General Virology, 86(6): 1759-1769. DOI: https://www.doi.org/10.1099/vir.0.80822-0

Dimitrov KM, Abolnik C, Afonso CL, Albina E, Bahl J, Berg M, Briand FX, Brown IH, Choi KS, Chvala I et al. (2019). Updated unified phylogenetic classification system and revised nomenclature for Newcastle disease virus. Infection, Genetics and Evolution, 74: 103917. DOI: https://www.doi.org/10.1637/10298-070212-Reg.1

Dimitrov KM, Ramey AM, Qiu X, Bahl J, and Afonso CL (2016). Temporal, geographic, and host distribution of avian paramyxovirus 1 (Newcastle disease virus). Infection, Genetics and Evolution, 39: 22-34. DOI: https://www.doi.org/10.1016/j.meegid.2016.01.008

Eichler R, Lenz O, Garten W, and Strecker T (2006). The role of single N-glycans in proteolytic processing and cell surface transport of the Lassa virus glycoprotein GP-C. Virology Journal, 3(1): 41. DOI: https://www.doi.org/10.1186/s12985-022-01864-5

Eid AAM, Hussein A, Hassanin O, Elbakrey RM, Daines R, Sadeyen JR, Abdien HMF, Chrzastek K, and Iqbal M (2022). Newcastle disease genotype VII prevalence in poultry and wild birds in Egypt. Viruses, 14(10): 2244. DOI: https://www.doi.org/10.3390/v14102244

Elbestawy AR, Ellakany HF, Abd El-Hamid HS, Zedan RE, Gado AR, Sedeik ME, Abd El-Hack ME, Saadeldin IM, Alowaimer AN, Ba-Awadh HA et al. (2019). Muscovy ducks infected with velogenic Newcastle disease virus (genotype VIId) act as carriers to infect in-contact chickens. Poultry Science, 98(10): 4441-4448. DOI: https://www.doi.org/10.3382/ps/pez276

Elbestawy A, Ellakany H, Sedeik M, Gado A, Abdel-Latif M, Noreldin A, Orabi A, Radwan I, and El-Ghany WA (2023). Superior efficacy of apathogenic genotype I (V4) over lentogenic genotype II (LaSota) live vaccines against Newcastle disease virus genotype VII.1.1 in pathogen-associated molecular pattern-H9N2 vaccinated broiler chickens. Vaccines, 11(11): 1638. DOI: https://www.doi.org/10.3390/vaccines11111638

Ferreira L, Munoz-Barroso I, Marcos F, Shnyrov VL, and Villar E (2004). Sialidase, receptor-binding and fusion-promotion activities of Newcastle disease virus haemagglutinin-neuraminidase: A mutational and kinetic study. Journal of General Virology, 85(Pt 7): 1981-1988. DOI: https://www.doi.org/10.1099/vir.0.79877-0

Gupta R and Brunak S (2002). Prediction of glycosylation across the human proteome and the correlation to protein function. Pacific Symposium on Biocomputing, pp. 310-322. Available at: https://pubmed.ncbi.nlm.nih.gov/11928486/

Hall TA (1999). Biodiet: A user-friendly biological sequence alignment editor and analysis. Nucleic Acids Symposium Series, 41(2): 95-98. DOI: https://www.doi.org/10.14601/phytopathol_mediterr-14998u1.29

Hidaka C, Soda K, Nomura F, Kashiwabara Y, Ito H, and Ito T (2021). The chicken-derived velogenic Newcastle disease virus can acquire high pathogenicity in domestic ducks via serial passaging. Avian Pathology, 50(3): 234-245. DOI: https://www.doi.org/10.1080/03079457.2021.1889461

Hu S, Wang T, Liu Y, Meng C, Wang X, Wu T, and Liu X (2010). Identification of a variable epitope on the Newcastle disease virus hemagglutinin-neuraminidase protein. Veterinary Microbiology, 140(1-2): 92-97. DOI: https://www.doi.org/10.1016/j.vetmic.2009.07.029

Huang Z, Panda A, Elankumaran S, Govindarajan D, Rockemann DD, and Samal SK (2004). The hemagglutinin-neuraminidase protein of Newcastle disease virus determines tropism and virulence. Journal of Virology, 78(8): 4176-4184. DOI: https://www.doi.org/10.1128/jvi.78.8.4176-4184.2004

Iorio RM, Syddall RJ, Sheehan JP, Bratt MA, Glickman RL, and Riel AM (1991). Neutralization map of the hemagglutinin-neuraminidase glycoprotein of Newcastle disease virus: Domains recognized by monoclonal antibodies that prevent receptor recognition. Journal of Virology, 65(9): 4999-5006. DOI: https://www.doi.org/10.1128/jvi.65.9.4999-5006.1991

Jin Z, Wei Q, Bi Y, Li Y, Huo N, Mou S, Wang W, Liu H, Yang Z, Chen H et al. (2021). Identification of a potential neutralizing linear epitope of hemagglutinin-neuraminidase in Newcastle disease virus. Virology Journal, 18: 8. DOI: https://www.doi.org/10.1186/s12985-020-01483-y

Jindal N, Chander Y, Chockalingam AK, de Abin M, Redig PT, and Goyal SM (2009). Phylogenetic analysis of Newcastle disease viruses isolated from waterfowl in the Upper Midwest Region of the United States. Virology Journal, 6: 191. DOI: https://www.doi.org/10.1186/1743-422X-6-191

Kiani MH, Bozorgmehrifard MH, Hosseini H, Charkhkar S, and Ghalyanchilangeroudi A (2021). Hemagglutinin-neuraminidase sequence and phylogenetic analysis of two Newcastle disease virus isolated from chickens in Iran. Archives of Razi Institute, 76(1): 31-39. DOI: https://www.doi.org/10.22092/ari.2019.124844.1289

Kim LM, King DJ, Curry PE, Suarez DL, Swayne DE, Stallknecht DE, Slemons RD, Pedersen JC, Senne DA, Winker K et al. (2007). Phylogenetic diversity among low-virulence Newcastle disease viruses from waterfowl and shorebirds and comparison of genotype distributions to those of poultry origin isolates. Journal of Virology, 81(22): 12641-12653. DOI: https://www.doi.org/10.1128/JVI.00843-07

Kim SH, Subbiah M, Samuel AS, Collins PL, and Samal SK (2011). Roles of the fusion and hemagglutinin-neuraminidase proteins in replication, tropism, and pathogenicity of avian paramyxoviruses. Journal of Virology, 85(17): 8582-8596. DOI: https://www.doi.org/10.1128/JVI.00652-11

Kim SH, Yan Y, and Samal SK (2009). Role of the cytoplasmic tail amino acid sequences of Newcastle disease virus hemagglutinin-neuraminidase protein in virion incorporation, cell fusion, and pathogenicity. Journal of Virology, 83(19): 10250-10255. DOI: https://www.doi.org/10.1128/jvi.01038-09

Lamb RA and Parks GD (2007). Paramyxoviridae: The viruses and Their Replication. In: D. M. Knipe, P. M. Howley (Editors), Fields Virology. Lippincott, Williams, and Wilkins., Philadelphia, pp. 1449-1496. Available at: https://www.scholars.northwestern.edu/en/publications/paramyxoviridae-the-viruses-and-their-replication-2

Lien YY, Lee JW, Su HY, Tsai HJ, Tsai MC, and Hsieh CY (2007). Phylogenetic characterization of Newcastle disease viruses isolated in Taiwan during 2003-2006. Veterinary Microbiology, 123(1-3): 194-202. DOI: https://www.doi.org/10.1016/j.vetmic.2007.03.006

Liu M, Shen X, Cheng X, Li J, and Dai Y (2015). Characterization and sequencing of a genotype VIId Newcastle disease virus isolated from laying ducks in Jiangsu, China. Genome Announce, 3(6): e01412-15. DOI: https://www.doi.org/10.1128/genomea.01412-15

McGinnes L, Sergel T, and Morrison T (1993). Mutations in the transmembrane domain of the HN protein of Newcastle disease virus affect the structure and activity of the protein. Virology, 196(1): 101-110. DOI: https://www.doi.org/10.1006/viro.1993.1458

McGinnes LW, Sergel T, Chen H, Hamo L, Schwertz S, Li D, and Morrison TG (2001). Mutational analysis of the membrane proximal heptad repeat of the Newcastle disease virus fusion protein. Virology, 289(2): 343-352. DOI: https://www.doi.org/10.1006/viro.2001.1123

McLellan JS, Ray WC, and Peeples ME (2013). Structure and function of respiratory syncytial virus surface glycoproteins. Challenges and opportunities for respiratory syncytial virus vaccines. Springer., Berlin, Heidelberg, pp. 83-104. Available at: https://link.springer.com/chapter/10.1007/978-3-642-38919-1_4

Melanson VR and Iorio RM (2004). Amino acid substitutions in the F-specific domain in the stalk of the Newcastle disease virus HN protein modulate fusion and interfere with its interaction with the F protein. Journal of Virology, 78(23): 13053-13061. DOI: https://www.doi.org/10.1128/jvi.78.23.13053-13061.2004

Meng C, Rehman ZU, Liu K, Qiu X, Tan L, Sun Y, Liao Y, Song C, Yu S, Ding Z et al. (2018). Potential of genotype VII Newcastle disease viruses to cause differential infections in chickens and ducks. Transboundary Emerging Diseases, 56(6): 1851-1862. DOI: https://www.doi.org/10.1111/tbed.12965

Miller PJ and Koch G (2013). Newcastle disease. In: D. E. Swayne, J. R. Glisson, L. R. McDougald, L. K. Nolan, D. L. Suarez, V. Nair (Editors), Disease of poultry, 13th Edition. Wiley-Blackwell., Ames, pp. 89-107. Available at: https://www.ars.usda.gov/research/publications/publication/?seqNo115=286542

Miller PJ, Decanini EL, and Afonso CL (2010). Newcastle disease: E volution of genotypes and the related diagnostic challenges. Infection, Genetics and Evolution, 10(1): 26-35. DOI: https://www.doi.org/10.1016/j.meegid.2009.09.012

Moharam I, Razik AA, Sultan H, Ghezlan M, Meseko C, Franzke K, Harder T, Beer M, and Grund C (2019). Investigation of suspected Newcastle disease (ND) outbreaks in Egypt uncovers a high virus velogenic ND virus burden in small-scale holdings and the presence of multiple pathogens. Avian Pathology, 48(5): 406-415. DOI: https://www.doi.org/10.1080/03079457.2019.1612852

Morrison TG (2003). Structure and function of a paramyxovirus fusion protein. Biochimica et Biophysica Acta, 1614(1): 73-84. DOI: https://www.doi.org/10.1016/s0005-2736(03)00164-0

Munir M, Linde AM, Zohari S, Ståhl K, Baule C, Holm K, Engström B, and Berg M (2010). Complete genome analysis of an avian paramyxovirus type 1 strain isolated in 1994 from an asymptomatic black-headed gull (*Larus ridibundus*) in southern Sweden. Avian Diseases, 54(2): 923-930. DOI: https://www.doi.org/10.1637/9086-092409-RESNOTE.1

Moustapha A, Talaki E, Akourki A, and Ousseini M (2023). Newcastle disease virus in poultry: Current status and control prospects. Worlds' Veterinary Journal, 13(2): 240-249. DOI: https://www.doi.org/10.54203/scil.2023.wvj26

Naguib MM, Höper D, Elkady MF, Afifi MA, Erfan A, Abozeid HH, Hasan WM, Arafa AS, Shahein M, Beer M et al. (2021). Comparison of genomic and antigenic properties of Newcastle disease virus genotypes II, XXI and VII from Egypt do not point to antigenic drift as selection marker. Transboundary Emerging Diseases, 69(2): 849-863. DOI: https://www.doi.org/10.1111/tbed.14121

Nagy A, Ali A, Zain El-Abideen MA, Kilany W, and Elsayed M (2020). Characterization and genetic analysis of recent and emergent virulent Newcastle disease viruses in Egypt. Transboundary Emerging Diseases, 67(5): 2000-2012. DOI: https://www.doi.org/10.1111/tbed.13543

Panda A, Elankumaran S, Krishnamurthy S, Huang Z, and Samal SK (2004). Loss of N-linked glycosylation from the hemagglutinin-neuraminidase protein alters virulence of Newcastle disease virus. Journal of Virology, 78(10): 4965-4975. DOI: https://www.doi.org/10.1128%2FJVI.78.10.4965-4975.2004

Qin ZM, Tan LT, Xu HY, Ma BC, Wang YL, Yuan XY, and Liu WJ (2008). Pathotypical characterization and molecular epidemiology of Newcastle disease virus isolates from different hosts in China from 1996 to 2005. Journal of Clinical Microbiology, 46(2): 601-611. DOI: https://www.doi.org/10.1128/jcm.01356-07

Quinones-Kochs MI, Buonocore L, and Rose JK (2002). Role of N-linked glycans in a human immunodeficiency virus envelope glycoprotein: effects on protein function and the neutralizing antibody response. Journal of Virology, 76(9): 4199-4211. DOI: https://www.doi.org/10.1128/jvi.76.9.4199-4211.2002

Radwan MM, Darwish SF, El-Sabagh IM, El-Sanousi AA, and Shalaby MA (2013). Isolation and molecular characterization of Newcastle disease virus genotypes II and VIId in Egypt between 2011 and 2012. Virus Genes, 47(2): 311-316. DOI: https://www.doi.org/10.1007/s11262-013-0950-y

Ragab ESM, Ibrahim M, El-Naggar RF, Hussien EGS, El-Kanawati ZR, and AboElkhair MA (2022). Isolation and molecular characterization of Newcastle disease virus genotype VII circulating in Egypt (2017-2020). Journal of Current Veterinary Research, 4(2): 45-55. DOI: https://www.doi.org/10.21608/jcvr.2022.267507

Rima B, Balkema-Buschmann A, Dundon WG, Duprex P, Easton A, Fouchier R, Kurath G, Lamb R, Lee B, Rota P et al. (2019). ICTV virus taxonomy profile: Paramyxoviridae. Journal of General Virology, 100(12): 1593-1594. DOI: https://www.doi.org/10.1099/jgv.0.001328

Sallam HM, Saleh MM, and Zanaty AM (2022). Phylogenetic analysis of virulent Newcastle Disease virus recently isolated from broiler farms. Alexandria Journal of Veterinary Sciences, 75(2): 37-45. Available at: https://openurl.ebsco.com/EPDB%3Agcd%3A6%3A7841190/detailv2?sid=ebsco%3Aplink%3Ascholar&id=ebsco%3Agcd%3A160914981&crl=c

Selim KM, Selim A, Arafa A, Hussein HA, and Elsanousi AA (2018). Molecular characterization of full fusion protein (F) of Newcastle disease virus genotype VIId isolated from Egypt during 2012-2016. Veterinary World, 11(7): 930-938. DOI: https://www.doi.org/10.14202/vetworld.2018.930-938

Sergel TA, McGinnes LW, and Morrison TG (2001). Mutations in the fusion peptide and adjacent heptad repeat inhibit folding or activity of the Newcastle disease virus fusion protein. Journal of Virology, 75(17): 7934-7943. DOI: https://www.doi.org/10.1128/jvi.75.17.7934-7943.2001

Sergel-Germano T, McQuain C, and Morrison T (1994). Mutations in the fusion peptide and heptad repeat regions of the Newcastle disease virus fusion protein block fusion. Journal of Virology, 68(11): 7654-7658. DOI: https://www.doi.org/10.1128/jvi.68.11.7654-7658.1994

Spackman E, Senne DA, Bulaga LL, Myers TJ, Perdue ML, Garber LP, Lohman K, Daum LT, and Suarez DL (2003). Development of real-time RT-PCR for the detection of avian influenza virus. Avian Diseases, 47(s11): 1079-1082. DOI: https://www.doi.org/10.1637/0005-2086-47.s3.1079

steward M, Vipond IB, Millar NS, and Emmerson PT (1993). RNA editing in Newcastle disease virus. Journal of General Virology, 74(Pt 12): 2539-2547. DOI: https://www.doi.org/10.1099/0022-1317-74-12-2539

Tamura K, Stecher G, Peterson D, Filipski A, and Kumar S (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution, 30(12): 2725-2729. DOI: https://www.doi.org/10.1093/molbev/mst197

Wang Y, Yu W, Huo N, Wang W, Guo Y, and Wei Q (2017). Comprehensive analysis of amino acid sequence diversity at the F protein cleavage site of Newcastle disease virus in fusogenic activity. PLoS One, 12(9): e0183923. DOI: https://www.doi.org/10.1371/journal.pone.0183923

Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, Heer FT, de Beer TAP, Rempfer C, Bordoli L et al. (2018). SWISS-MODEL: Homology modelling of protein structures and complexes. Nucleic Acids Research, 46(W1): W296-W303. DOI: https://www.doi.org/10.1093/nar/gky427

Wise MG, Suarez DL, Seal BS, Pedersen JC, Senne DA, King DJ, Kapczynski DR, and Spackman E (2004). Development of a real-time reverse-transcription PCR for detection of Newcastle disease virus RNA in clinical samples. Journal of Clinical Microbiology,

42: 329-338. DOI: https://www.doi.org/10.1128/jcm.42.1.329-338.2004

World health organization (WHO) (2002). WHO manual on animal influenza diagnosis and surveillance. Geneva, Switzerland. Available at: https://iris.who.int/bitstream/handle/10665/68026/WHO_CDS_CSR_NCS_2002.5.pdf;jsessionid=C83CF8E27914682992A7D325B2B69114?sequence=1

Xiang B, Han L, Gao P, You R, Wang F, Xiao J, Liao M, Kang Y, and Ren T (2017). Spillover of Newcastle disease viruses from poultry to wild birds in Guangdong province, southern China. Infection, Genetics and Evolution, 55(1): 99-204. DOI: https://www.doi.org/10.1016/j.meegid.2017.09.020

Xue C, Cong Y, Yin R, Sun Y, Ding C, Yu S, Liu X, Hu S, Qian J, Yuan Q et al. (2017). Genetic diversity of the genotype VII Newcastle disease virus: Identification of a novel VIIj sub-genotype. Virus Genes, 53: 63-70. DOI: https://www.doi.org/10.1007/s11262-016-1404-0

Yan Y, Rout SN, Kim SH, and Samal SK (2009). Role of untranslated regions of the hemagglutinin-neuraminidase gene in replication and pathogenicity of Newcastle disease virus. Journal of Virology, 83(11): 5943-5946. DOI: https://www.doi.org/10.1128/jvi.00188-09

JWPR
Journal of World's Poultry Research
2024, Scienceline Publication
J. World Poult. Res. 14(2): 236-243, 2024
Research Paper
DOI: https://dx.doi.org/10.36380/jwpr.2024.24
PII: S2322455X2400024-14
ISSN: 2322-455X

Effect of Ginger (*Zingiber officinale*) and Cinnamon (*Cinnamon zeylanicum*) on Production, Fatty Acid Profile, and Meat Quality of Broiler Chickens

Dhananjay Gaikwad[1,2]*, and Yeshwant Fulpagare[2]

[1]*Amity Institute of Organic Agriculture, Amity University, Noida, Uttar Pradesh, India*
[2]*Department of Animal Husbandry and Dairy Science, PGI, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India*

*Corresponding Author's E-mail: dsgaikwad7@gmail.com

Received: April 02, 2024, Revised: April 28, 2024, Accepted: May 20, 2024, Published: June 30, 2024

ABSTRACT

Phytogenic feed additives play an important role in broilers' nutrition, contributing to the improvement of the performance and quality of meat. The study aimed to evaluate the effect of Ginger (*Zingiber officinale*) and Cinnamon (*Cinnamon zeylanicum*) on broiler chicken production, fatty acid profile, and meat quality. In the present study, 140-day-old Vencob-400 broiler chicks were divided into 7 groups, including the control group (with no additives, T0), and T1 to T6 groups receiving varying concentrations of cinnamon and ginger. Accordingly, the chickens' diet in T1 was supplemented with 1.0% cinnamon, T2 with 2.0% cinnamon, T3 with 3.0% cinnamon, T4 with 1.0% ginger, T5 with 2.0% ginger, and T6 with 3.0% ginger, all calculated based on dry matter. The carcass traits, weight of immune organs, organoleptic tests, and fatty acid profile of meat (breast and thigh) were recorded after the age of 42 days. The findings indicated that the breast and thigh had the highest organ weights in group T4 compared to other groups, however, the neck, back, drumstick, wing, and heart were not affected. The inclusion of 2% cinnamon (T2) and 1% ginger (T4) in the diet, significantly enhanced the color, texture, flavor, juiciness, and overall acceptability of the meat, compared to the diet of the control group. Adding a supplement of 2% cinnamon or 1% ginger powder to the diet of broiler chickens significantly decreased the percentage of total saturated fatty acid and increased the total unsaturated fatty acid (breast and thigh). The improvement in fatty acid composition is beneficial for the quality of the broiler meat. Based on these findings, it is recommended to supplement the diet of the broiler with either 2% cinnamon or 1% ginger powder to improve the carcass parameters and quality of the meat.

Keywords: Broiler meat, Characteristic, Cinnamon, Ginger, Quality

INTRODUCTION

The poultry industry has become an increasingly important part of the agriculture sector due to its rapid growth in recent times. The Indian poultry industry has made significant progress since its establishment and is currently becoming a promising sector with an impressive growth rate of 8.51% and 7.52% in the production of eggs and broilers, respectively (BAHS, 2019).

Poultry meat is a popular choice in society due to its affordability, easy availability, and versatility in cuisine. The poultry industry has emerged as a top provider of nutritious animal proteins worldwide. Poultry meat and eggs offer various benefits over other animal-based food sources. Poultry meat stands out in terms of protein content, amino acid balance, energy, and micronutrients compared to other animal products (Bohrer, 2017).

In the poultry industry, antibiotics are often used to prevent diseases and reduce mortality rates, improving meat production. However, this practice also leads to the growth of drug-resistant bacteria (Haque et al., 2020). Using antibiotics in animal feed can increase body weight gain and feed conservation ratio by up to 4% (Cowieson and Kluenter, 2019). The harmful effects of antibiotics on beneficial intestinal microflora populations and the development of drug-resistant bacteria have led many countries to prohibit their use in animal feed. Despite these concerns, some areas still allow the use of antibiotics in animal feed. These negative consequences make it evident that improvements must be made in this area (Andremont,

To cite this paper: Gaikwad D and Fulpagare Y (2024). Effect of Ginger (*Zingiber officinale*) and Cinnamon (*Cinnamon zeylanicum*) on Production, Fatty Acid Profile and Meat Quality of Broiler Chickens. *J. World Poult. Res.*, 14(2): 236-243. DOI: https://dx.doi.org/10.36380/jwpr.2024.24

2000). Currently, natural promoters should be used in poultry feed to maintain human health and safety, according to Selaledi et al. (2020).

Cinnamon and ginger are used as natural feed additives in poultry nutrition (Saeed et al., 2018). These additives are known as phytogenic feed additives and are used as replacements for antibiotic growth promoters (Gaikwad et al., 2019; Singh and Gaikwad, 2020; Ali et al., 2021). Ginger (*Zingiber officinale*), which belongs to the Zingiberaceae family, is a widely popular spice used for centuries as a traditional herbal medicine (Khaki et al. 2010). Ginger and its extract have been found to possess several beneficial properties, including antioxidant, anti-inflammatory, antimicrobial, radio-protective activities analgesic, and hepatoprotective (Mao et al., 2019). The main bioactive components responsible for these properties are gingerols, which are a group of phenolic compounds that include 6-, 8-, and 10-gingerol. Among them, 6-gingerol is the major component (Alsherbiny et al., 2019). Cinnamon bark contains a bioactive component called cinnamaldehyde. However, some types of cinnamon have other main components besides cinnamaldehyde. Plants contain cinnamaldehyde in the pathway of shikimate acid, which helps in the formation of lignin. Cinnamaldehyde is created from phenylalanine through cinnamic acid and then converted into cinnamyl alcohol during the lignin formation process (Ravindran et al., 2003). This information is relevant to a study that was proposed on broilers with the objectives of analyzing the fatty acid profile of meat and quality parameters of broiler meat.

MATERIALS AND METHODS

Ethical approval

Ethical clearance for the study was granted by the Board of Study (BOS) committee. This study was approved in the BOS, MPKV, Rahuri, Maharashtra state (India).

Study site

The study was conducted at the Poultry Unit, Veterinary Polyclinic, and AI Center at Mahatma Phule Agriculture University, Rahuri-413722, Dist. Ahmednagar, Maharashtra, India. It is located 30 km north of Ahmednagar on State Highway No. 14 and is 569 meters above sea level on the 190 47’ to 190 57’ north latitude and 7460 19’ East longitude. The trial was conducted with 140, day-old ‘Vencob-400’ broiler chicks, obtained from M/S Venkateshwara Hatchery, Pvt. Ltd., Pune (India).

Experimental diet and feed supplements

Cinnamon and ginger were purchased from the local market and after drying and grinding; it was mixed in commercial broiler ration as per different treatment levels. For the experiment, a commercial (Godrej© India) broiler starter and finisher crumbles (Chemical composition presented in Table 1) were used.

Table 1. Percent chemical constitution of experimental broiler chickens feed on a dry matter basis

Nutrients	Diet	
	Starter	Finisher
Crude Protein (%)	23	20
Crude Fibre (%)	4.6	3.78
Ether Extract (%)	4.8	4.3
Total Ash (%)	7.2	6.85
Nitrogen Free Extract (%)	60.4	65.15
Acid Insoluble Ash (%)	1.25	1.44
Metabolizable Energy (Kcal/kg)	2863.811	2939.75

Housing management

The Chickens that were part of the experiment (42 days) were raised using a deep litter system, a method of housing them in a way that allowed for the accumulation of litter on the floor of their living space. This litter consisted of organic materials, such as straw, wood shavings, and sawdust, providing the birds with a comfortable and sanitary environment. This rearing method was employed for a duration of up to 6 weeks, during which the birds were monitored closely to ensure optimal growth and development. The pens (7.5 square feet per Pen [1.1 sq. ft. per bird]), brooders, waterers, and feeders were thoroughly cleaned, washed, and disinfected before the arrival of chicks (Humidity 50-70%). Twenty chicks in each treatment group were reared and brooded separately on a deep litter system up to the age of 6 weeks. The brooding was carried out during the first 3 weeks. The brooding temperature was regulated to 26.6 to 35°C.

All the birds irrespective of their treatments were fed maize crumble for the first 4 days of their age. Then, the commercial broiler ‘starter’ crumbles were offered from day 5 to week 3 of age followed by broiler ‘finisher’ crumbles till week 6. The birds of different groups were fed separately throughout the experimental period. A weighed quantity of feed was offered, and the leftovers were collected and weighed the next day to determine the daily feed consumption. Fresh and clean water was offered

ad libitum to all the birds. All the chicks were vaccinated with the `F` strain of `Lassota` (India) vaccine on day 8 of hatching and vaccination against 'Gumboro' (India) disease was given on day 18 of hatching.

Adequate health coverage was provided to all the birds. At the end of day 42, eight birds from each treatment group were randomly picked up; blood samples (2 ml) were collected from the wing vein for measuring serum biochemistry (serum total cholesterol, high-density lipoprotein, low-density lipoprotein, serum triglyceride, hemoglobin, serum glucose, and serum total protein [mg/dl]).

Experimental design and measurements

A group of 140 one-day-old "Vencob-400" broiler chicks was used in a trial conducted by M/S Venkateshwara Hatchery, Pvt. Ltd., Pune. The commercial chicks were split into seven treatment groups, with 20 chicks in each group and 5 chicks per replicate. The trial involved feeding the chicks different dietary treatments, including a basal diet with no additives (T0 - Control), T1, T2, T3, T4, T5, and T6. The T1 to T6 treatment groups received 1.0%, 2.0%, and 3.0% cinnamon (bark powder) and 1.0%, 2.0%, and 3.0% ginger (root powder) of dry matter, respectively.

Terminal procedures, measurements, and sample collection

Four birds from each dietary group were selected based on their body weight, which was close to the mean for carcass studies 42 days after hatching. Before being slaughtered, the birds were kept off feed for 8 hours but were allowed to drink water. The carcass parameters, such as eviscerated weight, blood loss, cut-up part yields (such as breast, thigh, drumsticks, back, neck, and wing), and yield of various organs (such as liver, heart, and gizzard), were recorded and expressed as a percentage of live weight. The weights of lymphoid organs, including the bursa of Fabricius, spleen, and thymus, were recorded on day 42 from four birds in each treatment and expressed as a percentage (relative yield) of live weight.

After slaughtering birds at 42 days of age, samples of fresh chicken meat were collected for each treatment (T0-T6). A panel of semi-trained individuals was formed to evaluate the organoleptic quality of the meat samples using the nine-point Hedonic Scale developed by Peryam and Pilgrim in 1957. The evaluation parameters included color, appearance, tenderness, juiciness, flavor, and overall acceptance. The judges were not informed about the code numbers assigned to each treatment to prevent bias. In addition, they were required to wash their mouth between the use of two different samples, and the time was kept consistent throughout the investigation. To conduct the organoleptic tests, plain meat from four different treatment groups was cooked separately in four pressure cookers with 1% common salt for 10 minutes. The judges tasted the cooked meat and rated each parameter on a scale of 1 to 9. The observations were statistically analyzed. The fatty acid composition of the broiler meat was determined using NIR Spectrometer (India).

Statistical analysis

The statistical significance of the data obtained from various treatments was analyzed using standard methods and a completely randomized design (Snedecor and Cochran, 1994). The SPSS software package version 16.0 was used for statistical analysis of all data. In cases where variables had unequal observations, the least square design method, and Duncan's multiple range test were used for analysis. Chickens were used as experimental units to analyze growth, blood biochemistry, and carcass characteristics. On the other hand, replicate observations were used for analyzing the significance of feed intake and feed utilization. Results were considered significant at the 95% level ($p < 0.05$) for comparison.

RESULTS AND DISCUSSION

The effects of the cinnamon and ginger powder on carcass characteristics and immune response on day 42 of age are shown in Table 2. This experiment showed that the cinnamon and ginger powder supplemented group showed significant improvement in breast, thigh, gizzard, liver, and lymphoid organs (bursa, spleen, and thymus) values of live weight ($p < 0.05$), compared to the control group and the neck, back, drumstick, wing, gizzard, and heart not significantly influenced by the dietary treatments. The findings of the current study were in agreement with the results reported by Eltazi (2014), who found that adding cinnamon powder to the diets of broilers resulted in significantly higher liver and gizzard percentages compared to the control diet ($p < 0.05$). However, Onu (2010) reported that including ginger in the basal diet of broiler chicks did not lead to significant differences in carcass characteristics. In the same line, Eltazi (2014) reported that the highest percentage of commercial cuts (breast and thigh) was obtained by supplementation of 1% ginger powder. This result agreed with Sang-Oh et al. (2013) reported that CNP-supplemented groups show significantly heavier spleen of the thymus. The relative

weight of the bursa, spleen, and thymus remained higher in CNP (2%) and GRP (1%) supplemented birds among various dietary treatments than in control. As broiler birds mature, their thymus and bursa increase in size, while their immune responses depend on the spleen and peripheral lymph nodes. The Bursa of Fabricius is a consistent organ in chickens and is often used to study the development and maturity of B-lymphocytes. Recent research suggests that cinnamon powder can prevent harmful inflammation caused by the immune system response, while increasing the weight of immune organs, thereby promoting the growth of broilers by suppressing inflammation. The research suggests that adding 2.0% cinnamon and 1.0% ginger to the diet can serve as growth promoters, leading to increased profits per bird. These findings were reported by Gaikwad et al. (2019).

Table 2. Effect of supplementation of cinnamon and ginger on carcass traits (%) in broiler chickens at 6 weeks of age

Treatments	T_0	T_1	T_2	T_3	T_4	T_5	T_6
Neck	3.81 ± 0.16	3.89 ± 0.15	3.93 ± 0.18	3.88 ± 0.06	3.96 ±0.20	3.82 ± 0.14	3.82 ± 0.09
Back	15.93 ± 0.24	15.93 ± 0.35	16.20 ± 0.04	15.59 ± 0.43	16.36 ± 0.30	15.71 ± 0.25	15.99 ± 0.30
Drumstick	10.20 ± 0.17	10.27 ± 0.21	10.05 ± 0.09	10.14 ± 0.15	10.14 ± 0.28	10.09 ± 0.29	10.07 ± 0.28
Breast	20.99 ± 0.46^{a}	21.54 ± 0.17^{ab}	23.25 ± 0.39^{bcd}	22.91 ± 0.44^{cd}	24.13 ± 0.97^{d}	22.99 ± 0.28^{bcd}	22.42 ± 0.15^{abc}
Thigh	18.07 ± 0.01^{a}	18.52 ± 0.06^{a}	19.81 ± 0.05^{c}	18.78 ± 0.07^{a}	19.53 ± 0.04^{bc}	18.69 ± 0.02^{ab}	18.64 ± 0.05^{bc}
Wing	8.68 ± 0.29	9.12 ± 0.01	9.16 ± 0.88	9.14 ± 0.01	9.18 ± 0.53	9.13± 0.58	8.89 ± 0.22
Gizzard	1.71 ± 0.01^{a}	1.74 ± 0.01^{ab}	1.76 ± 0.01^{bc}	1.74 ± 0.02^{bc}	1.77 ± 0.01^{c}	1.74 ± 0.01^{ab}	1.73 ± 0.01^{ab}
Liver	3.61 ± 0.01^{b}	3.57 ± 0.04^{ab}	3.72 ± 0.05^{ab}	3.57 ± 0.02^{ab}	3.82 ± 0.07^{a}	3.59 ± 0.01^{b}	3.57 ± 0.04^{ab}
Heart	0.49 ± 0.00	0.49 ± 0.01	0.50 ± 0.01	0.49 ± 0.01	0.50 ± 0.01	0.49 ± 0.01	0.48 ± 0.01
Bursa of Fabrics	0.12 ± 0.01^{a}	0.14 ± 0.01^{ab}	0.21 ± 0.01^{d}	0.16 ± 0.01^{bc}	0.22 ± 0.01^{d}	0.19 ± 0.03^{cd}	0.17 ± 0.01^{bc}
Spleen	0.12 ± 0.01^{a}	0.19 ± 0.01^{b}	0.21 ± 0.01^{b}	0.19 ± 0.01^{b}	0.18 ± 0.01^{b}	0.18 ± 0.02^{b}	0.19 ± 0.01^{b}
Thymus	0.13 ± 0.01^{a}	0.15 ± 0.00^{ab}	0.20 ± 0.01^{d}	0.16 ± 0.00^{b}	0.19 ± 0.01^{cd}	0.17 ± 0.02^{bc}	0.16 ± 0.01^{b}

[abcd] Values bearing different superscript letters differed significantly ($p < 0.05$), T_0: a basal diet with no additives, T1: Basal diet + 1.0% cinnamon (bark powder), T_2: Basal diet + 2.0% cinnamon (bark powder), T_3: Basal diet + 3.0% cinnamon (bark powder), T_4: Basal diet + 1.0% ginger (root powder), T_5: Basal diet + 2.0% ginger (root powder), T_6: Basal diet + 3.0% ginger (root powder) of dry matter

Table 3. Effect of supplementation of cinnamon and ginger on sensory score in boiled broiler chicken meat at 6 weeks

Treatments	T_0	T_1	T_2	T_3	T_4	T_5	T_6
Colour	7.64	7.86	7.94	7.86	7.83	7.70	7.45
Flavour	7.35^{bc}	7.25^{c}	8.10^{a}	7.60^{bc}	8.10^{a}	7.85^{a}	7.76^{ab}
Tenderness	7.40^{b}	7.71^{ab}	8.01^{a}	7.49^{b}	7.98^{a}	7.44^{b}	7.39^{b}
Juiciness	7.15^{d}	7.59^{bc}	7.77^{b}	7.44^{bcd}	8.26^{a}	7.61^{bc}	7.22^{cd}
Acceptability	7.40^{e}	7.79^{bcd}	8.15^{a}	7.69^{cde}	8.25^{a}	8.00^{ab}	7.65^{de}

[abcde] Values bearing different superscript letters differed significantly ($p < 0.05$), T_0: a basal diet with no additives, T1: Basal diet + 1.0% cinnamon (bark powder), T_2: Basal diet + 2.0% cinnamon (bark powder), T_3: Basal diet + 3.0% cinnamon (bark powder), T_4: Basal diet + 1.0% ginger (root powder), T_5: Basal diet + 2.0% ginger (root powder), T_6: Basal diet + 3.0% ginger (root powder) of dry matter

The taste and flavor of boiled chicken meats were found to be significantly improved in groups that were supplemented with CNP and GRP when compared to the control group shown in Table 3. The group that was supplemented with 2.0% CNP and 1.0% GRP had the highest score among all the treatment groups. These findings were consistent with a study by Sang-Oh et al. (2013), which also showed improved acceptability of boiled chicken meats in CNP-supplemented groups. It is possible that the cinnamon powder groups experienced an increase in flavor score due to the essential oils present in the muscle tissues of the meat. Cinnamon powder contains cinnamaldehyde, which is the primary essential oil and makes up 89.47% of cinnamon powder (Kim and Kim, 2000). The researchers also looked at how different diets affected the subjective scores for broiler chicken meat. The results showed that the inclusion of cinnamon and ginger powder in the broiler diet significantly increased the measured scores for juiciness, flavor, and texture except for the color of the meat. Singh et al. (2019) found that the combination of herbs, including cinnamon, improved the flavor, tenderness, and overall acceptability score of meat. Adedeji et al. (2021) reported that the addition of cinnamon significantly affected the color, flavor, tenderness, juiciness, and overall acceptability of meat ($p < 0.05$). In another study by Hengl et al. (2017)

the sensory quality of chicken breast and drumstick meat, including color, structure, juiciness, tenderness, odor, and taste acceptability, was enhanced by adding XTRACT® (carvacrol, cinnamaldehyde, and capsicum oleoresin) as feed additives for broiler chicken. The study conducted by Eltazi (2014) found that there were no significant differences in the tenderness, juiciness, flavor, and color of the meat among different dietary treatments. Furthermore, the score given for all attributes was above the moderate acceptability level ($p < 0.05$).

Table 4. Effect of supplementation of cinnamon and ginger on fatty acids profile of broiler breast meat (g/100g) in broiler chickens at 6 weeks of age

Fatty Acid	T_0	T_1	T_2	T_3	T_4	T_5	T_6	p
C14:0	00.73 ± 0.01	00.71 ± 0.01	00.62 ± 0.05	00.72 ± 0.01	0.65 ± 0.03	0.71 ± 0.01	00.73 ± 0.00	**
C15:0	00.18 ± 0.01	00.18 ± 0.01	00.19 ± 0.01	0.14 ± 0.01	00.17 ± 0.01	00.18 ± 0.01	00.17 ± 0.01	**
C16:0	20.10 ± 1.03	20.55 ± 0.57	20.75 ± 0.35	22.06 ± 0.8	21.11 ± 0.7	21.5 ± 0.74	19.93 ± 0.82	NS
C17:0	00.24 ± 0.01	00.22 ± 0.01	00.23 ± 0.01	00.18 ± 0.01	00.2 ± 0.01	00.18 ± 0.01	00.20 ± 0.01	**
C18:0	12.1 ± 0.96	10.25 ± 0.43	9.91 ± 0.12	08.87 ± 0.32	9.82 ± 0.26	9.79 ± 0.18	11.36 ± 0.90	**
C20:0	0.35 ± 0.01	0.31 ± 0.01	0.31 ± 0.01	00.24 ± 0.02	0.26 ± 0.02	0.25 ± 0.00	00.23 ± 0.01	**
Σ SFA	33.70 ± 0.29	32.23 ± 1.01	32.00 ± 0.37	32.21 ± 0.51	32.22 ± 0.88	32.60 ± 0.65	32.61 ± 0.76	NS
C16:1n-7	2.06 ± 0.05	3.47 ± 0.3	3.07 ± 0.07	3.04 ± 0.08	3.07 ± 0.07	3.07 ± 0.07	3.07 ± 0.07	**
C18:1n-9	22.54 ± 0.48	21.72 ± 0.19	22.77 ± 0.2	21.6 ± 0.61	22.43 ± 0.16	22.1 ± 0.73	21.77 ± 0.81	NS
C20:1	0.18 ± 0.01	0.19 ± 0.01	0.2 ± 0.01	0.22 ± 0.02	0.2 ± 0.01	0.2 ± 0.01	0.2 ± 0.01	NS
C14:1	0.16 ± 0.03	0.16 ± 0.02	0.17 ± 0.01	0.17 ± 0.02	0.17 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	NS
Σ MUFA	24.94 ± 0.54	25.54 ± 0.18	26.21 ± 0.13	25.03 ± 0.69	25.87 ± 0.21	25.54 ± 0.71	25.21 ± 0.88	NS
C18:2n-6	27.98 ± 0.87	28.77 ± 1.00	27.7 ± 0.46	29.4 ± 0.68	28.38 ± 0.55	28.72 ± 0.41	28.03 ± 1.53	NS
C18:3n-3	01.55 ± 0.26	1.55 ± 0.27	1.47 ± 0.21	1.49 ± 0.24	1.65 ± 0.29	1.19 ± 0.12	1.48 ± 0.23	NS
C: 20:4n-6	10.72 ± 0.31	10.72 ± 0.32	11.44 ± 0.24	10.71± 0.31	10.74 ± 0.38	10.77 ± 0.46	11.21 ± 0.21	NS
C20:5n-3	0.67 ± 0.04	0.68 ± 0.04	0.67 ± 0.04	0.7 ± 0.06	0.68 ± 0.04	0.65 ± 0.03	0.77 ± 0.05	NS
C20:3n-6	0.44 ± 0.03	0.5 ± 0.05	0.52 ± 0.03	0.45 ± 0.03	0.46 ± 0.02	0.53 ± 0.04	0.7 ± 0.04	**
Σ PUFA	41.36 ± 1.13	42.23 ± 0.88	41.79 ± 0.33	42.76 ± 1.27	41.91 ± 0.39	41.86 ± 0.44	42.18 ± 1.47	NS

[abcde] Values bearing different superscript letters differed significantly ($p < 0.05$); NS: Non-significant, p: p-value, **: significant difference, T1: Basal diet + 1.0% cinnamon (bark powder), T_2: Basal diet + 2.0% cinnamon (bark powder), T_3: Basal diet + 3.0% cinnamon (bark powder), T_4: Basal diet + 1.0% ginger (root powder), T_5: Basal diet + 2.0% ginger (root powder), T_6: Basal diet + 3.0% ginger (root powder) of dry matter.

Table 5. Effect of supplementation of cinnamon and ginger fatty acids profile of broiler thigh meat (g/100g) in broiler chickens at 6 weeks of age

Fatty acid	T_0	T_1	T_2	T_3	T_4	T_5	T_6	P
(C14:0)	0.62 ± 0.04	0.53 ± 0.04	0.34 ± 0.02	0.37 ± 0.01	0.58 ± 0.04	0.59 ± 0.03	0.61 ± 0.02	**
(C15:0)	0.19 ± 0.01	0.19 ± 0.01	0.16 ± 0.01	0.16 ± 0.02	0.13 ± 0.00	0.25 ± 0.07	0.27 ± 0.10	NS
(C16:0)	18.02 ± 0.45	15.94 ± 0.47	16.13 ± 0.47	16.05 ± 0.22	19.17 ± 0.25	17.55 ± 1.08	16.81 ± 0.27	**
(C17:0)	0.30 ± 0.01	0.44 ± 0.07	0.25 ± 0.02	0.25 ± 0.02	0.26 ± 0.05	0.33 ± 0.05	00.45 ± 0.03	**
(C18:0)	13.40 ± 0.6	11.41 ± 1.09	9.76 ± 0.97	10.63 ± 0.59	12.43 ± 0.74	13.2 ± 0.73	14.56 ± 0.47	**
(C20:0)	0.51 ± 0.05	0.45 ± 0.03	0.25 ± 0.05	0.50 ± 0.10	0.37 ± 0.06	0.42 ± 0.09	0.38 ± 0.08	NS
Σ SFA	33.05 ± 1.02^{b}	28.96 ± 1.28^{a}	26.9 ± 1.43^{a}	27.96 ± 0.69^{a}	32.94 ± 0.89^{b}	32.35 ± 1.58^{b}	33.08 ± 0.18^{b}	**
(C16:1n-7)	2.92 ± 0.04	2.78 ± 0.31	2.76 ± 0.32	2.49 ± 0.31	2.6 ± 0.30	2.13 ± 0.08	2.66 ± 0.17	NS
(C18:1n-9)	24.18 ± 1.16	28.25 ± 0.79	28.62 ± 0.5	29.13 ± 1.41	24.92 ± 1.6	25.86 ± 1.00	24.37 ± 0.42	**
(C20:1)	0.35 ± 0.02	0.38 ± 0.03	0.40 ± 0.00	0.38 ± 0.04	0.29 ± 0.04	0.41 ± 0.01	0.41 ± 0.03	NS
(C14:1)	0.27 ± 0.01	0.31 ± 0.01	0.30 ± 0.04	0.31 ± 0.02	0.3 ± 0.03	0.3 ± 0.03	0.37 ± 0.01	NS
Σ MUFA	27.71 ± 1.12^{a}	31.72 ± 0.92^{c}	32.08 ± 0.27^{c}	30.84 ± 0.49^{bc}	28.11 ± 1.27^{ab}	28.71 ± 0.94^{ab}	27.81 ± 0.55^{a}	**
(C18:2n-6)	27.24 ± 0.62	27.7 ± 0.35	27.38 ± 0.88	27.76 ± 0.37	27.09 ± 0.55	26.44 ± 0.84	25.94 ± 0.71	NS
(C18:3n-3)	1.53 ± 0.24	1.25 ± 0.05	1.35 ± 0.16	1.61 ± 0.17	1.18 ± 0.08	1.23 ± 0.11	1.23 ± 0.12	NS
(C: 20:4n-6)	9.19 ± 0.58	9.48 ± 0.78	11.42 ± 0.26	11.18 ± 0.20	10.14 ± 0.55	10.56 ± 0.26	11.03 ± 0.36	NS
(C20:5n-3)	0.59 ± 0.03	0.61 ± 0.17	0.65 ± 0.12	0.45 ± 0.06	0.31 ± 0.09	0.44 ± 0.13	0.64 ± 0.04	NS
(C20:3n-6)	0.70 ± 0.01	0.28 ± 0.04	0.21 ± 0.04	0.20 ± 0.04	0.23 ± 0.04	0.27 ± 0.04	0.27± 0.01	**
Σ PUFA	39.24 ± 1.4	39.32 ± 0.62	41.02 ± 1.2	41.2 ± 0.59	38.95 ± 0.7	38.95 ± 0.84	39.11 ± 1.01	NS

[abcde] Values bearing different superscript letters differed significantly ($p < 0.05$); NS: Non-significant, p: p-value, **: significant difference, T_0: Basal diet with no additives, T1: Basal diet + 1.0% cinnamon (bark powder), T_2: Basal diet + 2.0% cinnamon (bark powder), T_3: Basal diet + 3.0% cinnamon (bark powder), T_4: Basal diet + 1.0% ginger (root powder), T_5: Basal diet + 2.0% ginger (root powder), T_6: Basal diet + 3.0% ginger (root powder) of dry matter

Tables 4 and 5 demonstrate the effect of supplementing cinnamon and ginger on the fatty acids profile of broiler breast and thigh meat (g/100g) in broilers that are 6 weeks old. Chickens that were fed diets containing cinnamon and ginger showed a significant decrease in the percentage of total saturated fatty acids (SFA, $p < 0.05$) and an increase in the total unsaturated fatty acids (in both breast and thigh, $p < 0.05$) compared to those on the control diet.

The group that was given cinnamon supplements had a significantly ($p < 0.05$) lower total SFA ratio in their thigh meat and a significantly higher PUFA ratio. Different dietary fatty acid profiles may lead to changes in body fat deposition in broilers due to variations in lipid synthesis or lipid oxidation rates. According to research, *Coriander sativum* can reduce lipid absorption and increase lipid breakdown, which may result in a lipolytic effect (Chithra and Leelamma, 1997). This effect on lipid metabolism could potentially explain the decrease in saturated fatty acid levels in meat. Conversely, unsaturated fatty acids in meat lipids would increase due to a decrease in fatty acid oxidation in the tissue. Research has shown that cinnamon, an essential oil, has antioxidant properties, which could explain the increase in unsaturated fatty acids in meat (Yu et al., 1994; Case et al., 1995; Lee et al., 2001; Lee et al., 2007). The present study supports the idea that cinnamon has antioxidant properties. Cinnamon is believed to block the process of lipid peroxidation in tissues, particularly in polyunsaturated fatty acids (Dalkilic et al., 2009). The study showed a significant increase in the levels of polyunsaturated fatty acids in both the serum and thigh meat. The outcomes correspond with the research conducted by Ciftci et al. (2010) where it was observed that the ratio of total saturated fatty acids (SFA) declined, while the ratio of total unsaturated fatty acids (PUFA) and ω-6b fatty acids increased significantly in both serum and thigh meat of the cinnamon groups. Additionally, an improvement in meat quality was also reported.

CONCLUSION

Including 2% cinnamon or 1% ginger of dry matter in the diet of broiler chickens has been found to enhance the quality of meat. This addition results in improved color, appearance, flavor, texture, juiciness, and overall acceptability when compared to the meat from chickens on a controlled diet. It has been determined that feeding broilers chickens with this level of cinnamon or ginger powder can lead to better feed efficiency, growth, and an improved fatty acid profile in the meat. These benefits can ultimately result in maximum returns. To achieve the best results, it is recommended to include these levels of cinnamon or ginger in the ration of broilers.

DECLARATIONS

Acknowledgments

The authors of the article wish to express their gratitude to the Post Graduate Institute at Mahatma Phule Agriculture University in Rahuri, India.

Availability of data and materials

All data generated or analyzed during the current study are included in this published article.

Funding

Financial support by Post Graduate Institute of Mahatma Phule Agriculture University, Rahuri, Maharashtra, India.

Authors' contributions

Dr. Dhananjay S. Gaikwad conducted the field trials and collected all the data for analysis. Dr. Yeshwant Fulpagare supervised and guided the research as well as participated in data analysis and corrections. All authors read and approved the final edition of the manuscript.

Competing interests

The authors assert that they have no competing interests.

Ethical considerations

The current regulations regarding ethical concerns, such as plagiarism, consent to publication, misconduct, data fabrication and/or falsification, double posting and/or submission, and redundancy have been carefully considered and complied with by the authors to prevent any violations. They have taken necessary measures to ensure that none of these concerns have been overlooked or violated.

REFERENCES

Adedeji OS, Oyetoro BA, and Oki HA (2021). Effect of dietary cinnamon powder on the organolopetic properties of cockerel chickens. Greener Journal of Agricultural Sciences, 11(3): 157-162. Available at: https://gjournals.org/GJAS/Publication/2021/3/PDF/092121091%20Adedeji%20et%20al.pdf

Ali A, Ponnampalam EN, Pushpakumar G, Cottrell JJ, Suleria HA, and Dunshea FR (2021). Cinnamon: A natural feed additive for poultry health and production-A review. Animals, 11(7): 2026. DOI: https://www.doi.org/10.3390/ani11072026

Alsherbiny MA, Abd-Elsalam WH, El badawy SA, Taher E, Fares M, Torres A, Chang D, and Li CG (2019). Ameliorative and protective effects of ginger and its main constituents against natural, chemical and radiation-induced toxicities: A comprehensive review. Food and Chemical Toxicology, 123: 72-97. DOI: https://www.doi.org/10.1016/j.fct.2018.10.048

Andremont A (2000). Consequences of antibiotic therapy to the intestinal ecosystem. Annales Francaises d'anesthesie et de Reanimation, 19(5): 395-402. DOI: https://www.doi.org/10.1016/s0750-7658(00)90209-0

Basic animal husbandry statistics (BAHS) (2019). Ministry of Fisheries, Animal Husbandry, and Dairying. Government of India. Available at: https://dahd.nic.in/sites/default/filess/BAHS%20%28Basic%20Animal%20Husbandry%20Statistics-2019%29_0.pdf

Bohrer BM (2017). Review: Nutrient density and nutritional value of meat products and non-meat foods high in protein. Trends in Food Science & Technology, 65: 103-112. DOI: https://www.doi.org/10.1016/j.tifs.2017.04.016

Case GL, He L, Mo H, and Elson CE (1995). Induction of geranyl pyrophosphate pyrophosphatase activity by cholesterol-suppressive isoprenoids. Lipids, 30(4): 357-359. DOI: https://www.doi.org/10.1007/BF02536045

Chithra V and Leelamma S (1997). Hypolipidemic effect of coriander seeds (*Coriandrum sativum*): Mechanism of action. Plant Foods for Human Nutrition, 51: 167-172. DOI: https://www.doi.org/10.1023/A:1007975430328

Ciftci M, Simsek UG, Yuce A, Yilmaz O, and Dalkilic B (2010). Effects of dietary antibiotic and cinnamon oil supplementation on antioxidant enzyme activities, cholesterol levels and fatty acid compositions of serum and meat in broiler chickens. Acta Veterinaria Brno, 79(1): 33-40. DOI: https://www.doi.org/10.2754/avb201079010033

Cowieson AJ and Kluenter AM (2019). Contribution of exogenous enzymes to potentiate the removal of antibiotic growth promoters in poultry production. Animal Feed Science and Technology, 250: 81-92. DOI: https://www.doi.org/10.1016/j.anifeedsci.2018.04.026

Dalkilic B, Ciftci M, Guler T, Cerci IH, Ertas ON, and Guvenc M (2009). Influence of dietary cinnamon oil supplementation on fatty acid composition of liver and abdominal fat in broiler chicken. Journal of Applied Animal Research, 35(2): 173-176. DOI: https://www.doi.org/10.1080/09712119.2009.9707011

Eltazi S (2014). Effect of using ginger powder as natural feed additive on performance and carcass quality of broiler chicks. Assiut Veterinary Medical Journal, 60(2): 87-95. DOI: https://www.doi.org/10.21608/avmj.2014.170756

Gaikwad DS, Fulpagare YG, Bhoite UY, Deokar DK, and Nimablkar CA (2019). Effect of dietary supplementation of ginger and cinnamon on growth performance and economics of broiler production. International Journal of Current Microbiology and Applied Science, 8(3): 1849-1857. DOI: https://www.doi.org/10.20546/ijcmas.2019.803.219

Haque MH, Sarker S, Islam MS, Islam MA, Karim MR, Kayesh ME, Shiddiky MJ, and Anwer MS (2020). Sustainable antibiotic-free broiler meat production: Current trends, challenges, and possibilities in a developing country perspective. Biology, 9(11): 411. DOI: https://www.doi.org/10.3390/biology9110411

Hengl B, Đidara M, Pavić M, Lilić S, and Šperanda M (2017). Antioxidative status and meat sensory quality of broiler chicken fed with XTRACT® and zeolite dietary supplementation. Pakistan Journal of Agriculture Research, 54(4): 897-902. DOI: https://www.doi.org/10.21162/PAKJAS/17.4153

Khaki AA, Khaki A, Ahmadi-Ashtiani HR, Rastegar H, Rezazadeh S, Babazadeh D, Zahedi A, and Ghanbari Z (2010). Treatment effects of ginger rhizome & extract of carrot seed on diabetic nephropathy in rat. Journal of Medicinal Plants, 9(33): 75-80. Available at: https://jmp.ir/article-1-516-fa.pdf

Kim NM and Kim YH (2000). Effect of ethanol concentration on extraction of volatile components in cinnamon. The Korean Journal of Food And Nutrition, 13(1): 45-52. Available at: https://koreascience.kr/article/JAKO200011920136821.pdf

Lee JS, Choi MS, Jeon SM, Jeong TS, Park YB, Lee MK, and Bok SH (2001). Lipid-lowering and antioxidative activities of 3, 4-di (OH)-cinnamate and 3, 4-di (OH)-hydrocinnamate in cholesterol-fed rats. Clinica Chimica Acta, 314(1-2): 221-229. DOI: https://www.doi.org/10.1016/S0009-8981(01)00700-8

Lee MK, Park YB, Moon SS, Bok SH, Kim DJ, Ha TY, Jeong TS, Jeong KS, and Choi MS (2007). Hypocholesterolemic and antioxidant properties of 3-(4-hydroxyl) propanoic acid derivatives in high-cholesterol fed rats. Chemico-Biological Interactions, 170(1): 9-19. DOI: https://www.doi.org/10.1016/j.cbi.2007.06.037

Mao QQ, Xu XY, Cao SY, Gan RY, Corke H, Beta T, and Li HB. (2019). Bioactive compounds and bioactivities of ginger (*Zingiber officinale* Roscoe). Foods, 8(6): 185. DOI: https://www.doi.org/10.3390/foods8060185

Onu PN (2010). Evaluation of two herbal spices as feed additives for finisher broilers. Biotechnology in Animal Husbandry, 26(5-6): 383-392. DOI: https://www.doi.org/10.2298/BAH1006383O

Peryam DR and Pilgrim FJ (1957). Hedonic scale method of measuring food preferences. Food Technology, 11: 9-14. Available at: https://psycnet.apa.org/record/1959-02766-001

Ravindran PN, Nirmal-Babu K, and Shilaja N (2003). Cinnamon and cassia genus Cinnamomum. CRC Press., Washington DC, p. 384. DOI: https://www.doi.org/10.1201/9780203590874

Saeed M, Kamboh A, Syed SF, Babazadeh D, Suheryani I, Shah QA, Umar M, Kakar I, Naveed M, Abd El-Hack ME et al. (2018). Phytochemistry and beneficial impacts of cinnamon (*Cinnamomum zeylanicum*) as a dietary supplement in poultry diets. World's Poultry Science Journal, 74(2): 331-346. DOI: https://www.doi.org/10.1017/S0043933918000235

Sang-Oh P, Chae-Min R, Byung-Sung P, and Jong H (2013). The meat quality and growth performance in broiler chickens fed diet with cinnamon powder. Journal of Environmental

Biology, 34(1): 127-133. Available at: https://pubmed.ncbi.nlm.nih.gov/24006819/

Selaledi LA, Mohammed Hassan Z, Manyelo TG, and Mabelebele M (2020). The current status of the alternative use to antibiotics in poultry production: An African perspective. Antibiotics, 9(9): 594. DOI: https://www.doi.org/10.3390/antibiotics9090594

Singh J and Gaikwad DS (2020). Phytogenic feed additives in animal nutrition. In: J. Singh, A. Yadav (Editors), Natural bioactive products in sustainable agriculture. Springer., Singapore, pp. 273-289. DOI: https://www.doi.org/10.1007/978-981-15-3024-1_13

Singh J, Kaur P, Sharma M, Mehta N, Singh ND, Sethi APS, and Sikka SS (2019). Effect of combination of garlic powder with black pepper, cinnamon and aloe vera powder on the growth performance, blood profile, and meat sensory qualities of broiler chickens. Indian Journal of Animal Sciences, 89(12): 1370-1376. Available at: https://hero.epa.gov/hero/index.cfm/reference/details/reference_id/8018590

Snedecor GW and Cochran WG (1994). Statistical methods, 8th Edition. The Iowa state college perss., Ames, IOWA, Oxford and I. B. H. publication Co., Calcutta.

Yu SG, Abuirmeileh NM, Qureshi AA, and Elson CE (1994). Dietary .beta.-ionone suppresses hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity. Journal of Agricultural and Food Chemistry, 42(7): 1493-1496. https://www.doi.org/10.1021/jf00043a019

JWPR
Journal of World's Poultry Research
2024, Scienceline Publication
J. World Poult. Res. 14(2): 244-254, 2024
Research Paper
DOI: https://dx.doi.org/10.36380/jwpr.2024.25
PII: S2322455X2400025-14

ISSN: 2322-455X

Efficiency of Recycled Plastic Bedding Material and Gender in Improvement of Productive Traits, Physiological, and Immunological Parameters of Hybrid Broiler Chickens

Mohamed A. EL-Masry[1], Magdy S. Hassan[2], AbdelSatar A. Arafa[3]*, Tarek M. El-Afifi[4], Ahmed M. Bealish[2], Magdy M. Ouda[2], Ibrahim A. Fathey[2], Hanan A. Fahmy[3], and Hanaa K. Abd El-Atty[2]

[1]*Misr Organic Food Industry Company, Dokki, Giza, Egypt,* [2]*Animal Production Research Institute (APRI), Agriculture Research Center (ARC), Dokki, Giza, Egypt;* [3]*Animal Health Research Institute (AHRI), Agriculture Research Center (ARC), Dokki, Giza, Egypt;* [4]*Regional Center for Food and Feed, Agriculture Research Center (ARC) Giza, Egypt.*

*Corresponding author's E-mails: abd.arafa@hotmail.com; abd.arafa@gmail.com, abd.arafa@ahri.gov.eg

Received: March 31, 2024, Revised: April 23, 2024, Accepted: May 15, 2024, Published: June 30, 2024

ABSTRACT

Litter management is important for poultry housing husbandry and affects chicken performance. The present study evaluated the effect of bedding material and gender on the productive, physiological, and immunological performance of a new hybrid chicken (WINZY Line 105) under cold stress for 56 days of age. A total of 540 one-day-old broiler chicks were divided into two groups. The groups, including 270 males (M) and 270 females (F) were further divided into two sub-groups, including sawdust litter (SL) and plastic slatted floor (PSF), three replicates, and 45 chickens each. Broiler chickens were raised during the winter with an average temperature of 10^{o}C for 56 days. Productive, physiological, and immunological performance parameters were measured. The obtained results indicated that M reared on PSF (M x PSF) recorded the highest values in body weight (BW), body weight gain (BWG), carcass characteristics, and the best values in feed conversion compared with other interaction groups during all experimental periods. In addition, F reared on PSF had higher BW, carcass, and thigh percentages, low feed intake (FI), and best feed conversion compared to females reared on SL. The M reared on PSF had the highest hepatic enzymes except AST which was higher in F reared on PSF than other treatments. However, renal function biomarkers (Creatine, Uric acid, Urea) were higher in both M and F that were reared on SL than those reared on PSF. Moreover, there was a significant interaction detected for antibody titters against avian influenza (H5) and Newcastle disease at 21 days of age suggesting that the highest values observed for M reared on PSF (M x PSF) compared with other interaction groups during the experimental period, and it was higher in F that reared on PSF than F reared on SL at 21 days of age. It can be concluded that plastic slatted floors could be an alternative to substitute wood shavings to raise broiler chickens since it was efficient from the perspective of environmental conditions and production rates.

Keywords: Bedding material, Environmental adaptation, Hybrid chicken, Immunological parameter, Productive trait, Plastic slatted floor, Performance

INTRODUCTION

Bedding management is critical for animal welfare, especially in intensive production systems. Litter protects the chickens from the rigid ground, and facilitates the evaporation of urine, excreta, and spilled water. To improve the health and welfare of chickens, they should be kept on a dry floor. A variety of factors are used in broiler production to meet these goals. The main factors are the type of litter material used, housing system, litter control methods, and bird water balance (Collett, 2012).

The purpose of bedding material included excreta and moisture absorption, aerobic decomposition of excrement, and heat insulation (Shepherd and Fairchild, 2010). Additionally, it influences the body weight (BW) and immunity of broiler chicks, which reflects on growth rate and carcass quality like carcass body weight percent. Broilers reared on sand and paper roll floors perform similarly to those reared on wood shavings (Bilgili et al., 2009; Toghyani et al., 2010).

Broiler performance can be enhanced through practical litter management to ensure a healthy atmosphere

To cite this paper: EL-Masry MA, Hassan MS, Arafa AA, El-Afifi TM, Bealish AM, Ouda MM, Fathey IA, Fahmy HA, and Abd El-Atty HK (2024). Efficiency of Recycled Plastic Bedding Material and Gender in Improvement of Productive Traits, Physiological, and Immunological Parameters of Hybrid Broiler Chickens. *J. World Poult. Res.*, 14(2): 244-254. DOI: https://dx.doi.org/10.36380/jwpr.2024.25

in their houses by controlling ammonia concentrations, and increasing immunological responses against various diseases (Beker et al., 2004; Miles et al., 2004; De Jong et al., 2014; Wei et al., 2015). In addition, heat exhaustion puts broiler breeding in danger (Liverpool-Tasie et al., 2019).

The most common materials used as litter in commercial broiler production are wood shavings and sawdust; recently, plastic floors have been effective in providing a healthy environment and higher production rates. Wood shavings and plastic floors demonstrated better performance with males than females at 42 days of age including weight increase, feed intake, and feed conversion (Almeida et al. 2017).

Climate change negatively impacted both direct and indirect agricultural production systems and people's food security, especially in dry areas like Sub-Saharan Africa (Thompson et al., 2010). The Food and Agricultural Organization (FAO, 2011) advised increasing public awareness of how climate change affects food security and nutrition, the mechanisms affecting food security, and how to adapt to climate change.

This study aims to investigate the effect of two different bedding materials (sawdust litter and plastic slatted floors) on the growth performance and immunological parameters of both males and females of the new hybrid chickens WINZY Line 105.

MATERIALS AND METHODS

Ethical approval

The animal study protocol was conducted with permission and approved by the Review Board of Animal Production Research Institute (APRI), Agriculture Research Center, Ministry of Agriculture, Dokki, Giza, Egypt. All experiments were conducted according to the relevant guidelines and regulations of the Ethical Committee coded: 202110

Broiler chickens

A new hybrid breed broiler that originated from a crossing between the local native Egyptian breed (Fayomy) PP line and the French line breed (Sasso) GG line. The average body weight for males is 2.2 kg, while for females it was 1.750 kg at 52 days of age. This line was characterized by a sex phenotype difference where the color of females was reddish brown and can be easily differentiated from the yellowish-grey color of males at one day old.

Experimental design

The current study was conducted in collaboration with Misr Organic Food Industry Company, Animal Production Research Institute Animal Health Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt. This study was conducted at the facility of poultry breeding station, Anshas, Sharkaia, Egypt. The chicks were hatched from the fifth generation of cross-type chickens (WINZY line 105) of both sexes. A total of 540 chicks were reared from one day old up to 56 days old. The chicks were divided into two groups of 270 males (M) and 270 females (F). Groups were further divided into two sub-groups based on the type of litter: sawdust litter (SL) and plastic slatted floor (PSF), with 3 replicates of 45 chickens each under the same management conditions including ventilation system, lighting program, temperature inside houses, feeding formulae, and vaccination programs. The composition and calculated analysis of the basic diet was done according to the NRC (1994) and presented in Table 1. The chickens, house facility, and plastic-slated floor were supplied by Misr Organic Food Industry Company.

Floor design

The floors were distributed in rooms at the same height in each room. Broiler chickens were reared on two types of bedding material (sawdust litter and plastic slatted floor). Sawdust litter was distributed with height (7 cm) and considered as control groups. The plastic slatted floor was designed with narrow holes measured 2-3 millimeters thick. Water and feed diet were provided *ad libitum*. Broiler chickens in this experiment were reared during the winter season (December 2021 and January 2022) under extreme cold conditions. The ambient temperature in the daytime reached 10°C and fell to 2°C at night. The chicks were reared in a semi-closed, controlled house with a nearly fixed temperature inside to maintain chickens during growth.

Effective operation

Live body weight (BW) and feed intake (FI) were recorded weekly, consequently body weight gain (BWG) and the feed conversion (FC) ratio were calculated too. Daily mortalities were counted, and a weekly mortality rate was computed.

Features of slaughter and carcass

After eight weeks, chickens were kept fasting for 5-6 hours to keep the corps of the chickens empty at slaughtering time, then three chickens from each treatment were slaughtered by slitting the jugular vein, de-feathered,

eviscerated, weighted, and the percentages of live body weights were calculated. The breast, thigh, and giblets were excised, weighed, and expressed as percentages to live body weights.

Table 1. Composition of basal diet of the hybrid broiler (WINZY Line 105) affected by gender and plastic-based bedding material from day one to eight weeks of age

Ingredients (%)	Starter (1-21 days)	Grower (22-42 days)	Finisher (43-56 days)
Yellow corn	56.00	61.65	65.25
Soya bean meal 44%	32.50	25.00	21.00
Corn gluten meal 62%	6.00	7.00	7.00
Cotton seed oil	2.00	2.50	3.25
Di-calcium phosphate	0.80	1.00	1.00
Limestone	1.85	2.00	1.75
Salt	0.25	0.25	0.25
Mineral and vitamin mix*	0.30	0.30	0.30
DL-Methionine	0.15	0.15	0.10
L-lysine	0.15	0.15	0.10
Total	100	100	100
Calculated analysis			
Crude protein (%)	23.08	20.88	19.29
ME (kcal/kg diet)	3004.95	3104.62	3201.57
Crude fiber (%)	3.61	3.24	3.08
Calcium (%)	1.00	1.00	0.97
Available phosphorus (%)	0.55	0.56	0.54
Methionine (%)	0.53	0.52	0.46
Lysine (%)	1.23	1.07	0.95
Methionine+Cysteine (%)	0.91	0.87	0.79

*Vitamin and mineral mix. mix: each 3kg contains: Vit. A, 12000000 IU; Vit. D3, 2000000 IU; Vit. E, 10 g; Vit. K, 2.0 g; Vit. B1, 1g; Vit. B2, 5g; Vit. B6, 1.5 g; Vit. B12, 10 mg; Folic acid, 1g; Biotin, 50mg; Pantothenic acid, 10 g; Nicotinic acid, 30 g; Choline chloride, 250 g; Mn, 60g; Fe 30, g; Zn, 50 g; Cu, 10g; I, 1 g; Co 100 mg; Se, 100 mg; Anti-oxidant, 10 g, and complete to 3.0 kg by Calcium Carbonate. The data in the table was calculated according to NRC (1994).

Blood parameters

Blood parameters were measured at the Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Giza, Egypt. Blood testing was carried out to evaluate physiological performance in the tested groups. Blood samples were taken from 18 chickens/treatment after slaughtering at 56 days of age from each group in collecting tubes containing heparin as an anticoagulant to obtain whole blood as 1 mL per individual bird. Then, it was centrifuged for 15 min at 3000 rpm and stored at -20°C. Blood testing parameters conducted in this study included: liver function testing the concentrations of two enzymes, aspartate transaminase (AST) and alanine transaminase (ALT), and measuring glucose level, alkaline phosphatase (ALP), and kidney function testing creatinine (CRE), uric acid and urea by calorimetric methods using commercial kits of Bio Diagnostic Co., Egypt, following the manufacturer's instructions.

Immunological parameters

On days 10 and 21, blood samples were collected to determine immunological parameters including antibody titters against avian influenza virus (H5) and Newcastle disease (ND). Blood samples were taken on days 10 and 21 from 18 chickens from each group using sterile plastic syringes to collect blood from the wing vein as 1 mL per individual bird then left for complete agglutination to separate serum. The collected sera were transferred to Eppendorf tubes and centrifuged at 3000 rpm to separate clear sera. Serum samples were tested to determine the immunological response after vaccination by measuring antibody titers against avian influenza virus (AIV-H5) and Newcastle disease virus (NDV) using Hemagglutination inhibition test (HI) according to standard protocol (WOAH, 2021).

Statistical analysis

The experiment data were statistically examined by analysis of variance according to Mead (2002) using ANOVA procedures of SAS (SAS, 2011). The statistical model was used as the following formula.

$Y_{ij} = \mu + Z_i + S_j + (ZS)\ ij + e_{ij}$

Where Y_{ij} is an observation, μ is the overall mean, Zi is the effect of the sex groups, (i is equal to 1 and 2), Sj is the effect of bedding materials (j is equal to 1 and 2), (ZS)ij is the interaction effect between sex and bedding materials (ij = 1, 2….+4), eij refer to random error.

The differences between means were tested by using Duncan's multiple-range test procedures (Duncan, 1955), and p-values less than 0.05 were considered significant. The percentage values were subjected to sine transformation before analyzing variance. Means were present after recalculating from the transformed value to percentages.

RESULTS

Body weight and body weight gain

The effects of sex and bedding material on BW and BWG are illustrated in Table 2. During all the experimental periods, males (M) had significantly higher BW and BWG values than females (F). However, the bedding material has a low impact on the BW. Chickens raised on plastic slatted floors (PSF) had higher BWGs at different ages (1-8 weeks) than chickens kept on sawdust litter (SL). The results indicated that a significantly higher interaction was found for BW and BWG, indicating that the highest values were observed for M reared on PSF compared with those of the other interaction groups during all experimental periods and this interaction was higher for F reared on PSF than for F reared on SL ($p < 0.05$).

Feed intake and feed conversion

The effect of sex and bedding type on FI and FC are shown in Table 3. Significant increases in FI and FC among males and females were found during all the experimental periods ($p < 0.05$). Compared with females, males presented a higher FI during all experimental periods and improved FC by 0.2% during the whole 8 weeks and 0.25% during (4-8 weeks). No significant differences were found in FI during all experimental periods for chickens reared on either floor type (SL or PSF), while chickens reared on PSF had higher FCs by 0.08% and 0.06%, respectively, than chickens housed on SL at 4–8 and 1–8 weeks of age ($p < 0.05$). The interaction effects demonstrated that the FI of males raised on both types of floors did not differ significantly. However, compared to females raised on SL, those raised on PSF had lower FI and higher FC. For all the experimental periods, the FC values for males raised on PSF were higher than those for the other interaction groups ($p < 0.05$).

Carcass traits of several internal organs

As shown in Table 4, males had significantly higher live body weight (LBW) than females including carcass 2.3%, breast 0.97%, and thigh 0.8%, while giblet percentages were not significantly different ($p < 0.05$). Compared with those raised on sawdust litter, the chickens raised on plastic slatted floors had a substantially higher percentage of carcass by 1.55%, breast by 0.83%, and thigh by 0.98% ($p < 0.05$). The interaction effects indicated no significant differences in LBW between males reared on both floor types (SL and PSF; $p < 0.05$). However, females reared on PSF had higher LBW (carcass by 1.2% and thigh by 1.18%) than females reared on SL. Among the males reared on SL, those reared on PSF had the highest LBW (carcass by 1.9%, breast by 0.84% and thigh by 0.79%; $p < 0.05$). Furthermore, there was no significant difference in giblet percentages among the groups ($p > 0.05$).

Blood parameters

The hepatic and renal function biomarkers affected by the sex and rearing system are presented in Table 5. Alanine transaminase (ALT) and glucose levels were significantly higher in males than in females. However, renal function biomarkers (CRE, uric acid, and urea) were significantly higher in F than in M ($p < 0.05$). Chickens that were reared on PSF had the highest hepatic function biomarkers AST and ALT and the lowest renal function biomarkers ($p < 0.05$). The interaction effects indicated also that M reared on PSFs had the highest hepatic function biomarkers, except for AST, which was higher in F reared on PSFs than in those of other groups ($p < 0.05$). However, renal function biomarkers were higher in both M and F chicks reared on SL.

Immunological performance

According to the data in Figure 1, M had significantly higher antibody titers against avian influenza AI (H5) and ND at the 10^{th} and 21^{st} days of age than females ($p < 0.05$). Moreover, chickens reared on PSF had higher antibody titters against AI (H5) and ND on the 21^{st} day of age than those reared on SL. The data revealed that there was a higher interaction effect on the antibody titters against AI (H5) and ND at 10^{th} and 21^{st} days of age, suggesting that the highest values were observed for M reared on PSF compared with those of the other interaction groups during the whole experimental period ($p < 0.05$).

Table 2. Body weight and body weight gain of the hybrid broiler (WINZY Line 105) affected by gender and plastic-based bedding material from day one to eight weeks of age

Items		Body weight			Weight gain		
		1d	4 W	8 W	1d-4 W	4-8 W	1d-8 W
Gender	M	33.64±0.68	594.48±15.52^{a}	1813.78±22.04^{a}	553.36±10.30^{a}	1234.61±22.70^{a}	1787.97±23.88^{a}
	F	33.67±0.26	510.30±10.01^{b}	1508.50±21.81^{b}	476.63±9.89^{b}	998.20±16.30^{b}	1474.83±22.12^{b}
	P Value	0.956	0.006	0.001	0.001	0.001	0.001
Bedding materials	SL	33.66±0.48	544.00±45.27	1646.33±166.40	510.34±45.23	1102.33±122.16^{b}	1612.67±166.40^{b}
	PSF	33.65±0.55	560.78±46.03	1683.78±171.31	519.65±38.38	1130.48±130.75^{a}	1650.13±171.31^{a}
	P Value	0.983	0.609	0.055	0.590	0.028	0.041
Interaction between gender and bedding materials							
Gender	**Bedding materials**						
M	SL	33.64±0.76	585.44±10.52^{a}	1800.67±7.40^{b}	551.80±3.54^{a}	1215.22±11.08^{b}	1767.02±8.70^{b}
	PSF	33.64±0.76	603.51±1.33^{a}	1842.56±6.38^{a}	554.91±9.41^{a}	1254.00±10.22^{a}	1808.91±6.43^{a}
F	SL	33.68±0.07	502.56±6.57^{c}	1492.00±10.00^{d}	468.87±11.12^{b}	989.44±6.59^{c}	1458.32±10.93^{d}
	PSF	33.66±0.41	518.04±5.12^{bc}	1525.00±9.95^{c}	484.38±10.93^{b}	1006.9±5.06^{c}	1491.34±11.68^{c}
P Value.		0.973	0.022	0.007	0.002	0.003	0.002

$^{a, b, c, d}$ Means bearing different superscript letters within the same column were significantly different ($p < 0.05$). d: Day, W: week, M: Males, F: Females, SL: Sawdust litter, PSF: Plastic slatted floor.

Table 3. Feed intake and feed conversion of the hybrid broiler chickens affected by gender and plastic-based bedding material from day one to eight weeks of age

Items		Body weight			Weight gain		
		1 d – 4 wk.	4 wk.- 8 wk.	1d- 8wk.	1d-4 W	4-8 W	1d -8 W
Gender	M	1253.85±11.37^{a}	2799.30±43.03^{a}	4053.15±51.94^{a}	2.27±0.05^{b}	2.27±0.04^{b}	2.27±0.04^{b}
	F	1137.58±20.33^{b}	2511.04±50.61^{b}	3648.62±54.25^{b}	2.39±0.06^{a}	2.52±0.08^{a}	2.47±0.07^{a}
	P Value	0.023	0.007	0.005	0.032	0.008	0.006
Bedding materials	SL	1189.67±66.67	2666.94±130.0	3856.62±199.52	2.34±0.08	2.43±0.10^{a}	2.40±0.12^{a}
	PSF	1201.76±60.33	2643.40±183.22	3845.16±222.11	2.32±0.09	2.35±0.12^{b}	2.34±09^{b}
	P Value	0.678	0.057	0.122	0.321	0.046	0.033
Interaction between gender and bedding materials							
Gender	**Bedding materials**						
M	SL	1253.09±5.12^{a}	2784.69±30.3^{a}	4037.79±28.66^{a}	2.27±0.04^{c}	2.29±0.02^{c}	2.29±0.03^{c}
	PSF	1254.61±11.18^{a}	2813.91±45.39^{a}	4068.52±62.22^{a}	2.26±0.05^{c}	2.24±0.04^{d}	2.25±0.05^{d}
F	SL	1126.25±6.51^{b}	2549.19±14.55^{b}	3675.44±17.17^{b}	2.40±0.05^{a}	2.58±0.03^{a}	2.52±0.02^{a}
	PSF	1148.91±14.61^{b}	2472.89±25.62^{c}	3621.80±66.54^{c}	2.37±0.06^{b}	2.46±0.05^{b}	2.43±0.05^{b}
P Value.		0.037	0.008	0.009	0.042	0.009	0.008

$^{a, b, c, d}$ Means bearing different superscript letters within the same column were significantly different ($p < 0.05$). d: Day, W: Week, M: Males, F: Females, SL: Sawdust litter, PSF: Plastic slatted floor

Table 4. Carcass traits of the hybrid broiler (WINZY Line 105) affected by gender and plastic-based bedding materials from day one to eight weeks of age

Items		Body weight (gm)	Carcass (%)	Breast (%)	Thigh (%)	Giblets (%)
Gender	M	1901.67±12.69[a]	78.25±1.95[a]	25.45±1.08[a]	28.02±0.54[a]	4.86±0.09
	F	1771.67±11.19[b]	76.28±1.88[b]	24.48±0.64[b]	27.22±1.12[b]	4.78±0.15
	P Value	0.006	0.033	0.022	0.036	0.151
Bedding materials	SL	1820±14.62	76.49±2.23[b]	24.55±0.78[b]	27.13±1.09[b]	4.80±0.16
	PSF	1853.33±54.04	78.04±1.51[a]	25.38±0.64[a]	28.11±0.48[a]	4.84±0.09
	P Value	0.336	0.047	0.041	0.040	0.611
Effect of interaction between gender and bedding materials						
Gender	**Bedding materials**					
M	SL	1898.33±30.12[a]	77.31±1.43[bc]	25.03±1.02[bc]	27.62±0.12[b]	4.83±0.10
	PSL	1905.00±32.23[a]	79.19±0.99[a]	25.87±0.88[a]	28.41±0.46[a]	4.88±0.09
F	SL	1741.67±12.52[c]	75.67±2.85[d]	24.07±0.56[d]	26.63±1.23[c]	4.76±0.22
	PSL	1801.67±14.46[b]	76.89±0.88[cd]	24.90±1.12[c]	27.81±0.45[b]	4.79±0.08
P Value.		0.024	0.031	0.042	0.032	0.345

[a, b, c, d] Means bearing different superscript letters within the same column were significantly different ($p < 0.05$). d: Day, W:week, M: Males, F: Females, SL: Sawdust litter, PSF: Plastic slatted floor

Table 5. Blood parameters of the hybrid broiler (WINZY Line 105) as affected by gender and plastic-based bedding material from day one to eight weeks of age

Items		AST (U/L)	ALT (U/L)	Glucose (mg/100ml)	ALK (U/L)	CRE (mg/dl)	Uric acid (mg/dl)	Urea (mg/dl)
Gender	M	67.10±4.19	59.55±2.02[a]	81.05±3.54 [a]	110.44±4.94	0.81±0.08[b]	3.80 ±.62[b]	4.81±1.16[b]
	F	65.70±4.33	56.38±2.17[b]	78.14 ±1.40[b]	109.37±4.2	0.89±0.11[a]	4.23 ±.49[a]	5.11±0.86[a]
	P Value	0.422	0.007	0.004	0.654	0.002	0.009	0.005
Bedding materials	SL	64.07±3.76[b]	56.92±2.16[b]	78.07±2.86[b]	105.78±0.88[b]	0.93±0.06[a]	4.31±.60[a]	5.29±0.71[a]
	PSF	68.73±3.72[a]	59.02±2.51[a]	81.11±2.61[a]	114.03±1.26[a]	0.77±018[b]	3.72±.42[b]	4.63±1.11[b]
	P Value	0.003	0.023	0.033	0.005	0.004	0.003	0.003
Effect of interaction between gender and bedding materials								
Gender	**Bedding materials**							
M	SL	66.47±3.31[b]	58.77±1.00[b]	78.76±2.83[bc]	105.98±0.65[b]	0.88±0.03[b]	4.48±.68[a]	5.23±0.68[a]
	PSF	67.73±3.5[b]	60.33±1.71[a]	83.33±1.38[a]	114.90±0.66[a]	0.73±0.06[d]	3.98±0.39[b]	4.39±.54[c]
F	SL	61.67±1.70[c]	55.07±1.28[c]	77.38±1.19[c]	105.57±1.04[b]	0.98±0.03[a]	4.14±0.41[ab]	5.34±.88[a]
	PSF	69.73±3.31[a]	57.70±2.28[bc]	78.89±1.14[b]	113.16±1.05[a]	0.80±0.02[c]	3.46±0.28[c]	4.88±.74[b]
P Value.		0.023	0.016	0.008	0.007	0.006	0.005	0.006

[a, b, c, d] Means bearing different superscript letters within the same column were significantly different ($p < 0.05$). d: Day, W: Week, M: Males, F: Females, SL: Sawdust litter, PSF: Plastic slatted floor

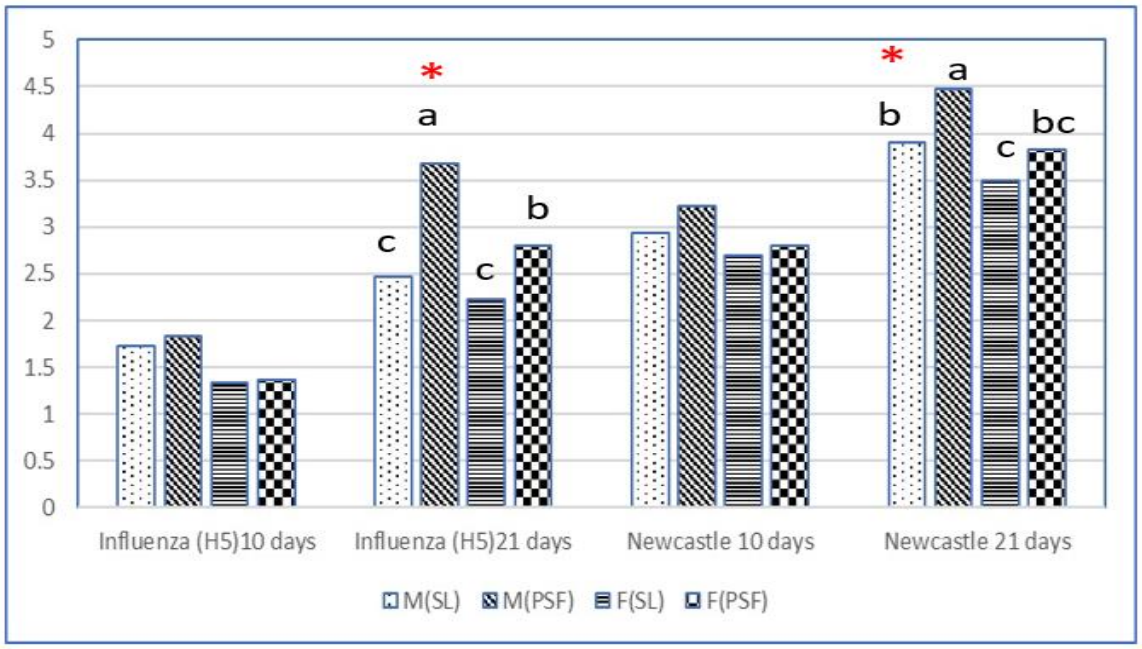


Figure 1. Antibody titers against avian influenza and Newcastle disease at days 10th and 21st of the age in hybrid broiler chickens (WINZY Line 105) as affected by gender and plastic-based bedding materials from day one to eight weeks of age. M (SL): Male (Sawdust Litter). M (PSF): Male (Plastic Slatted Floor). F (SL): Female Sawdust Litter. F (PSF): Female (Plastic Slatted Floor). *: $p < 0.05$.

DISCUSSION

Litter or bedding material is an important constituent of the poultry industry and growth performance, it primarily affects growth parameters such as BW and BWG (Almeida et al. 2017). In this study, the chickens reared on the plastic-slated floor had better growth performance than those in the wood shavings. The perforated plastic floors were considered to be a good alternative to wood shavings when rearing broiler chickens both sex male and female (Almeida et al. 2018). They were effective at promoting a better-quality environment and superior production rates.

Females and males grown on plastic floor had a BWG significantly higher than that reported in wood shavings. Similar results were obtained in a previous study; when comparing groups reared on different floor materials, the growth of chickens reared on a plastic-slatted floor was higher in terms of final BW, BWG, and growth rate than other flooring systems (Almeida et al., 2018; Çavuşoğlu et al. 2018).

Feed intake in females of Winzy line 105 was recorded as 3621.8g and 3675.4g at 8 weeks of age for rearing on PSF and SL respectively, while for males was 4068.5g and 4037.8g respectively. In a previous study, males grown on plastic floors were heavier than males raised on wood shavings, and females raised on plastic floors were also heavier than those reared on the wood shavings floor.

Manning et al. (2007) indicated the level of water usage may be influenced by the litter quality and the degree of absorbency of the litter material.

The higher final BW and total FI of broilers in the groups raised in fully or partially slatted flooring designs were significantly higher than those of broiler chickens in the deep litter flooring system (Topal and Petek, 2021).

Carcass body weight for females reared on PSF was higher than SL in both sexes. That was agreed with Kralik et al. (2015), who indicated that sex had a higher significant impact on the live weight, carcass weight, and weight of the main body parts. it is also preferable for individuals of younger ages, to be reared on a plastic-slated flooring system (Passini et al., 2012). Almeida et al. (2017) verified that females raised on plastic flooring had a heavier carcass weight than those raised on wood shavings, but male broiler chickens had a heavier carcass weight than females. Slat flooring systems showed preference in younger ages at slaughter (Çavuşoğlu et al., 2018). Poultry reared on plastic floor had higher live weights and carcass weights for males than females (Almeida et al., 2018).

Abo Ghanima et al. (2020) investigated the effects of three litter-rearing systems including wood-shaving litter,

perforated plastic slate-rearing systems, and cage-rearing systems, they reported that chickens raised on wood-shaving litter had lower dressing percentages than chickens raised on plastic-slatted floors. They reported that the style of flooring had no significant impact on the relative weights of the liver and heart. Broilers raised on different types of floors indicated nonsignificant differences in LBW and giblets, according to Farghly et al. (2021a; 2021b).

Compared to chickens reared on sawdust, chickens reared on perforated plastic floors had significantly heavier carcasses. According to Al-Nasseri et al. (2021), an increase in the live body weight of chickens reared on plastic floors due to general improvement in environmental conditions, including improved air quality and heat relief due to decreased dust content and decreased moisture in the floor, leading to increased performance and growth parameters. The plastic-slated floor system allows the chickens' feet to be in direct contact with the ground, this allows high heat conductivity in the environment and facilitates airflow around the chickens. Blood parameters like AST and ALT were higher in both sexes reared on PSF than SL. These results support those of Wang et al. (2015), who reported that chickens reared in litter-based systems had lower apparent ileal_digestible energy intake than those reared on the Net rearing system during the first 3 weeks of age.

The results also indicated that monitoring blood parameters was important for assessing stress and immune response to stressors in poultry (Saeed et al., 2019; Nwaigwe et al., 2020).

The increased significant interaction effect on the antibody titters against AI (H5) and ND on days 10 and 21 of age was observed in this study**.** Results suggested that the highest values were observed for M reared on PSF compared with those of the other interaction groups during the whole experimental period. There were higher antibody titers in female individuals reared on PSF than in female individuals reared on SL on the 21st day of age which indicated the desirable effect of PSF on immunity than the SL.

The level of AIV-H5 and NDV antibodies in chickens reared on PSF compared with those reared on sawdust litter SL revealed that antibody titers against NDV and AI were elevated in the PSF groups. These results indicated a significant increase in the level of antibodies against both viruses and increased their vaccination response and ability for stress control. Previous results suggested that rearing chickens in cages and on plastic-slated floors could enhance immunity (Sogunle et al., 2008).

CONCLUSION

The plastic slatted floors were effective in terms of environmental conditions and production rates, and they are suitable replacements for wood sawdust during the growing period of broilers chickens. This flooring system would enhance production rates and immunity under adverse climatic conditions in chickens. The production efficiency of the hybrid breed used in this study was enhanced using a plastic slated floor compared to the traditional sawdust floor, especially for body weight gain and antibody titers against ND and AI.

DECLARATIONS

Ethical considerations

The article was written originally by authors from the obtained original data and it was not submitted or published totally or even partially in other publications. The text article is checked by a well-known plagiarism checker software before submission to the journal.

Acknowledgments

The authors are grateful to Misr Organic Food Industry Company for its funding support for this study.

Authors' contributions

Mohamed EL-masry (ME) and Magdy Hassan (MH) designed, performed, and followed up the experiment. Ahmed Bealish (AB), Magdy Ouda (MO), and Ibrahim Fathey (IF) ran the experiment and conceived the study. Tarek El-Afifi (TE) and Abdelsatar Arafa (AA) did the laboratory work. Hanaa Abd El-Atty (HA) wrote the original draft. Hanaa Abd El-Atty (HA), Abdelsatar Arafa (AA), and Hanan Fahmy (HF) participated in the manuscript draft review, coordination, and editing. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no conflict of interest.

Funding

Misr Organic Food Industry Company, Egypt funded this study by providing the hybrid chicken of the 5th generation (WINZY Line 105) and germinated feed (barley + sorghum).

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

Abo Ghanima MM, Abd El-Hack ME, Othman SI, Taha AE, Allam AA, and Eid Abdel-Moneim AM (2020). Impact of different rearing systems on growth, carcass traits, oxidative stress biomarkers, and humoral immunity of broilers exposed to heat stress. Poultry Science, 99(6): 3070-3078. DOI: https://www.doi.org/10.1016/j.psj.2020.03.011

Almeida EA, De Souza LFA, Sant'Anna AC, Bahiense RN, Macari M, and Furlan RL (2017). Poultry rearing on perforated plastic floors and the effect on air quality, growth performance, and carcass injuries-experiment 1: Thermal comfort. Poultry Science, 96(9): 3155-3162. https://www.doi.org/10.3382/ps/pex131

Almeida EA, Sant'Anna AC, Crowe T, Macari M, and Furlan RL (2018). Poultry rearing on perforated plastic floors and the effect on air quality, growth performance, and carcass injuries- Experiment 2: Heat stress situation. Poultry Science, 97(6): 1954-1960. DOI: http://www.doi.org/10.3382/ps/pey048

Al-Nasseri ANI, Taha AT, and Hasan ATK (2021). Effects of different broiler flooring `systems on surface temperature, air quality and carcass characters of broilers. IOP Conference Series of Earth and Environmental Science, 735: 012011. Available at: https://ui.adsabs.harvard.edu/abs/2021E%26ES..735a2011A/abstract

Beker A, Vanhooser SL, Swartzlander JH, and Teeter RG (2004). Atmospheric ammonia concentration effects on broiler growth and performance. Journal of Applied Poultry Research, 13(1): 5-9. DOI: https://www.doi.org/10.1093/japr/13.1.5

Bilgili SF, Hess JB, Blake JP, Macklin KS, Saenmahayak B, and Sibley JL (2009). Influence of bedding material on footpad dermatitis in broiler chickens. The Journal of Applied Poultry Research, 18(3): 583-589. DOI: https://www.doi.org/10.3382/JAPR.2009-00023

Çavuşoğlu E, Petek M, Abdourhamane İM, Akkoc A, and Topal E. Effects of different floor housing systems on the welfare of fast-growing broilers with an extended fattening period. Archives Animal Breeding, 61(1): 9-16. DOI: https://www.doi.org/10.5194/aab-61-9-2018, 2018

Collett SR (2012). Nutrition and wet litter problems in poultry. Animal Feed Science and Technology, 173(1-2): 65-75. DOI: https://www.doi.org/10.1016/j.anifeedsci.2011.12.013

De Jong IC, Gunnink H, and van Harn J (2014). Wet litter not only induces footpad dermatitis but also reduces overall welfare, technical performance and carcass yield in broiler chickens. Journal of Applied Poultry Research, 23(1): 51-58. DOI: https://www.doi.org/10.3382/japr.2013-00803

Duncan DB (1955). Multiple range and multiple F tests. Biometrics, 11(1): 141. DOI: https://www.doi.org/10.2307/3001478

Food and agriculture organization of the United Nations (FAO) (2011). Climate change mitigation finance for smallholder agriculture, pp. 1-88. Available at: https://www.fao.org/climatechange/29763-0daebeae838c70f713da780982f16e8d9.pdf

Farghly MFA, Mahrose KM, Cooper RG, Metwally KA, Abougabal MS, and El-Ratel IT (2021a). Use of available crop by-products as alternative bedding materials to wheat straw for rearing broilers. Animal: An International Journal of Animal Bioscience, 15(7): 100260. DOI: https://www.doi.org/10.1016/j.animal.2021.100260

Farghly MFA, El-Kelawy AMI, Kassab AY, and Emam MS (2021b). Use of palm wastes as alternative flooring materials in broiler chickens houses under prevailing conditions in new valley. Journal of Desert and Environmental Agriculture, 1(1): 59-69. DOI: https://www.doi.org/10.21608/jdea.2021.61866.1006

Kralik G, Kralik Z, Kušec ID, Škrtić Z, and Kralik I (2015). Influence of dietary histidine, hybrid line and gender on chicken meat quality and carnosine concentration. The Journal of Poultry Science, 52(4): 295-303. DOI: https://www.doi.org/10.2141/jpsa.0140201

Kumar M, Ratwan P, Dahiya SP, and Nehra AK (2021). Climate change and heat stress: Impact on production, reproduction and growth performance of poultry and its mitigation using genetic strategies. Journal of Thermal Biology, 97: 102867. DOI: https://www.doi.org/10.1016/j.jtherbio.2021.102867

Lamarca DSF, Pereira DF, Magalhães MM, and Salgado D (2018). Climate Change in Layer Poultry Farming: Impact of heat waves in region of Bastos, Brazil. Brazilian Journal of Poultry Science, 20(4): 657-664. DOI: https://www.doi.org/10.1590/1806-9061-2018-0750

Lien R J, Conner DE, and Bilgili SF (1992). The use of recycled paper chips as litter material for rearing broiler chickens. Poultry Science, 71(1): 81-87. DOI: https://www.doi.org/10.3382/ps.0710081

Liverpool-Tasie LSO, Sanou A, and Tambo JA (2019). Climate change adaptation among poultry farmers: Evidence from Nigeria. Climatic Change, 157: 527-544. DOI: https://www.doi.org/10.1007/s10584-019-02574-8

Manning L, Chadd SA, and Baines RN (2007). Water consumption in broiler chicken: A welfare indicator. World's Poultry Science Journal, 63(1): 63-71. DOI: https://www.doi.org/10.1017/S0043933907001274

Mead R (2002). Statistical methods in agriculture and experimental biology, 3rd Edition. Chapman and Hall/CRC., New York, pp. 1-488. DOI: https://www.doi.org/10.1201/9780203738559

Miles DM, Branton SL, and Lott BD (2004). Atmospheric ammonia is detrimental to the performance of modern commercial broilers. Poultry Science, 83(10): 1650-1654. DOI: https://www.doi.org/10.1093/ps/83.10.1650

Mohammed HH, Abdelaty AI, Saleem AY, Youssef MI, and Abdel-Hamid SE (2019). Effect of bedding materials on duck's welfare and growth performance. Slovenian Veterinary Research, 56(Suppl 22): 149-56. DOI: https://www.doi.org/10.26873/SVR-752-2019

National research council (NRC) (1994). Nutrient Requirements of Poultry, 9th Revised Edition. The National Academies Press., Washington, DC. Available at: https://www.agropustaka.id/wp-content/uploads/2020/04/agropustaka.id_buku_Nutrient-Requirements-of-Poultry_Ninth-Revised-Edition-1994-NRC.pdf

Nwaigwe CU, Ihedioha JI, Shoyinka SV, and Nwaigwe CO (2020). Evaluation of the hematological and clinical biochemical markers of stress in broiler chickens. Veterinary World, 13(10): 2294-2300. DOI: https://www.doi.org/10.14202/vetworld.2020.2294-2300

Oh SM, Yoon SY, Lee JY, Jeon SM, Oh DY, Ha JJ, Song YH, and Kim JS (2019). Effects of mixed or split-sex feeding on growth performance and behavior of Korean Native Chicken (KNC). Annals of Animal Resource Sciences, 30(3): 105-110. DOI: https://www.doi.org/10.12718/AARS.2019.30.3.105

Passini R, De Araujo MAG, De Almeida EA, and Yasuda VM (2012). Evaluation of reflective painting of the roof and artificial ventilation on performance and carcass yield of broilers. Revista Brasileira de Zootecnia, 41: 1769-1774. DOI: http://www.doi.org/10.1590/S1516-35982012000700029

Saeed M, Abbas G, Alagawany M, Kamboh AA, Abd El-Hack ME, Khafaga AF, and Chao S (2019). Heat stress management in poultry farms: A comprehensive overview. Journal of Thermal Biology, 84: 414-425. DOI: https://www.doi.org/10.1016/j.jtherbio.2019.07.025

Shepherd EM and Fairchild BD (2010). Footpad dermatitis in poultry. Poultry Science, 89(10): 2043-2051. DOI: https://www.doi.org/10.3382/ps.2010-00770

Sogunle OM, Egbeyale LT, Bajomo TT, Bamigboje OV, and Fanim AO (2008). Comparison of the performance, carcass characteristics and haematological parameters of broiler chicks reared in cage and floor. Pakistan Journal of Biological Sciences, 11(3): 480-483. DOI: https://www.doi.org/10.3923/pjbs.2008.480.483

Thompson HE, Berrang-Ford L, and Ford JD (2010). Climate change and food security in Sub-Saharan Africa: A systematic literature review. Sustainability, 2(8): 2719-2733. DOI: http://www.doi.org/10.3390/su2082719

Toghyani M, Gheisari A, Modaresi M, Tabeidian SA, and Toghani M (2010). Effect of different litter material on performance and behavior of broiler chickens. Applied animal behaviour science, 122(1): 48-52. DOI: https://www.doi.org/10.1016/j.applanim.2009.11.008

Topal E and Petek M (2021). Effects of fully or partially slatted flooring designs on the performance, welfare and carcass characteristics of broiler chickens. British Poultry Science, 62(6): 804-809. DOI: https://www.doi.org/10.1080/00071668.2021.1934399

Wang Y, Ru YJ, Liu GH, Chang WH, Zhang S, Yan HJ, Zheng AJ, Lou RY, Liu ZY, and Cai HY (2015). Effects of different rearing systems on growth performance, nutrients digestibility, digestive organ weight, carcass traits, and energy utilization in male broiler chickens. Livestock Science, 176: 135-140. DOI: https://www.doi.org/10.1016/j.livsci.2015.03.010

Wei FX, Hu XF, Xu B, Zhang MH, Li SY, Sun QY, and Lin P (2015). Ammonia concentration and relative humidity in poultry houses affect the immune response of broilers. Genetics and Molecular Research, 14(2): 3160-3169. DOI: https://www.doi.org/10.4238/2015.April.10.27

World organization for animal health (WOAH) (2021). Terrestrial animal health code. Avian influenza (including infection with high pathogenicity avian influenza viruses). Volume I, Paris, France, Chapter 3.3.4, pp. 1-28. Available at: https://www.woah.org/fileadmin/Home/fr/Health_standards/tahm/3.03.04_AI.pdf

World organization for animal health (WOAH) (2021). Terrestrial animal health code. Newcastle disease (infection with Newcastle disease virus). Volume I, Paris, France, Chapter 3.3.14, pp. 1-23. Available at: https://www.woah.org/fileadmin/Home/fr/Health_standards/tahm/3.03.14_NEWCASTLE_DIS.pdf

Yang KY, Ha JJ, Roh H, Cho C, Oh SM, and Oh D (2019). Effects of litter type and gender on behavior characteristics and growth performance of Korean Hanhyup broiler. Korean Journal of Poultry Science, 46(3): 155-160. DOI: http://www.doi.org/10.5536/KJPS.2019.46.3.155

JWPR
Journal of World's Poultry Research
2024, Scienceline Publication
J. World Poult. Res. 14(2): 255-263, 2024
Research Paper
DOI: https://dx.doi.org/10.36380/jwpr.2024.26
PII: S2322455X2400026-14
ISSN: 2322-455X

Comparative Study of Various Diagnostic Methods for Detection of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in Egyptian Chicken Flocks

Marwa Emam[1*], Yousreya Mohamed Hashem[2], Elshaimaa Ismael[3], Mahmoud El Hariri[4], and Jakeen El-Jakee[4]

[1]*VACSERA Holding Company for Biological Products and Vaccines. Cairo, Egypt*
[2]*Animal Health Research institute, Agriculture research center, Giza, Egypt*
[3]*Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt*
[4]*Microbiology Department, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt*

*Corresponding author's E-mail: dr.memam2009@gmail.com

Received: March 27, 2024, Revised: April 25, 2024, Accepted: May 11, 2024, Published: June 30, 2024

ABSTRACT

The significance of *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) in the poultry industry underscores the critical importance of diagnosing avian mycoplasmosis within the field of veterinary medicine. The present study aimed to compare various diagnostic methods for detecting MG and MS in Egyptian Chicken Flocks. A total of 360 samples were collected from breeder, layer, and broiler chickens from four governorates in Egypt. Conventional isolation methods and polymerase chain reaction (PCR) were used for the direct detection of MG and MS, while serum plate agglutination test (SPA) and Enzyme-linked immunosorbent assay (ELISA) were used for detecting antibodies against MG and MS. The highest detection rate of MG was found in commercial layers, followed by breeders, and broilers. Regarding MS, the highest detection rate was found in breeders, followed by commercial layers, and broilers. By comparing the used diagnostic methods, MG and MS were determined by the SPA test (40% and 31.1% respectively), ELISA test (31.7% and 23.6%), PCR (16.7% and 11.7%), and by the conventional culture method (10.8% and 3.9%). It could be concluded that the serological methods and PCR gave better sensitivity than culture methods and can be used in the diagnosis of avian mycoplasmosis.

Keywords: Chicken, *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, Sensitivity

INTRODUCTION

Mycoplasma infection is a critical problem in veterinary medicine and in the poultry production industry (Qasem et al., 2015). Infections with *Mycoplasma gallisepticum* (MG) in poultry are linked with multiple disease conditions, including those affecting the respiratory and reproductive systems (Al-Baqir et al., 2023). *Mycoplasma gallisepticum* and *Mycoplasma synovia* (MS) are considered one of the most important avian *Mycoplasma* species in the commercial poultry industry (Felice et al., 2020). Chronic respiratory disorders are usually driven by MG infections that are characterized by sneezing and coughing besides nasal and ocular discharges (Raviv and Ley, 2013; Ghadimipour et al., 2018) while MS infections occur as subclinical upper respiratory tract infections and also air sac disease may occur. *Mycoplasma synoviae* may also cause an acute to chronic infectious disease in chickens called infectious synovitis (Ghadimipour et al., 2018). Enormous economic losses in the poultry industry can be caused by both *MG* and *MS* infections through weight gain loss and reduced meat quality in broilers, resulting in a severe drop in egg production in layers, and increasing embryo mortality in breeders (Messa Júnior et al., 2017). Isolation of the organism in a cell-free medium or direct detection of its DNA in infected tissues or swab samples and also serological diagnostic tests are widely used to detect the existence of MG or MS (OIE, 2008).

After an initial serological screening of suspected birds, mycoplasmosis diagnosis can be confirmed

by polymerase chain reaction (PCR) and culture (Muhammad et al., 2018). Identification of MG and MS through detecting their DNA (PCR) in field samples or by cultures (OIE, 2008). Identification of *Mycoplasma* isolates can be done through Mycoplasma media, biochemical, serological, or molecular tests, as well as serological analysis of host sera using Serum plate agglutination test (SPA), hemagglutination inhibition (HI) test, or ELISA (El-Ashram et al., 2021). It is preferable to use serological tests for flock screening rather than for testing individuals. The goal of the present study was to compare the occurrence of MG and MS in chicken flocks using serology, molecular, and culture methods.

MATERIALS AND METHODS

Ethical approval

The samples were collected from birds according to ethical guidelines of the Institutional Animal Care and Use Committee (IACUC) at the Faculty of Veterinary Medicine and Cairo University.

Sampling procedure

The samples collected from commercial layer, broiler breeder, and broiler farms from Elgarbeya, Elfayoum, Eldakahliya, and Giza governorates with clinical signs suggestive of MG or MS infections were investigated from February 2019 to the end of December 2019. Tracheal swabs (n = 360) were collected for isolation by culture and PCR detection. Additionally, blood samples (n = 360) were collected (2 ml) from the same examined chickens in an EDTA tube to record antibodies against MG and MS using serum plate agglutination (SPA) and enzyme-linked immunosorbent assay (ELISA).

Culture detection

The collected samples were cultivated into Pleuropneumonia like organism (PPLO) broth and agar (USA) media supplemented with *Mycoplasma* Enrichment Supplement FD075 at 37^0C in a moist 10% CO_2 for 3-5 days (Kleven, 2003). Traditional identification methods, such as digitonin sensitivity (Freundt, 1983), glucose fermentation (Ernø and Stipkovits, 1973), arginine hydrolysis (Fenske and Kenny, 1976), and film and spot formation test (Krieg and Holt, 1984) were performed.

Serological tests detection

Blood samples (2 ml) were collected aseptically from the wing vein using sterile disposable syringes, and left to clot then sera were separated by centrifugation and stored at 4^oC till used. The SPA test was performed by mixing 30 ul of serum with an equal volume of standard crystal violet *MG* antigen and MS antigen (Intervet, MSD animal health, USA) as well and then left for 2 minutes at room temperature (Heleili et al., 2012). Positive sera samples were inactivated at 56^0C for 30 minutes and serial dilutions were retested to ensure positivity in the SPA test (OIE, 2008).

Recombinant protein-based indirect ELISA was used to detect antibodies against MG and MS based on indirect ELISA. It was used to detect anti-MG antibodies in chicken sera (ID Screen® *MG* Indirect, IDvet) commercial test kit (France) and anti-MS antibodies in chicken sera (ID Screen® *Mycoplasma synoviae* Indirect, ID vet) commercial test kit (France). The procedures were followed according to manufacturer instructions.

PCR detection

DNA was extracted from tracheal swab samples suspended in 1 ml of PCR-grade Phosphate buffer saline (PBS) in a 1.5 ml snap-cap Eppendorf tube. The suspension was centrifuged for 30 minutes at 14,000 g at 4°C. Using a Pasteur pipette, the supernatant was carefully extracted and the pellet was then suspended in 25 µl PCR-grade water. The tube and the contents were boiled for 10 minutes and then placed on ice for 10 minutes before centrifugation at 14,000 g for 5 minutes. The supernatant contained the DNA. *Mycoplasma gallisepticum* and MS were detected using *16S* rRNA primers (OIE, 2008, Table 1). Each PCR tube was filled with a 45µl volume of the reaction mixture followed by the addition of 5 µl of DNA sample. The tubes were put in thermal cycles and ran through the following cycles, 40 cycles, 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 60 seconds, 1 cycle (final extension), 72°C for 5 minutes and soak at 4°C. Conventional 2% agarose gel electrophoresis was used to detect the Electrophoresis PCR products were detected by. Gels were observed using an ultraviolet transilluminator and photographed (Sambrook et al., 1989).

Statistical analysis

The results were analyzed using PASW Statistics, Version 18.0 software (SPSS Inc., Chicago, IL, USA). Data was displayed in tables as descriptive statistics (frequencies). Chi-square (χ^2) test for independence and Fisher's Exact test were used to examine the relation between the diagnostic method and the rate of positive results detected, as well as the relation between the type of poultry production and region and the detection rate of *Mycoplasma* spp. infection. A *p*-value < 0.05 was regarded as statistically significant.

Table 1. Primers used for *Mycoplasma gallisepticum* and *Mycoplasma synoviae* detection by PCR

Microorganism	Gene	Primer 5'- 3'	Amplicon size	Reference
Mycoplasma gallisepticum	*16S*rRNA	F-GAG-CTA-ATC-TGT-AAA-GTT-GGT-C R-GCT-TCC-TTG-CGG-TTA-GCA-AC	185 bp	OIE (2008)
Mycoplasma synoviae	*16S*rRNA	F-GAG-AAG-CAA-AAT-AGT-GAT-ATC-A R-CAG-TCG-TCT-CCG-AAG-TTA-ACA-A-	207 bp	

RESULTS

The present study indicated that the highest detection *MG* rate was identified in commercial layers aged 15-40 weeks, followed by breeders aged 50-70 weeks, and then broilers aged 30-39 days (Table 2). For MS, the highest detection rate was identified in breeders followed by commercial layers and broilers (Table 2). By comparing different methods for diagnosis of *Mycoplasma* infection (Tables 2 and 3 and Figure 1), the highest detection rates of *MG* and *MS* were recorded by serological tests including the SPA test (40% and 31.1%, respectively) and ELISA test (31.7% and 23.6%). These were followed by PCR (16.7% and 11.7%) and then by conventional culture methods (10.8% and 3.9%). The occurrence rates of *MG* and *MS* were higher in Eldakahliya than in Elgarbeya governorate among layers and breeders, while no infection was recorded among the Giza layer farm by the different methods of diagnosis. The lowest detection rate was recorded in the Elfayoum broiler farm.

Table 2. Comparative techniques for detection of *Mycoplasma gallisepticum* among the examined chickens

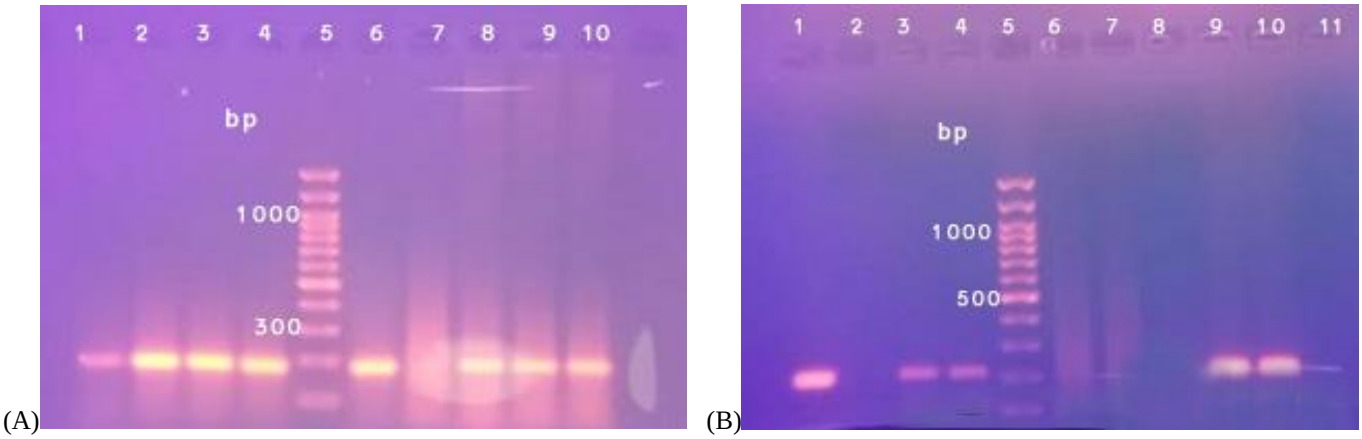
Parameters	Number of examined samples	Diagnostic method				*P*-value
		Culture No (%)	PCR No (%)	SPA No (%)	ELISA No (%)	
Type of poultry						
Broiler (30-39 days)	40	2 (5.0%)	4 (10.0%)	8 (20.0%)	7 (17.5%)	0.173
Layer (15-40 weeks)	170	21 (12.4%)	31 (18.2%)	74 (43.5%)	60 (35.3%)	< 0.0001*
Breeder (50-70 weeks)	150	16 (10.7%)	25 (16.7%)	62 (41.3%)	47 (31.3%)	< 0.0001*
	***P*-value**	0.403	0.454	0.022*	0.093	
Governorates						
Eldakahliya	200	27 (13.5%)	40 (20.0%)	95 (47.5%)	75 (37.5%)	< 0.0001*
Elgarbeya	100	10 (10.0%)	16 (16.0%)	41 (41.0%)	32 (32.0%)	< 0.0001*
Elfayoum	40	2 (5.0%)	4 (10.0%)	8 (20.0%)	7 (17.5%)	0.173
Giza	20	0	0	0	0	-
	***P*-value**	0.263	0.278	0.005*	0.047*	
Total	360	39 (10.8%)	60 (16.7%)	144 (40.0%)	114 (31.7%)	< 0.0001*

No: Number of positive results; *Indicate significance at $p < 0.05$. PCR: Polymerase Chain reaction, SPA: Serum Plate agglutination), ELISA: Enzyme Linked Immune Sorbent Assay

Table 3. Comparative techniques for detection of *Mycoplasma synoviae* among the examined chickens

Parameters	Number of examined samples	Diagnostic method				*P*-value
		Culture No (%)	PCR No (%)	SPA No (%)	ELISA No (%)	
Type of poultry						
Broiler (30-39 days)	40	1 (2.5%)	3 (7.5%)	6 (15.0%)	4 (10.0%)	0.277
Layer (15-40 weeks)	170	5 (3.0%)	17 (10.0%)	49 (28.8%)	38 (22.4%)	< 0.0001*
Breeder (50-70 weeks)	150	8 (5.3%)	22 (14.7%)	57 (38.0%)	43 (28.7%)	< 0.0001*
	***P*-value**	0.484	0.295	0.014*	0.041*	
Governorates						
Eldakahliya	200	10 (5.0%)	28 (14.0%)	77 (38.5%)	61 (30.5%)	< 0.0001*
Elgarbeya	100	3 (3.0%)	11 (11.0%)	29 (29.0%)	20 (20.0%)	< 0.0001*
Elfayoum	40	1 (2.5%)	3 (7.5%)	6 (15.0%)	4 (10.0%)	0.277
Giza	20	0	0	0	0	-
	***P*-value**	0.724	0.463	0.009*	0.009*	
Total	360	14 (3.9%)	42 (11.7%)	112 (31.1%)	85 (23.6%)	< 0.0001*

*Indicate significance at p <0.05. PCR: Polymerase Chain reaction, SPA: Serum Plate agglutination), ELISA: Enzyme-Linked Immune Sorbent Assay



(A) (B)

Figure 1. Agarose gel electrophoresis of amplified PCR products of *16SrRNA* gene among *Mycoplasma gallisepticum* and *Mycoplasma synoviae*. **A:** Agarose gel electrophoresis of amplified PCR products of *16SrRNA* gene among *Mycoplasma gallisepticum* at 185 bp. Lane 5: 100 bp DNA marker (Thermoscientific), Lane 6: Positive control, Lane 7: Negative control, Lanes 1-4 and 8-10: *Mycoplasma gallisepticum* positive isolates. **B:** Agarose gel electrophoresis of amplified PCR products of *16SrRNA* gene among *Mycoplasma synoviae* isolates at 207 bp. Lane 5: 100 bp DNA marker (Thermoscientific), Lane 1: Positive control, Lane 2: Negative control, lanes 3, 4, 9, and 10: *Mycoplasma synoviae* positive isolates, Lanes 6-8: *Mycoplasma synoviae* negative isolates

DISCUSSION

The poultry industry plays a vital role for mankind through food supply (Fathy et al., 2017). Mycoplasma infection is considered a complicated and multifactorial disease causing economic problems to the welfare of poultry corporations in many parts of the world (Ibrahim et al., 2021). Highly significant avian *Mycoplasma* species in the poultry industry are *MG* and *MS* resulting in huge economic losses (Felice et al., 2020). *Mycoplasma gallisepticum* is a serious poultry pathogen causing enormous economic losses in the poultry industry as it causes a reduction in egg production, hatchability, and downgrading of carcasses (Ibrahim et al., 2018). Emam et al. (2020) revealed that the prevalence of *MG* among the examined birds was 9.85%, while *MS* prevalence was 1.6%. The present results indicated that the highest detection rate of isolation by culture of *MG* was obtained in commercial layers, followed by breeders, due to the long life span while the lowest detection rate is identified

in broilers. Abbas et al. (2018) reported that the lowest prevalence observed in broilers is due to the short life cycle before marketing leading to a minimum period of exposure. This finding is in correspondence with Osman et al. (2009) who indicated that the most affected birds originate from the layer flocks as the highest prevalence of *MG* isolation (33.3%) was observed in layer flocks followed by 30.5% observed in broiler breeders and 4.9% in broilers. It was observed that the detection rate of *MG* and *MS* is higher in Eldakahliya than in Elgarbeyag governorate in layers and breeders and no infection was detected in a layer farm in Giza by the different methods of diagnosis. The lowest detection rate was identified in a broiler farm in Elfayoum.

Moreover, using age-based analyses, the most positive period was 46 weeks, followed by 40 weeks, 34 weeks, 27 weeks, and at least 20 weeks, in order of decreasing seropositivity (Demirbilek et al., 2020). *Mycoplasma synoviae* can spread vertically and horizontally (Stipkovits and Kempf, 1996). It is anticipated that horizontal transmission is the most effective transmission route for infected breeder flocks (Seifi and Shirzad, 2012).

SPA results showed that the highest detection rate of *MS* was found in breeders, followed by commercial layers due to long life span which is in agreement with Seifi and Shirzad (2012) who recorded 47.8% seropositivity by SPA in breeders above 60 weeks of age and also with results of Feberwee et al. (2008) with 60% seropositivity in breeders ≥ 52 weeks of age. It was found that the lowest detection rate was identified in broilers (15%) due to a short life span.SPA tests mainly measure type M immunoglobulin (IgM) which can be detected in serum within a week of infection and persist 70-80 days, while ELISA detects IgG (IgY in birds), which can be detected 7-10 days after infection and persist for up to six months (Bradbury and Morrow, 2008).

El-Jakee et al. (2019) investigated the seroprevalence of *MG* antibodies in 12 broiler breeder flocks and it was 52.92% (634/1198) using ELISA, while in hatched chicks from broiler breeder flocks, the serum plate agglutination test identified antibodies against MG in 52.86% (74/140) of the collected serum samples. The current study results indicated that positive samples were lower with ELISA for *MG* (31.7%) and *MS* (23.6%) than with SPA for *MG* (40%) and *MS* (31.1%) which agrees with Feizi et al. (2013), who recorded 33.33% with ELISA and 42.22% with SPA for *MG* and also with Osman et al. (2009), who recorded 41.9% with ELISA and 54.8% with SPA for *MG* and also with Luciano et al. (2011) who recorded (26.46%) positive in SPA and (4.21%) positive in HI and (21.06%) positive in ELISA and they observed weak statistical relation between all serological tests (SPA, HI, and ELISA). Ali et al. (2015) recorded that of 563 samples, 64.47% and 56.13% showed a complete prevalence of MG antibodies in ELISA and SPA tests respectively.

Despite the lower positivity of ELISA, it gave higher specificity than the SPA test for the detection of specific antibodies (Reda and Elsamie, 2012) as SPA is considered less specific than ELISA but the higher detection of positive birds by SPA may also be attributed to new infections (birds which developed an IgM response but did not have time to develop an IgY response to infection). Indirect ELISA was done by Bari and Shareef (2023) to evaluate the prevalence of MG antibodies in serum samples which were collected from 20 broiler flocks in Duhok governorate and all the serum-positive reactors to MG were 52.48%. The highest prevalence of *Mycoplasma* recorded by serological tests may be attributed to false positive results which can be related to the use of inactivated vaccines, recent infection with different *Mycoplasma* species which leads to cross-reactions, lack of heat inactivation, and age of birds (Feizi et al., 2013). In addition, the presence of antiglobulin-like factors and sera from chickens infected with infectious bursal disease viruses that cross-react in MG SPA tests can result in nonspecific reactions, Moreover, different degrees of temporary immunosuppression might permit a more prominent invasion of MG, and successively positive serological response (Asgharzade et al., 2013).

Using serological tests is recommended by OIE for screening only in flocks' diagnoses and not for individual birds' diagnoses as serological tests are rapid and easily performed. As serology gives information on the positive/negative status of the flock towards MG/MS it does not mean that mycoplasmas are still present in the flock (memory effect of serology, infection that may have happened several months before). Moreover, researchers must not depend on serological tests only for the diagnosis of *Mycoplasma* due to different sensitivities and specificities of serological tests. Isolation of microorganisms by culture method and/or molecular technique as PCR is a must to ensure the diagnosis (OIE, 2008).

By comparing different methods for diagnosis of *Mycoplasma* infection, it was found that the highest detection rate of MG and MS was detected by serological tests followed by PCR, with the lowest prevalence detected by the conventional culture method. Accordingly, both PCR and ELISA methods were considered superior

to the culture method for detecting avian mycoplasmosis (Qasem et al., 2015). Results of TaqMan RT-PCR showed an 81.25% detection rate, whereas the conventional polymerase chain reaction assay detected 51.92% positive cases (Elbehiry et al., 2016).

The lowest prevalence of MG (10.8%) and MS (3.9%) detected by the conventional culture method is probably because the culture of *Mycoplasma* species is fastidious and time-consuming as isolation takes a long time. Moreover, the detection of *Mycoplasma* species in medicated birds and chronic cases is very difficult due to low concentrations of mycoplasmas in these cases and culture is less sensitive than PCR (Gondal et al., 2015). The prevalence of MG by culture (10.8%) is lower than that detected by PCR technique (16.7%) and in correspondence with Gondalet al. (2015) who recorded a lower prevalence for culture (27.3%) than that for PCR detection (49.74%).

PCR is an alternative to the traditional isolation technique (Ferguson et al., 2005; Hess et al., 2007; Evans and Leigh, 2008) as it is more specific than the culture method. This is attributed to the fastidious nature of microorganisms, the high sensitivity of PCR tests, and the capability of PCR to amplify DNA from dead or alive pathogens. Application of molecular methods (PCR) on a large scale is used for accurate diagnosis of avian mycoplasmosis that aids in disease eradication programs to minimize the economic losses in poultry farms (Marouf et al., 2020). PCR is the most sensitive and reliable tool for the diagnosis of avian mycoplasmosis in field samples (Muhammad et al., 2018).

The culture technique is the gold standard test and PCR is a confirmative test but it does not differentiate between dead and live cells. Therefore, Culture must be performed in parallel with PCR to ensure greater diagnostic security.

CONCLUSION

In the present study, the highest detection rate of MG and MS is observed in layers and breeders, respectively while the lowest prevalence for both MG and MS are observed in broilers in Egypt poultry farms. Serological methods and PCR from tracheal samples gave better sensitivity than culture methods and can be used in the diagnosis of avian mycoplasmosis. Future research is recommended to identify the best prevention programs, hygienic measurements, effective treatments, and vaccination for the prevention and control of *M. gallisepticum* and *M. synoviae* in poultry production in Egypt.

DECLARATIONS

Funding

This study received no funding.

Availability of data and materials

The data that support the findings of this study are available on request from the corresponding author.

Ethical considerations

The authors considered farmers' ethical concerns and consent before conducting the study. This article was written originally without any copy from other articles.

Acknowledgments

The authors would like to show their deep gratitude to the Microbiology Department, Faculty of Veterinary Medicine, Cairo University, and the Mycoplasma Department, Animal Health Research Institute-Agriculture Research Center for their continuous support of our study.

Authors contributions

Dr. Marwa Emam collected the data, participated in the design of the work protocol, and performed the laboratory work. Dr. Mahmoud El Hariri and Dr. Yousreya Mohamed Hashem found the research idea, shared the performed data, and designed the work protocol. Dr. Elshaimaa Ismael performed the statistical analysis of the study. Dr. Jakeen El Jakee supervised the findings of the work. All authors discussed the results and contributed to the final manuscript.

Competing interests

The authors declare that they have no competing interests.

REFERENCES

Abbas N, Suleman M, Khan NA, Ijaz A, Rauf M, and Rahman S (2018). Prevalence of *Mycoplasma gallisepticum* in poultry and wild life birds suspected of chronic respiratory disease in Northern Pakistan. Pakistan Journal of Zoology, 50(3): 799-1198. DOI: http://www.doi.org/10.17582/journal.pjz/2018.50.3.1071.1077

Al-Baqir A, Hassanin O, Al-Rasheed M, Ahmed MS, Mohamed MHA, El Sayed MS, Megahed M, El-Demerdash A, Hashem Y, and Eid A (2023). Mycoplasmosis in poultry: An evaluation of diagnostic schemes and molecular analysis of Egyptian *Mycoplasma gallisepticum* Strains. Pathogens, 12(9): 1131. DOI: https://www.doi.org/10.3390/pathogens12091131

Ali Z, Rahman M, and Sultana S (2015). Seroprevalence of *Mycoplasma gallisepticum* antibody by ELISA and serum plate agglutination test

of laying chicken. Veterinary World, 8(1): 9-14. DOI: https://www.doi.org/10.14202%2Fvetworld.2015.9-14

Asgharzade S, Zaeri S, Hasanzade M, Ahmadi M, and Talebi AR (2013). Detection of *Mycoplasma gallisepticum* in experimentally infected broiler chickens using Culture, SPA, ELISA, and PCR methods. Comparative Clinical Pathology, 22: 1051-1055. Available at: https://link.springer.com/article/10.1007/s00580-012-1524-4

Bari NS and Shareef AM (2023). Seroprevalence of *Mycoplasma gallisepticum* in commercial broiler chickens in duhok governorate. Egyptian Journal of Veterinary Science, 54(2): 253-261. Available at: https://ejvs.journals.ekb.eg/article_269732.html

Bradbury JM and Morrow C(2008). Avian mycoplasmas. Poultry diseases. WB Saunders, pp. 220-234. DOI: https://www.doi.org/10.1016/B978-0-7020-2862-5.50025-8

Demirbilek SK, Ardicli Ö, and Carli KT(2020). Comparison of *Mycoplasma gallisepticum* infection in different samples and ages of chicken breeder flocks. Brazilian Journal of Poultry Sciences, 22(2): eRBCA-2020. DOI: https://www.doi.org/10.1590/1806-9061-2020-1271

El-Ashram S, Hashad ME, Abdel-Alim GA, Abdelhamid T, and Deif NH (2021). Seroprevalence of mycoplasmosis in broiler, layer, and native chickens in Giza, Egypt. PLoS One, 16(7): e0254220. DOI: https://www.doi.org/10.1371/journal.pone.0254220

Elbehiry A, Al-Dubaib M, and Marzouk E (2016). Serological, rapid molecular characterization and antibiotic resistance for field isolates of *Mycoplasma gallisepticum* in chicken in Saudi Arabia. Alexandria Journal of Veterinary Sciences, 49(2): 70-79. Available at: https://www.cabidigitallibrary.org/doi/pdf/10.5555/20163196471

El-Jakee J, Marouf SH, Amin BH, and Hedia RH (2019). Characterization of mycoplasmae isolated from characterization of mycoplasmae isolated from chicken. Bioscience Research, 16(2): 1843-1853.

Emam M, Hashem YM, El-Hariri M, and El-Jakee J (2020) Detection and antibiotic resistance of *Mycoplasma gallisepticum* and *Mycoplasmasynoviae* among chicken flocks in Egypt. Veterinary World, 13(7): 1410-1416. DOI: https://www.doi.org/10.14202/vetworld.2020.1410-1416

Ernø H and Stipkovits L (1973). Bovine *Mycoplasma*. Cultural and biochemical studies. Acta Veterinaria Scandinavica, 14(3): 463-449. DOI: https://www.doi.org/10.1186/bf03547431

Evans JD and Leigh SA (2008). Differentiation of *Mycoplasma gallisepticum* vaccine strains ts-11 and 6/85 from commonly used *Mycoplasma gallisepticum* challenge strains by PCR. Avian Diseases, 52(3): 491-497. DOI: https://www.doi.org/10.1637/8187-120307-resnote.1

Fathy M, El-Safty MM, El-Jakee J, Abd-Alla H, and Mahmoud H (2017). Study the effect of *Mycoplasma* contamination of eggs used in virus titration, and efficacy of some live attenuated poultry viral vaccines. Asian Journal of Pharmaceutical and Clinical Research, 10(1): 216-222. DOI: https://www.doi.org/10.22159/ajpcr.2017.v10i1.14930

Feberwee A, De Vries TS, and Landman WJ (2008). Seroprevalence of *Mycoplasma synoviae* in Dutch commercial poultry farms. Avian Pathology, 37(6): 629-633. DOI: https://www.doi.org/10.1080/03079450802484987

Feizi A, Nikpiran H, Bijanzad P, Moggadam ARJ, and Hosseini H (2013). Comparative evaluation of serological test in diagnosis of *Mycoplasma gallisepticum* infection in Iranian North-west rural Poultry. Advances in Bioresearch, 4(3): 50-53. Available at: https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=75bc3be55b27bd476eec43c45ea11b3fd4b01c7b

Felice V, Lupini C, Mescolini G, Silveira F, Guerrini A, Catelli E, and Di Francesco A (2020). Molecular detection and characterization of *Mycoplasma gallisepticum*and *Mycoplasma synoviae* strains in backyard poultry in Italy. Poultry Science, 99(2): 719-724. DOI: https://www.doi.org/10.1016/j.psj.2019.12.020

Fenske JD and Kenny GE (1976). Role of arginine deiminase in growth of *Mycoplasma hominis*. Journal of Bacteriology, 126(1): 501-510. DOI: https://www.doi.org/10.1128/jb.126.1.501-510.1976

Ferguson NM, Hepp D, Sun S, Ikuta N, Levisohn S, Kleven SH, and García M (2005). Use of molecular diversity of *Mycoplasma gallisepticum* by gene-targeted sequencing (GTS) and random amplified polymorphic DNA (RAPD) analysis for epidemiological studies. Microbiology, 151(Pt 6): 1883-1893. DOI: https://www.doi.org/10.1099/mic.0.27642-0

Freundt E (1983). Culture media for classic mycoplasmas. Methods in Mycoplasmology, 1: 127-135. DOI: https://www.doi.org/10.1016/B978-0-12-583801-6.50029-9

Ghadimipour R, Gharibi D, and Mayahi M (2018). Detection of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* among commercial poultry in Khouzestan province, Iran. Archives of Razi Institute, 73(2): 139-146. DOI: https://www.doi.org/10.22092/ari.2018.116164

Gondal M, Rabbani M, Muhammad K, YaqubT, Babar ME, Sheikh AA, Ahmad A, Shabbir MZ, and Khan MI (2015). Characterization of *Mycoplasma gallisepticum* isolated from commercial poultry flocks. Journal of Animal and Plant Sciences, 25(1): 108-113. Available at: https://www.thejaps.org.pk/docs/v-25-01/16.pdf

Heleili N, Ayachi A, Mamache B, and ChelihiAJ (2012). Seroprevalence of *Mycoplasma synoviae* and *Mycoplasma gallisepticum* at Batna Commercial poultry farms in Algeria. Veterinary World, 5(12); 709. DOI: https://www.doi.org/10.5455/vetworld.2012.709-712

Hess M, Neubauer C, and Hackl R (2007). Interlaboratory comparison of ability to detect nucleic acid of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* by polymerase chain reaction. Avian Pathology, 36(2): 127-133. DOI: https://www.doi.org/10.1080/03079450701203082

Ibrahim FF, Abd El-Ghany WA, El-Rawy EM, Shaker MM, and El-Jakee J (2021). Efficacy assessment of avian *Pasteurellamultocida* and *Mycoplasmagallisepticum*local vaccines. Journal of Animal Health and Production, 9(3): 213-221. DOI: http://www.doi.org/10.17582/journal.jahp/2021/9.3.213.221

Ibrahim FF, Abd El-Ghany WA, El-Rawy EM, Shaker MM, and El-Jakee J (2018). The protective efficacy of locally prepared combined inactivated *Mycoplasma gallisepticum* and *P. multocida* vaccine in chickens. Bioscience Research, 15(2): 702-707.

Kleven SH (2003). Recent developments in diagnosis and control (2003 Special). Poultry World, *Mycoplasma* Special. Available at: https://www.poultryworld.net/home/recent-developments-in-mycoplasma-diagnosis-and-control-2003-special/

Krieg NR and Holt JG (1984). Bergey's manual of systematic bacteriology, vol I. Williams and Wilkins., Baltimore London.

Luciano RL, Cardoso ALSP, Stoppa GFZ, Kanashiro AMI, de Castro AGM, and Tessari ENC (2011). Comparative study of serological tests for *Mycoplasma synoviae* diagnosis in commercial poultry breeders. Veterinary Medicine International, 2011: 304349. DOI: https://www.doi.org/10.4061/2011/304349

Marouf S, Moussa, IM, Salem, H, Sedeik M, Elbestawy A, Hemeg HA, Dawoud T, Mubarak AS, Mahmoud H, Alsubki RA et al. (2020). A picture of *Mycoplasma gallisepticum and Mycoplasma synoviae* in poultry in Egypt: Phenotypic and genotypic characterization. Journal of King Saud University-Science, 32(3): 2263-2268. DOI: https://www.doi.org/10.1016/j.jksus.2020.02.036

Messa Júnior A, Taunde P, Zandamela AF, Junior AP, Chilundo A, Costa R, and Bila CG (2017). Serological screening suggests extensive presence of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in backyard chickens in Southern Mozambique. Journal of Veterinary Medicine, 2017: 2743187. DOI: https://www.doi.org/10.1155/2017/2743187

Muhammad F, Hussain J, Fareed SK, Ahmad Khan T, Ahmad Khan S, and Ahmad A (2018). Diagnosis of avian mycoplasmas: A comparison between PCR and culture technique. Archives of Razi Institute, 73(3): 239-244. DOI: https://www.doi.org/10.22092/ari.2017.108217.1085

Office international des epizooties (OIE) (2008). Avian mycoplasmosis (*Mycoplasma gallisepticum, Mycoplasma synoviae*). OIE Terrestial Manual. OIE., Paris, Chapter 2.3.5 pp. 482-495.

Osman KM, Aly MM, Amin ZMS, and Hasan BS (2009). *Mycoplasma gallisepticum*: an emerging challenge to the poultry industry in Egypt. Revue scientifiqueet technique, 28(3): 1015. DOI: https://www.doi.org/10.20506/rst.28.3.1940

Qasem JA, Al-Mouqati SA, Al-Ali EM, and Ben-Haji A (2015). Application of molecular and serological methods for rapid detection of *Mycoplasma gallisepticum* infection (Avian mycoplasmosis). Pakistan Journal of Biological Sciences, 18(2): 81-87.DOI: https://www.doi.org/10.3923/pjbs.2015.81.87

Raviv Z and Ley DH (2013). Mycoplasmosis: *Mycoplasma gallisepticum* infection. In: D.E. Swayne, J.R. Glisson, L.R. McDougald, L.K. Nolan, D.L. Suarez, V. Nair (Editors). Diseases of poultry, 13th Edition. wiley-Blackwell., Ames, pp. 877-93.

Reda LM and El-Samie LA (2012). Some studies on the diagnosis of *Mycoplasma gallisepticum* in chicken. Lung, 26(180): 14-24. Available at: https://api.semanticscholar.org/CorpusID:212547585

Sambrook J, Fritsch EF, and Maniatis T (1989). Molecular cloning: A laboratory manual, 2nd Edition. Cold Spring Harbor Laboratory Press., NewYork.

Seifi S and Shirzad MR (2012). Incidence and risk factors of *Mycoplasma synoviae* infection in broiler breeder farms of Iran. Veterinary World, 5(5): 265. DOI: https://www.doi.org/10.5455/vetworld.2012.265-268

Stipkovits L and Kempf I (1996). Mycoplasmoses in poultry. Revue Scientifique et Technique, 15(4): 1495-1526. DOI: https://www.doi.org/10.20506/rst.15.4.986

Instructions for Authors

SUBMIT AN ARTICLE

Manuscript as Original Research Paper, Short Communication, Case Reports and Review or Mini-Review are invited for rapid peer-review publishing in *the Journal of World's Poultry Research*. Considered subject areas include: Husbandry and management; construction, environment and welfare; exotic and wild birds; Biochemistry and cellular biology; immunology, avian disease control; layer and quail management; nutrition and feeding; physiology, genetics, reproduction and hatching; technology, processing and food safety... view full aims and scope

- JWPR EndNote Style
- Manuscript Template (MS Word)
- Sample Articles
- Declaration form
- Publication Ethics

Submission

The manuscript and other correspondence should preferentially be submit online. Please embed all figures and tables in the manuscript to become one single file for submission. Once submission is complete, the system will generate a manuscript ID and will send an email regarding your submission. Meanwhile, the authors can submit or track articles via editor@jwpr.science-line.com or editorjwpr@gmail.com. All manuscripts must be checked (by English native speaker) and submitted in English for evaluation (in totally confidential and impartial way).

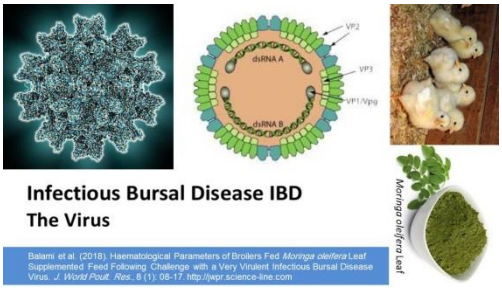
Supplementary information:
The online submission form allows supplementary information to be submitted together with the main manuscript file and covering letter. If you have more than one supplementary files, you can submit the extra ones by email after the initial submission. Author guidelines are specific for each journal. Our Word template can assist you by modifying your page layout, text formatting, headings, title page, image placement, and citations/references such that they agree with the guidelines of journal. If you believe your article is fully edited per journal style, please use our MS Word template before submission.

Supplementary materials may include figures, tables, methods, videos, and other materials. They are available online linked to the original published article. Supplementary tables and figures should be labeled with a "S", e.g. "Table S1" and "Figure S1". The maximum file size for supplementary materials is 10MB each. Please keep the files as small possible to avoid the frustrations experienced by readers with downloading large files.

Submission to the Journal is on the understanding that:
1.The article has not been previously published in any other form and is not under consideration for publication elsewhere;
2.All authors have approved the submission and have obtained permission for publish work.
3.Researchers have proper regard for conservation and animal welfare considerations. Attention is drawn to the *'Guidelines for the Treatment of Animals in Research and Teaching*'. Any possible adverse consequences of the work for populations or individual organisms must be weighed against the possible gains in knowledge and its practical applications. If the approval of an ethics committee is required, please provide the name of the committee and the approval number obtained.

Ethics Committee Approval
Experimental research involving animals should have been approved by author's institutional review board or ethics committee. This information can be mentioned in the manuscript including the name of the board/committee that gave the approval. The use of animals in experiments will have observed the Interdisciplinary Principles and Guidelines for the Use of Animals in Research, Testing, and Education by the New York Academy of Sciences, Ad Hoc Animal Research Committee.

Graphical Abstract
Authors should provide a graphical abstract (a beautifully designed feature figure) to represent the paper aiming to catch the attention and interest of readers. Graphical abstract will be published online in the table of content. The graphical abstract should be colored, and kept within an area of 12 cm (width) x 6 cm (height) or with similar format. Image should have a minimum resolution of 300 dpi and line art 1200dpi.
Note: Height of the image should be no more than the width. Please avoid putting too much information into the graphical abstract as it occupies only a small space. Authors can provide the graphical abstract in the format of PDF, Word, PowerPoint, jpg, or png, after a manuscript is accepted for publication. For preparing a Professional Graphical Abstract, please click **here**.



Presentation of the article

Main Format

First page of the manuscripts must be properly identified by the title and the name(s) of the author(s). It should be typed in Times New Roman (font sizes: 17pt in capitalization for the title, 10pt for the section headings in the body of the text and the main text, double spaced, in A4 format with 2cm margins. All pages and lines of the main text should be numbered consecutively throughout the manuscript. Abbreviations in the article title are not allowed.
Manuscripts should be arranged in the following order:
1. TITLE (brief, attractive and targeted);

2. Name(s) and Affiliation(s) of author(s) (including post code) and corresponding E-mail; ORCID: 0000-0000-0000-0000
3. ABSTRACT
4. Key words (separate by semicolons; or comma,)
5. Abbreviations (used in the manuscript)
6. INTRODUCTION
7. MATERIALS AND METHODS
8. RESULTS
9. DISCUSSION
10. CONCLUSION
11. DECLARATIONS
12. REFERENCES
13. Tables
14. Figure captions
15. Figures

Results and Discussion can be presented jointly.
Discussion and Conclusion can be presented jointly.

Article Sections Format

Title should be a brief phrase describing the contents of the paper. The first letter of each word in title should use upper case. The Title Page should include the author(s)'s full names and affiliations, the name of the corresponding author along with phone and e-mail information. Present address(es) of author(s) should appear as a footnote.

Abstract should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. The abstract should be 150 to 350 words in length. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited.

Following the abstract, about 3 to 8 key words that will provide indexing references should be listed.

Introduction should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines.

Materials and Methods should be complete enough to allow experiments to be reproduced. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Capitalize trade names and include the manufacturer's name and address. Subheadings should be used. Methods in general use need not be described in detail. The ethical approval for using animals in the researches should be indicated in this section with a separated title.

Results should be presented with clarity and precision. The results should be written in the past tense when describing findings in the author(s)'s experiments. Previously published findings should be written in the present tense. Results should be explained, but largely without referring to the literature. Discussion, speculation and detailed interpretation of data should not be included in the results but should be put into the discussion section.

Discussion should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

Conclusion should be brief and tight about the importance of the work or suggest the potential applications and extensions. This section should not be similar to the Abstract content.

Declarations including Ethics, Consent to publish, Competing interests, Authors' contributions, and Availability of data and materials are necessary.

Acknowledgments of persons, grants, funds, etc should be brief.

Tables should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph forms or repeated in the text.

Figure legends should be typed in numerical order on a separate sheet. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or PowerPoint before pasting in the Microsoft Word manuscript file. Use Arabic numerals to designate figures and upper case letters for their parts (Figure 1). Begin each legend with a title and include sufficient description so that the figure is understandable without reading the text of the manuscript. Information given in legends should not be repeated in the text.

Declarations section - Please include declarations heading

Please ensure that the sections:
-Ethics (and consent to participate)
-Consent to publish
-Competing interests
-Authors' contributions
-Availability of data and materials
are included at the end of your manuscript in a Declarations section.

Consent to Publish
Please include a 'Consent for publication' section in your manuscript. If your manuscript contains any individual person's data in any form (including individual details, images or videos), consent to publish must be obtained from that person, or in the case of children, their parent or legal guardian. All presentations of case reports must have consent to publish. You can use your institutional consent form or our consent form if you prefer. You should not send the form to us on submission, but we may request to see a copy at any stage (including after publication). If your manuscript does not contain any individual persons data, please state "Not applicable" in this section.

Authors' Contributions
For manuscripts with more than one author, JWPR require an Authors' Contributions section to be placed after the Competing Interests section.
An 'author' is generally considered to be someone who has made substantive intellectual contributions to a published study. To qualify as an author one should 1) have made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) have been involved in drafting the manuscript or revising it critically for important intellectual content; and 3) have given final approval of the version to be published. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content. Acquisition of funding, collection of data, or general supervision of the research group, alone, does not justify authorship.
We suggest the following format (please use initials to refer to each author's contribution): AB carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. JY carried out the immunoassays. MT participated in the sequence alignment. ES participated in the design of the study and performed the statistical analysis. FG conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.
For authors that equally participated in a study please write 'All/Both authors contributed equally to this work.' Contributors who do not meet the criteria for authorship should be listed in an acknowledgements section.

Competing Interests
Competing interests that might interfere with the objective presentation of the research findings contained in the manuscript should be declared in a paragraph heading "Competing interests" (after Acknowledgment section and before References). Examples of competing interests are ownership of stock in a company, commercial grants, board membership, etc. If there is no competing interest, please use the statement "The authors declare that they have no competing interests.".
Journal World's Poultry Research adheres to the definition of authorship set up by The International Committee of Medical Journal Editors (ICMJE). According to the ICMJE authorship criteria should be based on 1) substantial contributions to conception and design of, or acquisition of data or analysis and interpretation of data, 2) drafting the article or revising it critically for important intellectual content and 3) final approval of the version to be published. Authors should meet conditions 1, 2 and 3. It is a requirement that all authors have been accredited as appropriate upon submission of the manuscript. Contributors who do not qualify as authors should be mentioned under Acknowledgements.

Change in authorship
We do not allow any change in authorship after provisional acceptance. We cannot allow any addition, deletion or change in sequence of author name. We have this policy to prevent the fraud.

Acknowledgements
We strongly encourage you to include an Acknowledgements section between the Authors' contributions section and Reference list. Please acknowledge anyone who contributed towards the study by making substantial contributions to conception, design, acquisition of data, or analysis and interpretation of data, or who was involved in drafting the manuscript or revising it critically for important intellectual content, but who does not meet the criteria for authorship. Please also include their source(s) of funding. Please also acknowledge anyone who contributed materials essential for the study.
Authors should obtain permission to acknowledge from all those mentioned in the Acknowledgements. Please list the source(s) of funding for the study, for each author, and for the manuscript preparation in the acknowledgements section. Authors must describe the role of the funding body, if any, in study design; in the collection, analysis, and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication.

Data Deposition
Nucleic acid sequences, protein sequences, and atomic coordinates should be deposited in an appropriate database in time for the accession number to be included in the published article. In computational studies where the sequence information is unacceptable for inclusion in databases because of lack of experimental validation, the sequences must be published as an additional file with the article.

References:

A JWPR reference style for **EndNote** may be found **here**.

1. All references to publications made in the text should be presented in a list with their full bibliographical description. DOI number or the link of article should be added to the end of the each reference.
2. In the text, a reference identified by means of an author`s name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author`s surname should be mentioned, followed by 'et al`. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like 'a` and 'b` after the date to distinguish the works.
3. References in the text should be arranged chronologically (e.g. Kelebeni, 1983; Usman and Smith, 1992 and Agindotan et al., 2003). The list of references should be arranged alphabetically on author's surnames, and chronologically per author. If an author's name in the list is also mentioned with co-authors, the following order should be used: Publications of the single author, arranged according to publication dates - publications of the same author with one co-author - publications of the author with more than one co-author. Publications by the same author(s) in the same year should be listed as 1992a, l992b, etc.
4. Names of authors and title of journals, published in non-latin alphabets should be transliterated in English.
5. A sample of standard reference is "1th Author surname A, 2th Author surname B and 3th Author surname C (2013). Article title should be regular and 9 pt. Journal of World`s Poultry Research, Volume No. (Issue No.): 00-00." DOI:XXX."
6. Journal titles should be full in references. The titles should not be italic.
7. References with more than 10 authors should list the first 10 authors followed by 'et al.'
8. The color of references in the text of article is blue. Example: (Preziosi et al., 2002; Mills et al., 2015).

9. At least 35% of the references of any submitted manuscript (for all types of article) should include scientific results published in the last five years.

-Examples (at the text- blue highlighted)

Abayomi (2000), Agindotan et al. (2003), Vahdatpour and Babazadeh (2016), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; Chukwura, 1987a,b; Tijani, 1993, 1995), (Kumasi et al., 2001).

--Examples (at References section)

a) For journal:

Lucy MC (2000). Regulation of ovarian follicular growth by somatotropin and insulin- like growth factors in cattle. Journal of Dairy Science, 83: 1635-1647.

Kareem SK (2001). Response of albino rats to dietary level of mango cake. Journal of Agricultural Research and Development. pp 31-38. DOI:XXX.

Chikere CB, Omoni VT and Chikere BO (2008). Distribution of potential nosocomial pathogens in a hospital environment. African Journal of Biotechnology, 7: 3535-3539. DOI:XXX.

Tahir Khan M, Bhutto ZA, Abbas Raza SH, Saeed M, Arain MA, Arif M, Fazlani SA, Ishfaq M, Siyal FA, Jalili M et al. (2016). Supplementation of different level of deep stacked broiler litter as a source of total mixed ration on digestibility in sheep and their effects on growth performance. Journal of World`ˢ Poultry Research, 6(2): 73-83. DOI: XXX

b) For symposia reports and abstracts:

Cruz EM, Almatar S, Aludul EK and Al-Yaqout A (2000). Preliminary Studies on the Performance and Feeding Behaviour of Silver Pomfret (Pampus argentens euphrasen) Fingerlings fed with Commercial Feed and Reared in Fibreglass Tanks. Asian Fisheries Society Manila, Philippine 13: 191-199.

c) For edited symposia, special issues, etc., published in a journal:

Korevaar H (1992). The nitrogen balance on intensive Dutch dairy farms: a review. In: A. A. Jongebreur et al. (Editors), Effects of Cattle and Pig Production Systems on the Environment: Livestock Production Science, 31: 17-27.

d) For books:

AOAC (1990). Association of Official Analytical Chemists. Official Methods of Analysis, 15th Edition. Washington D.C. pp. 69-88. Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

e) Books, containing sections written by different authors:

Kunev M (1979). Pig Fattening. In: A. Alexiev (Editor), Farm Animal Feeding. Vol. III. Feeding of Different Animal Species, Zemizdat, Sofia, p. 233-243 (Bg).

In referring to a personal communication the two words are followed by the year, e.g. (Brown, J. M., personal communication, 1982). In this case initials are given in the text.

Nomenclature and Abbreviations:

Nomenclature should follow that given in NCBI web page and Chemical Abstracts. Standard abbreviations are preferable. If a new abbreviation is used, it should be defined at its first usage. Abbreviations should be presented in one paragraph, in the format: "term: definition". Please separate the items by ";".
E.g. ANN: artificial neural network; CFS: closed form solution; ...

Abbreviations of units should conform with those shown below:

Decilitre	dl	**Kilogram**	kg
Milligram	mg	**hours**	h
Micrometer	mm	**Minutes**	min
Molar	mol/L	**Mililitre**	ml
Percent	%		

Other abbreviations and symbols should follow the recommendations on units, symbols and abbreviations: in "A guide for Biological and Medical Editors and Authors (the Royal Society of Medicine London 1977).
Papers that have not been published should be cited as "unpublished". Papers that have been accepted for publication, but not yet specified for an issue should be cited as "to be published". Papers that have been submitted for publication should be cited as "submitted for publication".

Formulae, numbers and symbols:

1. Typewritten formulae are preferred. Subscripts and superscripts are important. Check disparities between zero (0) and the letter 0, and between one (1) and the letter I.
2. Describe all symbols immediately after the equation in which they are first used.
3. For simple fractions, use the solidus (/), e.g. 10 /38.
4. Equations should be presented into parentheses on the right-hand side, in tandem.
5. Levels of statistical significance which can be used without further explanations are *P < 0.05, **P < 0.01, and ***P < 0.001
6. In the English articles, a decimal point should be used instead of a decimal comma.
7. In chemical formulae, valence of ions should be given, e.g. Ca2+ and CO32-, not as Ca++ or CO3.
8. Numbers up to 10 should be written in the text by words. Numbers above 1000 are recommended to be given as 10 powered x.
9. Greek letters should be explained in the margins with their names as follows: Aα - alpha, Bβ - beta, Γγ - gamma, Δδ - delta, Eε - epsilon, Zζ - zeta, Hη - eta, Θθ - theta, Iι - iota, Kκ - kappa, Λλ - lambda, Mμ - mu, Nν - nu, Ξξ - xi, Oo - omicron, Ππ - pi, Pρ - rho, Σσ - sigma, Tτ - tau, Yυ - ipsilon, Φφ - phi, Xχ - chi, Ψψ - psi, Ωω - omega.

Review/Decisions/Processing

Firstly, all manuscripts will be checked by one of the plagiarism finding tools (iThenticate, PlagScan and or Docol©c). A double-blind reviewing model is used by JWPR for non-plagiarized papers. The manuscript is edited and reviewed by the English language editor and three reviewers selected by section editor of JWPR respectively. Also, a reviewer result form is filled by reviewer to guide authors. Possible decisions are: accept as is, minor revision, major revision, or reject. See sample of evaluation form. The estimated time from submission to first decision is 5.2 weeks and the estimated time from submission to final decision is 6.6 weeks. The estimated time for final publication of accepted manuscript is 6 weeks

To submit a revision please click here, fill out the form, and mark "Revised" attach the revision (MSword) and submit when completed.
After review and editing the article, a final formatted proof is sent to the corresponding author once again to apply all suggested corrections during the article process. The editor who received the final revisions from the corresponding authors shall not be hold responsible for any mistakes shown in the final publication. Manuscripts with significant results are typically reviewed and published at the highest priority.

Plagiarism
There is a zero-tolerance policy towards plagiarism (including self-plagiarism) in our journals. Manuscripts are screened for plagiarism by one of the plagiarism finding tools (iThenticate, PlagScan and or Docol©c), before or during publication, and if found they will be rejected at any stage of processing. See sample of Docol©c-Report.

Declaration
After manuscript accepted for publication, a declaration form will be sent to the corresponding author who that is responsible to coauthors' agreements to publication of submitted work in JWPR after any amendments arising from the peer review.

Date of issue
The journal will be issued on 25th of March, June, September and December, each year.

Publication charges
No peer-reviewing charges are required. However, the publication costs are covered through article processing charges (APCs). There is a modest APC of 150 Euro(€) editor fee for the processing of each primary accepted paper (1000-4000 words) to encourage high-quality submissions. APCs are only charged for articles that pass the pre-publication checks and are published. A surcharge will be placed on any article that is over 4000 words in length to cover the considerable additional processing costs. Payment can be made by credit card, bank transfer, money order or check. Instruction for payment is sent during publication process as soon as manuscript is accepted. Meanwhile, this journal encourages the academic institutions in low-income countries to publish high quality scientific results, free of charges.

WORD COUNT	PRICE*
1000-4000 words	€150
over 4000 words	€230

* The prices are valid until 30th June 2024.

The Waiver policy
The publication fee will be waived for invited authors, authors of hot papers, and corresponding authors who are editorial board members of the *Journal of World's Poultry Research*. The Journal will consider requests to waive the fee for cases of financial hardship (for high quality manuscripts and upon acceptance for publication). Requests for waiver of the submission fee must be submitted via individual cover letter by the corresponding author and cosigned by an appropriate institutional official to verify that no institutional or grant funds are available for the payment of the fee. Letters including the manuscript title and manuscript ID number should be sent to: editor [at] jwpr.science-line.com. It is expected that waiver requests will be processed and authors will be notified within two business day.

The OA policy
Journal of World's Poultry Research is an open access journal which means that all content is freely available without charge to the user or his/her institution. Users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author. This is in accordance with the *BOAI definition of Open Access*.

Scienceline Language Editing Services
We suggest that authors whose first language is not English have their manuscripts checked by a native English speaker before submission. This is optional, but will help to ensure that any submissions that reach peer review can be judged exclusively on academic merit. We offer a Scienceline service, and suggest that authors contact as appropriate. Please note that use of language editing services is voluntary, and at the author's own expense. Use of these services does not guarantee that the manuscript will be accepted for publication, nor does it restrict the author to submitting to Scienceline journals. You can send the article/s to the following Emails: daryoushbabazadeh@gmail.com ; info@science-line.com
For more information about editing services please visit here.

Paper Submission Flow

Submission Preparation Checklist

Authors are required to check off their submission's compliance with all of the following items, and submissions may be returned to authors that do not adhere to the following guidelines.
The submission has not been previously published, nor is it before another journal for consideration (or an explanation has been provided in Comments to the Editor).
The submission file is in Microsoft Word, RTF, or PDF document file format.
Where available, URLs for the references have been provided.
The text is single-spaced; uses a 12-point font; and all illustrations, figures, and tables are placed within the text at the appropriate points, rather than at the end.
The text adheres to the stylistic and bibliographic requirements outlined in the Author Guidelines.

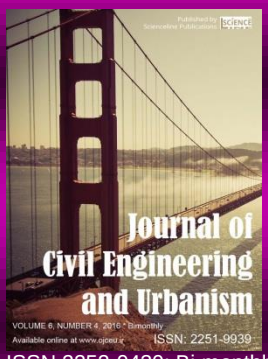
SCIENCELINE PUBLISHING CORPORATION

Scienceline Publication Ltd is a limited liability non-profit non-stock corporation incorporated in Turkey (Company No. 0757086921600001). Scienceline journals that concurrently belong to many societies, universities and research institutes, publishes internationally peer-reviewed open access articles and believe in sharing of new scientific knowledge and vital research in the fields of life and natural sciences, animal sciences, engineering, art, linguistic, management, social and economic sciences all over the world. Scienceline journals include:

Online Journal of Animal and Feed Research

ISSN 2228-7701; Bi-monthly
View Journal | Editorial Board
Email: editors@ojafr.ir
Submit Online >>

Journal of Civil Engineering and Urbanism

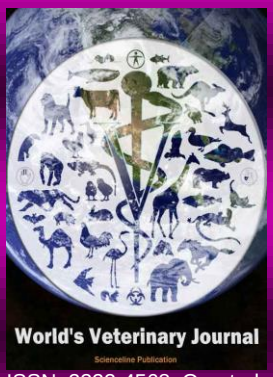


ISSN 2252-0430; Bi-monthly
View Journal | Editorial Board
Email: ojceu@ojceu.ir
Submit Online >>

Journal of Life Sciences and Biomedicine

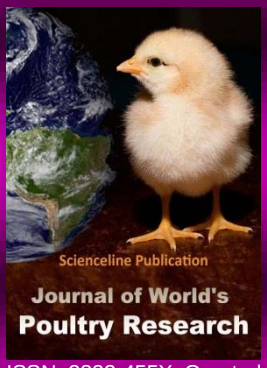
ISSN: 2251-9939; Bi-monthly
View Journal | Editorial Board
Email: editors@jlsb.science-line.com
Submit Online >>

World's Veterinary Journal



ISSN: 2322-4568; Quarterly
View Journal | Editorial Board
Email: editor@wvj.science-line.com
Submit Online >>

Journal of World's Poultry Research



ISSN: 2322-455X; Quarterly
View Journal | Editorial Board
Email: editor@jwpr.science-line.com
Submit Online >>

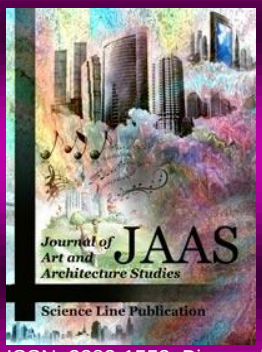
Asian Journal of Medical and Pharmaceutical Researches

ISSN: 2322-4789; Quarterly
View Journal | Editorial Board
Email: editor@ajmpr.science-line.com
Submit Online >>

Journal of Educational and Management Studies

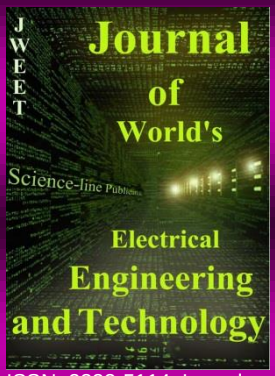
ISSN: 2322-4770; Quarterly
View Journal | Editorial Board
Email: info@jems.science-line.com
Submit Online >>

Journal of Art and Architecture Studies



ISSN: 2383-1553; Biannually
View Journal | Editorial Board
Email: jaas@science-line.com
Submit Online >>

Journal of World's Electrical Engineering and Technology



ISSN: 2322-5114; Irregular
View Journal | Editorial Board
Email: editor@jweet.science-line.com
Submit Online >>

Asian Journal of Social and Economic Sciences

ISSN: 2383-0948; Quarterly
View Journal | Editorial Board
Email: ajses@science-line.com
Submit Online >>

Journal of Applied Business and Finance Researches

ISSN: 2382-9907; Quarterly
View Journal | Editorial Board
Email: jabfr@science-line.com
Submit Online >>

Scientific Journal of Mechanical and Industrial Engineering

ISSN: 2383-0980; Quarterly
View Journal | Editorial Board
Email: sjmie@science-line.com
Submit Online >>

Scienceline is a non-profit organisation inspired by research funders and led by scholars. Our mission is to help researchers accelerate discovery and innovation by operating a platform for research communication that encourages and recognises the most responsible behaviours in science.

Scienceline Publications, Ltd is a limited liability non-profit non-stock corporation registered in the State of Erzurum, Turkey, with company number 0757086921600001, and branch number 18677/25379 at the address: Scienceline Publications, Ltd., Ömer Nasuhi Bilmen Road, Dönmez Apart., G1/6, Yakutiye, Erzurum 25100, Turkey